Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania, USA☆

Marlise Franke-Snyder a, David D. Douds Jr a,*, Larisa Galvez a, John G. Phillips a, Peggy Wagoner b, Laurie Drinkwater b, Joseph B. Morton c

a USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA
b Rodale Institute, 611 Siegfriedale Road, Kutztown, PA 19530, USA
c West Virginia University, 401 Brooks Hall, Morgantown, WV 26506, USA

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Abstract

We compared the composition and structure of the communities of arbuscular mycorrhizal (AM) fungi associated with maize (Zea mays L.) and soybeans (Glycine max (L.) Merr.) in a conventional (CON) and two low-input (LI) farming systems to better understand the relationship among AM fungi present in different agricultural systems. One LI system utilized animal manure (LI-AM) and the other green manure (LI-GM) as the nitrogen source. Spores were extracted from rhizosphere soil samples by wet-sieving to perform microscopic identification of the species and to assess frequency of occurrence. These data were used to calculate species richness, Shannon and Wiener index of diversity, and indices of dominance among other ecological measures. The results indicated that 15 consecutive years of farming under the three management practices did not cause many differences among the fungal communities. The majority of the 15 fungal species found throughout the site were present in all treatments. Sporulation of particular fungal species differed among farming systems and/or among hosts, but the general structure of AM fungal communities (according to most ecological measures) was similar for all treatments. Trap cultures were set up for the different treatments and grown for three cycles to try to recover species with low or no sporulation in natural conditions. These results also supported our conclusion about the homogeneity of the communities in the different farming system/plant host combinations, because only one species (Glomus constrictum) that was not found in the field samples sporulated in trap culture pots. Given that differences in sporulation may reflect differential rates of growth, three undescribed species plus Glomus mosseae and Glomus etunicatum were better established, both in the field and in trap cultures, than the other 10 species present in these soils. Also, Gigaspora gigantea accounted for more than 60% of the total volume of spores produced in each treatment, with the exception of conventional plots planted with maize where spore biovolumes were spread much more evenly among several fungal species suggesting that carbon allocation relationships were much more balanced in these plots. The focus of future studies at these sites will be a comparison of the efficacy among the communities in terms of enhancement of plant growth. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Arbuscular mycorrhizal fungus spores; Dominance; Biovolume; Sustainable agriculture

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* Corresponding author. Fax: +215-233-6581.
E-mail address: ddouds@arserrc.gov (D.D. Douds Jr).
1. Introduction

The role of microbial communities in the maintenance of soil quality and assistance of plant growth is important even in agroecosystems where human interference is pronounced. They are involved in processes such as acquisition and recycling of nutrients important for plant growth, antagonism of detrimental organisms, and participation in the formation and maintenance of soil structure (Visser and Parkinson, 1992; Bever, 1994; Schimel, 1995; Pankhurst et al., 1996).

Arbuscular mycorrhizal (AM) fungi are important components of the microbiota, performing several of the tasks listed above, and especially are appreciated for their role in nutrient transfer from soil to plants (Bolan, 1991; Jakobsen et al., 1992a,b; George et al., 1995). Recently, more attention has been given to their role in soil aggregation (Bethlenfalvay, 1992; Miller and Jastrow, 1992; Schreiner and Bethlenfalvay, 1995; Wright and Upadhyaya, 1996).

We are still developing our understanding of the relationships among members of mycorrhizal fungal communities. Biodiversity has been used as a means to characterize and compare the number and relative abundance of organisms within and among communities (Whittaker, 1965; Peet, 1974; Ludwig and Reynolds, 1988; Magurran, 1988; Visser and Parkinson, 1992). Dominance weights abundance and favors the most common species (Peet, 1974; Magurran, 1988). There are known limitations to the use of these ecological indices (O’Donnell et al., 1994; Pankhurst et al., 1996; Zak and Visser, 1996), which may become accentuated when used for characterization of AM fungal communities because of the unique biology of this group of fungi. Though there has been exciting recent progress in the application of molecular techniques to identify genotypic diversity of AM fungi in roots and soil (Helgason et al., 1999), mycorrhizologists presently are limited to the use of spores to identify and count the fungi to perform diversity studies (Morton et al., 1995; Merryweather and Fitter, 1998). Spore morphology and numbers, however, may not necessarily reflect the vegetative community structure (Douds and Millner, 1999). These facts influence interpretation of such studies.

There are many questions that can be investigated by the study of microbial diversity. Some researchers have addressed the need to compare the microbial communities of agroecosystems maintained with conventional, high chemical input practices, to those under reduced-input farming (Hassink et al., 1991; Workneh and van Bruggen, 1994; Hooker and Black, 1995; Kennedy and Smith, 1995; Buyer and Kaufman, 1996). Several studies have showed greater numbers of AM fungus spores and more inoculum of AM fungi in soils under low-input as compared to conventional, chemical-based agriculture (Limonard and Ruissen, 1989; Vivekanandan and Fixen, 1991; Douds et al., 1993; Kurle and Pfleger, 1994). There is need for comparison of AM fungal community structure between soils under the two management regimes at the same site (Kurle and Pfleger, 1996).

We determined diversity, dominance and biovolume patterns for the communities of AM fungi from soils farmed by conventional, high-input methods and by two types of low-input agriculture based on spore populations. The conventional (CON) maize and soybean rotation incorporated inorganic fertilizers and chemical control of weeds. Both low-input systems use rotary hoeing, cultivation and crop rotations for weed control. One low-input system used animal manure as a nitrogen source (LI-AM), and a rotation of maize, soybeans, small grains and hay. The other incorporated green manure as the nitrogen source (LI-GM) in a rotation of maize, soybeans, small grains and leguminous cover crops. These systems are part of the Rodale Institute’s Farming Systems Trial (Liebhardt et al., 1989). The objectives of the Farming Systems Trial when it was established in 1981 were to define yield-limiting factors that occur during the transition from conventional to low-input farming, identify methods of minimizing yield reductions, and identify physical, chemical, and biological processes that occur during conversion to low-input methods. Comparing the mycorrhizal fungi in these systems gave us information about sporulation patterns associated with farming practices and about the composition of these communities.

2. Materials and methods

2.1. Sampling and experimental design

Soil samples were collected from the Farming Systems Trial field (6.1 ha) (Liebhardt et al., 1989; Drinkwater et al., 1998) at the Rodale Institute Experimental Farm in Kutztown, PA on 13 October 1995. This trial
was established in 1981 to monitor input and yield differences among three farming systems. The site had previously been utilized solely for conventional agriculture (Doran et al., 1987). The dominant soil type was Comly silt loam (fine-loam, mixed, mesic Typic Fragiudalf).

Treatments consisted of three farming systems (FS), i.e., a conventional, a low-input with animal manure, and a low-input with green manure in which two crops, maize (*Zea mays* L.) (MZ) and soybeans (*Glycine max* (L.) Merr.) (SOY), were grown that year. Each FS × crop combination was replicated in 6.1 × 91.5 m plots within four blocks in the 6.1 ha field. Seven rhizosphere soil sub-samples were collected at evenly-spaced intervals along one row within each plot. These were combined to yield four pooled samples (one per block) for each of the six FS × crop combinations. Soil samples were placed in zip lock plastic bags and stored at 4°C until analysis. Given the complex nature of the analyses to be performed, samples were taken at only one sampling date, in the fall, when populations of mycorrhizal spores were high and in good condition (Douds et al., 1993; Kirchner et al., 1993; Koske et al., 1997).

### 2.2. Spore extraction and taxonomic analysis

Spores were isolated from 350 cm³ of soil from the pooled samples via wet sieving (Gerdemann and Nicolson, 1963) and centrifugation (Jenkins, 1964). A very fine sieve (38 μm opening) was used to collect the small spores usually present in these soils, and the coarse material remaining on the top sieve (425 μm opening) also was checked for sporocarps and very large spores. Spores were separated into groups according to general morphological similarities under a Zeiss stereomicroscope and the diameter of spores was measured. Permanent slides of all spores were prepared by placing them in polyvinyl alcohol-lactic acid-glycerin (PVLG) mixed with Melzer's reagent (1:1 vol/vol) (Franke, 1992). Spores were cracked open under the cover slip to allow for observation of spore wall and inner wall characteristics. Spores were identified to species according to classical morphological analysis under a Zeiss compound microscope and their frequencies were recorded. Important taxonomic characters (Franke, 1992) included number and type of layers of the spore wall and their staining reaction to Melzer’s reagent; characteristics of inner walls, when present; morphology of the subtending hypha at point of attachment; and color and size range of spores. Spores that could be identified were counted, even if parasitized or nonviable, given the assumption that the spores had been produced during the past growing season. Spores did not seem to persist in the soil for longer periods of time according to observations from the preliminary work performed at these sites.

### 2.3. Trap cultures

Greenhouse pot cultures also were established with the soil samples to recover spores from AM fungal species present in the soil, including some which may not have sporulated at the time of sampling. We repeated these trap cultures for three consecutive cycles, modifying the culture conditions slightly each time. At first, a portion of the mix of the four-replicate composite soil samples from each treatment was diluted with an equal volume of a sterilized growing mix used in our greenhouse experiments (vermiculite:soil:surface:sand at 1:3/4:3/4:1, vol/vol), potted in 15.25 cm diameter pots, and seeded with maize. These trap cultures were allowed to grow for 5 months, after which cores of growth medium were taken from each pot and spores were extracted as described above. All species present were recorded. The remaining soil mix and the plants of each pot were allowed to slowly dry at room temperature. The dried medium was then stored at 4°C for 1 month and the second generation trap culture was started, again using a 1:1 mix of the cold-stored first generation trap cultures with the sterilized growth medium described above. The host used this time was sorghum–sudangrass CV. ‘Sudax’ [*Sorghum bicolor* (L.) Moench] × [*Sorghum sudanense* (Piper) Stapf]. These cultures grew for 3 months, and the same harvesting procedure was followed. A third generation of trap cultures was established by taking the center core of the second generation cultures, representing roughly half of the total pot volume, placing it into a new pot, surrounding the core with sterilized sand, and sowing new Sudax seeds. These cultures were grown in the greenhouse for 8 months and spores then were extracted from both portions of the medium.

### 2.4. Ecological measures of diversity

Species richness, Shannon and Wiener index of diversity, evenness, Simpson’s index of dominance,
Table 1
Diversity measures used to describe communities of AM fungi

<table>
<thead>
<tr>
<th>Species richness</th>
<th>Measured as species density, i.e. the number of species in a specified collection area&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon–Wiener index of diversity (H')</td>
<td>( H' = -\sum p_i \ln p_i )</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>( E = H'/H_{max} )</td>
</tr>
<tr>
<td>Biovolume (Biovol)</td>
<td>( \text{Biovol} = 4/3 \pi r^3 )</td>
</tr>
<tr>
<td>Simpson’s index (D)</td>
<td>( D = \sum [n_i(n_i-1)/N(N-1)] )</td>
</tr>
<tr>
<td>Modified Berger–Parker index (d)</td>
<td>( d = \text{Biovol}<em>{max}/\text{Biovol}</em>{total} )</td>
</tr>
</tbody>
</table>

<sup>a</sup> \( p_i \) is the proportion of individuals found in the \( i \)th species, estimated as \( n_i/N \), where \( n_i \) is the number of individuals in the \( i \)th species and \( N \) is the total number of individuals. \( H_{max} \) is the maximum diversity and it is calculated as \( \ln S \), where \( S \) is the number of species recorded. \( r \) is radius calculated from the diameters of a sample of spores of a species. \( \text{Biovol}_{max} \) is the biovolume of the species that presents the greatest biovolume in a treatment and \( \text{Biovol}_{total} \) is the total spore biovolume in the treatment.

species biovolume (based on spore volumes) and modified Berger and Parker index of dominance (based on biovolume data) were calculated (see Table 1 for formulae). Certain species were combined into groups for calculation of biovolume: one containing *Glomus occultum* Walker (isolate FST10/95), *Glomus aggregatum*-like (FST10/95) and *Glomus microaggregatum* Koske, Gemma & Olexia (FST10/95) and another with the *Glomus spurcum*-like (FST10/95) and the Small, yellow *Glomus* (FST10/95) isolates (Table 2). This was necessary because these species could not be differentiated under the stereomicroscope and therefore were measured together. *Glomus microaggregatum* produced very small spores (from 10 to 40 μm in diameter) singly in the soil or in large numbers in very tight clusters inside other empty, older mycorrhizal fungus spores. The calculations are made counting only the loose, individual spores, unless otherwise specified. We divided the volume of the old spores by the mean volume of *G. microaggregatum* spores to estimate the number of spores found in the clusters within old spores.

Analysis of variance (ANOVA) was performed for diversity and dominance indices and Duncan’s test was applied for comparison of means when appropriate. Percent values were transformed by arcsin \( \sqrt{x} \) and spore counts were transformed by \( \log (x+1) \) or \( \sqrt{x+1} \).

Table 2
Main morphological characteristics of undescribed AM fungal species found in study soils

<table>
<thead>
<tr>
<th>Undescribed species designation</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Glomus spurcum</em>-like</td>
<td>Small hyaline spores (≈50 μm), with a very thin subtending hypha (≈1–2 μm diameter); spore wall has thick and flexible laminae, which tend to form an even, thick layer (&gt; 2.5 μm) on spores cracked under the cover slip; there seems to be an outer mucilaginous layer, but very young spores have not been seen to confirm this observation</td>
</tr>
<tr>
<td>Small yellow <em>Glomus</em></td>
<td>Small bright yellow spores (≈40–50 μm) with straight subtending hypha; simple spore wall, with an outer thin, hyaline layer attached to the thick laminated layer</td>
</tr>
<tr>
<td><em>Glomus aggregatum</em>-like</td>
<td>Spores similar to <em>Glomus aggregatum</em>, also appearing inside older spores; a mucilaginous layer surrounding the spores; mycorrhizal association of this type of spores has not yet been observed in a single-species pot culture</td>
</tr>
<tr>
<td><em>Glomus E-1</em></td>
<td>Unique <em>Glomus</em>, crystalline appearance, ≈95–160 μm diameter (average 141 μm); subtending hypha very small and fragile, connected to outer layer of spore wall only; presence of inner wall layers, innermost stains mauve in Melzer’s reagent; multiple germination, through the spore wall</td>
</tr>
<tr>
<td><em>Glomus C-1</em></td>
<td>Hyaline spores, ≈(45)-55-(85) μm, with a very fragile subtending hypha which tends to break off easily; transparent outer spore wall forming wrinkles around a more rigid ‘unit’ layer (laminations hard to distinguish), the inner layer stains pink in Melzer’s reagent:PVLG mix; spore contents tend to form small lumps of material, which turn yellow in Melzer’s reagent:PVLG mix; mycorrhizal association of this type of spores has not yet been observed in a single species pot culture</td>
</tr>
</tbody>
</table>
3. Results

Most spores retrieved possessed recognizable morphological characteristics which permitted their identification. Spores with indistinctive features for character analysis were not considered. Data on root colonization of field plants and infectivity potential of soils under these farming systems at this site can be found in Douds et al. (1993).

3.1. Species composition

Fifteen species of AM fungi were found. Only one species belonged to the genus *Gigaspora* (Gerd. & Trappe) Walker & Sanders, i.e. *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe (FST10/95), and one species belonged to the genus *Scutellospora* Walker & Sanders, i.e. *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders (FST10/95). All others belonged to the genus *Glomus* (Tul. & Tul.): *Glomus claroideum* Schenck & Smith (FST10/95); *Glomus constrictum* Trappe (FST10/95); *Glomus etunicatum* Becker & Gerdemann (FST10/95); *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe (FST10/95); *Glomus geosporum* (Nicol. & Gerd.) Walker (FST10/95); *Glomus microaggregatum* Koske, Gemma & Oleksa (FST10/95); *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe (FST10/95); *Glomus occultum* Walker (FST10/95) and five other unknown species (see Table 2 for a brief description of characters), here designated *Glomus aggregatum*-like (FST10/95), *Glomus C-1* (FST10/95), *Glomus E-1* (FST10/95), *Glomus spurcum*-like (FST10/95), and Small Yellow *Glomus* (FST10/95). *Glomus C-1* (Table 2) was presumed to be a Glomalean fungus in this study, even though it could not be successfully cultured in greenhouse pots. More intense attempts to culture this specimen will be needed to confirm its mycorrhizal status, but general similarity with *Glomus*-like subtending hypha (very fragile and rarely remaining attached to the spores), and the presence of somewhat flexible spore wall layers, led us to include this putative species as part of the AM fungal community. *Glomus E-1*, another undescribed species, has sporulated in association with bahiagrass (*Paspalum notatum* Flugge) in two greenhouse cultures. Other attempts to culture this fungus were unsuccessful, even when pre-germinated spores were used. All other undescribed species were successfully cultured. We also have single-species cultures of *G. gigantea*, *G. mosseae*, *G. claroideum*, *G. etunicatum* and *G. geosporum*. *Glomus occultum* and *G. microaggregatum* appear in mixed-species cultures. *Glomus fasciculatum* and *S. pellucida*, rare species in the FST communities, were not retrieved in enough numbers to establish cultures.

3.2. Ecological measures

Species richness was ≈10 for all treatments (Table 3). Similarly, no significant differences were found among Shannon and Wiener’s diversity indices and evenness of all treatments (Table 3), reflecting the general resemblance of the proportional abundances of spores of the different species among the six communities. No difference was found in Simpson’s index of dominance (Table 3), indicating that the general pattern of spore abundance (from the most to the least dominant) was consistent under the various farming treatments. Significant differences were detected in the modified Berger and Parker indices (Table 3). This volume-based index reflects differences in dominance of large versus small-spored species among the six communities. Most of the spores collected were less than 80 μm in diameter, and just a few were over 180 μm. The biovolume of each AM fungal species as a percentage of the total volume of spores in each pooled sample was calculated in the six treatments (Table 4). Over 50% of the total spore volume belonged to *G. gigantea* spores for all communities except CON/MZ where spore biovolume was more evenly distributed among several species. This phenomenon is reflected by the Berger and Parker index, i.e. dominance is strong in all treatments except CON/MZ. Table 4 also demonstrates the rarity of some AM fungal species at this site/sampling point. The numbers between parenthesis in Table 3 represent the values of the indices when the large clusters of *G. microaggregatum* spores are included in the calculations. Shannon and Wiener’s indices became much smaller due to the dominance of these small spores (notice higher Simpson’s values on the other hand), especially in the low-input systems. Dominance as measured by spore volume was still high, but Berger and Parker values became more evenly distributed.
Table 3
Diversity measurements of AM fungal communities in conventional and low-input agricultural systems

<table>
<thead>
<tr>
<th>ecological parameters for Maize</th>
<th>Treatments</th>
<th>ecological parameters for Soybean</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species richness&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CON&lt;sup&gt;b&lt;/sup&gt;</td>
<td>LI-CG</td>
<td>LI-AN</td>
</tr>
<tr>
<td>9.75 A±0.96 (10.25)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.50 A±1.00 (10.50)</td>
<td>10.25 A±0.50 (10.75)</td>
<td>9.75 A±0.50 (10.0)</td>
</tr>
<tr>
<td>Shannon and Wiener diversity index (H&lt;sub&gt;I&lt;/sub&gt;)</td>
<td>1.57 A±0.28 (1.57)</td>
<td>1.61 A±0.43 (0.51)</td>
<td>1.95 A±0.13 (0.95)</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>0.69 A±0.09 (0.67)</td>
<td>0.69 A±0.20 (0.22)</td>
<td>0.84 A±0.06 (0.40)</td>
</tr>
<tr>
<td>Simpson’s index (D)</td>
<td>0.30 A±0.08 (0.31)</td>
<td>0.31 A±0.18 (0.77)</td>
<td>0.17 A±0.03 (0.61)</td>
</tr>
<tr>
<td>Modified Berger and Parker index (d)</td>
<td>0.36 B±0.07 (0.36) b&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.71 A±0.25 (0.57) ab</td>
<td>0.64 A±0.22 (0.55) ab</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Table 1 for definitions and formulae.
<sup>b</sup> Farming systems: CON, conventional; LI-CG, low-input, with green manure; LI-AN, low-input, with animal manure.
<sup>c</sup> Expressed as species density, i.e. the number of species in 350 cm<sup>3</sup> of soil.
<sup>d</sup> Mean of four pooled samples ±SEM, numbers followed by the same letter in a row are not significantly different (Duncan’s multiple range test; p<0.05).
<sup>e</sup> Means in parentheses are for data set containing the numerous G. microaggregatum spores found within other dead spores (see text); no statistical differences found for the four first ecological parameters in the table.
<sup>f</sup> Values followed by the same letter in this row are not statistically different (Duncan’s test at p=0.1).
Table 4
Percentage of biovolume of AM fungal species and groups of species in conventional and low-input agricultural systems

<table>
<thead>
<tr>
<th>AMF species or groups</th>
<th>Biovolume (%)</th>
<th>Maize</th>
<th>Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CON</td>
<td>LI-CG</td>
</tr>
<tr>
<td>Gigaspora gigantea</td>
<td>17.24 B(^{a}) ± 5.98</td>
<td>68.81 A± 14.37</td>
<td>60.86 A± 13.77</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>19.97 A± 6.12</td>
<td>17.79 A± 7.43</td>
<td>18.24 A± 4.85</td>
</tr>
<tr>
<td>Glomus etunicatum</td>
<td>20.79 A± 5.98</td>
<td>0.93 A± 0.55</td>
<td>4.99 A± 2.80</td>
</tr>
<tr>
<td>Glomus geosporum</td>
<td>2.48 B± 1.37</td>
<td>2.72 B± 0.75</td>
<td>4.06 B± 1.95</td>
</tr>
<tr>
<td>Glomus occultum, G. aggregatum-like</td>
<td>12.57 A± 9.84</td>
<td>0.67 A± 0.2</td>
<td>1.38 A± 0.40</td>
</tr>
<tr>
<td>Glomus C-1</td>
<td>10.36 A± 4.23</td>
<td>5.34 A± 3.84</td>
<td>5.86 A± 4.61</td>
</tr>
<tr>
<td>Glomus E-1</td>
<td>2.69 A± 0.83</td>
<td>0.15 B± 0.03</td>
<td>0.46 B± 0.11</td>
</tr>
<tr>
<td>Glomus C-1</td>
<td>0.00 A</td>
<td>0.74 A± 0.74</td>
<td>0.43 A± 0.43</td>
</tr>
<tr>
<td>Glomus fasciculatum</td>
<td>0.00 A</td>
<td>0.00 A</td>
<td>0.00 A</td>
</tr>
<tr>
<td>Glomus E-1</td>
<td>0.00 A</td>
<td>0.24 A± 0.25</td>
<td>0.00 A</td>
</tr>
</tbody>
</table>

a Farming systems: CON, conventional; LI-GM, low-input, with green manure; LI-AM, low-input, with animal manure.

b Means of four pooled samples ±SEM. Values followed by the same letter in a row are not significantly different (Duncan’s test at \( p \leq 0.05 \)).

3.3. Spore abundance

Though these communities appear to be similar when compared by single-value ecological indices which do not account for biovolume, these measures do not show the differences in abundance of spores of individual species of AM fungi among the six communities. Both farming system and crop had a significant \( p \leq 0.05 \) effect on the abundance of three species (Table 5). These differences can also be followed in the spore frequency chart (Fig. 1), comparing the six communities.

The greatest number of spores in all treatments came from groups of *G. microaggregatum* which ranged from...
Fig. 1. Frequency of appearance of spores of 11 species of AM fungi within soils farmed via conventional (CON), low-input with green manure (LI-GM), or low-input with animal manure (LI-AM) systems following a crop of maize (A) or soybean (B). Each bar represents the mean of four replicate subplots ±SEM. GLC-1, *Glomus* C1; LAGR, *Glomus aggregatum*-like; LSPR, *Glomus spurcum*-like; LMSS, *Glomus mosseae*; LETC, *Glomus etunicatum*; GGGT, *Gigaspora gigantea*; LMAG, *Glomus microaggregatum*; LCLR, *Glomus claroideum*; LGSP, *Glomus geosporum*; LOCT, *Glomus occultum* and SYG, small, yellow *Glomus*. See Table 2 for characteristics of GLC-1, LAGR, LSPR, and SYG. Total number of spores present in 350 cm$^{-3}$ soil for the three farming systems: maize, 420 ± 29 (CON), 228 ± 106 (LI-GM), and 453 ± 120 (LI-AM); soybean, 205 ± 30 (CON), 167 ± 42 (LI-GM), and 156 ± 25 (LI-AM).

50 to over 7000 spores (by estimation) as clusters inside a small number of dead spores of other species. It is unlikely that each spore in these clusters would act as an effective propagule, but rather the group would act as one propagule (therefore the presentation of data in Tables 3 and 4 without these spores). So, if these large numbers of spores were disregarded due to their spatial constraints, the following would be the most common species in each treatment (Fig. 1): *Glomus* C-1, *G. aggregatum*-like, *G. spurcum*-like, and *G. etunicatum* in CON/MZ; *Glomus* C-1 and *Glomus spurcum*-like in CON/SOY; *Glomus* C-1, *G. mosseae*, *G. etunicatum* and *Glomus spurcum*-like in LI-AM/MZ; *Glomus* C-1 and *G. aggregatum*-like in LI-AM/SOY; *Glomus* C-1 and *G. mosseae* in LI-GM/MZ; and no specific dominance in LI-GM/SOY.

3.4. Trap cultures

One new species was recovered from the trap cultures. *Glomus constrictum* Trappe sporulated in the third trap culture generation of CON/MZ and CON/SOY, LI-GM/SOY and LI-AM/SOY. Spores of *G. mosseae* and *G. claroideum* tended to dominate the soil/sand media of the first generation traps and in general, fewer species were found than in the field samples. *Glomus* E-1 appeared only in CON/SOY. Second generation traps were dominated by *G. mosseae*, *G. etunicatum* and *G. aggregatum*-like spores. Species richness varied from 9 to 11. In general, diversity was lower in the trap cultures than in the field, while dominance (Simpson’s index) was higher. The trap culture communities also tended to be less even. The relatively large spores of *G. mosseae* dominated the total spore volume (data not shown). *Glomus mosseae* and *G. etunicatum* spores were the most abundant in the third generation traps, followed by *G. aggregatum*-like spores. Spores of *G. constrictum* were also fairly common.

4. Discussion

The biology of AM fungi presents some limitations for the study of diversity. Assumptions for the computation of ecological indices involve the ability to define an individual of the group of interest and to fit it into a classification scheme (Peet, 1974). As in many plants, distant fungal tissues may be connected, and the multinucleate thallus of AM fungi encompasses a wide variety of genotypes not necessarily separated into functional or morphological units so that the delimitation of an individual is practically unfeasible (Morton et al., 1995; Zak and Visser, 1996; Clapp et al., 1999). This is complicated yet by the fact that these fungi only grow as endosymbionts in association with
host plant roots. The morphology of vegetative hyphae cannot efficiently and precisely be used to distinguish among AM fungal species. Their spores, on the other hand, are discrete units. Spores have complex and conserved morphological traits that are developmentally controlled, which enable us to group them into species (Franke and Morton, 1994; Morton, 1995). Therefore, unlike with plants, it is the reproductive phase of AM fungi which is used to quantify diversity. Spores and vegetative mycelium, however, behave as quite independent entities (Morton, 1993), making it difficult to relate the meaning of diversity patterns (based on spores) to functional ones (allocated to the mycelia). However, some inferences are appropriate (see below).

Taxonomy of AM fungi is in its early stages, and there is still some confusion on how to recognize and analyze characters, especially among the morphologically simpler spores and undescribed species as they are found (Morton, 1993; Allen et al., 1995). There are two tools that are valuable in overcoming the challenges in studies such as the present one. One tool is the comparison of spores found in field samples with those obtained in controlled conditions in pot and trap cultures. This allows for the appreciation of the complete set of characters present in well preserved and abundant specimens. In our case, spores from pot cultures established during other studies from the Rodale sites (Douds et al., 1993; Galvez et al., 1995) were available for examination prior to analysis of field-collected spores in this study. Another tool is the examination of characters of known species. Spore characteristics can be viewed either through the mycorrhizal collections of individual scientists or through the main international culture collections, such as International Culture Collection of Arbuscular and Vesicular-arbuscular Mycorrhizae (INVMAM) or European Bank of Glomaltes (BEG) to gain experience in distinguishing the important characters of AM fungal spores. Also, when previously undescribed species are found, these tools promote characterization by their unique features, even if a formal name is not conferred to them (a sound characterization is more valuable than the inaccurate placement of a specimen into a pre-existing taxon). We were able to overcome the greatest barriers in dealing with field-collected material by using the above guidelines and made taxonomic decisions with confidence.

An objective of the Farming Systems Trial was to identify physical, chemical, and biological processes that occur during conversion from conventional to low-input farming methods (Liebhardt et al., 1989). Our objective was to investigate whether the 15 consecutive years of these three farming systems induced changes in the AM fungal communities. Differences were not as pronounced as anticipated. Sporulation of particular species differed among farming systems and/or among hosts, but the general structure of AM fungal communities was similar for all treatments. Wander et al. (1995) sampled the soil at this site in 1991, 10 years after the implementation of the three different farming systems. They investigated the phospholipid fatty acid profiles of soil samples from management treatment replicates, and did not find differences in the structure of the microbial communities among the management systems. Buyer and Kaufman (1996) examined fast-growing, aerobic, culturable soil bacteria and fungi associated with maize rhizosphere at this site. Bacteria were identified by phospholipid fatty acid analysis and fungi by traditional taxonomical keys. Diversity, evenness and total counts for these microorganisms also were not significantly different among the conventional and the two low-input systems. A seasonal effect was detected, however, from June to August. Similar conclusions resulted from principal component analysis of the relative abundance of these bacteria and fungi. Buyer and Drinkwater (1997) analyzed structure and function of microbial species in composite soil samples (by fatty acid methyl ester analysis and Biolog plates, respectively) in a later study at the same location, and derived principle components which were then compared by analysis of variance and canonical discriminant analysis. This time they found that the conventional treatment differed from the low-input system with green manure in regard to the sampled microbial communities, but mentioned that treatment effects remained generally smaller than temporal ones. Kirchner et al. (1993) also found that microbial populations and activities in the Piedmont of North Carolina were similar between CON and LI systems, even though they had significant seasonal variations. These studies arrived at fairly similar conclusions, but noted that large variability within treatments may have caused the observed homogeneity.

The great majority of AM fungal species found in this study were present in all six treatments. Species
richness values reflected not only quantitative, but also qualitative similarities among treatments. Prior to the separation of the field into the farming systems experiment, the land was used for the production of maize and wheat with conventional practices (Doran et al., 1987). The fungal communities that once inhabited these fields were probably different from the ones present after cultivation, but the fungal species that survived and adapted to agricultural practices have persisted under conventional and low-input farming with either host. This may indicate that these farming systems are not so different when it comes to the ability of the fungi to survive and occupy the root niches available. All three systems have high amounts of available phosphorus (≈160–185 mg kg\(^{-1}\)) whether naturally occurring or accumulated from past applications, and nitrogen inputs are about 91 kg ha\(^{-1}\) per year for CON, 87 kg ha\(^{-1}\) per year for LI-AM and less for LI-GM (51 kg ha\(^{-1}\) per year). All systems are moldboard plowed, more frequently for LI-GM and less for LI-AM. Even though the LI systems have greater crop diversity and longer periods of coverage with living plants than CON (Douds et al., 1993), and mechanical weed control is used in contrast to herbicides, it is possible that the elevated nutrient status and disturbance of the soil in all plots played a more influential role in homogenizing fungal diversity than any differences due to farming system. Other factors may also contribute to the system-wide occurrence of AM fungal species. For example, propagules might disseminate from one narrow plot to another in this 6.1 ha field, via wind-blown disturbed soil after tillage.

Trap cultures can be very helpful in unveiling community members that are undetected in the initial extraction of spores from field soil (Morton et al., 1995). When three successive culture cycles of soil from the Arizona Sonoran desertscrub were analyzed, up to seven species of AM fungi, not present in the first round, appeared by the third (Stutz and Morton, 1996). Our trap cultures revealed only one species not detected in the field survey (\textit{G. constrictum}). This indicates that the species composition of the field samples was an accurate reflection of the richness of these communities and a good representation of the AM fungal community for the period of time and conditions sampled. Trap cultures, however, tended to encourage preferential sporulation by a few species, even when different conditions were applied, as observed by others (Bever et al., 1996; Koske et al., 1997).

Diversity and dominance indices, with exception of Berger and Parker values, also suggested uniformity among treatments (Table 3). Shannon and Wiener and evenness values indicated that the patterns of species composition and spore distribution were similar in the six treatments, and when farming systems or crops were analyzed as main effects (data not shown). Similarly, Simpson’s index did not discriminate among the dominance patterns between the treatments, even though Table 5 shows that sporulation by \textit{G. gigantea}, \textit{G. mosseae}, \textit{G. sparcum}-like and Small Yellow \textit{Glomus} was influenced by farming system, host, and/or the interaction of both. The frequency data presented in Fig. 1 help visualize these trends. The Berger and Parker values reflected dominance patterns based on differences in biovolumes. Carbon allocated to the fungus for sporulation can represent a significant drain to the plant as part of the carbon cost of the symbiotic relationship (Harris et al., 1985; Jakobsen and Rosendahl, 1990; Graham et al., 1991). Fixed carbon for sporulation may come directly from the host, or carbon may be pre-stored in hyphae before its onset (Gazey et al., 1992; Pearson and Schweiger, 1993). Sixty to seventy-five percent of the total spore volume belonged to \textit{G. gigantea} in all treatments but one (Table 4). The only treatment with fairly evenly distributed spore volumes was CON/MZ, which had a significantly lower Berger and Parker index. The partitioning of carbon between extraradical hyphae and spores, and its implications for the efficacy of mycorrhizas, should be studied for members of AM fungal communities in CON and LI agriculture. \textit{Gigaspora gigantea} and the community in CON/MZ are excellent candidates for these types of investigations.

The species richness at this site compares favorably with that of many agricultural soils (Douds and Millner, 1999). Though the 15 species found here is less than the 26 species found by Ellis et al. (1992) in soils farmed with a sorghum–soybean rotation in Nebraska, USA, it is greater than the 3–6 reported by Talukdar and Germida (1993) in Saskatchewan, Canada.

The mean Shannon–Weiner diversity index (\(H’\)) for soils in this study was 1.76. This is greater than the diversity of AM fungus communities in agricultural soils under a range of management regimes in Minnesota,
USA \( (H' = 0.57-0.64, \text{Kurle and Pfleger (1996); and } \)
\( H' = 0.42-1.59, \text{Johnson et al. (1991)}) \) and soils from \textit{Theobroma cacao} plantations in Venezuela \( (H' = 0.6-0.78, \text{Cuenca and Meneses, 1996}) \). The community of AM fungi in Polish soils in which \textit{Triticosecale} was
grown after a variety of precrops had somewhat greater Shannon–Weiner indices \( (H' = 1.81-2.22, \text{Blaszkowski, 1995}) \). \text{Kurle and Pfleger (1996) and Vestberg et al. (1999}) also noted no significant increase in diversity of AM fungi in populations due to the application of low-input agricultural management.

Variations in the patterns of spore abundance seen here, however, are liable to happen in different seasons as well as from year to year in a crop rotation. Douds et al. (1993) showed that the number of AM fungus spores in major species-groupings at this site tended to be lower in CON than in the LI systems from 1989 to 1991. Several other factors also have been previously shown to influence mycorrhizal populations in general, such as duration of fallowing period (Thompson, 1987), age of plantations (Wacker et al., 1990), soil factors, crop species, fumigation, and others (Cuenca et al., 1990; Johnson et al., 1992a,b; An et al., 1993; Douds et al., 1995, 1997; Hendrix et al., 1995; Miller et al., 1995).

Three species were present in very low frequency and/or abundance; \textit{S. pellucida}, \textit{Glomus E-1} and \textit{G. fasciculatum}. \textit{Scutellospora pellucida}, found in all treatments except CON/MZ, appeared at less than one spore per 350 cm\(^2\). However, soils collected from around maize roots at this site during the summer of 1997 contained more than 10 times as many spores of \textit{S. pellucida} (data not shown). \textit{Glomus E-1}, retrieved only from LI-GM/MZ in the field and CON/SOY in trap culture, is common at the Compost Utilization Trial, another study site within the Rodale Institute Experimental Farm (data not shown). \textit{Glomus fasciculatum} was only found once (at LI-GM/SOY) in several years of research in these sites. These dynamics indicate that biotic and abiotic factors interact to induce a fungus to sporulate and that these fungi may persist without sporulating. This underscores the need for continued development of molecular methods to identify members of the AM fungal community by vegetative structures.

Results from Bever et al. (1996) supported the idea that differential sporulation commonly observed in AM fungal communities results more from differential rates of growth of the fungi than from different timing in allocation of resources to sporulation. If growth rates are reflected in the sporulation patterns seen in this experiment, then some species, namely \textit{Glomus C-1}, \textit{G. aggregatum}-like, \textit{G. sparcum}-like, \textit{G. mosseae} and \textit{G. etunicatum}, are better established and spread within the roots than others. This is supported by the fact that these were also the species most prevalent in the trap cultures (with exception of \textit{Glomus C-1}). Spores of the remaining species were few or absent in the trap cultures, probably due to more intense competition and exclusion dynamics brought about by the constricted space of the pot environment.

The taxonomic diversity found cannot necessarily be linked to the functional role of these fungi. Inter- and intraspecific variation in efficacy as symbionts has been found. Isolates of species can become locally adapted (Bethlenfalvay et al., 1989; Stahl and Christensen, 1991; Allen et al., 1992; Morton and Bentivenga, 1994; Allen et al., 1995). So, even though all AM fungi occupy the same general niche, we cannot predict if functional redundancy (Walker, 1992) will occur among the fungi in the community. Future tests comparing symbiotic efficacy of these different fungi should indicate whether this uniformity also is reflected in the capabilities of the different communities to promote plant growth, or if there is functional diversity among these communities.

This ecological assessment has described the local AM fungal community composition and structure in relation to farming system and crop host. This study and others indicate that the microbial communities of CON and LI systems can have similar structure, even though absolute numbers of individuals of different species may vary. Future work with these communities should be to assess the functional roles of AM fungi from the different farming systems and determine if trends in symbiotic efficacy correlate with CON and LI practices (Johnson, 1993). Continued sampling of spores at this site may also be valuable to monitor large changes in species composition that may occur in the future.

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References


