

Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi

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Abstract

Effects of roots and of arbuscular-mycorrhizal (AM) fungi on the composition of soil bacterial colonies and the combined effects of AM fungus-rhizobacterium associations on plant and soil development are little-known. We grew sorghum (*Sorghum bicolor* L.) either nonsymbiotically or colonized by one of two isolates of the AM fungi *Glomus etunicatum*, *Glomus intraradices*, or *Glomus mosseae*. The isolates were either exotic or native to the test soil. Soils adhering (rhizosphere) or not adhering (hyphosphere) to the roots were sampled 45 days after planting. Total populations of bacteria were estimated by counting colony-forming units on a nonselective medium and grouped by colony and cell morphology. Rhizosphere populations of fluorescent pseudomonads were determined on P1 medium. Visually distinct isolates were selected for identification by Fatty-Acid-Methyl-Ester analysis; of these 25 were found to be separate species. Bacterial numbers were greater in rhizo- than in hyphosphere soil. Isolates of *Bacillus* and *Arthrobacter* were most frequent in hyphosphere and *Pseudomonas* in rhizosphere soils. More bacterial species were encountered in hyphosphere than in rhizosphere soil, and bacterial communities varied within and among AM treatments. The development of the AM mycelium in soil had little influence on the composition of the microflora in the hyphosphere, while AM root colonization was positively related with bacterial numbers in the hyphosphere and with the presence of *Pseudomonas* in the rhizosphere. The results suggest that qualitative effects of the AM fungal taxon on the hyphosphere, such as the nature of exudates, are more important to composition and proliferation of rhizobacteria than the quantitative development of AM soil mycelia.

Introduction

The interactions between functional groups of the soil microflora are a key to understanding the dynamic processes that characterize plant-soil relationships. Among these, the effects of rhizobacteria on the development and functioning of arbuscular mycorrhizal (AM) fungi (Linderman and Paulitz, 1990) are of particular interest because the latter form a living link between root and soil (Bethlenfalvay and Schüepp, 1994). AM fungi, in turn, affect the composition of bacterial communities (Meyer and Linderman, 1986b; Paulitz and Linderman, 1991), and fungi and bacteria in the mycorrhizosphere are thought to elicit in concert

such plant responses as resistance to stress and disease (Bethlenfalvay, 1992; Linderman, 1992).

The term mycorrhizosphere (Oswald and Ferchau, 1968) refers to the zone of influence of the mycorrhiza (fungus-root) in the soil. The mycorrhizosphere has two components. One is the rhizosphere, a thin layer of soil that surrounds the root and is under the joint, direct influence of the root, root hairs, and AM hyphae adjacent to the root. The other, the hyphosphere, is not directly influenced by the root. The hyphosphere is a zone of AM hypha-soil interactions (Marschner, 1995), and may be more or less densely permeated by the AM soil mycelium (8 to 20 km hyphae L⁻¹ soil, Schreiner et al., 1997). In the non-mycorrhiza literature, the root-free soil outside of the rhizosphere is referred to as 'bulk soil'. Here, we apply the term

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bulk soil only to the extra-rhizosphere soil of nonAM control plants that is free of AM hyphae.

Mycorrhizae may affect both the numbers and the composition of bacterial populations differently in their own rhizosphere and hyphosphere (Linderman, 1988). Studies on microbial interactions in the mycorrhizosphere have focussed on exotic populations of beneficial soil microorganisms, such as associative N₂-fixing bacteria (*Azospirillum*, Subba Rao et al., 1985), plant growth promoting rhizobacteria (Meyer and Linderman, 1986a) and phosphate-solubilizing bacteria (Toro et al., 1996). Relationships between native soil-microbe communities and AM fungi have received less attention (Pankhurst and Lynch, 1994), and it is not known whether members of a native soil bacterial flora show preference for native or exotic AM fungi.

Different bacterial populations may establish themselves under the influence of different AM plant-fungus combinations. Control of AM fungi over the nature and quantity of root exudates (Azaizeh et al., 1995) and subsequent competition between bacteria and AM fungi for this C source (Christensen and Jakobsen, 1993) may determine the size and composition of bacterial populations in the rhizosphere. In the hyphosphere, the AM mycelium is an important source of C for the microflora (George et al., 1995). Previous studies (see Olsson et al., 1996), however, did not relate differences between rhizo- and hyphosphere populations to the development of AM root colonization and soil hyphal density. Even less is known about preferences between AM-fungal isolates and functional groups or species of soil bacteria.

The objective of this experiment was to study 1. associative preference between groups of soil bacteria and isolates of AM fungi and to relate the results to bacterial colonization of the soil of nonAM plants, 2. the response of native soil bacteria to AM fungi native or exotic to the test soil, and 3. the relationship of the composition and proliferation of the bacterial community to the development of the AM soil mycelium.

Materials and methods

Experimental design

The experimental units (potted plants) were arranged on greenhouse benches in a completely randomized design with seven treatments and five replications. Treatments consisted of nonAM plants or of plants colonized by one of six AM fungi (a native or exotic

isolate of three species). The response variables were evaluated in two ways: as if all AM-fungal isolates were distinct and unrelated, and as a 3 × 2 factorial with species (3) and isolate (2) as factors. Analysis of variance, Fisher's protected LSD test, and regression analysis were used (Spreadware Statistics, Version 3, for Microsoft Excel).

Biological materials and soil

Sorghum (*Sorghum bicolor* L.) seeds were surface-sterilized (ethanol, 70%, v:v, 2 min), planted in 1.5 L plastic pots filled with a steam-pasteurized (75degC, 3 h.) mix of sand and sandy-loam soil (1:1, v:v, 2 kg pot⁻¹). The soil (pH 6.5, from the bank of the Willamette River near Corvallis, Oregon) contained (g kg⁻¹, after pasteurization): NH₄-N, 1.9; NO₃-N, 24.1; P (NaHCO₃-extractable), 0.01; P (total), 0.5; K, 176; Ca, 88; Mg, 8.8; Mg, 3.5; S, 0.8; and micronutrients (mg kg⁻¹) B, 0.1; Cu, 2.4; Fe, 70.0; Mn, 5.1; and Zn, 0.8 (Central Analytical Laboratory, Dept. Crop and Soil Science, Oregon State University).

Two isolates (native or exotic to the test soil) of each of three AM-fungal species were used as inocula. The exotic fungi were obtained from the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), Division of Plant Sciences, University of West Virginia, Morgantown, WV 26526-6057, USA. The native fungi were recently isolated from the Oregon State University Horticultural Farm. The fungi were: *Glomus etunicatum* Walker and Koske (native, GeN; exotic, GeE, INVAM # UT 183-1), *Glomus intraradices* (Schenck and Smith) Koske (native, GiN; exotic, GiE, INVAM # UT 126-2) and *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (native, GmN; exotic, GmE, INVAM # CA 110). They were pot-cultured on sorghum, wet-sieved, and washed on filter paper. Each test plant was inoculated with 150 spores placed near the root.

Growth and harvest conditions

Plants were grown in a greenhouse at Corvallis, Oregon, (December 1995 to January 1996). Temperatures were maintained between 18 and 28 degC. Sunlight was supplemented by 1000 W phosphor-coated metal halide lamps (General Electric) providing 16 h of photosynthetically active radiation (500 μmol m⁻² sec⁻¹) at soil-surface level. Plants were watered with tap water as needed, maintaining soil moisture content at approximately 25% (gravimetric).

Plants were harvested 45 days after planting. The soils were not root-bound, and were dried moderately for the harvest to facilitate their separation into rhizosphere- and hyphosphere-soil fractions. Upon removal from the pot, loose soil was shaken off the roots. Care was taken not to damage the roots in this process, so that the loose soil was free of root fragments. This extra-rhizosphere fraction was permeated by AM soil hyphae, and was considered to be hyphosphere soil. The roots remained encrusted by a distinct, cohesive layer of soil, 2 to 3 mm in thickness, which was considered to be the rhizosphere-soil fraction.

Bacterial assays

Two soil samples (1 g each) were taken from the homogenized rhizosphere or hyphosphere soil fractions of each experimental unit to estimate bacterial populations (Zuberer, 1994). The samples were suspended in 9 mL of one-quarter strength Ringer's solution (0.05% agar) maintained at 5 °C. Aliquots (100 μ L) of ten-fold dilutions were spread on duplicate plates on Tryptic-Soy agar medium (TSA) for enumerating total bacteria (Kirchner et al., 1993) and on P1 medium (Kato and Itoh, 1983) for enumerating fluorescent pseudomonads. Plates were incubated at 25 °C. Since nonselective media, such as TSA, may favor the assessment of fast-growing bacteria, colony-forming units (cfu) were counted after 3 days and also 5 days to allow for the development of slower-growing colonies.

Bacteria were streaked on TSA or P1 media from both the rhizosphere and hyphosphere soils of each treatment at each incubation time. All of the colonies that established themselves at 10^{-3} dilution were transferred to fresh media to purify the cultures. As distinguished by colony morphology, the cultures could be separated into seven major groups. However, not all of the 14 (7 rhizosphere and 7 hyphosphere) soil fractions yielded representative cultures for all of these seven morpho-groups. Group 6, for instance, was represented only by isolates from the GiE and the GiN treatments. The purified colonies were next examined microscopically to determine differences between them by cell morphology.

Morphologically identical colonies within a treatment sometimes contained different cell types, establishing new subgroups made up of potentially different species. On the other hand, colonies of like morphology within a treatment that contained the same cell type, were lumped as potentially one single species.

The total number of isolates distinct by both colony and cell morphology was 54; these were further examined taxonomically by fatty acid methyl-ester (FAME) analysis (Sasser, 1990). The assays were carried out by the Texas Plant Disease Diagnostic Laboratory (Texas Agricultural Extension Service; College Station, TX 77843) using the Fatty Acid-Microbial Identification System (Midi-Dos System, Version 4.15).

Mycorrhiza assay

Roots were washed, and cut into 1-cm segments. Samples (1 g) were cleared in KOH solution (5% KOH, w:v, 30 min, 90 °C), and stained with trypan blue (0.05%) in lacto-glycerol (water:glycerol:lactic acid, 1:1:1, 10 min, 90 °C). Total and AM-colonized root length was estimated by the grid-line intersect method (Giovannetti and Mosse, 1980).

Ten samples from pooled and mixed rhizo- and hyphosphere soils were used for a determination of AM fungal hyphal length. Samples (5 g) were suspended in 150 mL of a carrier solution (glycerol:water, 1:1, v:v) acidified with 1% HCl, and blended (20 sec) at high speed with a two-speed Waring blender. Suspensions were immediately decanted, and sieved (45 μ m) to eliminate fine debris. The hyphae were washed from the sieve, resuspended in 200 mL of the carrier solution and stirred (1 min). A 10 mL aliquot of the suspension was pipetted onto a GN-6 (Gelman, Ann Arbor, Michigan) membrane filter (0.45 μ m pore size, 47 mm diameter, grid-line interval 3 mm) attached to a vacuum apparatus. After vacuum-filtering the carrier solution, the membrane was covered with 10 mL of a staining solution (0.05% trypan blue in lacto-glycerol) for 10 min. The solution was filtered off, and the hyphae on the filter were rinsed with distilled water. Hyphal length was estimated by counting hypha-grid line intersections (Newman, 1966) after mounting the membrane onto a small Petri dish with 2 drops of carrier solution. Hyphal length was determined in the non-AM treatments and consisted of residual, dead AM or of nonAM hyphae. This value was subtracted from the hyphal length of the AM treatments.

Results

Bacterial populations in rhizosphere and hyphosphere soils of AM treatments

Rhizosphere and hyphosphere soils were populated largely by different bacteria. Taxonomic identification by FAME analysis revealed duplication among the 54 organisms originally selected from all treatments as visually distinct by cell and colony morphology. Of the 54 isolates, 25 could be identified as different species, although more accurate techniques may have revealed greater diversity. Only three of these 25, *Arthrobacter globiformis*, *Bacillus macerans*, and *Burkholderia cepacia* were found in both the rhizosphere and hyphosphere soils (Table 1). There were more species in the hyphosphere than in the rhizosphere soil (16 vs. 12). Of these, the genera *Arthrobacter* and *Bacillus* were represented by three and five species, respectively, in the hyphosphere, while three species of *Pseudomonas* occurred in rhizosphere soils.

The composition of the bacterial communities at the dilution levels tested varied greatly within AM treatments. For instance, we found only two species in the rhizospheres of the nonAM and the GiN roots, but six species in the rhizospheres of the GeN roots. Likewise, hyphosphere soils of GiN plants yielded only one species, while in those of GmN we found six and in those of GmE five species. Among AM treatments, a given species of bacterium was often represented in only one or two of the seven treatments. But two species were ubiquitous: *Burkholderia cepacia* was present in both rhizo- and hyphosphere soils of all AM (but not of nonAM) treatments, while *Pseudomonas fluorescens* was present in the rhizospheres of all treatments. Only six species of bacteria were associated with roots or in the bulk soil of nonAM plants, whereas 23 different species were associated with roots or hyphae of AM plants. Furthermore, only two bacteria (*Burkholderia pickettii* and *Pseudomonas corrugata*) found with nonAM plants were not found with any AM plants.

Root and rhizobacterium responses to AM-fungal species and isolates

Less abundant production of soil hyphae by Ge than by the other two AM fungi was associated with lower bacterial counts in its hyphosphere soil. However, low root colonization by Ge did not affect total bacterial counts in the rhizosphere (Table 2). The rela-

tively low development of Ge in roots and soil, however, was reflected by low counts of *P. fluorescens* in the rhizosphere. Association with Gm and its extensive mycelial network apparently inhibited host-plant root development. The AM-fungal isolates exotic to the soil developed more hyphae and stimulated greater root development than native isolates, but did not differ in the extent of root colonization. Exotic strains did not affect total bacterial counts in the rhizosphere and hyphosphere, but stimulated the proliferation of *P. fluorescens* (Table 2).

Significant AM-fungal species vs. isolate interactions for hyphal length were illustrated graphically in Figure 1. These data show that the simple effects of the Species Factor on the two levels of the Isolate Factor diverged in both direction and magnitude (Figure 1A), indicating statistically that the factors were not independent of one another. Biologically, this phenomenon may be interpreted as the ability of AM-fungal isolates within a morphospecies to respond differently to growth conditions. Here, native isolates, as a group, did not develop (respond to the same host soil) the same way as did the exotic isolates: GiN produced more soil hyphae than GiE, while GmN produced less hyphae than GmE. Likewise, the simple effects of the Isolate Factor on the Species Factor differed in magnitude for Ge and Gm, and in direction for Gi (Figure 1B). Significant interactions between AM species and isolate effects on the bacteria included differences in both direction and magnitude even in the absence of significant main effects.

Rhizobacterium numbers and AM-fungus development

Bacterial numbers of the rhizosphere were consistently 10- to 100-fold greater than those of the hyphosphere (Table 3). Bacterial numbers in the rhizosphere soils of nonAM plants tended to be lower than in AM-plant soils. Bacteria in the bulk soils of nonAM plants were not significantly different in number than in the hyphosphere soils of the AM plants, although hyphal density in the bulk soil was less than 5% of that found in the hyphosphere soils of the AM plants (Table 3). Hyphae in the bulk soil were adjudged to be nonAM, since the roots were not AM colonized.

The differences in the intensity of root or soil colonization by the different AM fungi did not significantly correlate with rhizobacterium numbers in either sphere (Figure 2). Bacterium numbers did not change significantly ($p = 0.554$) with root colonization in the

Table 1. Bacteria isolated from rhizosphere or hyphosphere soils from sorghum. Roots were colonized by the arbuscular-mycorrhizal (AM) fungi *G. etunicatum*, *G. intraradices*, or *G. mosseae*, or were nonsymbiotic (nonAM)

Bacteria	Treatment ^a						
	nonAM ^b	<i>G. etunicatum</i>		<i>G. intraradices</i>		<i>G. mosseae</i>	
		Native	Exotic	Native	Exotic	Native	Exotic
<i>Acidovorax avenae</i>	–	–	–	–	–	–	R
<i>Actinobacillus lignieresii</i>	–	–	–	–	–	H	–
<i>Agrobacterium radiobacter</i>	–	R	–	–	–	R	–
<i>Alcaligenes eutrophus</i>	–	–	–	–	–	–	H
<i>Alcaligenes faecalis</i>	–	R	–	–	–	–	–
<i>Arthrobacter globiformis</i>	H	–	–	–	R,H	–	R,H
<i>Arthrobacter ilicis</i>	H	–	–	–	–	H	–
<i>Arthrobacter urotoxicans</i>	–	–	–	–	–	–	H
<i>Bacillus brevis</i>	–	–	–	–	–	H	–
<i>Bacillus laterosporus</i>	–	H	–	–	–	–	–
<i>Bacillus macerans</i>	–	R,H	R,H	–	–	–	–
<i>Bacillus megaterium</i>	H	–	–	–	–	H	–
<i>Bacillus shaericus</i>	–	–	H	–	–	–	–
<i>Burkholderia cepacia</i>	–	R,H	R,H	R,H	R,H	R,H	R, H
<i>Burkholderia pickettii</i>	H	–	–	–	–	–	–
<i>Clavibacter michiganense</i>	–	R	–	–	–	R	–
<i>Enterobacter agglomerans</i>	–	–	R	–	R	–	–
<i>Micrococcus luteus</i>	–	H	–	–	–	–	–
<i>Phyllobacterium rubiaceum</i>	–	–	H	–	–	–	–
<i>Pseudomonas corrugata</i>	R	–	–	–	–	–	–
<i>Pseudomonas fluorescens</i>	R	R	R	R	R	R	R
<i>Pseudomonas putida</i>	–	–	–	–	–	–	H
<i>Pseudomonas rubrisubalbicans</i>	–	–	–	–	R	–	–
<i>Staphylococcus capitis</i>	–	–	R	–	–	–	–
<i>Variovax paradoxus</i>	–	–	–	–	–	H	–
Total isolates							
Rhizosphere	2	6	5	2	5	4	5
Hyphosphere	4	4	4	1	2	6	5

^aR or H indicate rhizo- or hyphosphere soil.

^bHyphosphere of nonAM roots corresponds to bulk soil.

rhizosphere (Figure 2A), nor with soil hyphal length ($p = 0.845$) in the hyphosphere (Figure 2B). However, there were significant differences in rhizosphere and hyphosphere bacterial numbers among AM treatments.

Discussion

The salient finding of our study was the lack of quantitative response (increase) by rhizobacteria to the development of AM mycelia in roots and soil. Instead, there was variation in the numbers of bacterial species associated with AM-fungal species and with AM-

fungal isolates within species. These results confirm the microflora preferences by AM plants vs. nonAM plants previously described by Meyer and Linderman (1986b) and Secilia and Bagyaraj (1987). The results are also similar to those of Olsson et al. (1996) who did not demonstrate significant effects, stimulatory or repressive, of the AM mycelium on the numbers of soil bacteria. Their results and ours suggest that the specific selection pressure on soil microbes from roots and hyphae of AM is not a quantitative one, but affects the species composition, perhaps due to root or hyphal exudation (Garbaye, 1991) into the mycorrhizosphere soil. Thus, a clear spatial separation of roots, mycor-

Table 2. Mycorrhiza formation and rhizobacterium populations in soils colonized by native or exotic isolates of different species of the arbuscular-mycorrhizal fungi *Glomus*. Response variables were averaged over species or isolate. Total bacterial counts as colony forming units (cfu) were made from rhizosphere or hyphosphere soils. The rhizobacterium *Pseudomonas fluorescens* was found in rhizosphere soil only. Numbers followed by the same letter (vertically) are not significantly different ($p \leq 0.05$, Fisher's protected LSD test)

Treatment	Root (m plant ⁻¹)	Soil hypha (m g soil ⁻¹)	Colonization (% of root)	Bacterium (log cfu g ⁻¹ dry soil)		
				Rhizosphere	Hyphosphere	<i>P. fluorescens</i>
Species						
<i>G. etunicatum</i>	62.5 b	2.2 c	32.5 b	7.3 a	5.4 b	3.2 b
<i>G. intraradices</i>	62.4 b	2.8 b	54.5 a	7.1 a	5.6 a	4.1 a
<i>G. mosseae</i>	44.5 a	3.7 a	49.5 a	7.1 a	5.6 a	4.5 a
Isolate						
Native	38.6 b	2.4 b	43.3 a	7.2 a	5.5 a	3.6 b
Exotic	74.3 a	3.4 a	41.7 a	7.2 a	5.5 a	4.2 a
ANOVA (probability values)						
Species	0.192	<0.001	0.001	0.108	0.012	<0.001
Isolate	0.001	<0.001	0.630	0.764	0.942	<0.001
Interactions (S × I)	0.254	<0.001	0.209	0.016	0.090	0.002

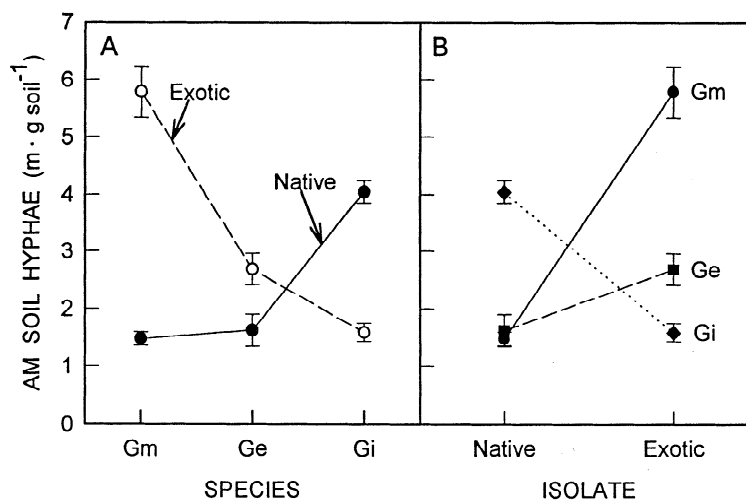


Figure 1. Graphical representation of two-factor interactions for the response variable AM soil hyphal length. **A.** Points denoting exotic or native isolates are connected to illustrate the failure of the Isolate Factor to be the same at each level of the Species Factor (difference in direction). **B.** Points denoting different fungal species are connected to illustrate differences in the magnitude (Gm vs. Ge) or in the direction (Gi vs. Gm and Ge) of the Species Factor in response to each level of the Isolate Factor.

rhizae and hyphae will be needed for a more rigorous analysis of the source of root or hyphal preferences for the bacterial microflora.

The study by Olsson et al. (1996) addressed the quantitative effects on bacteria in the rhizosphere vs. the hyphosphere, while we focussed on both bacterial numbers and on the associations between bacterial and AM fungal isolates. The variation in bacterial species

in association with AM plants compared to nonAM plants was significant, as was the occurrence of some bacterial species only in association with mycorrhizae formed by a specific fungus, or only in the hyphosphere or in the rhizosphere.

Our results indicate that AM hyphae enrich the bacterial flora of the mycorrhizosphere and confirm findings (Andrade, 1995) of a specific attraction between

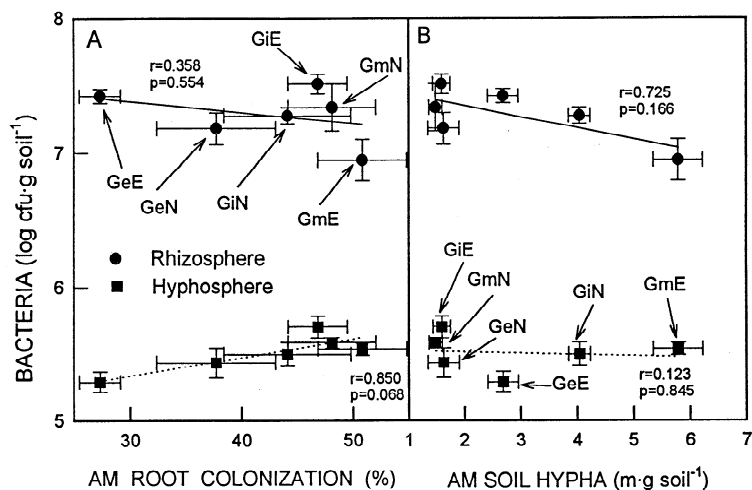


Figure 2. Bacterial populations in the rhizosphere and hyphosphere of sorghum roots. Colony-forming units (cfu) in response to: (A) root colonization or (B) soil hyphal length of a native or exotic isolate of the AM fungi *Glomus etunicatum* (GeN or GeE), *Glomus intraradices* (GiN or GiE), or *Glomus mosseae* (GmN or GmE).

Table 3. Bacterial proliferation and mycorrhiza development in the root zones of sorghum plants. Fungal hyphae in the bulk soil of plants not inoculated with arbuscular-mycorrhizal (AM) fungi were nonAM, judged by the absence of AM root colonization. Within each column, mean values followed by the same letter are not significantly different ($p < 0.05$) by Fisher's protected LSD test

Treatment	Bacteria (log cfu g ⁻¹ dry soil) ^a			Mycorrhiza	
	Rhizosphere	Hyphosphere	<i>P. fluorescens</i>	Root (m L ⁻¹)	Hypha (km L ⁻¹)
nonAM	6.9a	5.5ab	3.11a	45.2bc	0.1a
<i>G. mosseae</i>					
Native	7.3bc	5.6b	4.23b	22.5a	2.0b
Exotic	6.9a	5.5ab	4.71c	36.8ab	7.9e
<i>G. etunicatum</i>					
Native	7.2abc	5.4ab	3.16a	22.8a	2.1b
Exotic	7.4c	5.3a	3.24a	60.6c	3.6c
<i>G. intraradices</i>					
Native	7.0ab	5.5ab	3.38a	31.9ab	5.4d
Exotic	7.2abc	5.7b	4.76c	61.2bc	2.1b

^a Hyphosphere for the nonAM treatments signifies 'bulk soil'. *P. fluorescens* counts were made in rhizosphere soil.

AM hyphae and rhizobacteria, perhaps due to an exchange of nutritional compounds. The nature of a selective bacterial enrichment of the hyphosphere soil through AM fungi is speculative at this time, especially as higher levels of root exudation in nonAM vs. AM plants (Graham et al., 1981) can result in greater bacterial populations in nonAM soils (Schreiner et al., 1997). Bacterium-AM fungus preferences could result from fungus-bacterium competition for plant-derived

C and N compounds (Christensen and Jakobsen, 1993) and for minerals sequestered by plant-AM fungus-bacterium associations functioning in concert (George et al., 1995). Fluxes of C and N compounds from the root tissue into the soil may also be mediated by the AM mycelium (Pearson and Jakobsen, 1993), causing competition among types of rhizobacteria and affecting bacterial population composition without changing total bacterial numbers. The qualitative composition of

root, hyphal, or root and hyphal exudates may also differ between roots colonized by different AM fungi: a possible explanation for differences in the bacterial taxonomy, but not in bacterial numbers in our results.

Selective enrichment for specific bacteria in the mycorrhizosphere may result from the production of different hyphal exudates by different AM fungi that could favor the development of different bacteria in the hyphosphere, as described by Secilia and Bagyaraj (1987) for bacteria and actinomycetes in AM and nonAM pot culture comparisons. In that study (as in ours), some bacteria were found only in the mycorrhizosphere soils and not in the rhizosphere or bulk soil of nonAM plant. Our study allowed the separation of mycorrhizosphere bacteria into groups from the rhizo- or hyphospheres, resulting in the finding of preferential associations of AM-fungal isolates with bacteria not stimulated by nonAM roots. The source of bacteria in our study appears to be the bulk soil, in view of the diversity of species identified as AM-hyphal associates. Such close associations were recently demonstrated by the presence of specific bacteria on or within AM spore wall layers (Bianciotto et al., 1996; Walley and Germida, 1996). The bacterium found in spores by Bianciotto et al. (1996) was *Burkholderia*, a consistent associate of the hyphae of all the AM fungi in our study also.

It is possible that the bacteria associated with hyphae originated from the inoculated spores themselves, either internally or borne on the spore surfaces. Our inoculum spores were separated from soil by floatation and filtration, so that bacteria could have been present on their surfaces. Such surface bacteria, as well as selection from the large, undetected background soil bacterial populations, could explain the specificity we observed.

If the existence of preferential combinations between AM fungi and their associated bacterial partners were confirmed, it would support the concept of rhizobacteria and AM fungi functioning in concert to promote plant-growth (Linderman, 1988) and to stabilize soil (Bethlenfalvay and Schüepp, 1994). Efforts to manage the microbial components of the agrosystem should therefore focus on these microbial teams (e.g. 'mycorrhiza-helper bacteria', Garbaye, 1994), rather than on individual resident or introduced organisms (Andrade et al., 1995; Bethlenfalvay et al., 1996; Linderman, 1988;). Results such as those presented here conjure up new levels of complication in a system already well-known for its lack of manageability (Schroth and Weinhold, 1986). But the hierarchical

system of preferences between plants, AM fungi and rhizobacteria that has been delineated here may be a key to managing soil systems and to a better understanding of the factors that regulate mycorrhizosphere communities (Garland, 1996).

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