

Mycorrhizal fungi influence plant and soil functions and interactions

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Abstract

Potted soybean (*Glycine max* (L.) Merr.) plants were grown in P-fertilized (+P) or low-P soil (-P), or colonized in -P soil by one of the arbuscular mycorrhizal (AM) fungi *Glomus etunicatum* (Ge), *Glomus mosseae* (Gm), or *Gigaspora rosea* (Gr). Treatment effects on plant development, on the soil microflora, and on the status of water-stable soil aggregates (WSA) were evaluated for all 5 treatments or for the 3 AM treatments alone. Dry weights of the AM plants, as a group, were half-way between the dry weights of the +P and -P plants, but within the AM group, Gm plants had the highest pod dry weights and pod/stem and root/stem ratios and the lowest specific root lengths, while Ge plants had high stem dry weights and were highly nodulated. High reproductive development and coarse roots in the Gm plants were associated with the most extensive growth of AM soil hyphae (km pot⁻¹: Gm, 20; Gr, 12; Ge, 8), while nodulation was inversely related with AM-colonized root length. The soils colonized by AM fungi had significantly higher levels of WSA (size classes 1 to 2 and 2 to 4 mm), and within the larger size class, Gm soils had the highest percentage of WSA. Proliferation (plate counts) of Gram positive (G+) and Gram negative (G-) bacteria, *Arthrobacter* sp. (G+), and *Pseudomonas* sp. (G-) was greatest in the -P soils, but the bacterial populations of the +P and the AM soils were generally not significantly different. There were, however, differences among the AM treatments, where Gm soils had the lowest G- bacterial populations, while Ge soils had the highest populations of both G+ and G- bacteria. Correlations between plant and soil traits indicated that interactions within the plant-soil system were mediated by the AM fungi.

Introduction

Increasing emphasis on sustainability in agriculture is redefining the role of arbuscular mycorrhizal (AM) fungi in the plant-soil system (Bethlenfalvay and Linderman, 1992). No longer viewed as an association between host plant and fungal endophyte only, the AM symbiosis is being recognized to influence soil development as much as plant development; (Miller and Jastrow, 1992; Schreiner and Bethlenfalvay, 1995). As a living link between roots and the surrounding bulk soil, AM fungi integrate the interdependent functions of the plant-soil symbiosis (Bethlenfalvay and Schüepp, 1994).

To evaluate the effects of individual functions on the plant-soil system as a whole, attempts are made to determine inputs and responses of as many of its components as experimental design and available expertise permit (Abbott et al., 1995). In the majority of AM reports, however, plant growth response has been, and still is, the sole measure of AM-fungal input. Nevertheless, a small number of reports during the past decade show a growing awareness of the need to integrate AM research both into the greater context of soil biology and into the concept of stable and sustainable plant-soil systems (Allen, 1991; Gianinazzi and Schüepp, 1994; Sieverding, 1991). In particular, advances are being made to relate the interactions between AM plants, AM fungi and the soil microflora to plant health (Linderman, 1994), to plant ecology (Francis and Read,

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1995) and to soil structure (Miller and Jastrow, 1994), while the integration of the soil fauna (Ingham, 1992) with AM soil and plant effects in mycorrhiza-based soil food webs is still extant (Wardle, 1995).

Suppression of AM growth responses by natural soils (Ross, 1980), and the tenuous nature of inferences drawn from reductionist pot experiments to field conditions (Peterson et al., 1993) are well known. Yet, demonstrations of simultaneous effects of AM fungi on both the above- and below-ground development of agrosystems (Bethlenfalvai and Schüepp, 1994) are lacking even in the simplified microcosms of potted plants (Andrade et al., 1995). Among the interactions between functional groups of the soil microflora that affect both plant and soil development, those between rhizobacteria and AM fungi stand out, because of their mutual influence on each other (Garbaye, 1994; Paulitz and Linderman, 1991) and because they can influence, in concert, the plant-soil system in ways that depend on the specific composition of fungus-bacterium populations (Bethlenfalvai et al., 1997; Leyval et al., 1990; Meyer and Linderman, 1986).

The purpose of this experiment was to compare plant and soil responses to three AM-fungal isolates with P-sufficient or P-deficient nonAM plant-soil systems, and to determine if treatment-dependent changes in plant and soil parameters were interrelated.

Materials and methods

Experimental design

The experiment had five treatments with five replications and was repeated. Two of the treatments used nonsymbiotic plants, grown in P-fertilized (-AM, +P) or nonfertilized (-AM, -P) low-P soil without AM fungi. The other three treatments consisted of symbiotic plants colonized by one of three AM fungi (Ge, Gm, or Gr). The experimental units (potted plants) were placed at random on the greenhouse bench.

Biological materials and soil

Soybean (cv. Hobbit)¹ seeds were pre-germinated, selected for uniformity, planted in 1.5 L plastic pots, and thinned later (1 week) to one plant per pot. Pots were filled with a sandy-loam soil (2 kg pot⁻¹, obtained

from the bank of the Willamette River near Corvallis, OR), mixed with sand (1:1, v:v), and steam-pasteurized (75 °C, 3 h). The soil (pH 6.5) had a texture of 71% sand, 20% silt, and 9% clay. It contained (g kg⁻¹): NH₄-N, 1.9; NO₃-N, 24.1; P (NaHCO₃-extractable), 0.01; P (total), 0.5; K, 176; Ca, 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.

Soil inocula of the AM fungi *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (INVAM² #CA110), *Glomus etunicatum* Becker and Gerd. (INVAM # UT 183-1), and *Gigaspora rosea* Nicol. and Schenck (INVAM # FL 105) were used to colonize plants of the Gm, Ge, and Gr treatments, respectively. A saturating amount of the soil inoculum (3 000 spores or sporocarps per pot plus AM root and hyphal fragments) was used, making infectivity testing in this long-term experiment unnecessary. All fungi were exotic to the experimental soil: *G. mosseae* originated from the Anza-Borrego Desert in southern California, *G. etunicatum* of unknown origin was cultured at Native Plants, Inc., and *G. rosea* was isolated at the University of Florida (Gainesville) Horticultural Farm. We cultured the inocula on *Sorghum bicolor* L. roots. Unsterilized local soil and soils from the AM cultures were mixed, incubated in water on a shaker overnight, and sieved 6 times (45 μm sieve) to remove AM propagules. Equal amounts of this suspension, containing indigenous and inoculum-associated soil microbes, were applied to all pots to re-introduce the microflora.

Growth conditions

Plants were grown in a greenhouse at Corvallis, Oregon (November 1994 to March 1995) and were harvested after 135 d of growth, when seed pods were dry. The first and second experimental sets were separated by a 2 wk interval; the second set (repetition) was set up on a bench adjacent to the first. Automatic controls kept temperatures between 18 and 28 °C. 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. Hydroxyapatite (Ca₁₀[PO₄]₆[OH]₂, 1 g kg⁻¹ soil) was mixed into the soil of the -AM, +P treatment as P fertilizer. Plants were watered from

¹ Mention of cultivars or brand names does not imply endorsement by USDA-ARS.

² International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, Division of Plant and Soil Sciences, University of West Virginia, Morgantown, WV 26526-6057, USA.

below (saucers) with tap water for 3 wk. Then a nutrient solution consisting of (mM) $\text{Ca}(\text{NO}_3)_2$, 1; NH_4NO_3 , 1; K_2SO_4 , 1, MgSO_4 , 0.25; and micronutrients (≈ 0.25 -strength Hoagland's solution) was applied. The solutions were replenished as the saucers became dry. Since the larger plants of the +P and Ge treatments required more moisture than plants of the other treatments, they were given tap water between nutrient-solution applications. In this way, all plants received the same amount of nutrients. The N concentration of the solution was increased at the onset of flowering (42 d after planting) to 8 mM $\text{Ca}(\text{NO}_3)_2$. No nutrients were applied during the week prior to harvest while the pods were drying, to discourage vegetative growth.

Harvest and assays

Roots obtained in soil cores 8 d before harvest (128 d after planting) were washed, cleared in KOH solution (5% KOH, w:v, 30 min, 90 °C), and stained with trypan blue in lacto-glycerol (0.05%, 10 min, 90 °C). Root colonization (%) by the fungi was then estimated by the grid-line intersect method (Giovanetti and Mosse, 1980). Pods were harvested continuously as they dried to avoid loss of seeds due to dehiscing. Since the majority of the leaves had fallen by the time of pod maturity, total shoot weights were not obtained. The stems were weighed to provide a comparative measurement of vegetative vs. reproductive tissues.

Soil bacteria

Soil samples (30 g) taken from the pots at harvest were bagged and refrigerated (2 weeks) until the time of evaluation. They were sieved (sterilized 2.0 mm sieve) to remove rocks and roots. Duplicate samples of soil (≈ 10 g fresh weight) were placed in 95 mL of phosphate-buffered saline solution (Zuberer, 1994) with 25 g of glass beads (6.0 mm) and shaken at 150 rpm for 20 min. Serial dilutions (1:10) were made and aliquots (0.1 mL) were spread on duplicate plates. Plates were incubated at 27 °C and counted after 2 and 4 d.

To determine representative complements of bacteria in our experimental soil, counts of total bacteria, and of Gram positive (G+) and Gram negative (G-) bacteria were made. Because species of *Arthrobacter* (G+) and *Pseudomonas* (G-) are often the most common in soil (Hepper, 1975), the presence of these genera was also assessed. Total counts were made on one-tenth strength Trypticase soy agar (Kirchner et al.,

1993). The G+ and G- groups were plated on polymyxin B sulfate and crystal violet agar media, respectively (Gould et al., 1985; Wollum, 1982). Methyl red (Hagedorn and Holt, 1975) and NPCC (Simon et al., 1973) media were used to make plate counts of *Arthrobacter* and *Pseudomonas*, respectively.

Soil hyphae

Soil samples were frozen at harvest for subsequent determination of fungal hyphae. Hyphal length was measured by the filtration-gridline method (Sylvia, 1992) as modified below. Subsamples (≈ 2 g fresh weight) were thawed and placed into flasks (1 L), mixed with distilled water to give a total volume of 150 mL, and acidified with 50 mL of 1% HCl. At the same time, soil water content was determined on other subsamples to permit calculation of hyphal lengths based on soil dry weight. The suspension was shaken vigorously, decanted quickly (leaving only sand grains in the flask) into the flask of a commercial two-speed Warring¹ blender, and blended at high speed for 20 sec. Suspensions were immediately decanted, adjusted to 200 mL, stirred for 2 min with a magnetic stirrer, and allowed to stand (60 sec exactly) upon removal from the stir-plate. A subsample of the suspension (10 mL) was pipetted (tip opening 5 mm) onto a pre-wetted GN-6¹ (Gelman, Ann Arbor, Michigan) membrane filter (0.045 μm pore size, 47 mm diameter, grid-line interval 3 mm). The membrane was placed on a filter holder (Schleicher and Schnell, Keene, New Hampshire, 03431) attached to a vacuum apparatus.

After filtering, a staining solution (0.05% trypan blue in a mixture of lactic acid:glycerol:water, 1:1:1) was pipetted on the membrane and allowed to stand for 10 min. The staining solution was removed by filtering and the membrane was rinsed with distilled water. To allow hyphae and soil particles to cover grid lines and interspaces uniformly on the membrane, the sample was resuspended (vigorous use of squirt bottle) and filtered once more. Hyphal lengths were estimated by counting intersections of the grid and hyphae, after mounting the membrane onto a small petri dish with 2 drops of stain-free lacto-glycerol. Hyphae in the nonAM treatments were considered to be part of the mycelia of saprophytic, nonAM fungi.

Soil aggregation

Soil moisture content at the time of sampling can be a major source of variation in the structural stability of

soils (Perfect et al., 1990). To minimize variation in soil moisture content due to differences in plant sizes, the soils of all experimental units were wetted to field capacity by soaking from below at harvest. Shoots were excised, and the soil clumps (held together by the roots) were removed from the pots and allowed to dry uniformly (no transpiration, 3 d) to a stage where the soil was friable and could be easily crumbled by hand and shaken from the roots. Root fragments were removed and the soil was spread to air-dry. Soil samples (200 mL) were separated into size classes by hand-shaking (30 uniform strokes) on a set of (0.25, 1, 2, and 4 mm) sieves. Dry aggregates of each size class were weighed and mean weight diameters (MWD) were determined according to Kemper and Rosenau (1986). The MWD is an expression of the size distribution of aggregates: it is a weighting factor that is proportional to size.

Dry aggregates of the three size fractions (0.25 to 1, 1 to 2, and 2 to 4 mm) were placed on small sieves, vapor-wetted (30 min) in a humidifier, and washed in distilled water (stroke length 1.3 cm at 35 cycles min^{-1}) for 10 min in a sieving apparatus described by Kemper and Rosenau (1986). Sample sizes were 4 g for the two larger size classes and 3 g for the small size class, and were wet-sieved on 2, 1 or 0.25 mm sieves, respectively. Materials that passed through the sieves were considered to be the water-unstable fraction (WU). Materials that remained on the sieves were dispersed by wet-sieving in 0.2% NaOH and brushing with a rubber spatula. Sand particles that remained on the sieves after this second washing were discarded. The small particles that passed through the sieves were the water-stable (WS) fraction. The WU and WS materials were oven-dried (110 °C) and weighed. Water-stable soil aggregation (WSA) was calculated as a percentage of the stable and unstable materials [$\text{WSA} = 100 \times \text{WS}/(\text{WU}+\text{WS})$].

Statistics

A comparison of the data sets of the repeated experiments showed significant differences between Set 1 and Set 2 for less than 20% of the response variables. The data of the two sets were therefore combined and evaluated as one set with 10 replicates. Since the data of nonAM controls often differ widely from those of the AM treatments, they may bias the evaluation of responses to AM fungi alone, as a group. We therefore presented both the results of analyses of the entire five-treatment data set and those of the three AM treatments alone, whenever these two analyses differed in

the evaluation of the complete or partial data sets. Analysis of variance (ANOVA), orthogonal contrasts and linear regression were used. Actual probability values, rather than the arbitrary 5% level, are shown to permit individual interpretation of significance (Nelson, 1989). We interpreted differences to be biologically significant up to $p = 0.1$.

Results and discussion

Plant traits

The dry weights of AM-plant parts were approximately half-way between those of the nonAM +P and -P plants (Table 1). Analysis of variance showed significant differences for pod, stem and root dry weights not only for the entire 5-treatment data set with the high values for +P and low values for -P plants, but also for the 3 AM-plant comparisons (Table 1). Responses in plant development to the three fungi individually also differed: orthogonal contrasts showed greater pod dry mass in Gm plants, greater stem mass in Ge plants, and smaller root mass in Gr plants than in plants of the other two AM treatments.

Length and coarseness (specific root length, SRL) of roots varied both between AM and nonAM plants and among AM plants (Table 1) and indicated a coordination of growth between mycorrhiza development and root morphology (Miller et al., 1995). Root length was greatest in +P plants and smallest in -P plants among the five treatments, while +P plants had the coarsest (lowest SRL) and -P plants the finest (highest SRL) roots. Root/stem and pod/stem ratios were calculated as measures of the plants' allocation of biomass to the organs involved in soil relations or in yield.

Ge plants were considerably larger than Gm and Gr plants during early development and matched in shoot size the +P plants for the first 2 months of growth (visual observations). This effect may have been influenced by the rapid, early development of root colonization usually seen with this isolate of Ge (Bethlenfalvay, unpublished observations) and is perhaps due to its limited dormancy. We ascribe accelerated early shoot-growth response (Marschner, 1995) in Ge to an enhanced, early uptake of the limited amount of P in this P-deficient system. In the Gm plants, flowering and subsequent pod development apparently coincided with the (delayed) AM colonization of roots. The higher pod/stem ratios of Gm (vs. Ge) plants are therefore attributed to a diversion of limiting resources (P) to the

Table 1. Plant traits. Soybean was grown in P- fertilized (+P) or not fertilized (-P) soil, or inoculated (in -P soil) with one of the arbuscular mycorrhizal fungi *Glomus etunicatum* (Ge), *Glomus mosseae* (Gm), or *Gigaspora rosea* (Gr). Means of 10 replicates. Evaluations by analysis of variance (ANOVA) were based on the entire data set or on the three AM treatments alone. Treatment differences by orthogonal contrasts are shown only for the entire data set, as a separate evaluation of the three AM treatments did not provide additional information

Treatment	Pod (g)	Stem (g)	Nodule (#)	Root (g)	Root (m)	SRL ^a (m g ⁻¹)	Root/stem ratio	Pod/stem ratio
+P	38.0	26.1	275	3.4	561	166	0.13	1.46
-P	13.3	7.1	72	1.5	435	295	0.21	1.88
Ge	21.5	14.2	189	2.6	478	186	0.18	1.52
Gm	23.4	11.0	101	2.7	479	176	0.24	2.15
Gr	21.1	10.7	96	2.3	480	213	0.22	2.00
<i>ANOVA (probability values)</i>								
All treatments	<0.001	<0.001	<0.001	<0.001	0.032	<0.001	<0.001	<0.001
AM only	0.003	<0.001	0.047	0.009	0.999	0.144	<0.001	<0.001
<i>Contrasts (probability values)</i>								
+P vs. -P	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
Ge	<0.001	<0.001	0.068	<0.001	0.035	0.132	<0.001	0.520
Gm	<.0001	<0.001	<0.001	<0.001	0.039	0.461	<0.001	<0.001
Gr	<0.001	<0.001	<0.001	<0.001	0.039	0.008	<0.001	<0.001
-P vs. Ge	<0.001	<0.001	0.015	<0.001	0.256	<0.001	0.007	<0.001
Gm	<0.001	<0.001	0.535	<0.001	0.240	<0.001	0.001	0.006
Gr	<0.001	<0.001	0.611	<0.001	0.237	<0.001	0.630	0.194
Ge vs. Gm	0.065	<0.001	0.063	0.295	0.969	0.433	<0.001	<0.001
Gr	0.679	<0.001	0.049	0.027	0.962	0.220	0.002	<0.001
Gm vs. Gr	0.026	0.706	0.911	0.002	0.993	0.048	0.003	0.129

^aSpecific root length.

stronger sink (seed) tissue (Jarrell and Beverly, 1981). The low root/stem ratios of the Ge vs. the Gm and Gr plants are a reflection of the high stem dry weight of the former.

Symbiotic traits

Nodule numbers were highest in the +P and lowest in the -P plants, reflecting the well-known dependence of nodulation on P availability (Table 1). Differences in nodulation among AM plants were large, and nodule numbers were negatively related to AM root colonization (Table 2). The Ge plants with the highest nodule numbers also had the shortest colonized root lengths (44%), while high levels of AM root colonization (76%) in Gm and Gr plants were accompanied by low nodule counts, indicating antagonism between the two microsymbionts. Such negative relationships between rhizobia and AM fungi have been related to competition for nutrients (Bethlenfalvay, 1992) and to

selective compatibilities between the microsymbionts of the legume association (Azcón et al., 1991). Fungal hyphae in the soils of the two nonAM treatments were assessed and did not differ in +P and -P soils. Since nonAM hyphal length was only about 1% of that in the three AM soils (data not shown), nonAM fungal contributions to plant development and soil aggregation were considered to be negligible.

The data on soil colonization by AM hyphae (Table 2) illustrate why a measurement of root colonization alone does not suffice to explain plant or soil responses to AM fungi. Here, root lengths of all three AM treatments were similar (Table 1), AM root lengths were similar only for Gm and Gr plants (Table 2), but the length of soil hyphae differed significantly in all three AM treatments (ranging from 8 to over 20 km per pot, Table 2). These data indicate relationships between the development of the AM root or soil mycelia and corresponding plant responses: a well-developed soil mycelium (Gm) was associated with high seed yield

Table 2. Symbiotic traits. Soybean roots and the surrounding soil were colonized (col) by one of the arbuscular mycorrhizal (AM) fungi *Glomus etunicatum* (Ge), *Glomus mosseae* (Gm), or *Gigaspora rosea* (Gr). Means of 10 replicates. The AM data set was evaluated by analysis of variance (ANOVA). Individual treatments were compared by orthogonal contrasts

Treatment	AM root		AM soil hyphae		
	Length (m)	Col (%)	(m g ⁻¹ soil)	(km pot ⁻¹)	(m(AM rt ln) ^{-1a})
Ge	207	43.7	3.3	8.04	39.8
Gm	364	75.9	8.1	20.25	55.6
Gr	367	76.2	4.8	12.02	32.8
<i>ANOVA (probability values)</i>					
AM treatments	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Contrasts (probability values)</i>					
Ge vs. Gm	<0.001	<0.001	<0.001	<0.001	0.013
Ge vs. Gr	<0.001	<0.001	0.082	0.058	0.324
Gm vs. Gr	0.939	0.964	<0.001	<0.001	0.001

^aHyphal length (m) per colonized root length.

and coarse roots (low SRL), a sparse soil mycelium (Ge) with high stem development, and highly colonized roots (Gm and Gr) with high root/stem and pod/stem ratios (Tables 1 and 2). Our data confirm observations by Jakobsen et al. (1992) who related plant responses (P uptake) to different AM fungi to the variation in the development of their soil mycelia.

Soil bacteria

Counts of total and G+ bacteria were significantly higher in the -P control soils than in those of other treatments, while differences between the +P control and the AM treatments were not significant (Table 3). This was contrary to our expectation of prolific microbial colonization of AM mycelia in the bulk soil. Among AM treatments, Gr soils had significantly fewer counts of total and G+ bacteria (including G+ *Arthrobacter*) than Ge soils, while Gm soils were intermediate and not different from the other two AM treatments. Counts of G- bacteria showed a trend similar to that of G+ bacteria, in that the nonAM soils (especially -P) had the most colony-forming units (cfu). Unlike for the G+ bacteria, the difference between G- bacteria in the +P and -P treatments was not significant. Among AM treatments, differences were more pronounced for G- than G+ counts, and Ge soils had the highest G+ and G- populations, while Gm soils had the lowest counts of G- bacteria. Significant differences were noted between Ge and Gm soils in both total G- and *Pseudomonas* populations.

A comparison of treatment effects on bacterial populations indicated that proliferation of bacteria in -P relative to +P and AM soils (Table 3) could have been due to root exudation that often results from P-stress (Jones and Darrah, 1995) and tends to be alleviated by P fertilization or AM colonization (Johnson, 1993). Among AM treatments, factors other than plant-to-soil C transport may have affected bacterial populations, since similar plant development implied similar nutritional status. Differences in functional compatibility between AM fungi and their bacterial symbionts have been demonstrated (Meyer and Linderman, 1986; Secilia and Bagyaraj, 1987), and we infer that selective preferences between AM-fungal isolates and different groups of bacteria were the factor that determined the differences in root, hypha, and soil colonization by different groups of bacteria (Table 3). As mechanisms for these preferences we suggest control by AM fungi over the nature and quantity of root exudates (Azaizeh et al., 1995), subsequent competition between bacteria and AM fungi for this C source (Christensen and Jakobsen, 1993), and trophic attraction between the hyphae of specific isolates of AM fungi and specific groups or strains of rhizobacteria (Andrade, 1995).

Soil aggregation

Soils of the +P treatment had the highest level of dry aggregation (MWD, Table 4) and contained the root systems with the greatest total root length (Table 1), while there was no difference in these parameters

Table 3. Soil bacteria. Soybean was grown in P- fertilized (+P) or not fertilized (-P) soil, or inoculated (in -P soil) with one of the arbuscular mycorrhizal fungi *Glomus etunicatum* (Ge), *Glomus mosseae* (Gm), or *Gigaspora rosea* (Gr). Colony-forming units (cfu) are per g of dry soil. Means of 10 replicates. Evaluations by analysis of variance (ANOVA) and orthogonal contrasts were based on the entire data set or on the three AM treatments alone

Treatment	Total bacteria (cfu × 10 ⁸)	G+ bacteria (cfu × 10 ⁸)	G-bacteria (cfu × 10 ⁶)	<i>Arthrobacter</i> (cfu × 10 ⁶)	<i>Pseudomonas</i> (cfu × 10 ⁶)
+P	9.4	8.1	56.0	32.0	21.0
-P	48.0	47.1	91.0	41.0	17.0
Ge	8.0	7.8	16.0	6.1	15.0
Gm	5.7	4.9	3.4	3.1	2.7
Gr	4.4	3.4	6.9	1.7	6.5
<i>ANOVA (probability values)</i>					
All treatments	0.004	0.004	0.320	0.205	0.196
AM treatments	0.173	0.166	0.094	0.193	0.133
<i>Contrasts (probability values)</i>					
All treatments					
+P vs. -P	0.003	0.003	0.476	0.689	0.634
Ge	0.910	0.979	0.415	0.215	0.797
Gm	0.764	0.801	0.285	0.167	0.102
Gr	0.686	0.713	0.318	0.149	0.225
-P vs. Ge	0.002	0.003	0.130	0.104	0.464
Gm	0.001	0.001	0.079	0.078	0.037
Gr	0.001	0.001	0.091	0.068	0.094
Ge vs. Gm	0.851	0.821	0.797	0.884	0.165
Gr	0.770	0.733	0.852	0.949	0.337
Gm vs. Gr	0.917	0.908	0.943	0.949	0.662
AM treatments only					
Ge vs. Gm	0.230	0.121	0.011	0.174	0.063
Gr	0.067	0.023	0.058	0.054	0.197
Gm vs. Gr	0.501	0.420	0.458	0.544	0.549

among the other treatments. Roots growing in soil induce a compaction of the soil fabric adjacent to the roots (Emerson and Greenland, 1990), an effect that may be enhanced by constraints on the rooting volume (pots). Since the MWD is a linear function of the tensile strength of aggregates (Kay et al., 1988), which, in turn, is a function of compaction (Angers et al., 1987), the significant ($r = 0.783$, $p = 0.008$) correlation between MWD and total root length indicated that root pressure exerted on the soil was responsible for high MWD values in the +P soil.

More soil was either incorporated into water-stable aggregates (WSA) or stabilized by AM fungi than was the case in the nonAM treatments (Table 4). This effect was more pronounced for the 2-to-4 mm size class of WSA than for the 1-to-2 mm class, and for the smallest

size class (0.25 to 1 mm) differences due to treatment were not significant. The behavior of AM and nonAM soils differed within the two large WSA size classes (Table 4). The 1-to-2 mm class had less WSA in +P than in -P soils, but WSA was invariant in the AM soils ($p > 0.67$). On the other hand, in the 2-to-4 mm class no difference in WSA between +P and -P soils was noted, but WSA in Gm soil was significantly greater than that in the other two AM treatments.

This relationship of AM fungi with WSA size confirms findings that link fungi with the stability of larger soil aggregates (Burns and Davies, 1986; Harris et al., 1966; Tisdall and Oades, 1982), but it also points to differences in the capability of individual AM fungi to influence this process. Under our experimental conditions, AM hyphae were more important than root

Table 4. Soil aggregation. Soybean was grown in either P-fertilized (+P) or not fertilized (-P) soil, or inoculated (in -P soil) with one of the arbuscular mycorrhizal (AM) fungi *Glomus etunicatum* (Ge), *Glomus mosseae* (Gm), or *Gigaspora rosea* (Gr). Means of 10 replicates. Evaluations by analysis of variance (ANOVA) and orthogonal contrasts were based on the entire data set or on the three AM treatments alone

Treatment	Aggregation ^a			
	MWD (dry)	WSA (2 to 4 mm)	WSA (1 to 2 mm)	WSA ($\frac{1}{4}$ to 1 mm)
+P	2.32	51.9	69.8	87.9
-P	1.87	55.4	75.7	86.9
Ge	1.92	76.1	81.1	86.3
Gm	1.90	89.7	81.7	87.1
Gr	1.89	78.8	81.9	87.1
<i>ANOVA (probability values)</i>				
All treatments	<0.001	<0.001	<0.001	0.570
AM treatments	0.986	0.013	0.907	0.841
<i>Contrasts (probability values)</i>				
All treatments				
+P vs. -P	<0.001	0.536	0.003	0.931
Ge	<0.001	<0.001	<0.001	0.390
Gm	<0.001	<0.001	<0.001	0.182
Gr	<0.001	<0.001	<0.001	0.492
-P vs. Ge	0.496	0.001	0.007	0.344
Gm	0.742	<0.001	0.003	0.156
Gr	0.785	<0.001	0.002	0.440
Ge vs. Gm	0.742	0.022	0.775	0.628
Gr	0.682	0.637	0.695	0.861
Gm vs. Gr	0.935	0.065	0.916	0.510
AM treatments only				
Ge vs. Gm	0.984	0.006	0.672	0.679
Gr	0.964	0.551	0.909	0.880
Gm vs. Gr	0.977	0.023	0.757	0.573

^aThe mean weight diameter is calculated as $MWD = \sum_{i=1}^n \bar{x}_i w_i$. It is the summation over all n size fractions of the product of the mean diameter of each size fraction (\bar{x}_i) and the proportion of the total sample weight (w_i) occurring in the corresponding size fraction (Kemper and Rosenau, 1986). WSA, water-stable aggregation, %.

length or bacterial populations in stabilizing soil aggregates, as the highest level of soil hyphae (Table 6) and the lowest levels of bacteria (Table 5) or root length (Table 5) in the AM treatments was correlated with the best aggregation response.

Relationships between plant and soil traits

Soil aggregation and total soil bacterial counts were negatively correlated (Table 5), indicating C loss due to bacterial respiration. However, the data were not conclusive, and their evaluation may have been biased by

the high bacterial counts of the -P soils (Table 3). Considering only AM treatments, the relationship between the dominant G+ bacterial group and WSA was not significant ($p = 0.302$). The statistically significant WSA correlation with G- bacteria ($p = 0.003$) may have had little biological meaning due to the low preponderance of this group of bacteria in all soils. The highly significant positive correlation of WSA with the development of both soil and root hyphae (Tables 5 and 6) indicated that AM fungi play a role in the aggregative process of soil structure formation. Of the plant traits measured,

Table 5. Relationships between soil and plant traits of the 5-treatment data set evaluated by linear regression analysis. Response variables were: water-stable soil aggregates (WSA, 2 to 4 mm diameter,%), total bacteria (cfu), root length (m), specific root length (SRL, m g^{-1}), stem (g), and pod (g). Except for WSA (a dependent variable) all traits were considered to be interdependent. Numbers are regression coefficients (r) and probability values (p)

	WSA		Bacteria		Root length		SRL		Stem		Pod	
	r	p	r	p	r	p	r	p	r	p	r	p
Bacteria	-0.586	0.075										
Root length	-0.281	0.432	0.573	0.083								
SRL	-0.338	0.339	0.903	<0.001								
Stem	-0.436	0.208	-0.431	0.214	0.970	<0.001	-0.699	0.025				
Pod	-0.258	0.427	-0.565	0.088	0.995	<0.001	-0.803	0.005	0.964	<0.001		
Soil hyphae ^a	0.975	<0.001	-0.597	0.069	0.620	0.056	-0.580	0.079	-0.680	<0.001	0.894	<0.001
Root hyphae ^a	0.958	<0.001	-0.654	0.040	0.989	<0.001	0.133	0.715	-0.997	<0.001	0.347	0.326

^aCorrelations of plant and fungal traits are based on AM treatments only. Soil and root hyphae are the AM-fungal organs that colonize the soil or the root, respectively.

Table 6. Relationships between selected soil and plant traits among the three arbuscular mycorrhizal (AM) treatments. Response variables were: water-stable soil aggregates (WSA, 2 to 4 mm), Gram positive (G+, cfu) or Gram negative (G-, cfu) soil bacteria, soil hyphae (m g^{-1}), root hyphae (% colonization), and AM root length (m) and nodule numbers. Except for WSA (a dependent variable), all traits were considered to be interdependent. Numbers are regression coefficients (r) and probability values (p)

	Regression analyses with coefficients (r) and probabilities (p)											
	WSA		G+ bacteria		G- bacteria		Soil hypha		Root hypha		AM root length	
	r	p	r	p	r	p	r	p	r	p	r	p
G+ bacteria	-0.353	0.302										
G- bacteria	-0.834	0.003	0.817	0.004								
Soil hyphae	0.994	<0.001	-0.465	0.176	-0.890	0.001						
Root hyphae	0.650	0.042	-0.944	<0.001	-0.962	<0.001	0.731	0.016				
AM root ln	0.664	0.045	-0.947	<0.001	-0.959	<0.001	0.725	0.018	0.999	<0.001		
Nodule	-0.617	0.057	0.958	<0.001	0.949	<0.001	-0.700	0.024	-0.999	<0.001	-0.999	<0.001

only AM root length related (positively) with WSA (Table 6).

Soil bacteria were negatively correlated with both fungal and plant components of the mycorrhizae, except for specific root length (SRL, Tables 5 and 6). The highly significant positive correlation between bacteria and SRL (Table 5) indicated a close relationship between the fineness of roots and bacterial proliferation, probably because fine roots make contact with relatively larger volumes of rhizosphere soil than coarse ones. The experiment was not designed to evaluate nodulation, but we noted that nodulation was antagonized by mycorrhizae while favored by the bacterial microflora (Table 6). Although we did not determine the timing of colonization, early growth responses (evident by 4 weeks) indicated early establishment of Ge, but not of Gm and Gr. Thus, it appears that the extent of root colonization, and not the timing of mycorrhiza development, influenced nodule formation: Ge plants

had less AM root length (57%) and supported more nodules (52%) than Gm and Gr plants.

Root length was positively correlated with AM soil hyphae, and soils with high SRL had low soil hyphal densities (Table 5), suggesting a functional relationship between the fineness of roots and the need to support hyphae for nutrient uptake. The highly significant relationship between pod development and soil hyphae (Table 5) may be interpreted as increased hyphal development in response to nutrient demand associated with seed development (Bethlenfalvay et al., 1982), or as differences in plant-fungus compatibility (McGonigle and Fitter, 1990).

Conclusions

Interactions between plant and soil development were mediated by AM fungi in our reductionist microcosm of potted plants. Some of the data confirmed

findings that AM fungi improve soil aggregation and inhibit some soil bacteria (Andrade et al., 1995; Bethlenfalvay et al., 1997). Other data pointed to little-known aspects of mycorrhizal plant-soil relations: root-exudate-mediated changes in rhizosphere microflora in response to plant nutrient stress (Jakobsen and Rosendahl, 1990), fluctuations in soil aggregation with bacterial populations (Roldan et al., 1994), and nutrient allocation to above- and below-ground plant organs (Kothari et al., 1990). All of these phenomena were modified by the AM fungi in our experiment.

Our findings are pertinent to experimentation with AM fungi under controlled conditions and presage the difficulties of field work with them (Abbott et al., 1995; Fitter, 1985). Inclusion of the soil microflora (Puppi et al., 1994), soil fauna (Moore, 1994), pathogens (Linderman, 1994), and biocides (Ocampo, 1993) into work under controlled conditions cannot duplicate field conditions, but will lead to a better understanding of cause-effect relations between above- and below-ground events in AM research (Linderman, 1986). Thus, the addition of less-known aspects (here: soil hyphae, soil bacteria, and aggregate stability) to the standard components of AM experiments (root colonization, P nutrition, and plant response) is a step towards integration, understanding, and eventually utilization of the AM plant-soil system in agriculture (Bethlenfalvay and Linderman, 1992). Inferences to field applications that may be drawn from our data are: 1. AM fungi influence both plant and soil functions and mediate their interactions between above- and below-ground events; 2. inputs of different AM isolates to plant and soil responses are similar in some aspects but distinct in others; and 3. soil bacteria and AM fungi may form preferential associations.

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