

Mycorrhizae in sustainable agriculture. I. Effects on seed yield and soil aggregation

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Abstract. *Vesicular-arbuscular mycorrhizal (VAM) fungi colonize plant roots and the surrounding bulk soil. They transport mineral nutrients from the soil to the plant and carbon compounds from the plant to the soil, and have pervasive effects on plant form and function and on the composition of the soil microbiota. This experiment evaluated VAM effects on plants and soil to determine if VAM fungi mediate a relationship between changes in seed yield and soil aggregation. In a pot experiment with peas, an isolate of the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe did not significantly affect seed yield (8%), but improved soil aggregation by 400% in one soil, a gray silt-loam high in organic matter (OM) and phosphorus. In another soil, a yellow clay-loam low in OM and phosphorus, seed yield was enhanced significantly (57%), but there was only a small change (50%) in aggregation. The results suggest that carbon allocation between the plant (measured as seed yield) and the soil (measured as the formation of water-stable aggregates) is influenced by this VAM fungus. The soil appeared to gain carbon at the expense of carbon lost by the plant. Mycorrhizal fungi thus seem to affect two biologically controlled aspects of sustainable agriculture: plant production and soil quality.*

Key words: pea, plant growth, soil conservation, vesicular-arbuscular mycorrhizal fungi, water-stable soil aggregates

Introduction

The roots of most crop plants and weeds are colonized by beneficial vesicular-arbuscular mycorrhizal (VAM) soil fungi, forming symbiotic associations called mycorrhizae ("fungus roots") (Harley and

Smith, 1983). These fungi have a pervasive influence on plant form and function, often making the plant obligately dependent on its endophyte (Smith and Gianinazzi-Pearson, 1988; Graham et al. 1991). Because of their effects on plant growth and development, VAM fungi are considered integral parts of their host plant (Gianinazzi et al., 1982). It therefore is not surprising that until recently, research on VAM fungi focussed only on their effects on plants, either individual plants under controlled conditions (Reid, 1990) or plant communities both in natural (Barea and Jeffries, in press) and disturbed (Bagyaraj, 1992) ecosystems. This interest in the plant was enhanced by economic considerations, specifically the potential of VAM fungi to improve plant nutrition and yield (Bethlenfalvay, 1992), to protect plants from pathogens (Linderman, 1992), and to

improve plant resistance to environmental stress (Sylvia and Williams, 1992).

These goals of conventional, production-oriented agriculture (Cooke, 1982) continue to be valid, but there are now additional priorities. These priorities stem from the realization that the production base — a healthy soil that can support high-yielding plants — is a fragile resource in limited supply that must be protected and conserved to sustain agricultural production (Reganold et al., 1990). Mycorrhizal fungi, as colonizers not only of roots and the rhizosphere but also of the bulk soil, appear to play as great a role in conserving soil as in enhancing plant productivity. They mediate the movement of mineral nutrients from the soil to the plant and of carbon compounds from the plant to the soil. Although this exchange is known to benefit plants, only recently have its effects on soil aggregation been recognized (Tisdall and Oades, 1979) and fully appreciated (Tisdall, 1991; Miller and Jastrow, 1992). However, a relationship between VAM effects on plants and soil has not been demonstrated. The purposes of this experiment were to determine both host-plant and host-soil responses to a VAM fungus in a controlled and therefore simplified model setting, and to evaluate the VAM effect on the plant-soil system in the context of sustainable agriculture's simultaneous goals of plant production and soil conservation.

Materials and Methods

Experimental design

Soil type and VAM colonization were used as factors in a randomized 2×2 factorial design involving 2 soils, with the plants

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in each soil grown with or without VAM fungi. The experimental unit was one pot containing one plant, with each of the 4 treatments replicated 5 times. (This design was part of a larger experiment to be discussed in later reports.) The data were evaluated by analysis of variance, and significant main effects were further evaluated by student's t-test. Actual significance levels are presented (except that levels below 0.001 are reported simply as "p<0.001") instead of using an arbitrary 5% significance level, because this allows a more precise interpretation of each result's statistical significance (Nelson, 1989).

Biological materials and soil types

Pea (*Pisum sativum* L., cv. 'Lincoln') plants were inoculated with an isolate of the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe. This isolate was originally obtained from Barbara Mosse (Rothamsted Experimental Station, Hertfordshire, England), and cultured at the Estación Experimental del Zaidín (EEZ) for over 10 years on *Sorghum halepense* L. in EEZ soil. A saturating amount of inoculum was used, consisting of at least 1000 spores and sporocarps and 500 heavily colonized root fragments per pot.

The soils were obtained from the garden of the EEZ at Granada (Soil G) and from near Guadalupe, Extremadura (Soil E), Spain. Soil G, a gray silt-loam (43% sand, 48% silt, 9% clay) had a pH of 7.6, and organic matter (OM) and N contents of 11.4 and 2.8 g/kg, respectively. Other nutrients (in mg/kg) were: P (NaHCO₃-extractable), 43.1; Cu, 12; Fe, 18; Mn, 37; and Zn, 12. Soil E, a yellow clay-loam (42% sand, 40% silt, 18% clay) had a pH of 7.0, OM and N contents of 3.2 and 2.0 g/kg, respectively. Other nutrients (in mg/kg) were: P (NaHCO₃-extractable), 20.6; Cu, <1; Fe, 23; Mn, 53; and Zn, 2. The soils were autoclaved for 1 hour on 3 consecutive days. Sievings (30 µm) of the inoculum free of VAM propagules were added to all soils to equalize the microbiota.

Growth conditions

Plants were grown as greenhouse pot cultures from January to March 1993 in Granada, Spain. Temperatures were controlled to vary between 15 and 32°C, but because of occasional power failures the maximum day/night range was 41°C to 4°C. The pots were wrapped in reflective aluminum foil to shield the soil from the sun. They contained 350 g of soil mixed with 350 g of sand. The sand was sieved to a particle-size range of 2 to 3 mm. The VAM inoculum was mixed uniformly with the growth medium before planting. Pea seeds were pregerminated and selected for uniformity. Seedlings were thinned from 3 to 1 plants per pot after 1 week of growth. The plants were watered with a 4 mM solution of Ca(NO₃)₂ twice a week initially, then daily during the last 3 weeks of the experiment. They were harvested after 8 weeks of growth, when the pods were mature.

Assays

Roots were washed and fresh mass was determined. Samples were weighed and stained for VAM colonization (Phillips and Hayman, 1970). The length of the root sample was determined by counting the total (+VAM and -VAM) intersections (grid-line intersect method, Ambler and Young, 1977). Total root length was estimated from the ratio of the sample to total fresh mass. Colonized root length was calculated according to Giovanetti and Mosse (1980). Seeds were weighed after drying at 70°C for 2 days.

The growth medium was permitted to dry before harvest so that it could be easily crumbled and shaken loose from the roots. It was then air-dried and crushed with a dough roller. In this process, soil aggregates smaller than 2 mm were protected by the presence of the large (>2 mm) particles of sand. The crushed sand-soil mixture was sieved (2 mm openings) to separate and discard the coarse (>2 mm) sand. The remaining (dry, <2 mm) soil fraction was sieved again (1 mm openings) to separate it into 2 size classes (>1 mm and <1 mm). The soils of these two size classes were weighed and the large aggregates (>1 mm) that at this stage still contained fine (<2

mm) sand were reported as a percentage of total soil.

Water-stable soil aggregates (WSA) were determined according to Kemper and Rosenau (1986) with modifications. Dry aggregates (>1 mm) were spread in a thin, even layer (30 g) on a 1 mm sieve stacked above a 0.5 mm sieve of the same diameter (10 cm). The sieves were submerged rapidly into water (taking care first to eliminate air bubbles from below the 0.5 mm mesh) and agitated through a 10-cm water column with even, vertical strokes at 16 strokes per minute for 3 minutes. The WSA remaining on the sieves were washed onto preweighed filter paper with a wash bottle, dried at 110°C, and weighed. Next, the sand content of the WSA (size intervals 1 to 2 mm and 0.5 to 1 mm) was determined as follows. The sample was placed back on its sieves, submerged in a dilute NaCl solution, and washed to disperse the aggregated soil. The sand remaining on the sieves was dried, weighed, and subtracted from the original WSA to get the mass of sand-free aggregated soil.

The change in aggregation during the experiment was used as a second measure of the effect of VAM on WSA. The WSA status of the pre-experiment soil was determined (10 samples) by the same method as the post-experiment soils at harvest. The percentage difference between the two values was calculated as $[(WSA_{post} - WSA_{pre})/WSA_{pre}] \times 100$. Positive numbers show aggregation, negative numbers show slaking of the soil during the experiment.

Results

Seed yield of -VAM plants in the two soils depended on soil P content: in low-P Soil E, seed dry mass was lower (p<0.001) than in Soil G (Table 1). Colonization by the VAM fungus improved seed development by 57% (p<0.001) in Soil E plants, but had little effect (8%, p=0.120) in plants grown in Soil G. In the +VAM plants, the endophyte apparently equalized seed development: there was no difference in yield between plants growing in E or G soils (p=0.452).

Root length behaved like seed yield (Table 1), showing no VAM effect in Soil G

Table 1. Seed yield, root development, and soil aggregation status of potted pea plants.¹

Observations	Soil G			Soil E		
	+VAM	-VAM	p-value	+VAM	-VAM	p-value
Seed dry mass (g)	2.2	2.0	0.120	2.3	1.3	<0.001
Soil aggregates						
Dry aggregates	30.1	26.7	0.008	45.2	38.8	0.031
WSA <1 mm	6.9	5.4	0.043	5.8	4.4	0.028
WSA >1 mm	13.0	2.6	<0.001	22.3	11.3	0.003
WSA Ratio	1.9	0.5	<0.001	3.9	2.6	0.029
Root parameters						
Fresh mass (g)	5.4	4.5	0.001	4.7	3.2	<0.001
Length (m)	25.8	24.7	0.434	21.9	17.0	0.019
Colonized length (m)	4.7			13.9		

¹ Plants were grown in a silt-loam (G) or loam (E) soil and were colonized by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (+VAM) or left uncolonized (-VAM). Dry aggregates were greater than 1 mm. Water-stable soil aggregates (WSA) were in two size classes: 1 to 2 mm and 0.5 to 1 mm. p-value refers to the statistical significance of the difference between +VAM and -VAM means by a t-test.

($p=0.434$) but a marked increase ($p=0.019$) in +VAM over -VAM plants in Soil E. In contrast to seed yield, root lengths of the +VAM plants in the two soils were different ($p=0.043$). Only 18% of the root length in Soil G was colonized by the VAM fungus, compared with 63% in Soil E. Fresh root mass of +VAM and -VAM plants differed ($p<0.001$) within each soil type (Table 1). However, the difference in fresh root mass between the two soil types depended on VAM treatment (Soil G vs. Soil E: +VAM, $p=0.058$; -VAM, $p<0.001$).

The dry aggregation status of the soil can be interpreted as a measure of physical cohesion among soil particles and soil and fine sand. This cohesion was greater in Soil E than in Soil G in both the +VAM ($p<0.001$) and the -VAM ($p=0.004$) treatments (Table 1). Within soil type, dry aggregation was greater for the +VAM than the -VAM treatment (Soil E, $p=0.008$; Soil G, $p=0.031$).

The percentage of large (>1 mm) WSA for both +VAM and -VAM treatments was markedly greater ($p<0.01$ for both) in Soil E than in Soil G, perhaps because of the higher (two-fold) clay content of the former, or because of the greater length of colonized roots (Table 1). Differences in large WSA between +VAM and -VAM treatments also were large (Soil G, $p<0.001$; Soil E, $p=0.003$). Unlike with the

large WSA, the percentages of small WSA (Table 1) were similar between the two soil types within both VAM treatments (E vs. G: +VAM, $p=0.101$; -VAM, $p=0.077$). However, there were marked differences between the +VAM and -VAM treatments within the same soil type (Soil G, $p=0.043$; Soil E, $p=0.028$). Thus, the differences in small WSA between the +VAM and -VAM treatments were less than the differences in large WSA. This may be ascribed to an accelerated slaking of the large WSA in the -VAM treatments. That is, large WSA in the -VAM treatments were converted to small ones at a faster rate than in +VAM treatments. This process is reflected in the large/small WSA ratio (Table 1), which is a measure of the relative changes in WSA size.

The change in WSA status during the experiment was positive for the small WSA (Fig. 1). In Soil G, the increase was small ($p=0.324$) in the -VAM treatment, but large ($p<0.001$) in the +VAM treatment, as it was for both the +VAM and -VAM treatments in Soil E ($p<0.001$). The increase in small WSA was greater in Soil E than in Soil G in the soils of both the +VAM ($p=0.014$) and the -VAM ($p=0.002$) treatments. For the large WSA, however, there was a marked difference not only in the magnitude but also in the direction of change: in the +VAM treat-

ments WSA were formed, while in the -VAM treatments the soil disaggregated. The apparent increase in small WSA in the -VAM treatment probably resulted from the slaking of the larger aggregates: as macroaggregates disintegrate, the proportion of microaggregates increases (see Cambardella and Elliott, 1993). The severe slaking of the large -VAM aggregates was probably enhanced by the large day-night temperature differences and the concomitant shrinking/swelling of the soils. This process apparently was ameliorated in the +VAM treatments.

Discussion

We have used an artificial agrosystem (see Bethlenfalvay and Schüepp, 1994), simplified to contain only its most basic components (plant and sterile soil), to show some effects of a member of an ever-present but little-known class of soil organisms on both plant and soil. These organisms, the VAM fungi, are best known as plant symbionts, and have been shown to alter fundamentally the development, nutrition and physiology of plants (see Bethlenfalvay, 1992). Much less is known, however, of their role in soil development, even though their hyphae are known to transport mineral nutrients from

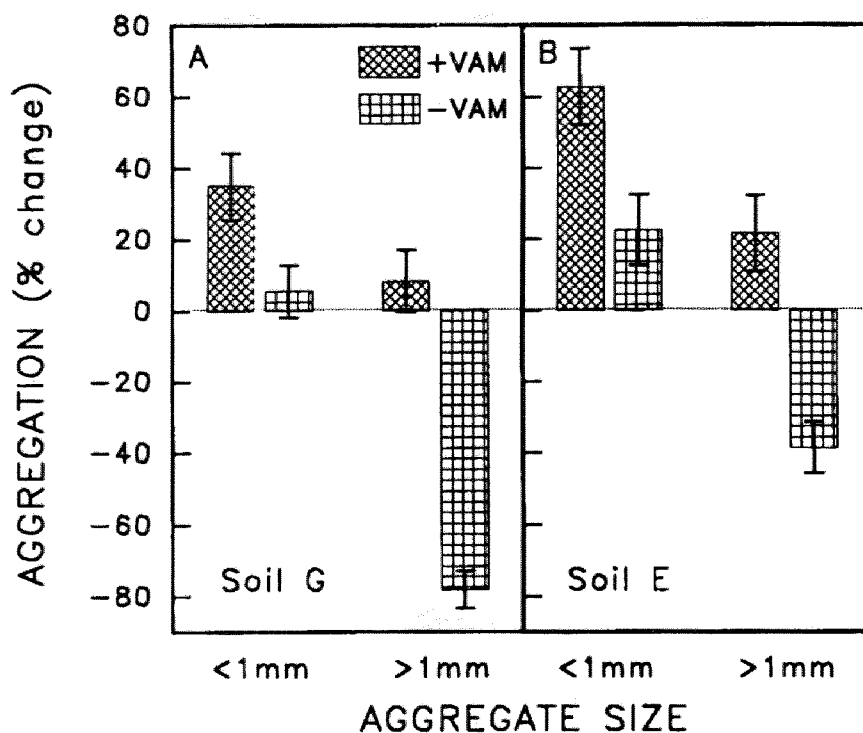


Figure 1. Percent change in water-stable soil aggregates (WSA) during the experiment. Soils were inoculated with a vesicular-arbuscular mycorrhizal fungus (+VAM) or not inoculated (-VAM). The sizes of WSA were 1 to 2 mm or 0.5 to 1 mm. Values reflect the means and standard errors of 5 replicates.

the soil to the plant and carbon compounds from the plant to feed the soil biota.

Our results, which focus on combined VAM effects on both plant and soil, reveal three salient points: 1) root colonization by VAM fungi affects soil aggregate status; 2) the VAM condition of the plant-soil system affects the relationship between plant and soil development; and 3) the concept of "mycorrhizal effectiveness" needs to be reevaluated. Defined in the past by VAM-mediated changes in host-plant growth (see Graham et al., 1991), VAM effectiveness in the context of sustainability must include VAM effects on the host soil. Simultaneous VAM-mediated enhancement of both plant and soil development is possible only when the production of carbohydrates is not limited by some external factor. When the supply of carbon is limiting, allocation priorities that probably are regulated at the symbiosis level (Andersen and

Rygiewicz, 1991) will direct its flow to the strongest sinks within the plant-fungus association (Wang et al., 1989; Eissenstat et al., 1993) or to the ultimate sink, the soil (Jakobsen and Rosendahl, 1990). If "soil quality is the key to sustainable agriculture" (Papendick and Parr, 1992), the role of soil as a sink for carbon becomes a useful one, even if it is at the expense of plant growth.

The data illustrate these considerations. In Soil G, there was no VAM effect in the conventional sense: the difference between the seed yields of +VAM and -VAM plants was small (8%, $p=0.120$; Table 1). However, the difference between the percentage of large WSA in the +VAM and -VAM treatments was large (400%, Table 1), and the -VAM soil disaggregated, whereas WSA increased in +VAM soil relative to the original soil (Fig. 1). Since root length was the same in the +VAM and -VAM

treatments (Table 1) and the soils were not root-bound, the aggregation effect cannot be ascribed to compaction through root pressure in a confined volume, but rather to the activity of the VAM mycelium (Thomas et al., 1993). This raises two questions: 1) whether the improved WSA status of the +VAM soil (carbon gained by the soil, Jakobsen and Rosendahl, 1990) was at the expense of carbon that could have contributed to seed development (carbon lost by the plant); and 2) how improved WSA status may benefit the development of subsequent crops. The level of colonization, although low, was sufficient (by our experience) to enhance plant growth.

In Soil E, there was a respectable 56% increase in seed yield of +VAM plants over the -VAM control, but the improvement in large WSA was only 50%. Why was carbon allocation between plant and soil controlled differently in Soil E (favoring plant over soil) compared with Soil G? There are several possible reasons: physiological changes (Koide and Schreiner, 1992) resulting from more intense colonization (63% vs. 18%); differences in soil texture (more clay, less organic matter) or mineral nutrients (less P, Cu, and Zn); or differences in the intensity of hyphal permeation of the soils. The experiment was not designed to answer these questions, but to point them out because of their relevance to alternative agricultural practices (Parr et al., 1992).

Summary and Conclusions

In one soil, root and soil colonization by a VAM fungus did not enhance seed yield, but markedly improved aggregation compared with the -VAM control. In another soil, the same VAM fungus improved soil aggregation only slightly but enhanced seed yield significantly. Thus, the VAM fungus affected the development of both its plant and soil hosts. The flux of carbon to the soil and its availability to the soil biota apparently were enhanced by the VAM soil mycelium, which accounts for the VAM effect on soil aggregation. Of interest are the conditions that stimulate the plant to allocate carbon preferentially to its

own development or to its fungal endophyte and ultimately to formation of soil structure. Also of interest is the role that the VAM fungus may play in influencing the carbon allocation process. Its ability to affect the balance of plant or soil development shows that it has the potential of a biological tool for use in both plant production and soil conservation.

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