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Response of sulphur cycling microorganisms to arbuscular mycorrhizal fungi in the rhizosphere of maize

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Abstract

Fluctuations in the number of anaerobic sulphur mineralizers, autotrophic sulphur oxidizers and sulphate-reducing microorganisms in pot cultures of arbuscular mycorrhizal and non-mycorrhizal plants were examined at 0, 15, 30, 45 and 60 days after planting using the most probable number enumeration method. Two arbuscular mycorrhizal (AM) fungi belonging to different *Glomus* species were assayed. Populations of S-cycling microorganisms varied along the growth period for both AM fungi colonized and uncolonized plants, with significant quantitative and qualitative changes in the mycorrhizal compared with the non-mycorrhizal root zone. The occurrence of autotrophic sulphur oxidizers in pot cultures colonized with *G. fasciculatum* was significantly higher than in non-mycorrhizal ones throughout the plant growth period. The population size of this group was similar in non-mycorrhizal and *G. mosseae* colonized soil. The population of anaerobic sulphur mineralizing microorganisms was significantly decreased by mycorrhizal colonization. In contrast, pot cultures colonized by *G. mosseae* or *G. fasciculatum* had a higher quantity of sulphate-reducing bacteria than the control. The fact that the autotrophic S oxidizers and sulphate-reducing root-associated microorganisms were selectively stimulated by each one of the AM fungi assayed is a noticeable aspect. The maximum activity of the microbial processes regarding specific groups was at 15 days for the mineralizing, 45 days for the oxidizing and 0 days for the reducing microorganisms. In the case of autotrophic S oxidizers, the maximum population was achieved by 30 days in *G. fasciculatum* colonized treatment. From the data on occurrence of physiological grouping of sulphur-cycling microorganisms, we can deduce that a different ecological situation existed in the rhizosphere developed by non-mycorrhizal plants compared with those colonized by each one of the mycorrhizal endophytes. © 1997 Elsevier Science B.V.

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1. Introduction

In general, soil productivity and nutrient cycling in the rhizosphere are influenced by the characteristics of the soil microbial populations. The significance of biological factors related to S cycling in the soil are still incompletely understood. In natural ecosystems, the activity of microorganisms could be used to evaluate soil productivity (Bethlenfalvay and Schüepp, 1994). Current interest in environmental problems associated with sulphur in the soil is mainly focused on the transformation processes, which are

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in part microbial (Maynard et al., 1985). Few studies have been done on the ecology of these microbial groups, and data about their survival and growth are important in understanding the processes of S transformation in nature.

Arbuscular mycorrhizal (AM) symbiosis is a widespread phenomenon. Mycorrhizal status may alter the number and activity of sulphur-cycling microorganisms through changes in the soil conditions. Negative as well as positive AM effects on total viable counts of bacteria have been reported as result of AM colonization (Secilia and Bagyaraj, 1987).

There are no studies on the interactions between sulphur-cycling microorganisms and AM fungi. For this reason, the present work was undertaken as part of an examination of the influence of roots colonized by two selected AM-forming species (*G. mosseae* and *G. fasciculatum*) in the numbers of anaerobic sulphur mineralizers, autotrophic sulphur oxidizers and sulphate-reducing microorganisms.

2. Materials and methods

2.1. Experimental design

The experiment consisted of three treatments: non-mycorrhizal control, and *G. mosseae*- or *G. fasciculatum*-colonized plants. Triplicate pots with one plant per pot were placed in a random complete block design. Measurements were taken at 0, 15, 30, 45 and 60 days after sowing and mycorrhizal inoculation.

2.2. Test soil and growth conditions

The test soil was collected from Guejar-Maitena, Granada Province, Spain. It was a cambisol type (14.91% clay, 26.37% loam and 58.71% sand), containing 0.8% organic matter and 14.8 mg P kg^{-1}, with pH (H_2O) 6.8 (Esteban et al., 1974). The N content was 2.6 g kg^{-1}, with 2.2 mg kg^{-1} of $N\text{–}NO_3^-$. The soil was sieved (< 2 mm), diluted with sand (soil:sand, 5:2 v/v) and steam-sterilized (1 h, on three consecutive days) before the reintroduction of the native microbial population except for propagules of arbuscular mycorrhiza fungi. The soil was re-inoculated with 20 ml per pot of an aliquot of a filtrate of a natural soil extract. The extract was obtained by mixing equal volumes of soil and water, and filtration through Watman No. 1 paper after shaking and decanting the suspension.

One seed was sown in each pot containing 1 kg of the re-inoculated soil–sand mixture. Plants were grown in a greenhouse under a 16-h light (21°C) and 8-h dark (15°C) cycle, with 59% relative humidity and a photosynthetic photon flux density of 700 $\mu mol\ m^{-2}\ s^{-1}$ for the compensating photophase. Throughout the experiments, pots were weighed every day and water loss was replaced by top watering to maintain soil moisture close to 100% of field capacity during the period of plant growth.

2.3. Mycorrhizal inoculation

Inoculum of each AM fungus was obtained from a stock culture raised with *Lactuca sativa*. After 6 months of plant growth, the shoots were removed and the underground part stored for 6–9 months in a polyethylene box at 5°C.

The mycorrhizal inoculum consisted of thoroughly mixed rhizosphere samples containing spores, mycelium and mycorrhizal root fragments of *Glomus mosseae* (Nicol. et Gerd.) Gerd. et Trappe and *G. fasciculatum* (Thax. sensu Gerd.) Trappe et Gerd. The AM fungal species were obtained (as previously described by Azcón, 1989) from the stock culture collection at the Zaidín Experimental Station. Five grammes of inoculum were put into the planting hole at the time of planting maize seeds.

2.4. Most probable number (MPN) enumeration method

Subsamples (20 g) of soil were taken from each pot with a hollow cylinder to 2 cm depth after 0, 15, 30, 45 and 60 days. The hole was closed with the soil of the same pot.

One soil sample (10 g wet weight) was placed in an oven at 70°C to obtain the soil moisture percentage. The other 10 g were diluted and homogenized by shaking in a 10-fold dilution series in 90 ml of sterile water plus a drop of Tween 80. This dilution series was used for immediate inoculation of three test tubes per dilution containing the appropriate medium (Alexander, 1982).

MacGradýs tables (Postgate, 1969) were used to calculate the MPN of organisms in the original sample.

2.5. Number of anaerobic sulphur-mineralizing microorganisms

Test-tubes were filled with 12 ml of medium containing 0.5 g K_2PO_4H, 1 g NH_4-clorure, 0.1 g $CaCl_2$, 1 g L-cysteine, 0.5 g Fe–NH_4–citrate, 5 ml Na–lactate at 60%, 20 ml soil extract and 100 ml distilled water (Pochon and Tardieux, 1962), and also 1 ml of the rhizosphere soil dilute inoculum in the absence of sulphate. After inoculation of the test tubes with appropriate dilutions, their caps were tightened to prevent the diffusion of atmospheric oxygen. The temperature of incubation was constant at 28°C. The tubes were examined every 3 days during a 29-day period for the presence of a black precipitate of FeS indicating active H_2S production. Other organics and sulphate or any inorganic form of reducible sulphur were excluded in the medium.

2.6. Numbers of autotrophic sulphur oxidizers

Test-tubes were filled with 10 ml of Starkey medium (Kuenen and Tuovinen, 1981) containing (g l^{-1}): K_2HPO, 3.5; $(NH_4)_2SO_4$, 0.3; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.018; $CaCl_2$, 0.25; flowers of sulphur, 5.0. Elemental sulphur served as the sulphur source in this medium, and 1 ml of rhizosphere soil dilution inoculum was incubated under anaerobic conditions. The sulphur oxidizer tubes and controls were incubated at 26–28°C in the dark to exclude phototrophic sulphur bacteria. Initial observations were made after 4 weeks. The presence of H_2SO_4 was examined by changes in pH. Weekly monitoring was carried out for 6 weeks.

2.7. Enumeration of sulphate-reducing bacteria

Screw-top tubes were filled with a solution of Lapage medium. Solution 1 (g per 980 ml distilled water): K_2HPO_4, 0.5; NH_4Cl, 1.0; Na_2SO_4, 1.0; $CaCl_2.2H_2O$, 0.1; $MgSO_4.7H_2O$, 2.0; Na–lactate (70%), 3.5; yeast extract, 1.0. Solution 2 (g per 10 ml distilled water): $FeSO_4.7H_2O$, 0.5 g (preferably acidic with sulphuric acid). Solution 3 (g per 10 ml distilled water): ascorbic acid, 0.1; Na–thioglycolate, 0.1. Lactate served as the carbon source and electron donor, and Na_2SO_4, $MgSO_4$ and $FeSO_4$ as sulphate sources (Pfennig et al., 1981), to displace about two-thirds of the total volume. After inoculation of the test tubes with 1 ml of the rhizosphere soil dilutions, the caps were tightened to exclude the diffusion of atmospheric oxygen during a 6-week incubation period. A black precipitation indicated the presence of FeS.

2.8. Determination of plant weight and mycorrhizal infection

The plants were harvested after 60 days. The dry weight of the shoots and roots were recorded after drying in an oven at 70°C.

The percentage of total root length infected was estimated by examining stained samples (Phillips and Hayman, 1970). The grid-line intersect method of Giovannetti and Mosse (1980) was used for microscopic examination.

Data were subjected to analysis of variance, and the means were differentiated with Duncańs multiple range test.

3. Results

Results on the population of anaerobic sulphur-mineralizing microorganisms are presented in Fig. 1. The maximum occurrence of anaerobic sulphur-mineralizing microorganisms occurred at 15 days of plant growth. *G. mosseae* and *G. fasciculatum* simi-

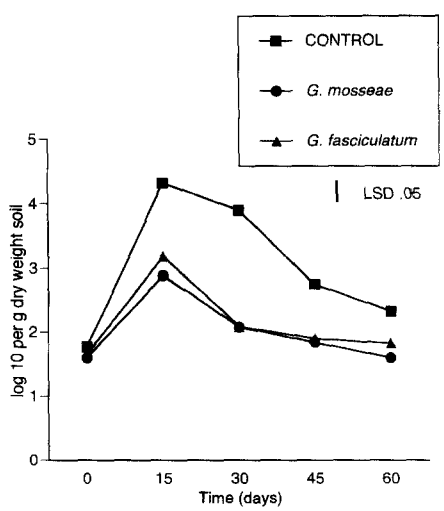

Fig. 1. Effect of endomycorrhizal fungi (*Glomus mosseae* or *G. fasciculatum*) on the sulphur-mineralizing microorganisms in maize rhizosphere soil.

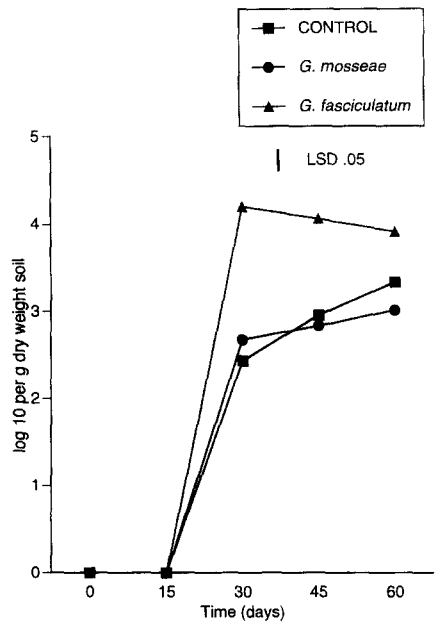

Fig. 2. Effect of endomycorrhizal fungi (*Glomus mosseae* or *G. fasciculatum*) on the autotrophic sulphur oxidizer population in maize rhizosphere soil.

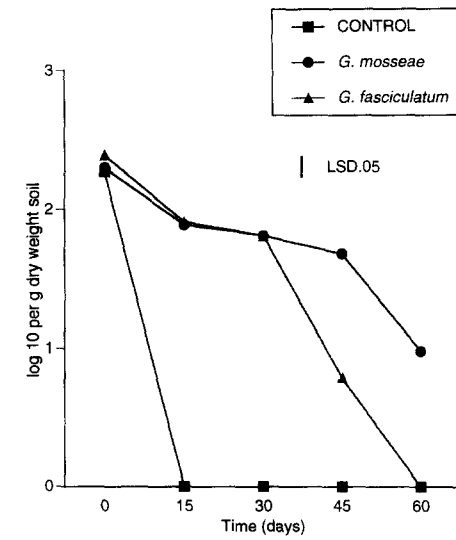

Fig. 3. Effect of endomycorrhizal fungi (*Glomus mosseae* or *G. fasciculatum*) on the sulphate-reducing bacteria in maize rhizosphere soil.

larly affected the populations of these microbial groups.

Initially (15 days), no autotrophic sulphur oxidizers were detected. After 30 days of plant growth a substantial increase in the population of autotrophic S oxidizers was found, particularly in the *G. fasciculatum*-colonized rhizosphere zone.

Autotrophic sulphur oxidizers in the rhizosphere soil of *Glomus fasciculatum* mycorrhizal pots were significantly more abundant than in the non-mycorrhizal control or *G. mosseae* pot cultures at 30, 45 and 60 days into the evaluation (Fig. 2). Soils from both non-inoculated and *G. mosseae*-inoculated pots showed similar bacterial populations.

Rhizosphere soil from AM-colonized plants harboured higher sulphate-reducing bacterial populations than non-mycorrhizal controls (Fig. 3). In non-mycorrhizal rhizosphere soil, no sulphate-reducing microorganisms were detected after 2 weeks of plant growth. Nevertheless, a population of this group was present in *G. fasciculatum*-colonized soil even at 45 days of plant growth. In the rhizosphere soil from *G. mosseae*-colonized plants, this effect was maintained even at 60 days of plant growth. Determinations at 45 and 60 days show that *G. mosseae* colonization had a consistent positive effect in enhancing the sulphate-reducing bacterial population.

Mycorrhizal inoculation positively affected plant growth, with *G. fasciculatum* being the most efficient endophyte. The degree (as a percentage) of mycorrhizal colonization was similar by both AM fungi (Table 1).

Table 1
Effect of arbuscular mycorrhizal fungi on the shoot and root growth and mycorrizal colonization of 60-day-old maize plants

Treatment	Shoot dry weight (g)	Root dry weight (g)	Mycorrhizal infection (%)
Control	0.23 a	0.21 a	0
G. mosseae	0.30 b	0.35 b	36.2
G. fasciculatum	0.40 c	0.42 c	39.4

Means followed by the same letter in each column are not significantly different ($P < 0.05$) as determined by Duncańs multiple range test.

4. Discussion

Populations of S-cycling microorganisms fluctuated during the plant growth period and were influenced by mycorrhizal colonization of maize plants. The physiological changes, including C compounds released to the medium between control and mycorrhizal plants during the growth period, may have been responsible for these differences.

AM colonization normally decreases the amount of plant root-derived organic matter available for bacterial growth by altering the permeability of root cells, thus affecting exudation (Rambelli, 1973; Schwab et al., 1983). These finding support the changes found in viable counts of total and specific bacterial groups reported by Ames et al. (1984) and Secilia and Bagyaraj (1987).

Under the experimental conditions of this study, the most abundant S-cycling population at initial time (0 days) was that of the sulphur-reducing microorganisms. This group maintained a high population during the period of plant growth only under mycorrhizal conditions. The metabolic activity of the mycorrhizal root cells affects the rhizosphere soil conditions over time, and that change seems to be involved in the highest number of sulphur-reducing microbial populations in AM treatments. Increases in rhizosphere microbial populations have often been attributed to soluble organic substances (sugar, organic acids and amino acids) exuded by roots (Foster, 1988; Kloepper et al., 1985; Lynch, 1989).

The population of autotrophic sulphur oxidizers was not detected in the first two evaluations (0 and 15 days); nevertheless, the mycorrhizal effect of *G. fasciculatum* particularly increasing the counts of this bacterial group was important. Autotrophic sulphur-oxidation is a biological process due, so far as is known, to a few genera of chemoautotrophic bacteria.

Previous studies have show that rhizosphere bacterial populations are differently affected by arbuscular–mycorrhizal colonization depending on the fungus involved (Azcón, 1989; Azcón-Aguilar and Barea, 1991). Meyer and Linderman (1986) also reported that arbuscular mycorrhiza affected specific groups of bacteria.

It is difficult to predict the outcome of interactions between bacteria and AM fungi in relation to the microbial activity as well as the meaning of this activity regarding the interaction of specific groups of microorganisms on plant growth. The interactions between mycorrhizal fungi and the S-cycling microbial population may be a consequence of changes in physical and/or chemical characteristics into the environment closed to mycorrhizal roots. AM fungi do not consume organic C from the rhizosphere due to their symbiotic condition, but they can potentially affect the rhizosphere hydrocarbonate status in several ways depending on their effect on the host's physiological processes (Secilia and Bagyaraj, 1987; Paulitz and Linderman, 1989; Ruiz-Lozano et al., 1995). The different physiology of roots colonized by AM fungi compared with non-mycorrhizal roots may alter root exudation, thereby influencing the growth conditions for the general microbiota (Christensen and Jakobsen, 1993).

In this study, each one of the mycorrhizal fungi affected each specific S-cycling microbial group in a different way compared with the control. In fact, the number of sulphur oxidizers was increased and mineralizing bacteria decreased in the mycorrhizal rhizosphere.

Regarding oxygen conditions in the rhizosphere zone, this area is influenced by the oxygen diffusion from the atmosphere, by the metabolic activity of inhabiting microbial populations, and also by root metabolism. All these oxygen sources are interacting during the plant growth period and are closely related to the metabolic changes in the rhizosphere zone.

The present data provide evidence supporting the theory that as result of mycorrhizal colonization, the ecological soil conditions change, affecting specifically the microbial population involved in S-cycling and presumably the amount of available S in the soil. However, it is impossible to establish a cause–effect correlation for the interactions observed and their involvement in plant growth and nutrition.

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References

Alexander, M., 1982. Most probable number method for microbial populations. In: Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Madison, WI, pp. 815–820.

Ames, R.N., Reid, C.P.P. and Ingham, E.R., 1984. Rhizosphere bacterial population responses to root colonization by a vesicular–arbuscular mycorrhizal fungus. New Phytol., 96: 555–563.

Azcón, R., 1989. Selective interaction between free-living rhizosphere bacteria and vesicular–arbuscular mycorrhizal fungi. Soil Biol. Biochem., 21: 939–944.

Azcón-Aguilar, C. and Barea, J.M., 1991. Interactions between mycorrhizal fungi and other rhizosphere microorganisms. In: M.F. Allen (Editor), Mycorrhizal Functioning. Chapman Hall, New York.

Bethlenfalvay, G.J. and Schüepp, H., 1994. Arbuscular mycorrhizas and agrosystem stability. In: S. Gianinazzi and H. Schüepp (Editors), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhaüser, Berlin, pp. 117–131.

Christensen, H. and Jakobsen, I., 1993. Reduction of bacterial growth by a vesicular–arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L.). Biol. Fertil. Soils, 15: 253–258.

Esteban, E., Huertas, F., Linares, J. and Aguilar, A., 1974. Potassium assimilable dams quelques rols types de la province de Granada (Espagne). Agrochimica, l0: 558–564.

Foster, R.C., 1988. Microenvironment of soil microorganisms. Biol. Fertil. Soil, 6: 189–203.

Giovannetti, M. and Mosse, B., 1980. An evaluation of techniques for measuring vesicular–arbuscular infection in roots. New Phytol., 84: 489–500.

Kloepper, J.W., Scher, F.M., Laliberte, M. and Zalesca, I., 1985. Measuring the spermosphere colonizing capacity (spermosphere competence) of bacterial inoculants. Can. J. Microbiol., 31: 926–929.

Kuenen, J.G. and Tuovinen, O.H., 1981. The genera *Thiobacillus* and *Thiomicrospira*. In: M.P. Starr and H. Stolp (Editors), The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Springer, New York, pp. 1023–1036.

Lynch, J.M., 1989. Development and interaction between microbial communities on the root surface. In: V. Vancura and F. Kunc (Editors), Interrelationships Between Microorganisms and Plants in Soil. Elsevier, Amsterdam, pp. 5–12.

Maynard, D.G., Stewart, J.W.B. and Bettany, J.R., 1985. The effects of plants on soil sulphur transformations. Soil Biol. Biochem., 17: 127–134.

Meyer, J.R. and Linderman, R.G., 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol. Biochem., 18: 191–196.

Paulitz, T.C. and Linderman, R.G., 1989. Interactions between fluorescent pseudomonas and VA mycorrhizal fungi. New Phytol., 113: 37–45.

Pfennig, N., Widdel, F. and Trüper, H.G., 1981. The dissimilarity of sulcate-reducing bacteria. In: M.P. Starr and H. Stolp (Editors), The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Springer, New York, pp. 1005–1022.

Phillips, D.A. and Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158–161.

Pochon, J. and Tardieux, Y., 1962. Techniques d'analyse en Microbiologie du sol. de la Tourelle (Editor), Paris.

Postgate, J.R., 1969. Viable counts and viability. In: J.R. Norris and D.W. Ribbons (Editors), Methods in Microbiology. Vol. 1. Academic Press, New York, pp. 611–618.

Rambelli, A., 1973. The rhizosphere of mycorrhizae. In: G.L. Marks and T.T. Koslowski (Editors), Ectomycorrhizae: Their Ecology and Physiology. Academic Press, New York, pp. 299–343.

Ruiz-Lozano, J.M., Azcón, R. and Gómez, M., 1995. Effects of arbuscular–mycorrhizal *Glomus* species on drought tolerance: Physiological and nutritional plant responses. Appl. Environ. Microbiol., 61: 456–460.

Schwab, S.M., Menge, J.A. and Leonard, R.T., 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sundangrass roots in relation to vesicular–arbuscular mycorrhiza formation. Plant Physiol., 73: 761–765.

Secilia, J. and Bagyaraj, D.J., 1987. Bacteria and actinomycetes associated with pot cultures of vesicular–arbuscular mycorrhizas. Can. J. Microbiol., 33: 1069–1073.