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Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants

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Abstract

Arbuscular mycorrhizal (AM) fungi as well as microbial-free inoculants used as phytostimulators (*Azospirillum*) or as biological control agents of fungi (*Pseudomonas* and *Trichoderma*) have shown beneficial effects on plant growth and health. The study of plants inoculated with biological control agents and AMF requires special attention because of the possibility that these fungal antagonists could also interfere with AM fungi. Our study was performed to test the effects of these inoculants upon mycorrhizal colonization in maize plants inoculated with *Glomus mosseae*, *Glomus deserticola* and natural AMF from the test soil. Populations of culturable bacteria and fungi in the rhizosphere soil were also examined since inoculation with AM fungi and other soil microorganisms can affect both quantitatively and qualitatively the microbial communities in the plant rhizosphere. Enzyme activities (esterase, phosphatase, trehalase and chitinase) were used as an index to detect changes in the microbial functioning in soil, as affected by mycorrhizal and other inoculation treatments. None of the microbial inoculants used, even those biocontrol agents of fungi, showed negative effects on AM establishment. Mycorrhizal colonization induced qualitative changes in the bacterial population depending on the inoculant combination involved. Esterase activity was particularly increased by *G. mosseae* (256%), phosphatase activity by natural AMF (166%), chitinase by *G. mosseae* (197%), *G. deserticola* (152%) and natural AMF (151%), and trehalase by *G. deserticola* (444%). As a result of mycorrhizal colonization and microbial inoculation, modifications of the microbial community structure and ecology were found. An understanding of these effects as part of ecosystem processes is essential for obtaining the maximum benefit for plant growth and health in the context of soil–plant system sustainability.

Keywords: AMF; Microbial inoculation; Rhizosphere populations; Soil enzyme activities

1. Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous component of most agroecosystems, where they provide several benefits to their host plant, including better phosphorus nutrition (Toro et al., 1998), increased drought tolerance (Ruíz-Lozano and Azcón, 1995), and increased disease resistance (Pozo et al., 1999). Many rhizosphere colonizing bacteria, including *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium*, and *Pseudomonas*, typically produce substances that stimulate plant growth or inhibit root pathogens (Glick,

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1995). *Trichoderma* spp. have been described as fungal biocontrol agents, acting by either, the production of antimicrobial compounds or parasitism of fungal plant pathogens (Handelsman and Stabb, 1996). The potential use of these microbial inoculants (phytostimulators or biocontrol agents) is been investigated in horticultural and agricultural systems, where they must be compatible with AM fungi (Barea, 1997). The use of biocontrol agents such as fluorescent *Pseudomonas* and *Trichoderma* requires particular attention because of the possibility that these antagonists of fungi interact not only with fungal plant pathogens but also mycorrhizal fungi. Free-living microbial inoculants could also stimulate mycorrhizal colonization (Vosátka and Gryndler, 1999). Conversely, mycorrhiza formation can affect the microbial population in the rhizosphere directly or indirectly through changes in root exudation (composition and quantity) patterns, or through fungal exudates. This is the so-called 'mycorrhizosphere effect' (Linderman, 1992). AM colonization can increase (Krishnaraj and Sreenivasa, 1992), not affect (Waschkies et al., 1994) or decrease (Ames et al., 1984) the total number of aerobic bacteria in the rhizosphere. In addition, AM colonization might influence the species composition of the soil microbial community by increasing some groups and decreasing others (Krishnaraj and Sreenivasa, 1992; Christensen and Jakobsen, 1993).

The ecological impact of microbial inoculants in soil has often been characterized in terms of size and composition of specific microbial groups. However, these approaches do not provide a comprehensive view of the impact of an inoculant on the functioning of the soil ecosystem (Doyle and Stotzky, 1993). Enzyme activities have been proposed as a tool to monitor changes in soil ecology resulting from the interactions between inoculants and indigenous microbial populations of soil (Doyle and Stotzky, 1993). Soil enzyme activity is often used as an index of total microbial activity in a soil as well as its fertility (Dhruva Kumar et al., 1992) and it may be useful for gaining a better understanding of the nature of perturbations caused to ecosystem function. Soil enzyme measurements have been successfully used by Mawdsley and Burns (1995) to assess perturbations caused by the introduction of a *Flavobacterium* species, and by Naseby and Lynch (1998) with the inoculation of *Pseudomonas fluorescens* strains. Qualitative and quantitative changes in microbial populations in soil rhizosphere due to AM formation have been estimated through the study of the different types of total culturable bacteria and the measurement of soil enzyme activity (Camprubí et al., 1995). Esterase (E.C. 3.1.1.1) indicates catabolic activity in soil, directly correlated with microbial activity (Inbar et al., 1991). Phosphatase (E.C. 3.1.3.1) mediates the release of inorganic phosphorus from organically bound phosphorus returned to soil (Dhruva Kumar et al., 1992). Chitinase (E.C. 3.2.1.14) catalyses the degradation of chitin, a major component of most fungal cell walls, and may be involved in the plants' defense mechanisms against invading pathogens (Spanu et al., 1989). Trehalase (E.C. 3.2.1.28) catalyses the hydrolysis of trehalose, a very common sugar in plant symbiosis (Mellor, 1992).

The purpose of this study was to examine the influence of different microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) on colonization of maize roots by AM fungi (especially biocontrol agents of fungi such as *Pseudomonas* and *Trichoderma*), and the effects of both AM fungi and microbial inoculation as functional inocula upon ecosystem processes such as bacterial populations and enzyme activities in rhizosphere soil.

2. Materials and methods

2.1. Experimental design

The experiment used a randomized complete-block design with two factors. One factor, AM colonization, consisted of two single AMF isolates, a mixed population of indigenous AMF (natural AMF) and a non-mycorrhizal control treatment. The second factor, microbial inoculation, consisted of the application of microbial inoculants (*Azospirillum*, *Pseudomonas* or *Trichoderma*) or not (uninoculated treatment). Five replicates of each one of the 16 different treatments were used.

2.2. Test soil

The soil was collected from the grounds of the Estación Experimental del Zaidín (Granada, Spain). It was a loamy soil (19% clay, 43% loam and 38%

sand) containing 2.1% organic matter, 1.3 g l^{-1} N, 9 mg l^{-1} P, 82 mg l^{-1} K, with a pH of 7.9. The soil was sieved (4 mm), diluted with quartz sand (1/1, v/v) and pasteurized (100°C, 1 h per day during three consecutive days) and then reinoculated with a soil and arbuscular mycorrhizal inoculum filtrate (10 ml/pot of a soil/water mixture (1/1, v/v) filtered through a Whatman No. 1 paper) to reintroduce the normal microbiota, except AM propagules. Pots (1500 cm^3 capacity) were filled with the pasteurized soil/sand mixture.

2.3. Host plant and biological treatments

Maize (*Zea mays* L. var. Potro) was used as the test plant. Surface sterilized seeds were pregerminated 24 h before use.

The AM fungal species used were *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, and *Glomus deserticola* (Trappe. Bloss. and Menge) belonging to the collection of the Estación Experimental del Zaidín (Ruíz-Lozano et al., 1995). The mycorrhizal inocula obtained from pot cultures of *Lactuca sativa* L. as the host plant and maintained by storage for 3–6 months in polyethylene bags at 4°C, consisted of soil, spores, hyphae and AM root fragments colonized to approximately 70% by *G. mosseae* and 80% by *G. deserticola*. The 20 g of each inoculum were added to each pot and mixed with the soil–sand mixture. When natural AMF (mixed population of indigenous AMF) were tested, unsterilized soil was used.

Three free-living microorganisms, belonging to the genera *Azospirillum*, *Pseudomonas* and *Trichoderma*, were assayed. *Azospirillum brasilense* Sp245, isolated from the root surface of wheat (Baldani et al., 1983), is known to produce 8.5–14 μg IAA ml^{-1} when grown in minimum medium plus tryptophan (100 μg ml^{-1}). *P. fluorescens* WCS 365 is an efficient biocontrol strain in the cucumber/*Pythium* sp. system (Simons et al., 1996). Both bacterial strains were grown in LB broth (Miller, 1972), incubated overnight at 28°C with shaking. Cells were collected by centrifugation (7000 rpm for 8 min), washed and resuspended in 1/4-strength Ringer's solution (Oxoid) to give an O.D. (600 nm) of 1.0. This O.D. is equivalent to approximately 10^9 cells ml^{-1}. The soaking of 4 g of maize seeds for 10 min in 10 ml cell suspension resulted in 7.2×10^7 cfu *Azospirillum*/seed and 1.6×10^8 cfu *Pseudomonas*/seed.

Trichoderma harzianum T12 was shown to be an efficient biocontrol agent against *Pythium* sp. (Naseby et al., 2000). It was grown in malt–yeast–agar (0.4% yeast extract, 1% malt extract, 0.4% glucose, 1.2% agar) plates for 10 days at 25°C. Conidia were scraped from the plates and resuspended in sterile distilled water, yielding 10^9 conidia ml^{-1}. The 2 ml of this conidial suspension were inoculated per pot.

2.4. Growth conditions

Plants were grown for 2 months in a greenhouse under controlled conditions at 25°C during the day and 19°C during the night, with a 16 h photoperiod (photosynthetic photon flux density of 400–700 μmol m^{-2} s^{-1}) and a relative humidity of 70–90%. Water was supplied daily as needed.

2.5. Measurements of the maize plants

Upon harvest, the dry weights of shoots and roots were recorded after drying in an oven at 65°C. At this time, the percentage of root length colonized by AM fungi was estimated by examining stained samples (Phillips and Hayman, 1970) microscopically (Brundrett et al., 1984).

2.6. Evaluation of Azospirillum, Pseudomonas, total saprotrophic fungi and total culturable bacteria in rhizosphere soil

Rhizosphere samples were obtained by collecting the soil adhering to the roots. The 10 g of soil samples were placed in an Erlenmeyer flask containing 90 ml of sterilized distilled water, and shaken for 30 min. Ten-fold series dilutions were prepared, and appropriate dilutions were plated in specific media.

For the isolation of *Azospirillum* spp. the RC medium (Rodríguez-Cáceres, 1982) was used, and for fluorescent *Pseudomonas*, S1 medium (1% sucrose, 1% glycerol, 0.5% casaminoacids, 0.12% *N*-lauroylsarcosine sodium salt, 0.1% $NaHCO_3$, 0.23% K_2HPO_4, 1.2% Agar, with 1% $MgSO_4{\cdot}7H_2O$ added after sterilization) was used.

The numbers of colony forming bacterial cells and total saprotrophic fungi were determined in PDA medium by plate dilution (Johnson and Curl, 1972). For the quantification of bacterial cfu, cycloheximide

(100 μg ml^{-1}) was added to the growing medium. The different types of bacteria were determined by examining colony morphology.

2.7. Determination of soil enzyme activities

The activities of four soil enzymes: esterase, phosphatase, chitinase and trehalase were studied in rhizosphere soil samples. For each enzymatic determination, controls with twice-autoclaved samples were included to account for non-enzymatic decomposition of the soil solution. The concentrations of enzymatic hydrolysis products were determined by spectrophotometry and compared with a standard curve.

Esterase activity was determined by measuring the hydrolysis of fluorescein diacetate (FDA) (Schnurer and Roswall, 1982; Inbar et al., 1991) and the fluorescein released was quantified by spectrophotometry. Soil samples (0.1 g) were placed in plastic tubes and 10 ml sterile potassium phosphate buffer (pH 7.6, 60 mM) were added. The reaction was started by adding 1 ml of a stock solution (1 mg ml^{-1} in acetone) of fluorescein diacetate (Sigma). The tubes were sealed with hermetic caps and incubated at 37°C for 4 h. The reaction was stopped by adding 10 ml acetone. The tubes were centrifuged at 3200 rpm for 10 min. The optical density of the supernatant was determined at 490 nm.

Neutral-alkaline phosphatase and chitinase activities were analyzed following a standard methodology adapted from Beam (1971). The 2 ml of a stock solution (1 mg ml^{-1}) of *p*-nitrophenyl phosphate (Sigma) were added to 0.1 g of soil to determine phosphatase activity, and 2 ml of a stock solution (1 mg ml^{-1}) of *p*-nitrophenyl-β-acetylglucosamine (Sigma) were added to 1 g of soil samples to determine chitinase activity. After 4 h incubation at 37°C, the reaction was stopped by adding 6 ml ethanol. After centrifugation at 3500 rpm for 10 min, 2 ml fractions of the supernatant were pipetted into test tubes containing 8 ml water, and color was developed by adding 1 ml of 0.2 N NaOH to each tube. The amount of *p*-nitrophenol released was determined by reading optical density at 420 nm.

Trehalase activity was determined by the method proposed by Smith and Rodríguez-Kabana (1982). This activity was expressed as reducing groups (glucose) released after incubation with trehalose. One gram of soil was placed in plastic tubes and 2 ml of 2% trehalose (w/v) (Sigma) in distilled sterile water added. The tubes were incubated at 36°C for 3 h. Afterwards, 10 ml distilled water were added and the soil–water suspension centrifuged at 3200 rpm for 20 min. The 1 ml of clear supernant was then analyzed for reducing sugars (Nelson, 1944).

2.8. Statistical analysis

Results were analyzed by two-way analysis of variance (ANOVA2). Significance was determined according to Fisher's Protected Least Significance Difference (PLSD) test. Percentage values were analyzed following arcsin square root (×100) transformation.

3. Results

3.1. Plant growth

The natural AMF produced a significant adverse effect on plant growth (Fig. 1A, B, D, E, G and H). Inoculation with *Azospirillum* increased both shoot and root growth but this effect was only significant in *G. deserticola*-mycorrhizal plants (Fig. 1A and B). Inoculation with *Pseudomonas* did not have an effect on plant growth (Fig. 1D and E) and *Trichoderma* only exerted a positive effect on shoots of non-mycorrhizal control plants (Fig. 1G and H).

3.2. AM colonization

None of the free-living microbial inoculants affected the percentage of AM colonization. Root colonization ability depended on the AMF species used, with *G. deserticola* being the most and the natural AMF being the least infective (Fig. 1C, F and I).

3.3. Populations of Azospirillum spp. and Pseudomonas spp. in the rhizosphere soil

The populations of *Azospirillum* were highest in the rhizosphere of maize roots inoculated with *Azospirillum* (Fig. 2A) and were also higher in the rhizosphere of mycorrhizal than non-mycorrhizal plants (Fig. 2A, D and G). Inoculation with *Pseudomonas* and *Trichoderma* tended to decrease the population of

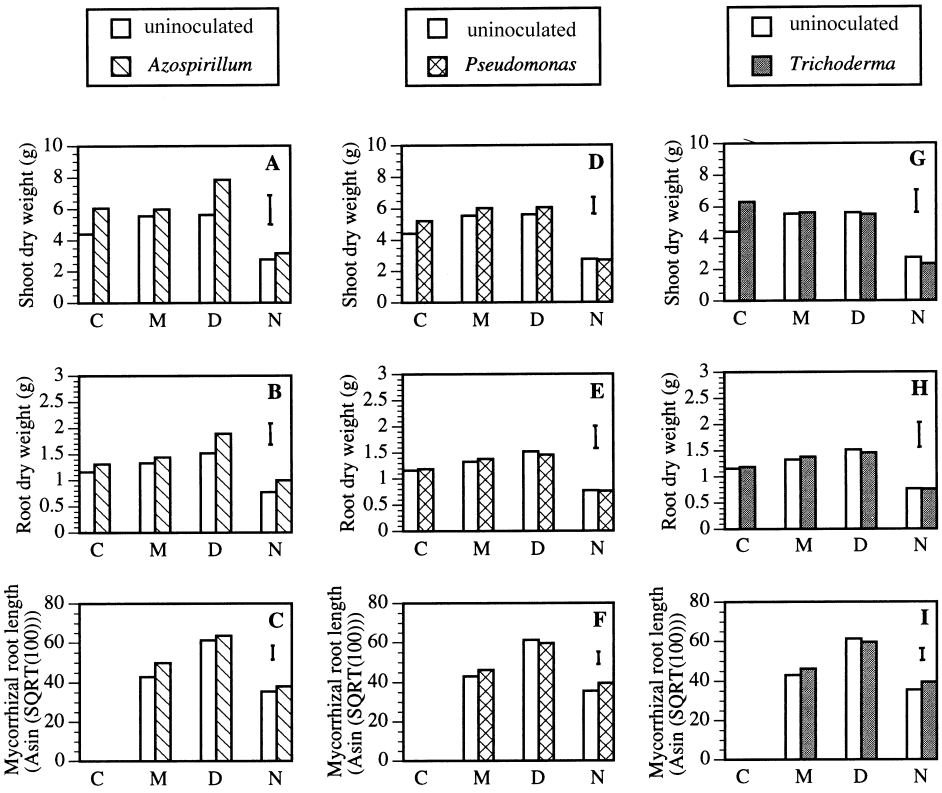

Fig. 1. Effect of AMF (C=non-mycorrhizal control; M=*G. mosseae*; D=*G. deserticola*; N=natural AMF) and other microbial inoculants (*Azospirillum brasilense* Sp245, *Pseudomonas fluorescens* WCS365 and *Trichoderma harzianum* T12) on: (A, D, G) shoot dry weight (g); (B, E, H) root dry weight (g) and (C, F, I) mycorrhizal root length (arcsin square root (×100) transformed). Bars represent LSD (least significant differences, $P \leq 0.05$) for comparisons between treatments.

Azospirillum in the rhizosphere of mycorrhizal maize plants but this effect was significant only in natural AMF-mycorrhizal plants (Fig. 2D and G).

The lowest populations of fluorescent *Pseudomonas* were found in the rhizosphere of mycorrhizal maize roots, especially in roots colonized by *G. deserticola*. The application of microbial inoculants (*Azospirillum*, *Pseudomonas* and *Trichoderma*) had no significant effect on the population levels of *Pseudomonas* (Fig. 2B, E and H).

3.4. Total saprotrophic fungi and bacterial populations

The population of total saprotrophic fungi was increased in all treatments inoculated with *Trichoderma* although this effect was less significant in natural AMF-colonized rhizospheres (Fig. 2I). Inoculation with *Azospirillum*, *Pseudomonas* and even mycorrhization did not affect the population of total saprotrophic fungi (Fig. 2C and F).

Populations of total culturable bacteria decreased in mycorrhizal inoculated rhizospheres. Microbial inoculation had no significant effect on this variable, but inoculation with *A. brasilense* Sp245 significantly reduced the populations of total bacteria in natural-AMF mycorrhizal rhizospheres (Table 1).

Besides these quantitative effects on bacterial populations, inoculants also induced qualitative changes on the distribution of the different bacterial groups. These changes were particularly due to mycorrhizal colonization although in some instances microbial inoculation exerted such an effect (Table 1). The different bacterial types found, still unidentified, are

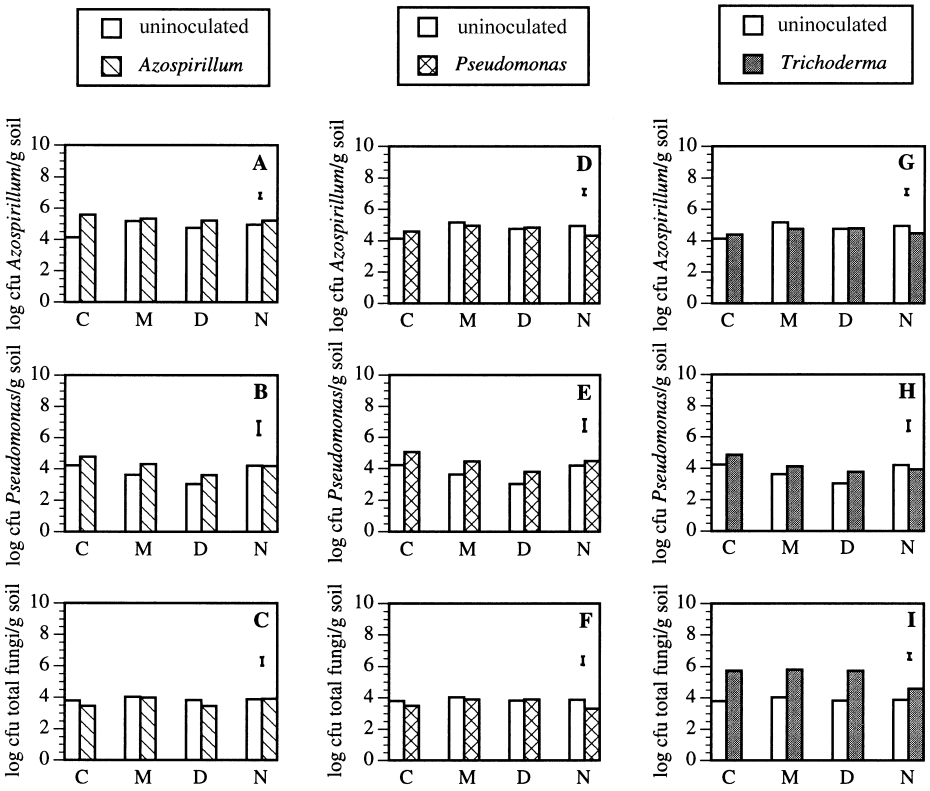

Fig. 2. Effect of AMF (C=non-mycorrhizal control; M=*G. mosseae*; D=*G. deserticola*; N=natural AMF) and other microbial inoculants (*Azospirillum brasilense* Sp245, *Pseudomonas fluorescens* WCS365 and *Trichoderma harzianum* T12) on: (A, D, G) log cfu *Azospirillum* spp./g dry soil; (B, E, H) log cfu *Pseudomonas* spp./g dry soil; (C, F, I) log cfu total fungi/g dry soil. Bars represent LSD (least significant differences, $P \leq 0.05$) for comparisons between treatments.

named by the letter B followed by a number (1–9). B1 decreased in the rhizosphere of maize roots colonized by *G. deserticola*, but it was almost undetectable in the rhizosphere of plants colonized by natural AMF. *Azospirillum* and *Pseudomonas* did not affect the relative distribution of B1, but *Trichoderma* decreased this value in *G. mosseae*-colonized rhizospheres. The proportion of B2 decreased in the rhizosphere of mycorrhizal plants, particularly in plants colonized by *G. mosseae* and natural AMF. *Trichoderma* increased the proportion of this bacterial type in the rhizosphere of plants colonized by *G. mosseae*, and *Pseudomonas* increased this proportion in non-mycorrhizal control plants. The proportion of B3 decreased in the rhizosphere of plants colonized by *G. mosseae* or by the natural AMF. *Trichoderma* decreased the proportion of B3 in the rhizosphere of non-mycorrhizal control plants. B4 was mainly detected in the rhizosphere of maize roots colonized by *G. mosseae*, but other inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) significantly decreased the proportion of this bacterial type in this mycorrhizal treatment. B5 and B6 were not affected by mycorrhizal colonization or *Azospirillum* inoculation, but *Trichoderma* increased the relative proportion of both B5 and B6 in non-mycorrhizal control plants, and *Pseudomonas* increased B5 in *G. mosseae*-colonized plants and B6 in non-mycorrhizal control plants. B7 was only affected by mycorrhizal colonization with natural AMF increasing its proportion. B9 was only detected in the rhizosphere of maize roots colonized by the natural AMF, but the application of *Trichoderma* significantly decreased this proportion.

Table 1
Total bacterial cfu (log) and relative proportions (arcsin square root (×100) transformed) of the different bacterial types found in the rhizosphere of maize colonized by AMF and other microbial inoculants (*Azospirillum brasilense* Sp245, *Pseudomonas fluorescens* WCS365 and *Trichoderma harzianum* T12)[a]

Inoculant	AMF	Log bacterial cfu	Relative proportions of bacterial types								
			B1	B2	B3	B4	B5	B6	B7	B8	B9
Uninoculated	C	6.82	23.80	13.14	53.95	0	4.34	0	19.45	5.63	0
	M	6.53	40.50	0	30.55	27.18	6.95	0	14.67	4.75	0
	D	6.25	9.83	8.58	49.79	0	5.24	0	36.16	0	0
	N	6.56	2.09	0	20.32	2.09	0	2.09	42.60	3.17	39.83
Azospirillum	C	7.24	28.46	10.54	40.77	5.41	8.68	3.87	30.30	4.48	0
	M	6.98	31.37	0	41.40	11.47	4.32	0	27.00	4.64	0
	D	6.22	21.11	13.93	44.49	0	17.51	3.67	23.23	0	0
	N	5.98	0	0	13.28	0	5.39	3.63	35.08	0	50.65
	LSD	0.5	18.7	10.8	29.9	10.2	15.3	4.1	22.4	5.7	13.0
Pseudomonas	C	7.17	21.32	19.21	38.18	0	8.24	3.24	35.62	2.12	0
	M	6.65	35.70	0	27.41	9.76	28.92	0	20.13	1.95	0
	D	6.09	6.94	4.99	51.17	0	9.78	0	34.75	0	0
	N	6.27	0	0	11.23	0	6.56	0	52.74	0	32.89
	LSD	0.5	18.6	5.5	23.4	12.6	18.7	2.4	21.5	5.5	10.8
Trichoderma	C	6.93	34.19	7.39	29.47	0	9.60	19.56	27.80	3.81	0
	M	6.40	14.05	19.94	30.98	9.99	8.07	2.35	31.42	19.22	0
	D	6.43	14.08	2.99	49.29	0	6.51	0	34.53	0	0
	N	6.60	0	0	23.12	0	2.62	0	50.11	2.01	30.16
	LSD	0.5	21.8	10.1	22.5	10.0	4.7	17.0	19.6	19.1	4.8

[a] C=non-mycorrhizal control; M=*G. mosseae*; D=*G. deserticola*; N=natural AMF; LSD=least significance difference ($P \leq 0.05$) for the different microbial inoculants vs. uninoculated treatments.

3.5. Soil enzyme activities

In addition to qualitative and quantitative changes produced by microbial inoculants on the microbial populations in the rhizosphere, there were also changes in the functioning of the system, as evaluated by analyzing soil enzyme activities. Esterase activity was higher in the rhizosphere of *G. mosseae*-colonized plants as compared with non-mycorrhizal controls (256% increase). The application of other inoculants (*Azospirillum*, *Pseudomonas* and *Trichoderma*) eliminated this significant effect in *G. mosseae*-colonized rhizospheres (Fig. 3A, E and I), but *Pseudomonas* significantly increased esterase activity in *G. deserticola* (175%) and natural AMF-colonized plants (209%).

Phosphatase activity was higher in the rhizosphere of natural AMF-colonized plants as compared to non-mycorrhizal plants (166% increase) (Fig. 3B, F and J). The inoculation with *Pseudomonas* and *Trichoderma* significantly decreased (87 and 89%, respectively) phosphatase activity in this treatment. On the other hand, inoculation with either *Azospirillum*, *Pseudomonas* or *Trichoderma* significantly increased phosphatase activity in the rhizosphere of *G. deserticola*-colonized plants (150, 142 and 188%, respectively) and *Trichoderma* also produced a similar effect upon this activity in the rhizosphere of *G. mosseae*-colonized plants (increased of 121%). Chitinase activity in the rhizosphere was higher for mycorrhizal than for non-mycorrhizal plants (197, 152 and 151% increase in *G. mosseae*, *G. deserticola* and natural AMF rhizospheres, respectively). Inoculation with *Azospirillum* and *Pseudomonas* did not have any effect upon chitinase activity in soil, but *Trichoderma* significantly increased this activity in the rhizosphere of non-mycorrhizal plants and natural AMF-colonized plants (151 and 121%, respectively) (Fig. 3C, G and K).

Trehalase activity showed great differences between replicates. In spite of this variability it was significantly higher in the rhizosphere of *G. deserticola*-colonized plants (444% compared to

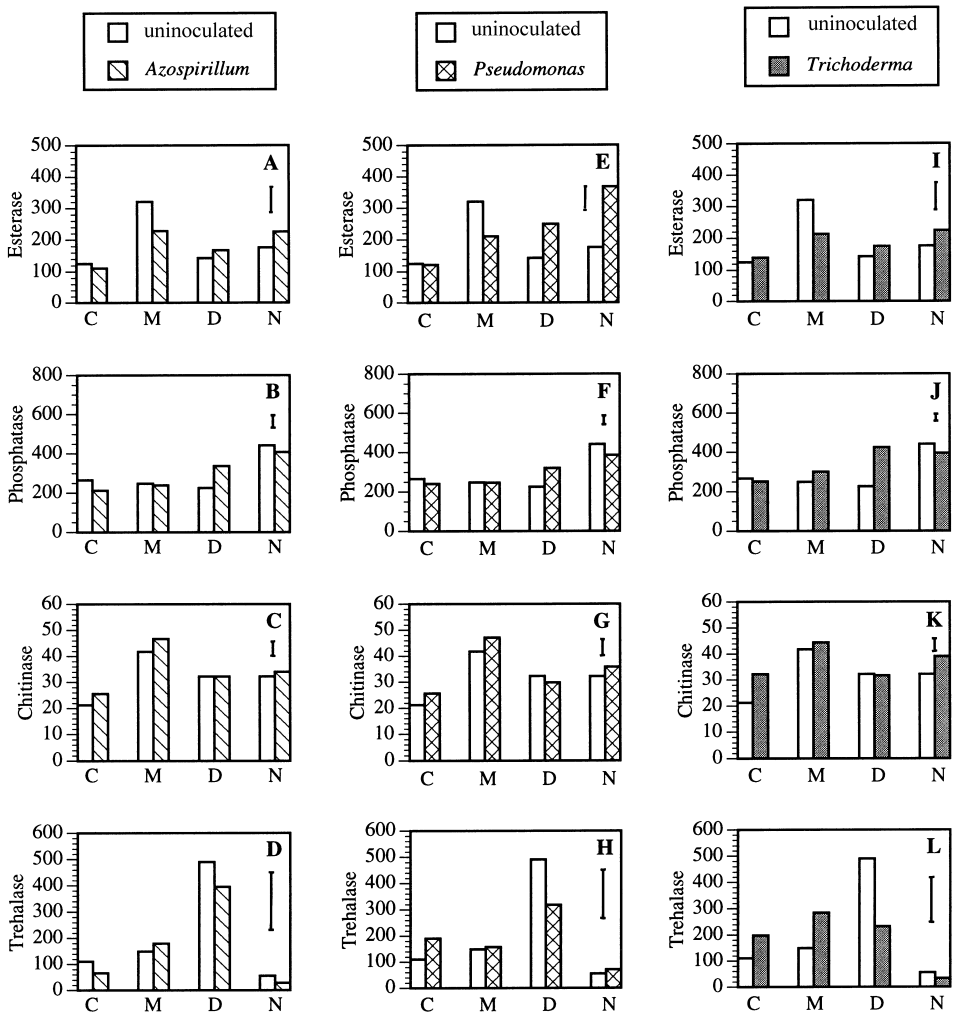

Fig. 3. Effect of AMF (C=non-mycorrhizal control; M=*G. mosseae*; D=*G. deserticola*; N=natural AMF) and other microbial inoculants (*Azospirillum brasilense* Sp245, *Pseudomonas fluorescens* WCS365 and *Trichoderma harzianum* T12) on soil enzyme activities: (A, E, I) esterase (μg fluorescein/g dry soil); (B, F, J) phosphatase (μg *p*-nitrophenol/g dry soil); (C, G, K) chitinase (μg *p*-nitrophenol/g dry soil); and (D, H, L) trehalase (μg glucose/g dry soil). Bars represent LSD (least significant differences, $P \leq 0.05$) for comparisons between treatments.

non-mycorrhizal plants). *Trichoderma* significantly decreased (47%) trehalase in this treatment. *Azospirillum* and *Pseudomonas* did not exert any significant effect on trehalase activity (Fig. 3D, H and L).

4. Discussion

In general, plants having a low degree of dependence on mycorrhizal associations are suitable for the testing of a non-nutritional mediated effect of AMF upon rhizosphere processes. Thus, the homogeneous soil microcosm assayed in this study, with the use of maize as the model plant producing large amounts of rhizosphere soil, allows for detection of the mutual effects of microbial inoculation and/or AMF colonization on rhizosphere populations and soil enzymology.

None of the microbial inoculants interfered with mycorrhizal colonization. Balota et al. (1995) described the stimulation of mycorrhizal root estab-

lishment by IAA-producing bacteria. They related this effect to the ability of growth promoting compounds to stimulate plant susceptibility to mycorrhizal colonization, spore germination or the growth of mycelium, thereby increasing the chance of contacts between fungal hyphae and plant roots and, consequently, to increase mycorrhizal establishment. So, the beneficial effects on plant growth observed as a result of the inoculation of *Azospirillum*, may not be related only to stimulation of colonization by *G. deserticola*, but an increase in the development of the extraradical mycelium could also be involved. The effects of biological control agents (*Pseudomonas* and *Trichoderma*) are of particular relevance because of the possibility that these antagonists of fungi could also negatively interfere with AMF. *P. fluorescens* WCS365 has not been shown to produce antifungal compounds (Bloemberg, personal communication). In spite of this it has been considered an effective biological control agent by inducing systemic resistance in inoculated plants (Simons et al., 1996). AMF colonization has been demonstrated to induce similar defense mechanisms in mycorrhizal plants (Gianinazzi-Pearson, 1996), however it seems logical that *P. fluoresecens* WCS365 would have no effect on AM colonization. The mechanisms of action of *Trichoderma* are known to be based on antibiosis, fungistasis and mycoparasitism. Rousseaeu et al. (1996) reported a mycoparasitism of the extramatical phase of *G. intraradices* by *T. harzianum*. Such results are difficult to generalize, because they may be linked to the aggressiveness of the *Trichoderma* strain used. Therefore, it could be that *T. harzianum* T12 was not aggressive enough to interact negatively with AMF.

The positive influence of IAA-producing bacteria on the growth of certain plant species has been previously described (Beyeler et al., 1997). Dual inoculation of *G. deserticola* and *A. brasilense* increased the growth of maize plants significantly as compared to the uninoculated control or any of these microorganisms inoculated singly. These results indicate, as previously reported, the existence of 'functional compatibilities' between saprotrophic and symbiotic microorganisms (Azcón et al., 1991). The plant response may be diagnostic for the success of the association which was dependent on the AM fungi involved. The effects of the inoculation of *Trichoderma* on plant growth cannot be generalized as positive, negative and no effects have been described in the literature. Naseby et al. (2000) described increases in plant growth due to a *Trichoderma* single isolate or in combination with the pathogenic fungus *Pythium*. The growth response of plants caused by *Trichoderma* depends on the ability of the fungus to survive and develop in the rhizosphere (Kleifeld and Chet, 1992). A possible mechanism for increased plant growth could be the stimulation of nutrient transfer from soil to root, as *Trichoderma* can colonize the interior of roots (Kleifeld and Chet, 1992). In the present study, colonization by AMF eliminated the positive effect caused by *Trichoderma* on plant growth. This could be the result of a possible interaction between both microorganisms (AMF and *Trichoderma*) in the root. Complex interactions with other components of the soil microbiota, as could be confirmed by quantitative and qualitative changes observed, could also be involved. Surprisingly, the natural AMF was ineffective in increasing plant growth in spite of their suitable colonizing ability.

AM colonization by *G. deserticola* in particular reduced the rhizosphere populations of fluorescent *Pseudomonas*. This effect on *Pseudomonas* has previously been reported by Waschkies et al. (1994). This mycorrhizosphere effect could be attributed to AMF causing nutrient leakage from roots (quantitative changes in root exudates) or specific changes in the quality of root exudates. Autoradiographic studies have shown that large amounts of host carbon are accumulated in fungal vesicles (Cox et al., 1975). *G. deserticola* produces a heavy mycorrhizal colonization with many vesicles, and therefore could change the allocation of carbon in the rhizosphere. This might explain the reduction in populations of fluorescent *Pseudomonas*, which are dependent on host carbon sources (Paulitz and Linderman, 1989). The populations of *Azospirillum* spp., however, increased in mycorrhizal treatments. These results agree with previous findings by other researches who observed better survival of *Azospirillum* on the roots of mycorrhizal plants (Pacovsky, 1989; Belimov et al., 1999). The increase in soil fungal populations with *Trichoderma* inoculum is in part due to the addition of an inoculum that had an additive effect on the total fungal population.

Each microbial inoculant was differentially affected by the specific mycorrhizal treatments, which supports the idea that mycorrhizal colonization produced

qualitative, more than quantitative, changes in the composition of root exudates (Kothari et al., 1991). These results indicate that the response of the rhizosphere populations to AM cannot be generalized, but here it seems not to be dependent on the host growth responses to the AM fungal species. However, methods requiring microbial growth can be hampered by the non-culturability of many microorganisms (Colwell et al., 1985).

Measurements of soil enzyme activities have been used as an indicator of the effect of such soil manipulation (Naseby and Lynch, 1998) and may be useful for gaining a better understanding of the nature of the perturbations caused to ecosystem function after microbial inoculations. Soil enzyme activities have also been used as an indicator of carbon leakage from roots (Naseby and Lynch, 1998; Naseby et al., 1999). The increased enzyme activities found in the rhizosphere of mycorrhizal plants indicate an increase in C and nutrient leakage from roots. So, the changes detected in the present study suggest a direct effect of AM colonization, as well as an indirect effect through changes in microbial composition in rhizosphere soil. The increased esterase activity in some *P. fluorescens* inoculated plants could be related to the metabolic versatility of this microbial group (Bolton et al., 1993). The P cycle enzyme activities are inversely related to P availability (Tadano et al., 1993) and when P is a limiting nutrient its demand increases, resulting in an increase in phosphatase activity, as occurred in natural AMF-colonized rhizospheres. Treatments which decrease the available phosphate, cause an overall increase in phosphatase activity (Azcón and Barea, 1997). Camprubí et al. (1995) found higher populations of chitinase producers in the rhizosphere of non-mycorrhizal plants, an effect which was not corroborated in the present study. The increase in chitinase activity, caused by mycorrhization and inoculation of *Trichoderma* could be related to biological control activities. Trehalase activity, a symbiotic determinant, was expected to be higher in mycorrhizal treatments (Mellor, 1992), but this was found only in plants inoculated with *G. deserticola* and *G. mosseae*, probably due to the better colonizing ability of these strains.

In summary, as a result of mycorrhizal colonization and microbial inoculation, modifications of the microbial community structure and ecology were found. An understanding of these effects as part of ecosystem processes is essential for obtaining the maximum benefit for plant growth and health in the context of soil-plant system sustainability.

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