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Effect of drought on the stability of rhizosphere soil aggregates of *Lactuca sativa* grown in a degraded soil inoculated with PGPR and AM fungi

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ABSTRACT

The effect of different arbuscular mycorrhizal (AM) fungi, *Glomus intraradices* (Schenk and Smith) or *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe, and plant growth-promoting rhizobacteria (PGPR) (*Pseudomonas mendocina* Palleroni), alone or in combination, on structural stability and microbial activity in the rhizosphere soil of *Lactuca sativa* L. was assessed under well-watered conditions and two levels of drought. Desiccation caused an increase in aggregate stability and water-soluble and total carbohydrates but there were no significant differences among treated soils and the control soil. The glomalin-related soil protein (GRSP) levels in both the <2 and 0.25–4 mm soil fractions increased with moderate water stress, whereas under severe water stress they did not differ with respect to those of well-watered soils. The values of GRSP in soils inoculated with PGPR and AM fungi were higher than in the control or fertilized soil under well-watered and severe-drought conditions, while under moderate-drought conditions all soils showed similar GRSP values. Soils inoculated with AM fungi and PGPR generally presented higher dehydrogenase and phosphatase activities than the control soil, independent of the water regime.

1. Introduction

Drought stress limits growth and production of crop plants, particularly in arid and semi-arid areas (Kramer and Boyer, 1997). The interaction of drought with other important or critical factors for ecosystem functioning, such as soil aggregation, needs to be studied to gain insight into the impact of the predicted drought enhancement on Mediterranean ecosystems (Roldán et al., 2006). Likewise, soil aggregate stability is one of the most important properties controlling plant growth in semi-arid Mediterranean environments which, in turn, protects the soil against water erosion. Mycorrhizal fungi are known to be important for soil aggregation under well-watered conditions (Miller and Jastrow, 2000). Analogous to the role of roots at larger spatial scales, in inducing wet–dry cycles in the rhizosphere that are important for aggregate formation, one could hypothesize the occurrence of this on the smaller scale of hyphae of mycorrhizal fungi; that is, on the scale of microaggregates (Rillig and Mummey, 2006). A key factor in the contribution of AM fungi to soil aggregation is the production of the glycoprotein glomalin (Gadkar and Rillig, 2006). Operationally defined by extraction and detection conditions (Wright and Upadhyaya, 1996), it is detected in large amounts in diverse soils as glomalin-related soil protein (GRSP; Rillig, 2004). As such, it is widely studied regarding its implication in carbon storage and its role in soil stability. But not only mycorrhizal fungi can participate in soil aggregation: Kohler et al. (2006) showed the participation of one of the plant growth-promoting rhizobacteria (PGPR), *Pseudomonas mendocina*, in both soil stabilisation and promotion of soil fertility. To the best of our knowledge, there are no reports on the effects of PGPR on aggregate stability under water-stress conditions.

The PGPR are a group of bacteria that can actively colonize plant roots and increase plant growth. Specific strains of *Pseudomonas* have been shown to increase the growth and yield of some agricultural crops (Kohler et al., 2006). PGPR produce plant growth-promoting compounds, including phytohormones (auxins, cytokinins and gibberellins) and siderophores (Saikia et al., 2006), and antibacterial peptides that inhibit pathogenic strains. Because of the potential of PGPR for improving plant nutrition and health, the use of these rhizobacteria in low-input agriculture has been addressed in several investigations (Vessey, 2003). To achieve maximum benefit from beneficial microorganisms, the influence of bacterial inoculants on mycorrhizal development should be studied for possible interactions. However, nothing is known about the interaction of PGPR and AM fungi with respect to soil aggregate stability under induced water stress.

We hypothesize that inoculation with AM fungi and/or PGPR can improve soil properties, even under drought stress. To assess

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this hypothesis, we determined the effects of microbial inoculation on soil structure and microbial activity in the rhizosphere soil of *Lactuca sativa*.

2. Materials and methods

2.1. Soil and plant

An agricultural soil, used to cultivate lettuce was collected near Murcia (SE Spain). The climate is semi-arid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm y^{-1}. The main characteristics of the agricultural soil used were: pH (1:5) 8.89; electrical conductivity 0.18 dS m^{-1}; TOC 1.80%; total N 2.01 g kg^{-1}; available P, 70 μg g^{-1}; extractable K, 440 μg g^{-1}; cationic exchange capacity, 15 cmol kg^{-1}.

The plant used in the experiment was lettuce (*L. sativa* L. cv. Tafalla). Seeds of lettuce were grown for 15 days in peat substrate under nursery conditions, without any fertilization treatment.

2.2. Microorganisms

The mycorrhizal fungi used were either *Glomus intraradices* (Schenk and Smith) or *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe, obtained from the collection of the experimental field station of Zaidín, Granada. The two *Glomus* species were multiplied in pots using a mixture of sterile sepiolite/vermiculite (1:1, v:v) as growing substrate and *Sorghum* sp. as host plant. Trap cultures were maintained under greenhouse controlled conditions for 4 months. AM fungal inoculum consisted of a mixture of rhizospheric soil from the trap cultures containing spores, hyphae and mycorrhizal root fragments and was stored in polyethylene bags at 5 °C. Both inocula were subjected to a most probable number test (Sieverding, 1991) to determine potential infectivity and to equalize application doses. Both sources of inoculum had a potential infectivity of about 35 infective propagules per gram inoculum.

The *P. mendocina* Palleroni strain was obtained from Probelte, S.A., Murcia, which was selected on the basis of its ability to produce siderophores. *P. mendocina* was grown in a medium (nutrient broth, Scharlau Chemie, Spain) composed of meat and yeast extracts, peptone and sodium chloride, for 2 days at room temperature on a Heidolph Unimax1010 shaker. The bacterial culture was centrifuged at 2287 × *g* for 5 min at 2 °C and the sediment was re-suspended in sterilised tap water. The bacterial suspension contained 10^9 colony forming units (CFU) ml^{-1}.

2.3. Microbial inoculation and water stress treatments

The experiment was a mesocosm assay, conducted as a randomized factorial design with two factors and fivefold replication. The first factor had seven levels: control soil, soil inoculated with the AM fungus *G. intraradices* or *G. mosseae*, soil inoculated with the bacterium *P. mendocina*, the combination of soil inoculated with the bacteria *P. mendocina* and with *G. intraradices* or *G. mosseae* and a soil fertilized with inorganic fertilizer. The second factor had three levels of water stress: well-watered, moderate and severe water stress. Five replicates per treatment were set up, making a total of 105 pots.

Seven hundred grams of substrate, consisting of soil and vermiculite at a ratio of 2:1 (v:v) sterilized by autoclaving at 105 °C for 60 min in three consecutive days, were placed in 1-l pots. *L. sativa* seedlings were transplanted to the pots (one per pot). The AM inoculum was mixed with the potting substrate, at a rate of 5% (v/v). The same amount of the autoclaved inoculum was added to non-AM plants, supplemented with a filtrate (Whatman no. 1 paper) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. *P. mendocina* was inoculated two times during the growth period. The dose of inoculum applied corresponded to 10^{10} CFU per plant. Fertilized plants received 10 ml of Long Ashton Nutrient Solution on two occasions (μg ml^{-1}): 175.9 N, 156.2 K, 160.2 Ca, 98.4 S, 11.7 Na, 5.4 Cl, 5.0 Fe-chelate sequestrene, 0.54 Mn, 0.54 B, 0.10 Cu, 0.06 Zn, 0.006 Mo and 40 P. All seedlings were grown for five weeks without any fertilizer treatment. One third of the pots were watered regularly with decalcified water maintaining substrate water potential equivalent to field capacity (−0.03 MPa). Another third of the pots were cultivated under a soil water potential of −0.2 MPa. The remaining pots were maintained at a substrate water potential close to wilting point (averaging −0.60 MPa). The experiment was conducted in a greenhouse, located in the SACE service at the Campus of Espinardo (University of Murcia, Spain). During the experiment, the mean temperature ranged 22 °C, and the relative humidity was between 60% and 80%. Midday photosynthetically active radiation (PAR) averaged 260 μE m^{-2} s^{-1}. Soil moisture was monitored gravimetrically before each watering. Water content in the substrate, calculated as a percentage of dry weight and corresponding with substrate water potential at field capacity and at permanent wilting, was determined according to the method of Richards (1941).

2.4. Soil water potential

Soil water potential was determined by a pressure plate apparatus and soil water content was measured by weighing the soil before and after drying at 110 °C for 24 h (Richards, 1941). A characteristic soil moisture curve was constructed and used to correlate soil water content and soil water potential (Ψ) by gravimetric measurement of soil water content in the pots.

2.5. Plant analyses

Five weeks after planting five plants per treatment were harvested and basal stem diameters were measured with callipers. The roots were washed free from soil under a stream of cold tap water and fresh and dry (105 °C, 5 h) weights of leaves and roots were recorded.

Roots were subsampled in three 2-cm cross-sections of the upper, middle, and lower root system. To assess colonization, roots were cleared with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970). The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovanetti and Mosse, 1980). Positive counts for AM colonization included the presence of vesicles or arbuscules or typical mycelium within the roots.

2.6. Soil chemical, biological and biochemical analyses

Total and water-soluble carbohydrates were determined by the method of Brink et al. (1960) using anthrone and measuring spectrophotometrically at 630 nm.

The percentage of stable aggregates was determined by the method described in Lax et al. (1994). A 4 g aliquot of soil sieved between 0.25 and 4 mm was placed on a small 0.250 mm sieve and wetted by spraying with water. After 15 min the soil was subjected to an artificial rainfall of 150 ml with energy of 270 J m^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (*T*), dried at 105 °C and weighted (P1). The soil was then soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a spatula to break the remaining aggregates. The residue remaining on the sieve, made up of plant debris and sand particles, was dried at 105 °C and

Table 1
Effect of inoculation with *G. intraradices*, *G. mosseae* and *P. mendocina* on shoot and root dry biomass of *L. sativa* seedlings grown at three levels of irrigation ($n = 5$).

	Well-watered (−0.03 MPa)	Stressed (−0.2 MPa)	Stressed (−0.6 MPa)
Shoot dry biomass (g dw)			
Control	1.48 ± 0.04f*	1.00 ± 0.01de	0.68 ± 0.02a
Fertilized	1.90 ± 0.04g	0.98 ± 0.02cde	0.72 ± 0.04a
P. mendocina	1.92 ± 0.01g	1.05 ± 0.01e	0.77 ± 0.02abcd
G. intraradices	1.42 ± 0.02f	1.03 ± 0.01e	0.73 ± 0.03ab
G. mosseae	1.46 ± 0.02f	0.97 ± 0.02bcde	0.64 ± 0.04a
P. mendocina + *G. intraradices*	1.44 ± 0.01f	1.09 ± 0.02e	0.72 ± 0.02a
P. mendocina + *G. mosseae*	1.62 ± 0.03f	0.98 ± 0.01cde	0.75 ± 0.03abc
Root dry biomass (g dw)			
Control	0.82 ± 0.02fgh	0.61 ± 0.02def	0.33 ± 0.01ab
Fertilized	0.80 ± 0.04efgh	0.54 ± 0.02bcd	0.30 ± 0.01a
P. mendocina	0.89 ± 0.02h	0.66 ± 0.02defg	0.35 ± 0.01ab
G. intraradices	0.64 ± 0.02defg	0.54 ± 0.00bcd	0.37 ± 0.02abc
G. mosseae	0.73 ± 0.03defgh	0.58 ± 0.01cd	0.32 ± 0.01a
P. mendocina + *G. intraradices*	0.73 ± 0.01defgh	0.62 ± 0.01defg	0.35 ± 0.02ab
P. mendocina + *G. mosseae*	0.84 ± 0.02gh	0.59 ± 0.01cde	0.35 ± 0.01ab
Colonization (%)			
Control	2.4 ± 0.1a	1.4 ± 0.2a	4.4 ± 0.8a
Fertilized	1.7 ± 0.1a	1.6 ± 0.5a	7.1 ± 0.5a
P. mendocina	2.8 ± 0.2a	2.6 ± 0.5a	1.0 ± 0.2a
G. intraradices	68.8 ± 1.1d	35.6 ± 3.7b	31.0 ± 1.4b
G. mosseae	63.6 ± 3.3cd	36.0 ± 0.9b	37.8 ± 2.5b
P. mendocina + *G. intraradices*	68.2 ± 2.0d	45.2 ± 2.4bc	49.4 ± 1.6bc
P. mendocina + *G. mosseae*	59.4 ± 0.1cd	33.4 ± 2.3b	48.4 ± 0.9bc

* Mean ± standard error. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Duncan's test.

weighted (P2). The mass of stable aggregates as a percentage of the total aggregates was calculated by $(P1 - P2) \times 100/(4 - P2 + T)$. Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through Whatman no. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two millilitres of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

The glomalin-related soil protein (GRSP) was extracted from soil samples sieved between 0.25 and 4 mm and to <2 mm with 20 mM sodium citrate (pH 7.0) at a rate of 250 mg of soil in 2 ml of buffer and autoclaving at 121 °C for 30 min (Wright and Anderson, 2000). The supernatant was removed and two additional sequential 1-h extractions were performed. All supernatants from a sample were combined, the volume was measured, an aliquot was centrifuged at 10,000 × *g* for 15 min to remove soil particles and Bradford-reactive total protein was measured.

2.7. Statistical analysis

Aggregate stability and percentage colonization were arcsin-transformed, and the other parameters were log-transformed to compensate for variance heterogeneity before analysis of variance. Amendments addition, water regime and their interactions effects on the measured variables were tested by a two-way analysis of variance and comparisons among means were made using Duncan's test calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS 14.0 for Windows.

3. Results

3.1. Plant growth and mycorrhizal colonization

Under non-stressing conditions (−0.03 MPa), inoculation with *P. mendocina* and inorganic fertilization increased shoot dry biomass significantly with respect to the control plants (Table 1). However, AM, PGPR and their combinations had no significant effect on root dry biomass. The fertilization and bacterial inoculation produced similar increases in plant growth (about 29% greater than the control plants). Water deficit caused a significant decrease in the shoot and root dry biomass of all plants (Table 1). Hence, *P. mendocina* and fertilization did not increase root dry biomass under water stress. The microbial inoculants did not have a significant effect on the growth parameters of lettuce plants grown at the moderate (−0.2 MPa) or severe (−0.6 MPa) water-stress levels.

In non-stressed plants, only the mycorrhizal inoculation treatments and its combination with PGPR had a significant effect on the level of colonization in lettuce roots and there were no significant differences between them (Table 1). Both water-stress levels decreased significantly the percentage colonization in plants inoculated with *G. intraradices* or *G. mosseae* (Table 1).

3.2. Aggregate stability and aggregation agents

Only water stress, but neither the microbial treatments nor fertilization, had a significant effect on aggregate stability (Table 2). Under well-watered conditions, only the aggregate stability value of the *G. mosseae*-inoculated soil was higher than that of the control soil (about 17% higher). Drought stress increased aggregate stability in all soils, except for the soil inoculated with *G. mosseae*, for which the levels of stable aggregates remained constant independent of the water regime (Table 3). In addition, it is worth noting that, under

Table 2
Two factors ANOVA (amendments and water stress) for all parameters studied of *L. sativa*. *P* significance values.

	Amendments (*A*)	Water stress (*W*)	Interaction (*A* × *W*)
Shoot biomass	<0.001	<0.001	<0.001
Root biomass	0.030	<0.001	0.180
Colonization	<0.001	<0.001	<0.001
Aggregate stability	NS	<0.001	NS
Total carbohydrates	0.002	<0.001	0.002
Water-soluble CH	0.005	NS	0.037
GRSP (<2 mm)	<0.001	<0.001	<0.001
GRSP (0.25–4 mm)	<0.001	<0.001	<0.001
Dehydrogenase	<0.001	<0.001	0.002
Phosphatase	<0.001	<0.001	NS

NS: not significant. CH: carbohydrates. GRSP: glomalin-related soil protein.

both moderate and severe drought, there were no significant differences among the soils inoculated with either AM fungi or PGPR and the control soil.

In the <2 mm soil fraction, the GRSP levels of the control soil were similar to those of the inoculated and fertilized soils, under non-stressing conditions (Table 3). Drought had a significant effect on the GRSP levels of soil (Table 2). With moderate drought (−0.2 MPa), GRSP abundance increased in almost all soils, the highest values being in the soils inoculated with either of the selected AM fungi, in the soil inoculated with *G. intraradices* and *P. mendocina* and in the fertilized soil. With severe drought, GRSP levels decreased significantly with respect to those of soils subjected to moderate drought, the lowest values occurring in the control soil.

The GRSP in the 0.25–4 mm soil fraction showed a similar trend to that of the <2 mm soil fraction (Table 3). Thus, the GRSP levels increased with moderate water stress (−0.2 MPa), whereas under severe water stress (−0.6 MPa) they did not differ with respect to those of well-watered soils. The values of GRSP in soils with microbial treatments (PGPR and mycorrhiza) were higher than in the control or fertilized soil under well-watered and severe-drought conditions, while with moderate drought all soils showed similar GRSP values (Table 3).

Total carbohydrates in the rhizosphere soil were affected by the level of water stress and the microbial treatments (Table 2). Under well-watered conditions, the total carbohydrates were increased in soils inoculated with either AM fungus, alone or in combination with the PGPR, with respect to the control soil (Table 4). Incrementing the level of water stress, the differences among these treatments and the control soil decreased, with no significant differences among them under severe drought.

The highest concentration of water-soluble carbohydrates was found in the soil inoculated with *G. intraradices*, under well-watered conditions (Table 4). There was a progressive increase in the abundance of water-soluble carbohydrates as the drought increased from −0.2 to −0.6 MPa. At both water-stress levels, no significant differences among the different treatments and the control soil were found for the content of water-soluble carbohydrates.

3.3. Enzymatic activities

Both water stress and amendments had a significant effect on dehydrogenase activity (Table 2). Without water stress, the highest dehydrogenase activity was found in the soil inoculated with either of the selected AM fungi, alone or in combination with *P. mendocina*, compared with the other soils (Table 5). Increasing drought raised the dehydrogenase activity slightly in almost all soils. In soils inoculated with either of the AM fungi, the increase took place under moderate stress (−0.2 MPa). The level of dehydrogenase activity in the control, fertilized soil or in the soil inoculated with *P. mendocina* alone increased only with severe stress (−0.6 MPa). Under such conditions, the control soil exhibited lower dehydrogenase activity than the soils inoculated with AM fungi.

In well-watered conditions, acid phosphatase activity was higher in soils treated with either of the microbial inoculations (PGPR, AM alone or in combination) than in the control or fertilized soils (Table 5). Drought stress increased phosphatase activity significantly in all soils (Table 2). The differences in phosphatase activity among the inoculated soils and non-inoculated soils

Table 3
Effect of inoculation with *G. intraradices*, *G. mosseae* and *P. mendocina* on aggregate stability and GRSP of rhizosphere soil of *L. sativa* seedlings grown at three levels of irrigation ($n = 5$).

	Well-watered (−0.03 MPa)	Stressed (−0.2 MPa)	Stressed (−0.6 MPa)
Aggregate stability			
Control	47 ± 1abc*	52 ± 1abcdef	57 ± 1efghi
Fertilized	45 ± 1ab	54 ± 2cdefgh	60 ± 2ghi
P. mendocina	45 ± 2a	52 ± 1abcdef	61 ± 2hi
G. intraradices	48 ± 1abc	54 ± 2bcdefgh	62 ± 2i
G. mosseae	55 ± 1defg	57 ± 2fghi	59 ± 2fghi
P. mendocina + *G. intraradices*	47 ± 1abcde	56 ± 2defghi	59 ± 1fghi
P. mendocina + *G. mosseae*	49 ± 1abcd	56 ± 2defghi	58 ± 1fghi
GRSP ($\mu g\ g^{-1}$ soil) (<2mm soil fraction)			
Control	84 ± 1abcd	96 ± 1ef	82 ± 1abc
Fertilized	79 ± 2a	108 ± 2g	90 ± 1def
P. mendocina	81 ± 1ab	99 ± 2f	97 ± 1ef
G. intraradices	89 ± 2cde	111 ± 2g	91 ± 1def
G. mosseae	80 ± 2a	111 ± 2g	90 ± 2def
P. mendocina + *G. intraradices*	89 ± 2cde	112 ± 3g	91 ± 2def
P. mendocina + *G. mosseae*	94 ± 2ef	96 ± 1ef	92 ± 3def
GRSP ($\mu g\ g^{-1}$ soil) (0.25–4 mm soil fraction)			
Control	75 ± 1a	112 ± 5gh	83 ± 1abc
Fertilized	80 ± 1ab	112 ± 5fgh	83 ± 1abc
P. mendocina	91 ± 1bcd	116 ± 4gh	95 ± 1de
G. intraradices	91 ± 1bcd	114 ± 3gh	100 ± 2def
G. mosseae	86 ± 2bc	109 ± 4fg	119 ± 2h
P. mendocina + *G. intraradices*	107 ± 2fgh	112 ± 2fgh	108 ± 2fgh
P. mendocina + *G. mosseae*	90 ± 1bcd	107 ± 1efg	109 ± 1fg

GRSP: glomalin-related soil protein.
* Mean ± standard error. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Duncan's test.

Table 4
Effect of inoculation with *G. intraradices*, *G. mosseae* and *P. mendocina* on carbon fractions of rhizosphere soil of *L. sativa* seedlings grown at three levels of irrigation ($n = 5$).

	Well-watered (−0.03 MPa)	Stressed (−0.2 MPa)	Stressed (−0.6 MPa)
Total carbohydrates ($\mu g\ g^{-1}$)			
Control	3847 ± 127a*	3476 ± 180a	4223 ± 17ab
Fertilized	6320 ± 120d	6326 ± 216d	5581 ± 126bcd
P. mendocina	3926 ± 83a	6054 ± 94cd	4633 ± 193ab
G. intraradices	4747 ± 94bcd	4495 ± 87ab	4387 ± 146ab
G. mosseae	4854 ± 73bcd	5156 ± 23bcd	5221 ± 129ab
P. mendocina + *G. intraradices*	5524 ± 68bcd	4570 ± 42abc	3857 ± 41a
P. mendocina + *G. mosseae*	5494 ± 218bcd	3680 ± 100a	4492 ± 31ab
Water-soluble carbohydrates ($\mu g\ g^{-1}$)			
Control	8 ± 0a	22 ± 1b	33 ± 1def
Fertilized	8 ± 0a	25 ± 0bc	35 ± 1f
P. mendocina	14 ± 1a	26 ± 1bcd	33 ± 2def
G. intraradices	22 ± 1b	25 ± 1bc	32 ± 1ef
G. mosseae	8 ± 0a	21 ± 1b	34 ± 1def
P. mendocina + *G. intraradices*	7 ± 1a	24 ± 1bc	34 ± 1def
P. mendocina + *G. mosseae*	12 ± 1a	26 ± 1bc	31 ± 0cde

* Mean ± standard error. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Duncan's test.

increased sharply as a consequence of water stress, particularly under moderate water stress.

4. Discussion

In the present study, drought stress improved the aggregate stability of the rhizosphere soil of lettuce plants, independently of the inoculation with either of the selected AM fungi, *G. intraradices* or *G. mosseae*, and/or with a PGPR, *P. mendocina*. Soil structure is influenced by soil water content and its variation with time, and plant growth can influence strongly the magnitude and frequency of wetting and drying cycles. Decreased water content typically increases contact points between primary particles and organic matter, resulting in increased soil cohesion and strength (Rillig and Mummey, 2006). Localised drying of soil, in close proximity to roots, promotes binding between root exudates and clay particles (Reid and Goss, 1982), directly facilitating microaggregate formation—which could be confirmed by increased aggregate stability under drought conditions. As is well known (Rillig and Mummey, 2006), mycorrhizal fungi can potentially influence soil aggregation in different ways, by means of the fungal mycelium itself or fungal mycelium products like glomalin-related soil protein or polysaccharides. In accordance with Wu et al. (2008), the moderate drought stress enhanced the GRSP concentration, which reached similar values in all soils. These results could support the hypothesis that the GRSP probably originated as a protective coating on fungal hyphae to keep water and nutrients from being lost prior to reaching the plant host (Nichols, 2008), particularly under such water-stress conditions. This substance also helps to stabilize soil aggregates by forming a protective, polymer-like lattice on the aggregate surface. In the present experiment, the mycorrhizal inoculation treatments did not produce increases either in aggregate stability or in the GRSP concentration with respect to the non-AM soils under drought conditions. These results contrast with those found by Wu et al. (2008) and with previous findings reported by Roldán et al. (2006), where the positive effect of the exotic AM fungi on the structural stability of the rhizosphere of *Juniperus oxycedrus* was more pronounced after soil drying. Under severe drought stress, the production of GRSP by AM fungi decreased with respect to the moderate stress level, although the concentration was higher in the AM-inoculated soils. However, there was no evidence for the major participation of glomalin produced by exotic AM fungi, with respect to that produced by local indigenous AM fungi from the soil, in the improvement of soil structural stability. This lack of change in soil aggregate stability may be attributed to the fact that the effectiveness of AM fungi depends greatly on the associated mycorrhizal fungus and the host plant species.

Some studies have shown that soil drying may represent a significant stress for the soil microbiota, provoking a substantial loss of its biomass and activity (Rosacker and Kieft, 1990). Decreasing the water content of soil restricts the diffusion of nutrients to

Table 5
Effect of inoculation with *G. intraradices*, *G. mosseae* and *P. mendocina* on enzyme activities of rhizosphere soil of *L. sativa* seedlings grown at three levels of irrigation ($n = 5$).

	Well-watered (−0.03 MPa)	Stressed (−0.2 MPa)	Stressed (−0.6 MPa)
Dehydrogenase (μg INTF g^{-1} soil 20 h^{-1})			
Control	84 ± 1abc*	89 ± 5abcd	96 ± 1bcde
Fertilized	83 ± 1ab	80 ± 1ab	106 ± 2cdefg
P. mendocina	84 ± 1a	73 ± 1a	111 ± 1defghi
G. intraradices	111 ± 2defghi	131 ± 5ij	128 ± 3hij
G. mosseae	102 ± 1bcdef	139 ± 2j	118 ± 3fghij
P. mendocina + *G. intraradices*	125 ± 2ghij	161 ± 7k	127 ± 1ghij
P. mendocina + *G. mosseae*	108 ± 2defgh	109 ± 3defghi	116 ± 1efghi
Phosphatase (nmol PNP g^{-1} h^{-1})			
Control	34 ± 4a	71 ± 3bc	69 ± 4ab
Fertilized	43 ± 6a	84 ± 5bc	97 ± 3bcd
P. mendocina	108 ± 10bcde	129 ± 5def	118 ± 4def
G. intraradices	135 ± 9def	198 ± 9g	127 ± 2def
G. mosseae	97 ± 12bcd	160 ± 11f	135 ± 1def
P. mendocina + *G. intraradices*	112 ± 5cdef	139 ± 7def	151 ± 7ef
P. mendocina + *G. mosseae*	120 ± 7cdef	149 ± 12ef	141 ± 10ef

* Mean ± standard error. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Duncan's test.

microorganisms (Harris, 1981). In this way, it has been reported that soil enzyme activity decreases with drought under field conditions (Sardans and Peñuelas, 2005; Sardans et al., 2008). In the present experiment, both dehydrogenase and phosphatase activities were increased by the soil drying, particularly in the soils inoculated with AM fungi and PGPR. One possible explanation for this observation is that the native soil community is physiologically and genetically well adapted to water-limited environments. Soil microorganisms have developed various mechanisms to survive desiccation in soil. For example, bacteria have been reported to change the structure of their membrane or to synthesize exopolysaccharides in order to increase their survival during periods of low external water potential. Polysaccharides are hydroscopic and therefore may maintain higher water content in the colony microenvironment than in the bulk soil as water potential declines. This increase in water content could increase nutrient availability within the bacterial colony. Thus, it has been shown that a *Pseudomonas* sp. strain isolated from soil increased its exopolysaccharide production during desiccation (Roberson and Firestone, 1992). The extracellular polysaccharides of bacteria can form, with the surrounding mineral particles, an organo-mineral sheath around the cells (Chenu, 1993), which leads to an increase in macroaggregates as an indirect, additional effect. It has been demonstrated that inoculation with an exopolysaccharide-producing rhizobacterium increased the mass of sunflower root-adhering soil under water-stress conditions, although the values reached were lower than under well-watered conditions (Alami et al., 2000). Indeed, the levels of total and water-soluble carbohydrates were higher after soil drying, with no significant differences between inoculated and non-inoculated soils. Thus, this labile organic fraction involved in the formation of stable aggregates during soil drying can be used also as a carbon and energy source by soil microflora, contributing to the enhancement of soil microbial activity as mentioned above.

On the other hand, AM fungi can influence soil microbial communities, including plant growth-promoting rhizobacteria involved also in soil aggregation, by exuding photosynthesis-derived carbon into the mycorrhizosphere (Hodge, 2000). However, how and where within the soil matrix these changes are mediated and their significance regarding soil aggregation are poorly understood. Unlike AM fungi, which exert a strong influence at the scale of macroaggregates, rhizobacteria would be expected to influence the formation and stabilisation of microaggregates in a more-direct manner. Thus, AM fungi-facilitated alteration of prokaryotic communities may influence indirectly aggregation processes at scales smaller than the macroaggregate. Nevertheless, any mutual influence of the PGPR and the AM fungi in soil aggregation could not be confirmed.

We conclude that the highest aggregate stability promoted by the soil autochthonous microbial population in response to drought stress was independent of inoculation with PGPR and/or AM fungi. The improvement in aggregate stability under stress conditions was related to increases in the glomalin-related soil protein and carbohydrate concentrations. The inoculations with AM fungi and/or PGPR were not sufficient to restore soil structure but were effective for re-activating soil microbial populations under drought-stress conditions.

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