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An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*

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ABSTRACT

A mesocosm experiment was conducted to examine the effect of an arbuscular mycorrhizal (AM) fungus (*Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe) and a plant growth-promoting rhizobacterium (PGPR) (*Pseudomonas mendocina* Palleroni), alone or in combination, on the structural stability of the rhizosphere soil of *Lactuca sativa* L. grown under two levels of salinity. The plants inoculated with *P. mendocina* had significantly greater shoot biomass than the control plants at both salinity levels, whereas the mycorrhizal inoculation was only effective in increasing shoot biomass at the moderate salinity level. The aggregate stability of soils inoculated with the PGPR and/or *G. mosseae* significantly decreased with increasing saline stress (about 29% lower than those of soils under non-saline conditions). Only the inoculated soils showed higher concentrations of sodium (Na) under severe saline stress. The severe salinity stress decreased the glomalin-related soil protein (GRSP) concentration, but the highest values of GRSP were recorded in the inoculated soils. Our findings suggest that the use of AM fungi and/or a PGPR for alleviating salinity stress in lettuce plants could be limited by their detrimental effect on soil structural stability.

1. Introduction

Secondary salinisation of agricultural soils by irrigation is a serious land degradation problem in arid and semi-arid areas, where evaporation greatly exceeds precipitation and salts dissolved in the ground water reach and accumulate at the soil surface through capillary movement. It has been estimated that more than 7 % of the earth is land occupied by saline soil (Tester and Davenport, 2003), rising up to 15 % in arid and semi-arid areas of the world, while salt-affected soils represent about 40 % of the world's irrigated lands (Zahran, 1997). Excessive amounts of salts, mainly sodium (Na) salts, in the soil solution cause numerous adverse phenomena such as destabilisation of soil structure, deterioration of soil hydraulic properties and a considerable reduction in crop yield (Lax et al., 1994; Kohler et al., 2009). Likewise, various authors (Rietz and Haynes, 2003) have also reported negative effects of salinity on soil microbial biomass carbon and enzyme activities.

In semi-arid environments, soil aggregate stability is one of the most important properties controlling the growth of plants which, in turn, protects the soil against water erosion. Thus, the improvement of soil structural stability is of great importance in rendering these degraded, saline soils suitable for agriculture. The contribution of microbial populations, either as free-living organisms or associated with plant roots, and their activities to soil aggregate stability has been stressed by Jastrow and Miller (1991). In particular, the symbiosis between arbuscular mycorrhizal (AM) fungi and plants has been shown to contribute to the stability of soil aggregates, including soils of high salinity such as salt marshes (Caravaca et al., 2005). Arbuscular mycorrhizal fungi primarily influence the stability of macroaggregates (>250 μm), which they are hypothesised to help stabilise via hyphal enmeshment aggregates (Miller and Jastrow, 2000) and by deposition of organic substances (Bearden and Petersen, 2000). A key factor in the contribution of AM fungi to soil aggregation is the production of the glycoprotein glomalin, which acts as an insoluble glue to stabilise aggregates (Gadkar and Rillig, 2006). Operationally defined by the 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) although the role of GRSP in the stabilisation of saline soils has not been confirmed.

Bacteria associated with the mycorrhizosphere have been suggested to be involved in plant growth and establishment of AM fungi (Larsen et al., 2009). The production of exopolysaccharides (EPSs) by bacterial populations in response to adverse environmental conditions, such as desiccation, has been shown to contribute to soil aggregation, leading to increased water retention

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in the rhizosphere (Kaci et al., 2005). In previous work, we demonstrated the effectiveness of inoculation with a plant growth-promoting rhizobacterium (PGPR), *Pseudomonas mendocina*, for both soil stabilisation and promotion of soil fertility under non-saline field conditions (Kohler et al., 2006). The study of the antagonistic or synergistic effects of different microbial inoculants, when co-inoculated, is a crucial step in the development of effective host-microorganism combinations. To the best of our knowledge, nothing is known about the interaction of PGPR and AM fungi with respect to soil aggregate stability under induced saline stress.

We hypothesise that inoculation with a PGPR, alone or in combination with an AM fungus, can improve soil physical properties, even under saline stress. To assess this hypothesis, we determined the combined effects of such microbial inoculations on the structural stability of the rhizosphere soil of *Lactuca sativa* under different conditions of soil salinity.

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 analytical characteristics of the agricultural soil used, determined by standard methods (Page et al., 1982), were: pH (1:5 in H_2O) 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 AM fungus used was *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe, obtained from the collection of the experimental field station of Zaidín, Granada. The *Glomus* species was 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. The 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. The inoculum was subjected to a most probable number test (Sieverding, 1991) to determine potential infectivity and to equalize application doses. The source of inoculum had a potential infectivity of about 35 infective propagules g^{-1} inoculum.

The PGPR strain *Pseudomonas mendocina* Palleroni was obtained from Probelte, S.A., Murcia, and was selected on the basis of its ability to produce siderophores. The *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 sterilized tap water. The bacterial suspension contained 10^9 colony forming units (CFU) mL^{-1}.

2.3. Microbial inoculation and salt stress treatments

The experiment was a mesocosm assay, conducted as a randomized factorial design with two factors and five-fold replication. The first factor had seven levels: control soil, soil inoculated with the AM fungus *G. mosseae*, soil inoculated with the bacterium *P. mendocina*, the combination of soil inoculated with the bacteria *P. mendocina* and with *G. mosseae* and a soil fertilized with inorganic fertilizer. The second factor had three levels of salt stress: non-salt stress, moderate and severe salt 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 AM fungi. The plants were inoculated with *P. mendocina* twice 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 nitrogen, 156.2 potassium, 160.2 calcium, 98.4 sulphur, 11.7 sodium, 5.4 chlorine, 5.0 iron-chelate sequestrene, 0.54 manganese, 0.54 boron, 0.10 copper, 0.06 zinc, 0.006 molybdenum and 40 phosphorus. Two concentrations (2 g and 4 g NaCl kg^{-1} soil) of saline solution were applied to the saline pots. The NaCl concentration was gradually increased until reaching the required salinity of NaCl for each concentration, applied over four consecutives days to avoid osmotic shock. A plastic bag was put underneath each pot to collect excess water due to drainage. This water was reapplied to the respective pot. All seedlings were grown for five weeks without any fertilizer treatment (except fertilized seedlings). 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 was 22 °C, and the relative humidity was between 60% and 80%. Midday photosynthetically active radiation (PAR) averaged 260 μE m^{-2} s^{-1}.

2.4. Plant analyses

Five weeks after planting five plants per treatment were harvested. 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 colonisation, roots were cleared with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970). The percentage of root length colonised by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980). Positive counts for AM colonisation included the presence of vesicles or arbuscules or typical mycelium within the roots.

2.5. Soil analyses

At the end of the experiment, rhizosphere soil samples were collected from the pots. To collect the rhizosphere soil the root system with adhering rhizosphere soil was placed into a plastic bag and shaken, thus separating the rhizosphere soil from the root system. Rhizosphere soil samples were air-dried at room temperature and sieved at 2 mm for physical-chemical and chemical analysis or at 0.2–4 mm for aggregate stability.

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 Jm^{-2}. The remaining soil on the sieve was 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 weighted (P2). The mass of stable aggregates as a percentage of the total aggregates was calculated by $(P1 - P2) \times 100/(4 - P2)$.

The glomalin-related soil protein (GRSP) was extracted from soil samples (sieved between 0.250 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 then autoclaved 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.

2.6. Statistical analysis

Aggregate stability and percentage colonisation were arcsin-transformed, and the other parameters were log-transformed to compensate for variance heterogeneity before analysis of variance. Amendments addition, saline stress and their interactions effects on the measured variables were tested by a two-way analysis of variance and comparisons among means were made using Tukey's test calculated at $P < 0.05$. Correlation analysis among all the soil and plant parameters measured was carried out using Pearson's rank correlation coefficients. Statistical procedures were carried out with the software package SPSS 14.0 for Windows.

3. Results

3.1. Growth parameters and mycorrhizal colonisation

Under non-saline conditions, shoot dry biomass of lettuce was increased by the inoculation with *P. mendocina*, while no significant effect was found with the mycorrhizal inoculation treatments (Table 1). The fertilization and bacterial inoculation produced similar increases in plant growth (about 30% greater than control plants). The microbial treatments had a significant effect on the root biomass of plants (Table 2). There was a negative interaction between the amendments and saline stress regarding growth parameters or mycorrhizal colonisation. Salinity decreased the dry weights of the shoots and roots for all lettuce plants (Table 1). However, the plants inoculated with *P. mendocina* had significantly greater shoot biomass than the control plants at both salinity levels, whereas the mycorrhizal inoculation only increased shoot biomass at the moderate salinity level.

Mycorrhizal inoculation produced active colonisation of the root system of the *L. sativa* seedlings (Tables 1 and 2). The level of colonisation in roots of mycorrhizal plants was not affected by bacterial inoculation but decreased significantly with increasing NaCl concentration in the soil (Table 1).

Table 1
Effect of inoculation with *G. mosseae* and *P. mendocina* on plant growth and mycorrhizal colonisation of *L. sativa* seedlings grown at three levels of salinity ($n = 5$).

	Without NaCl	2 g NaCl kg^{-1} soil	4 g NaCl kg^{-1} soil
Shoot dry biomass (g dw)			
Control	1.48 ± 0.04*c	0.84 ± 0.02e	0.83 ± 0.02e
Fertilized	1.90 ± 0.04a	1.18 ± 0.02d	1.27 ± 0.05cd
P. mendocina	1.92 ± 0.01a	1.18 ± 0.02d	1.22 ± 0.05cd
G. mosseae	1.46 ± 0.02c	1.21 ± 0.02d	1.05 ± 0.02de
P. mendocina + *G. mosseae*	1.62 ± 0.03ab	1.29 ± 0.03cd	1.22 ± 0.05cd
Root dry biomass (g dw)			
Control	0.82 ± 0.02ab	0.42 ± 0.01c	0.26 ± 0.01d
Fertilized	0.80 ± 0.04ab	0.66 ± 0.02b	0.44 ± 0.03c
P. mendocina	0.89 ± 0.02a	0.77 ± 0.01b	0.41 ± 0.02c
G. mosseae	0.73 ± 0.03b	0.78 ± 0.03ab	0.45 ± 0.02c
P. mendocina + *G. mosseae*	0.84 ± 0.02a	0.85 ± 0.02a	0.50 ± 0.02c
Colonisation (%)			
Control	2.4 ± 0.1c	2.6 ± 0.5c	5.2 ± 0.7c
Fertilized	1.7 ± 0.1c	3.8 ± 0.6c	2.4 ± 0.4c
P. mendocina	2.8 ± 0.2c	5.0 ± 0.7c	4.3 ± 0.4c
G. mosseae	63.6 ± 3.3a	45.6 ± 2.0b	34.0 ± 1.0b
P. mendocina + *G. mosseae*	59.4 ± 1.1a	37.6 ± 1.2b	41.8 ± 1.4b

*Mean ± standard error.
For each parameter, values sharing the same letter are not significantly different (Tukey, $P < 0.05$).

3.2. Soil physico-chemical properties

Salinity increased the electrical conductivity of all soils significantly (Table 3). No significant differences among the different treatments were found for this physico-chemical parameter in soils under non-saline and saline conditions.

Both salinity and amendments had a significant effect on the concentration of Na in the soil (Table 2). The concentration of Na in the *P. mendocina*-inoculated and control soils was increased significantly under moderate salinity (Table 3). At the highest salt level, the soils inoculated with the AM fungus and the PGPR alone or in combination had higher concentrations of soil Na than the control soil.

3.3. Aggregate stability and aggregation agents

Only the salinity and the interaction amendment × saline stress had a significant effect on aggregate stability (Table 2). The percentages of stable aggregates of the soils inoculated with *P. mendocina* and/or *G. mosseae* decreased at the highest salinity level (Table 4), particularly in the soils inoculated with the AM fungus (about 29% with respect to those soils under non-saline conditions). The lowest percentages of stable aggregates were found in the soil inoculated with the PGPR, alone or in combination with the AM fungus, under severe saline stress.

Under non-saline conditions, only the combined inoculation of *P. mendocina* and *G. mosseae* increased the GRSP levels in the <2 mm fraction (Table 4). Salinity had a significant effect on the soil GRSP levels (Table 2). The GRSP concentrations of the control and the *P. mendocina* + *G. mosseae*-inoculated soils were decreased markedly under moderate salinity with respect to that of the non-saline conditions. With severe saline stress, GRSP levels were decreased significantly in all soils with respect to those of non-stressed soils, although without significant differences among soils.

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

	Amendments (A)	Saline stress (S)	Interaction (A × S)
Shoot biomass	<0.001	<0.001	<0.001
Root biomass	<0.001	<0.001	<0.001
Colonisation	<0.001	<0.001	<0.001
Electrical conductivity	<0.001	<0.001	NS
Total Na	0.019	<0.001	0.008
Aggregate stability	NS	<0.001	<0.001
GRSP (<2 mm)	<0.001	<0.001	0.002
GRSP (0.25–4 mm)	<0.001	<0.001	<0.001

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

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

	Without NaCl	2 g NaCl kg^{-1} soil	4 g NaCl kg^{-1} soil
Electrical conductivity (1:5, μS cm^{-1})			
Control	310 ± 3*c	580 ± 4b	902 ± 15a
Fertilized	342 ± 6c	585 ± 8b	914 ± 22a
P. mendocina	306 ± 10c	587 ± 6b	761 ± 9ab
G. mosseae	357 ± 6c	640 ± 12b	949 ± 38a
P. mendocina + *G. mosseae*	330 ± 10c	664 ± 8b	886 ± 16a
Total Na (mg kg^{-1})			
Control	672 ± 10c	789 ± 15b	682 ± 11c
Fertilized	706 ± 15bc	577 ± 7c	695 ± 22bc
P. mendocina	675 ± 14c	858 ± 19ab	757 ± 14b
G. mosseae	733 ± 11b	743 ± 13b	805 ± 21b
P. mendocina + *G. mosseae*	742 ± 15b	802 ± 15b	1053 ± 20a

*Mean ± standard error.
For each parameter, values sharing the same letter are not significantly different (Tukey, $P < 0.05$).

Under non-saline conditions, both microbial inoculations increased the GRSP levels of the 0.25–4 mm soil fraction with respect to the control soil (Table 4). The GRSP showed a trend similar to that of the <2 mm soil fraction in response to saline stress. Thus, the GRSP levels of soils under severe salinity drastically decreased with respect to those of the non-saline soils but there were pronounced differences among the microbially-treated soils and the control or fertilized soils. Under such conditions, the highest values of GRSP were recorded in the soils inoculated with the AM fungus (about 23% higher than in the control soil), followed by the soils inoculated with the PGPR (Table 4).

3.4. Correlation among the different parameters

As shown in the Table 5, electrical conductivity was negatively correlated with both growth parameters such as shoot dry biomass ($r = -0.690$, $P < 0.01$) and root dry biomass ($r = -0.790$, $P < 0.001$), aggregate stability ($r = -0.570$, $P < 0.05$) and GRSP concentrations in the <2 mm fraction ($r = -0.813$, $P < 0.001$). Total Na was only positively correlated with the electrical conductivity ($r = 0.556$, $P < 0.05$).

There was a positive significant correlation between mycorrhizal colonisation and GRSP concentrations in the <2 mm fraction ($r = 0.526$, $P < 0.05$) and in the 0.25–4 mm soil fraction ($r = 0.543$, $P < 0.05$). The GRSP concentrations in the <2 mm fraction correlated positively with shoot dry biomass ($r = 0.652$, $P < 0.01$), root dry biomass ($r = 0.898$, $P < 0.001$) and aggregate stability ($r = 0.511$, $P < 0.05$).

Table 4
Effect of inoculation with *G. mosseae* and *P. mendocina* on aggregate stability and GRSP of rhizosphere soil of *L. sativa* seedlings grown at three levels of salinity ($n = 5$).

	Without salt stress	2 g NaCl kg^{-1} soil	4 g NaCl kg^{-1} soil
Aggregate stability (%)			
Control	47 ± 1*a	46 ± 1a	46 ± 1a
Fertilized	47 ± 1a	47 ± 1a	41 ± 1ab
P. mendocina	45 ± 2a	44 ± 2a	37 ± 1b
G. mosseae	55 ± 1a	49 ± 1a	39 ± 1b
P. mendocina + *G. mosseae*	49 ± 1a	49 ± 1a	36 ± 2b
GRSP (μg g^{-1} soil) (<2 mm soil fraction)			
Control	84 ± 2b	63 ± 1c	63 ± 1c
Fertilized	79 ± 1b	79 ± 0b	63 ± 1c
P. mendocina	81 ± 1b	76 ± 1bc	67 ± 1c
G. mosseae	80 ± 1b	77 ± 1b	66 ± 2c
P. mendocina + *G. mosseae*	94 ± 1a	82 ± 0b	65 ± 1c
GRSP (μg g^{-1} ± soil) (0.25–4 mm soil fraction)			
Control	75 ± 1b	69 ± 2bc	66 ± 2c
Fertilized	80 ± 1b	88 ± 1a	56 ± 1cd
P. mendocina	91 ± 1a	76 ± 0b	75 ± 1b
G. mosseae	86 ± 2a	74 ± 0b	81 ± 1b
P. mendocina + *G. mosseae*	89 ± 1a	99 ± 2a	81 ± 1b

*Mean ± standard error.
For each parameter, values sharing the same letter are not significantly different (Tukey, $P < 0.05$).
GRSP: glomalin-related soil protein.

4. Discussion

4.1. Influence of salinity on soil aggregate stability

The results of the present study show that salinity decreased the aggregate stability of the rhizosphere soil of lettuce plants inoculated with the AM fungus, *G. mosseae*, and/or with a PGPR, *P. mendocina*. However, the structural stability of the control soil was not affected by the salinity. Sodium is a highly-dispersive agent, causing the direct breakup of aggregates and indirectly affecting aggregation through decreased plant productivity (Bronick and Lal, 2005). Previous studies have described a negative relationship between soil aggregation and the percentage of Na saturation in the exchange complex (Lax et al., 1994). In this experiment, both microbiological treatments increased the concentration of soil Na compared with the non-inoculated control soil under severe salinity. This suggests that the decrease in structural stability of the inoculated soils could be related to the increased concentration of soil Na. In this regard, there was a negative correlation between soil aggregate stability and the soil Na concentration with a significance level of $P < 0.05$. Likewise, the lack of change in the concentration of Na in the non-inoculated, control soil would explain why the stable aggregates of this soil hardly varied with the salinity treatments. It should also be mentioned that the same bacterial and mycorrhizal inoculations were shown to reduce the Na uptake and/or increased the K uptake in leaves of lettuce, increasing the salinity tolerance of the plants (Kohler et al., 2009). Giri et al. (2007) reported that an AM fungus in *Acacia nilotica* accumulated salt and thus prevented transport of Na to shoot tissues. Cantrell and Linderman (2001) suggested that Na might be retained in intraradical AM fungal hyphae. In our case, the excess Na resulting from the increasing levels of salinity was retained in the soil of the plants inoculated with the AM fungus. Further investigations are required to elucidate this mechanism. In the case of *P. mendocina*, this PGPR strain can produce exopolysaccharides (Kohler et al., 2006) that bind cations, including Na, thus decreasing the content of Na available for plant uptake.

4.2. Influence of PGPR and AM fungus on soil aggregation

The mechanisms involved in aggregate stabilisation are based on the enmeshment of soil particles by hyphae and roots, and on the exudation of polysaccharides (Bearden and Petersen, 2000). In our study, the AM fungal hyphae did not seem to be involved in the stabilisation of aggregates because there was no significant correlation between the percentage of stable aggregates and the mycorrhizal colonisation of the lettuce plants. Both aggregate stability and the percentage of colonised root length in plants exposed to salinity and inoculated with the AM fungus were reduced significantly compared with such plants not exposed to salinity. The AM fungi may also produce a glycoprotein, glomalin, which can act as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000). Recently, it has been postulated that glomalin plays a primary role in fungal physiology and in secondarily-arising

Table 5
Pearson rank correlation matrix among all parameters ($n = 15$). Correlation coefficient (significance level).

	Shoot Biomass	Root biomass	Colonisation	Elect. conductivity	Total Na	Aggreg. Stability	GRSP (<2 mm)	GRSP (0.25–4 mm)
Shoot biomass	1.000	0.740**	NS	−0.690**	NS	NS	0.652**	NS
Root biomass		1.000	NS	−0.790***	NS	NS	0.898***	0.648**
Colonisation			1.000	NS	NS	NS	0.526*	0.543*
Electrical conductivity				1.000	0.556*	−0.570*	−0.813***	NS
Total Na					1.000	−0.512*	NS	NS
Aggregate Stability						1.000	0.511*	NS
GRSP (<2 mm)							1.000	0.677**
GRSP (0.25–4 mm)								1.000

*, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. NS: not significant.

effects in the soil environment that lead to the observed correlations of glomalin with soil aggregate stability (Purin and Rillig, 2007). The severe salinity decreased the GRSP concentrations in all soils, although the highest values of GRSP were recorded in the soils inoculated with the AM fungus. These results agree with those obtained by Johnson et al. (2008), who found a negative correlation between the concentration of GRSP and electrical conductivity. In the present study, the GRSP levels in the <2 mm fraction correlated negatively with electrical conductivity ($r = -0.813$, $P < 0.001$). However, there was no evidence for the participation of glomalin produced by the exotic AM fungus in the stabilisation of soil structural aggregates under saline conditions. The fact that the highest concentrations of GRSP in the aggregates fraction (0.25–4 mm) occurred in the inoculated soils under severe saline stress may be related to the occurrence of the highest levels of Na in these soils, because glomalin has been shown to be efficient in sequestering different toxic elements (González-Chávez et al., 2004).

Also, 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 aggregation processes indirectly, at scales smaller than the macroaggregate. Nevertheless, any mutual influence of the PGPR and the AM fungus on soil aggregation could not be confirmed.

4.3. Influence of plant growth on soil aggregate stability

The proliferation of roots is probably the major factor responsible for increasing structural stability. Plant roots contribute to soil aggregate stability directly, through the root material itself, and indirectly, through stimulation of microbial activities in the rhizosphere. Haynes and Francis (1993) found that soil structure improved considerably with increasing root biomass. In our study, the root biomass of control plants was markedly decreased as a consequence of salinity, while those of inoculated and fertilized plants were not decreased at the moderate salinity level and suffered a lesser reduction at the higher level. However, these observations did not correspond with a major percentage of stable aggregates in the soils inoculated with either microorganisms under such conditions. Furthermore, no significant correlation was found between soil stable aggregates and root biomass. Thus, the dispersive effect of Na was greater than the aggregating effect of the roots of lettuce and the GSRP, leading to a decrease in the percentage of soil stable aggregates under saline conditions.

5. Conclusions

Based on these findings, the inoculations with an AM fungus and/or a PGPR were effective with regarding the negative effect of salinity on the growth of lettuce plants, but were no more effective than fertilization. Both the AM fungus and the PGPR increased the negative effects of salinity on soil aggregate stability. The disruption of soil aggregates was related to the accumulation of Na in the inoculated soils. The increased microbial activity and concentration of GRSP promoted by the microbial inoculants were not sufficient to restore soil structure under conditions of salinity stress. The use of AM fungi and/or a PGPR for alleviating salinity stress in lettuce plants could be limited by their detrimental effect on soil structural stability.

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