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Geoderma 124 (2005) 375–382

GEODERMA

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Plant type mediates rhizospheric microbial activities and soil aggregation in a semiarid Mediterranean salt marsh

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Received 24 July 2003; received in revised form 28 May 2004; accepted 28 May 2004
Available online 15 July 2004

Abstract

This study was carried out in a Mediterranean salt marsh from semiarid Southeastern Spain, to determine the influence of eight halophytes (*Asteriscus maritimus* (L.) Less., *Arthrocnemum macrostachyum* (Moric.) Moris, *Frankenia corymbosa* Desf., *Halimione portulacoides* (L.) Aellen, *Limonium cossonianum* O. Kuntze, *Limonium caesium* (Girard) O. Kuntze, *Lygeum spartum* L., and *Suaeda vera* Forsskål ex J.F. Gmelin growing in a homogeneous area with regard to salt content, on the rhizosphere soil microbiological and biochemical properties (labile C fractions, biomass C, oxidoreductases and hydrolases) and aggregate stabilisation. Rhizosphere soil of *H. portulacoides* showed the highest values of water-soluble C, water-soluble carbohydrates, microbial biomass C and dehydrogenase, urease, protease-BAA and acid phosphatase activities. *S. vera* had the lowest microbial activity. The soil under *A. maritimus*, *L. cossonianum*, *L. spartum* and *H. portulacoides* had the highest percentages of stable aggregates (on average, about 52%) and the soil under *S. vera* the lowest (about 27% of stable aggregates). There was a good correlation between enzyme activities, the C-biomass, root colonisation of the eight halophytes and the levels of stable aggregates. Our results suggest that soil microbial activity and soil properties related to microbial activity, such as aggregate stability, are determined by the type of the halophytic species.
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Keywords: Halophyte; Microbial biomass; Enzyme activity; Labile C fractions; Aggregate stability; Salt marsh

1. Introduction

The Mediterranean-type salt marsh is one of those most affected by environmental degradation and erosive processes, due to its climatic characteristics such as a scarce and irregular rainfall and a long, dry and hot summer. Salt marshes need be protected and

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doi:10.1016/j.geoderma.2004.05.010

conserved because they provide important habitats for endemic and rare plant species and migratory water birds. Damage to soil and plants in arid and semiarid areas is not easily repaired because these are fragile and ecologically sensitive ecosystems (Pascual et al., 2000).

There is widespread agreement on the importance of measuring the soil biochemical and biological parameters related to microbial activity in order to evaluate soil quality and productivity (García et al., 1998), because microorganisms play a fundamental role in establishing biogeochemical cycles and facilitate the development of plant cover. On the other hand, microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on the maintenance of soil quality. In addition, microbial activity is involved in forming and stabilising the structure of a soil (Roldán et al., 1994). Soil microbial activity has frequently been assessed through biological and biochemical parameters such as biomass C and enzyme activities. Moreover, the labile C fractions, such as water-soluble C and water-soluble carbohydrates, can also be considered as indicators of soil microbiological activity (De Luca and Keeney, 1993). These C fractions are made up of biodegradable substrates and are used by soil microorganisms as nutrient and energy sources. It is therefore important to increase our knowledge of the influence of the microbiological activity associated with root-inhabiting microorganisms on the functioning of salt marshes. However, there are no data concerning the microbial biomass and activity in these ecosystems.

Soil structural stability plays an important role in the control of erosion in semiarid areas and in the implementation of vegetation cover restoration programmes, since it greatly affects plant growth and development, the soil being the physical habitat of roots (Caravaca et al., 2002). Likewise, plant cover contributes to the formation and stability of soil aggregates by supplying organic matter from plant remains. In this way, it is necessary to ascertain the changes in the stability of aggregates in relation to vegetation type.

In salt marshes, spatio-temporal gradients of salinity and edaphic moisture, as well as biological interactions, have been considered one of the most important factors influencing plant distribution in these environments (Álvarez Rogel et al., 2001). We hypothesise that vegetation type, in turn, can have a significant effect on patterns of microbial activity in a salt marsh soil, because vegetation controls above- and below-ground litter quantity and quality as well as the microclimate. For this study, we have focused on several Mediterranean halophytes, which were present in a homogenous area of a salt marsh as regards salt quantity and quality. The general objective of our study was to determine the influence of eight halophytes on rhizosphere microbiological and biochemical properties (labile C fractions, biomass C, oxidoreductases and hydrolases) and aggregate stabilisation in a Mediterranean salt marsh soil from semiarid Southeastern Spain.

2. Materials and methods

2.1. Study sites

The salt marsh studied is located adjacent to the La Mata saline lagoon (38°02′ 00″ N, 0°40′ 35″ W), SE Spain. The area is a Natural Protected Zone as established by the Environmental Agency of the Comunidad Autonoma of Valencia and it is included in the Ramsar Convention on Wetlands. The maximum waterlogging occurs in autumn and decreases gradually so that the salt marsh remains almost dry in March–April. The salt marsh floods again during the first rainfalls of October. The climate is semiarid Mediterranean (Peinado et al., 1992) with an average annual rainfall of 178 mm, mostly distributed in autumn and spring, and a mean annual temperature of 17.4 °C. The vegetation is mainly dominated by halophytic plant species distributed spatially according to the soil gradient, mainly salinity and humidity (Álvarez Rogel et al., 2001). The soil is a Hypercalcic Sodic Calcisols type FAO (1998) with a sandy texture.

2.2. Materials

The plants selected were *Asteriscus maritimus* (L.) Less., *Arthrocnemum macrostachyum* (Moric.) Moris, *Frankenia corymbosa* Desf., *Halimione portulacoides* (L.) Aellen, *Limonium cossonianum* O. Kuntze, *Limonium caesium* (Girard) O. Kuntze, *Lygeum spartum* L., and *Suaeda vera* Forsskål ex J.F. Gmelin,

which have a high ecological value in the Mediterranean salt marshes.

2.3. Sampling procedures

A field sampling survey was carried out in December 2002 in a homogenous area from the La Mata salt marsh, measuring approximately 1500 m^2. Some physical–chemical characteristics of the topsoil (0–20 cm) of the sampled area are shown in Table 1. For this survey, 40 individual plants similar in size (five replicates for each of the eight target halophytes) were randomly chosen. One rhizosphere soil sample per individual plant was collected, each sample consisting of five bulked subsamples (200 cm^3 soil cores) randomly collected at 0–20 cm depth. Soil strongly adhering to roots and collected at 0–4 mm from the root surface was defined as rhizosphere soil. Roots contained in each rhizosphere soil sample were separated and washed for determination of root biomass. Fresh and dry (105 °C, 5 h) weights of roots were recorded. The sampling was carried out in early December after the autumn rainy season, when the highest microbial activity could be expected (Lax et al., 1997). Rhizosphere soil was allowed to dry at room temperature and weighted. Five rhizosphere soil subsamples of each halophytic species (a subsample per replicate) were sieved at 2 mm and another five rhizosphere soil subsamples (a subsample per replicate) sieved to collect 0.2–4 mm aggregates for stability measurements. From subsamples sieved at 2 mm, an aliquot was stored at 2 °C for biological and biochemical analysis and another aliquot was used directly for physico-chemical and chemical analysis.

Table 1
Chosen soil physical–chemical characteristics of the topsoil (0–20 cm) from the La Mata salt marsh

Particle size distribution (%)	
<2 μm	5.7±1.9[a]
2–50 μm	14.4±3.1
50–2000 μm	79.9±2.1
pH	8.6±0.1
Electrical conductivity (dS m^{-1})	1.9±0.1
Total organic C (g kg^{-1})	4.7±0.4
Total N (g kg^{-1})	0.6±0.0
Na^+ (g kg^{-1})	2.8±0.2
Cl^- (g kg^{-1})	3.8±0.3
SO_4^{2-} (g kg^{-1})	1.6±0.0

[a] Mean±standard error (N=10).

2.4. Assessment of mycorrhization

The percentage of root length colonised by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

2.5. Soil physical analysis

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with an energy of 270 Jm^{-2}. The remaining soil on the sieve was put in a previously weighed capsule (T), dried at 105 °C and weighed ($P1$). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105 °C and weighed ($P2$). The percentage of stable aggregates with regard to the total aggregates was calculated by $(P1–P2)\times100/(4\text{-}P2+T)$.

2.6. Soil physical–chemical, biological and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In the previous aqueous extract, water-soluble carbon was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby, 1971). Water-soluble carbohydrates were determined as anthrone-reactive total reducing C by a colorimetric method (Brink et al., 1960). Chlorides and sulphates of the saturation extract were measured with a DIONEX chromatograph. Sodium of the saturation extract was determined by atomic absorption spectrophotometry. Total organic carbon was measured by pretreatment with (1:1) HCl to remove carbonates, followed by combustion at 1020 °C in a Carlo Erba NA 1500 analyser. Total nitrogen was determined by the Kjeldahl method (Jackson, 1960).

Microbial biomass C was determined using the fumigation–extraction method (Vance et al., 1987). Ten grams of soil at 60% of its field capacity were fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ for 24 h. After removal of residual $CHCl_3$, 40 ml of 0.5M K_2SO_4 solution was added and the sample was shaken for 1 h before filtration of the mixture. The K_2SO_4-extracted C was measured as indicated for water-soluble carbon and microbial biomass C was calculated as the difference between fumigated and non-fumigated samples divided for the calibration factor (K_{EC}).

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% 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT) in distilled water for 20 h at 22 °C in darkness. The iodo-nitrotetrazolium formazan (INTF) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two milliliters of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al., 1980).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two milliliters of 0.5 M pH 5.5 sodium acetate buffer (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).

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. Two milliliters of 0.1 M maleate buffer pH 6.5 and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM) according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969).

All analyses were made in triplicate and data are expressed on an oven-dry sample basis.

2.7. Statistical analysis

The data were tested for normality and subjected to analysis of variance, and comparisons among means were made using the Least Significant Difference (LSD) test, calculated at $P<0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3. Results and discussion

The eight target halophytes, growing in a Mediterranean salt marsh with a homogenous level of soil salt contents, differed greatly in the microbiological and biochemical properties related to microbial activity of their rhizosphere soil.

The labile C fraction, including water-soluble C and water-soluble carbohydrates, can be used as carbon and energy sources for soil microflora, and also has a structural function (Roldán et al., 1994). This C fraction has been considered as an indicator of soil microbiological activity (De Luca and Keeney, 1993). Also, the labile C fraction can be used as an indicator of early changes in soil organic matter (Bolinder et al., 1999). The soil under *H. portulacoides* showed the highest values of soluble C and microbial biomass C (Table 2), suggesting a high biological activity associated with the rhizosphere of this halophyte. The lowest microbial biomass C values were recorded in soil beneath *A. macrostachyum*, *L. spartum*, *F. corymbosa*, and *S. vera* plants.

Soil oxidoreductases, such as dehydrogenase, are studied frequently because of their relationship to microbial respiration. For instance, the work of García et al. (1994) showed that dehydrogenase activity indicated the status of soil microbial activity in semiarid areas subjected to degradation and desertification processes, which was “very low” in the most degraded soils. The microbial biomass associated with rhizosphere soil of *A. macrostachyum*, *L. caesium*, *L. spartum*, and *S. vera* had the lowest activity, as

Table 2
Carbon fractions in the rhizosphere soil of eight halophytes collected in the La Mata salt marsh (N=5)

	Water-soluble C (μg g^{-1})	Water-soluble CH (μg g^{-1})	Microbial biomass C (μg g^{-1})
A. macrostachyum	150±7ab*	21±3a	276±15abc
A. maritimus	164±7ab	27±1b	375±23c
L. caesium	123±6a	23±1ab	333±14bc
L. cossonianum	176±8b	38±1c	375±18c
L. spartum	120±4a	19±1a	253±11ab
F. corymbosa	159±9ab	21±2a	231±12a
H. portulacoides	334±18c	43±2cd	677±12d
S. vera	124±2ab	21±1a	248±11ab

Mean±standard error. CH=carbohydrates.
* Values in columns sharing the same letter do not differ significantly (P<0.05) as determined by the LSD test.

revealed by the values of dehydrogenase activity (Table 3). On the other hand, the rhizosphere soil under *H. portulacoides* showed the highest dehydrogenase activity followed, by *L. cossonianum* and *F. corymbosa*, which agrees with the results mentioned above in relation to the soluble C-fraction and microbial biomass C.

Measurement of soil hydrolases, such as urease, protease-BAA, acid phosphatase, and β-glucosidase, provides an early indication of changes in soil fertility, since they are related to the mineralisation of such important nutrient elements as N, P, and C (Ceccanti and García, 1994). Moreover, enzyme activity of sandy textured soils depends mostly on the activity of intracellular enzymes, which are as sensitive to environmental and management factors as overall microbial activity (Nannipieri, 1994). Urease and protease act in the hydrolysis of organic to inorganic nitrogen, the former using urea-type substrates and the latter simple peptidic substrates. Phosphatases catalyse the hydrolysis of organic phosphorus compounds to phosphates. β-Glucosidase hydrolyses β-glucosides in soil or in decomposing plant residues. Rhizosphere soil of *L. caesium* and *S. vera* generally had the lowest urease, protease, phosphatase, and β-glucosidase activities. With the exception of β-glucosidase activity, the highest values for hydrolases were recorded in the rhizosphere soil of *H. portulacoides* (Table 3). The variations in soil enzyme activity observed among the different halophytic species could be attributed either to different values of microbial biomass C or to different microbial communities associated with the rhizosphere soil. The hyphae of mycorrhizal fungi may release enzymes involved in the mineralisation of organic matter. Literature on the mycorrhizal colonisation of plants in saline habitats is controversial. Salt marsh plant species belonging to the families Chenopodiaceae, such as *A. macrostachyum*, *H. portulacoides* and *S. vera*, and Plumbaginaceae, such as *L. caesium*, are believed to be non-mycorrhizal (Smith and Read, 1997). However, the roots of eight tested halophytes were colonised, mainly those of *A. maritimus*, *H. portulacoides*, and *L. spartum* (Fig. 1). Particularly interesting is the relationship between mycorrhizal dependency and phosphatase activity, although the level of correlation depends on the plant species tested (Tarafdar and Marschner, 1994). In fact, the relationship between these two biological factors was positive for *A. maritimus*, and *H. portulacoides*, but mycorrhizal

Table 3
Soil biochemical properties in the rhizosphere soil of eight halophytes collected in the La Mata salt marsh (N=5)

	Dehydrogenase (μg INTF g^{-1} soil)	Urease (μmol NH_3 g^{-1} soil h^{-1})	Protease (μmol NH_3 g^{-1} soil h^{-1})	Phosphatase (μmol PNP g^{-1} soil h^{-1})	β-glucosidase (μmol PNP g^{-1} soil h^{-1})
A. macrostachyum	122.4±5.6ab*	1.02±0.09c	0.80±0.07bc	0.81±0.04d	0.22±0.02abc
A. maritimus	149.7±2.6bc	0.54±0.02b	0.91±0.03c	0.68±0.02cd	0.96±0.05e
L. caesium	111.1±6.7a	0.25±0.02a	0.32±0.01a	0.23±0.01b	0.19±0.01ab
L. cossonianum	187.4±9.1cd	0.92±0.06c	0.96±0.06c	0.43±0.03c	0.25±0.02bc
L. spartum	136.2±4.8ab	0.65±0.07b	0.51±0.02b	0.23±0.02b	0.31±0.02bcd
F. corymbosa	176.9±4.8cd	0.76±0.02bc	0.68±0.05bc	0.43±0.03c	0.34±0.02cd
H. portulacoides	207.6±1.9d	1.11±0.05d	2.00±0.13d	1.14±0.10d	0.47±0.03d
S. vera	123.3±3.1ab	0.30±0.01a	0.29±0.02a	0.11±0.02a	0.13±0.01a

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

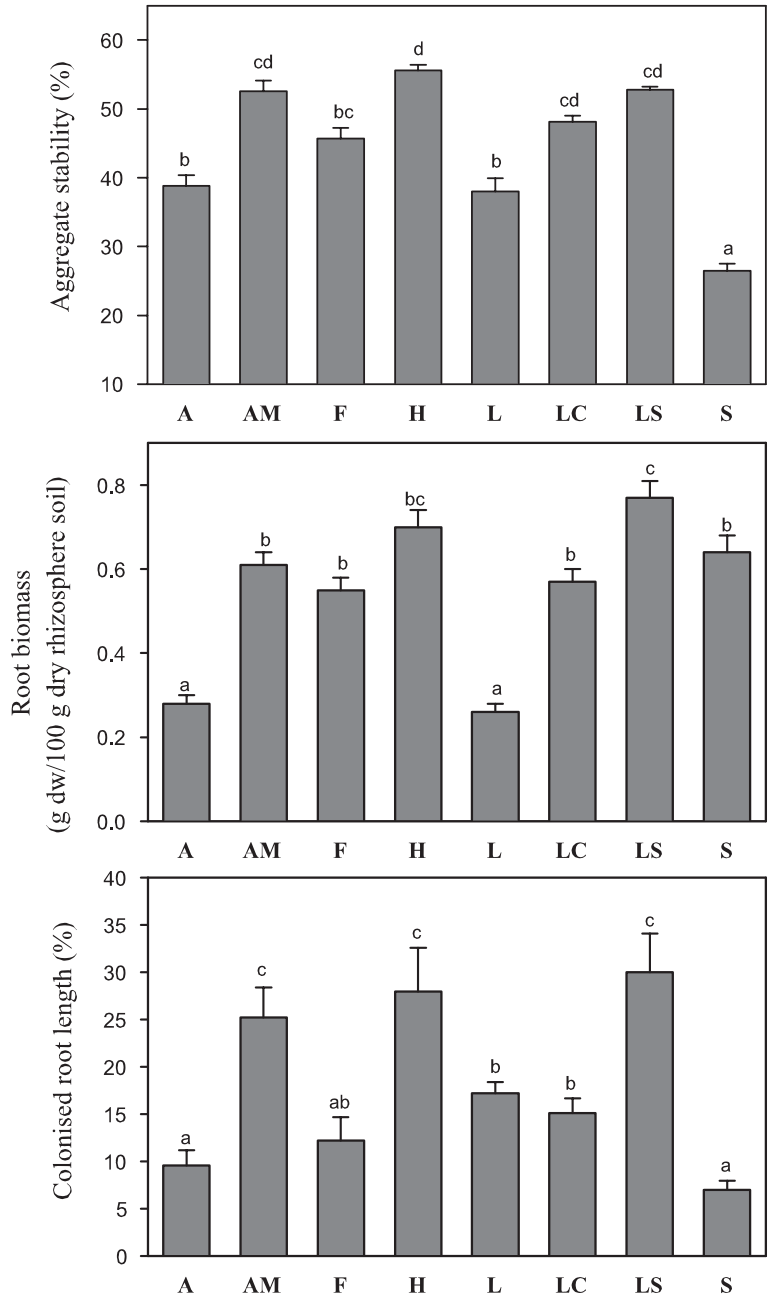

Fig. 1. Percentage of soil stable aggregates, root biomass and root colonisation in the eight halophytes collected in the La Mata salt marsh (A=*A. macrostachyum*; AM=*A. maritimus*; F=*F. corymbosa*; H=*H. portulacoides*; L=*L. caesium*; LC=*L. cossonianum*; LS=*L. spartum*; S=*S. vera*). Values with the same letter are not significant different at $P<0.05$, according to the LSD test.

colonisation had no effect on *L. spartum* (Table 3 and Fig. 1). On the other hand, the fact that the highest values for dehydrogenase and hydrolases activities were in the rhizosphere soil of *H. portulacoides* might be due to high levels of stable aggregates, which protect the organic fraction, on which enzymes are

Table 4
Pearson's coefficients of correlation between microbiological and biochemical parameters, root colonisation and aggregate stability of the rhizosphere soil of eight halophytes collected in the La Mata salt marsh (N=40)

Parameters	Aggregate stability
Water-soluble C	ns
Water-soluble CH	0.279*
Microbial biomass C	0.458***
Dehydrogenase	0.526***
Urease	0.446**
Protease	0.596***
Phosphatase	0.544***
β-glucosidase	0.581***
Colonisation	0.602***
Root biomass	0.393**

ns=not significant. CH=carbohydrates.
* Significant at P<0.05.
** Significant at P<0.01.
*** Significant at P<0.001.

immobilised, from microbial degradation (Nannipieri, 1994).

The present study points out to the influence of vegetation type on soil aggregate stability, as also shown by Cerdà (1998) in a non-saline, degraded Mediterranean area. The soil under *A. maritimus*, *L. cossonianum*, *L. spartum* and *H. portulacoides* had the highest percentages of stable aggregates (on average, about 52%), as shown in Fig. 1. The most unstable soil was under *S. vera* (about 27% of stable aggregates). The agents responsible for aggregate stability are mainly organic, and hence biological in origin, and usually develop in the rhizosphere (Oades, 1993). The mechanisms involved in aggregate stabilisation are based on the enmeshment of soil particles by hyphae and roots, and the exudation of polysaccharides (Tisdall et al., 1997). *L. spartum* develops soils with high aggregate stability because it is a plant species with a graminoid type, very dense root system (Fig. 1). In fact, root biomass and levels of soil water-soluble carbohydrates were related significantly to percentage stable aggregation in rhizosphere soil of the eight halophytes (Table 4). According to Roldán et al. (1994), the binding effect of polysaccharides is short-lived and the maintenance and increase of aggregate stability are attributable to the increases in microbial populations, and particularly to the proliferation of fungal mycelia. The symbiosis between arbuscular mycorrhizal fungi (AMF) and plants has been shown to increase the stability of soil aggregates (Bearden and Petersen, 2000). Mycorrhizae primarily influence the stability of macroaggregates (>250 μm) (Bearden and Petersen, 2000). Recent studies have indicated also that AMF produce a glycoprotein, glomalin, that acts as an insoluble glue, to stabilise aggregates (Wright and Anderson, 2000). The fact that in our study there was a close relationship between root colonisation and percentage of stable aggregates could suggest also the involvement of mycorrhizal fungi in soil aggregate stabilisation (Table 4). Because of the temporary nature of roots and hyphae, their abilities to act as stabilising agents should be related to the effects of root products on the activities of the soil biotic community (Miller and Jastrow, 1990). In the present study, there is a good correlation between enzyme activities, the microbial biomass C, and the levels of stable aggregates (Table 4). Thus, the microbiological activity associated with the rhizosphere soil of *S. vera* is ineffective with respect to increasing the stability of soil aggregates. Likewise, the low colonisation of roots of *S. vera* by mycorrhizal fungi could also contribute to develop of an unstable soil under this halophytic plant species, as indicated by Miller and Jastrow (1990).

In conclusion, our results suggest that in a homogenous area, with regard to salt content, from a Mediterranean salt marsh, rhizosphere soil microbial activity and rhizosphere soil properties related to microbial activity, such as aggregate stability, are determined by the type of halophytic species.

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