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Abstract: This study was carried out in a Mediterranean salt marsh in semiarid Southeast Spain to assess whether a spatial salinity gradient can affect the microbiological and biochemical properties (labile C fractions, biomass C, oxidoreductases, and hydrolases) of the rhizosphere soil of *Inula crithmoides* and the extent of arbuscular mycorrhizal (AM) colonization in its roots. There were no significant differences in the soluble C fractions (water-soluble C and water-soluble carbohydrates) or the microbial biomass C values of the rhizosphere soil of *I. crithmoides* among the different zones of the salt marsh. Dehydrogenase activity, hydrolases (urease, protease, phosphatase, and β-glucosidase) and the extent of AM colonization in *I. crithmoides* roots were greater in the higher salt marsh zones, corresponding to those of lower rhizosphere soil salinity. This study shows that soil salinity is inversely associated with some parameters related to soil microbial activity, such as dehydrogenase activity and some hydrolases, as well as the extent of colonization by AM fungi, which can improve plant performance. However, bioactive organic matter fractions that determine soil productivity are not related to soil salinity.

Key Words: water-soluble C fractions, oxidoreductases, hydrolases, Mediterranean salt marsh, semiarid areas

INTRODUCTION

Salt marshes are the second most biologically productive area in the world after rain forests (Alongi 1998). These wetlands are critical nursery areas for fish and invertebrates (Pomeroy and Wiegert 1981). In addition, the salt marsh vegetation acts as a sink, concentrating high levels of heavy metals and nutrients in the rhizosphere sediments (Keller et al. 1998). In Mediterranean-type salt marshes, the climatic conditions (long, dry, and hot summers with scarce but torrential rainfalls) in conjunction with anthropogenic impacts are potential threats to the conservation of these natural ecosystems. The ecological mechanisms that control plant growth, survival, and biodiversity need to be identified in order to carry out successful management of the conservation and restoration of Mediterranean-type salt marshes because plant cover is particularly threatened in these ecosystems (Álvarez Rogel et al. 2001

There has been 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. In addition, microbial activity is involved in the formation of the structure of a soil (Roldán et al. 1994). Soil microbial activity has been assessed frequently through biological and biochemical parameters such as biomass C and enzyme activities. However, the labile C fractions, such as water-soluble C and water-soluble carbohydrates, also can 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 the soil microorganisms as carbon and energy sources. However, there are no data concerning the labile C fractions of salt marshes

Mutualistic associations with soil microorganisms,

such as arbuscular mycorrhizae (AM), may improve plant salinity tolerance because of the recognized role that mycorrhizae have in plant performance (Carvalho et al. 2003). However, other results show that mycorrhizal infection can be suppressed by high soil salinity (Juniper and Abbott 1993). Ruíz-Lozano et al. (2000) reported that salt effects on AM might differ with either the species or the origin of the fungus. No previous assessment of arbuscular mycorrhizal status has been made in Mediterranean coastal marshes of Southeast Spain.

In salt marshes, the regular inundation with seawater leads to a partial or total submergence of vegetation, high soil salinity, and anoxia (Pennings and Callaway 1992). Generally, soil salinity and moisture decrease from the lower to the higher zone of a salt marsh, creating a zonal pattern in the vegetation (Álvarez Rogel et al. 2001). In these habitats, the activity of microorganisms in the root zones may respond to the temporal pattern of soil flooding and also to the type of plant. For this study, carried out in a Mediterranean salt marsh from semiarid Southeast Spain, we chose as the target species a common perennial halophyte of high ecological value (Peinado et al. 1992), *Inula crithmoides* L., which is present along the entire spatial-temporal gradient of salinity of this ecosystem. The aim of this study was to assess whether a spatial salinity gradient shows a corresponding gradient in microbiological and biochemical properties (labile C fractions, biomass C, oxidoreductases, and hydrolases) of the rhizosphere soil of *I. crithmoides* and the extent of AM colonization in its roots.

MATERIALS AND METHODS

Study Sites

The saltmarsh studied is located adjacent to the La Mata saline lagoon (38102′00″ N, 0140′35″ W), SE Spain. The area is a Protected Natural Zone, as established by the Environmental Agency of the Comunidad Autonoma of Valencia, and is included in the Ramsar Convention on Wetlands. The lagoon is separated from the Mediterranean Sea by upland of about 1000-m length, and it is connected with the sea by a channel that allows the water to enter. Maximum freshwater flooding 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 17.41°C. The halophytic vegetation is dominated by *Juncus maritimus* Lam., *Phragmites australis* (Cav.) Trin. ex Stende, *Limonium* spp., *I. crithmoides*, *Salicornia patula* Duval-Jouve, *Sarcocornia fruticosa* (L.) A.J. Scott, and *Arthrocnemum macrostachyum* (Moric.) Moris, distributed spatially according to soil salinity and humidity gradients (Álvarez Rogel et al. 2001). The predominant soils in the main plant communities are Hypercalcic, Sodic, and Mollic Solonchaks and Hypercalcic Sodic Calcisols (FAO 1998) with a sandy texture.

Sampling Procedures

One transect of 80-m length from the topographically lower part (border of the lagoon) to the upland salt marsh vegetation limit was drawn in an area of 800 m^2. The transect was divided, perpendicular to the slope (about 2.5% grade), into five plots (3×5 m^2 each plot) in order to sample at different levels of salinity. The plots were numbered consecutively from I (plot situated in the topographically lower part of the transect, which is subject to flooding periods) to V (plot situated in the topographically higher part of the transect). Thus, plots I and II are considered as the lowest marsh zones, and plots IV and V are considered as the highest marsh zones. In December 2002, five soil samples from each plot were collected (25 soil samples in total). Each sample consisting of four bulked subsamples (200 cm^3 soil cores) randomly collected at a fixed depth of 0–20 cm in the rhizospheres of four individual *I. crithmoides* plants of similar size and external appearance. Soil strongly adhering to roots and collected at 0–4 mm from the root surface was defined as rhizosphere soil. 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). Air-dried soil samples were sieved at 2 mm and stored at 21°C for biological and biochemical analysis.

Plant Analyses

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

Soil Physical-Chemical, Biological, and Biochemical Analyses

Soil pH and electrical conductivity were measured in a 1:5 (weight/volume) aqueous solution at 25°C. In soil aqueous extracts, 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 by the method of Brink et al. (1960).

Microbial biomass C was determined using a fumigation-extraction method (Vance et al. 1987). Ten g of soil moistened with distilled H_2O to 60% of its water-holding capacity were fumigated in a 125-mL Erlenmeyer flask with purified $CHCl_3$ for 24h. After removal of residual $CHCl_3$, 40 mL of 0.5M K_2SO_4 solution were added and the sample was shaken for 1 hr 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 by 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% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 221C in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 mL of methanol by shaking vigorously for 1 min and filtering through a Whatman NE 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 mL 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 mL 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 manner, although the substrate was added before the $CaCl_2$ and NaOH.

β-glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of PNP. Two mL of 0.1 M maleate buffer pH 6.5 and 0.5 mL of substrate was added to 0.5 g of sample and incubated at 37°C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethano (THAM) according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm (Tabatabai and Bremner 1969).

Table 1. Changes in physical-chemical properties of rhizosphere soil of *I. crithmoides* along a spatial salinity gradient in La Mata salt marsh (N = 5, df = 4).

Plots	pH (H_2O)	EC (dS m^{-1})
I	8.6 ± 0.1a*	9.0 ± 0.1a
II	8.8 ± 0.1bc	10.0 ± 0.1a
III	9.0 ± 0.1c	6.2 ± 0.0a
IV	8.9 ± 0.1c	2.1 ± 0.1b
V	8.8 ± 0.1bc	2.6 ± 0.2b
F-values	12.00	11.51

EC: electrical conductivity.
Mean ± SE
* Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

Statistical Analyses

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.

RESULTS AND DISCUSSION

Physical-Chemical Parameters

The pH of the soil varied little among salt marsh zones (Table 1). Electrical conductivity values ranged from 2.1 to 5.0 dS m^{-1} along the transect. The highest electrical conductivity value was observed in the lower marsh zones (plots I and II), although this value was lower than the upper limit tolerated by *I. crithmoides* (Aronson 1989).

The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins, and low molecular weight organic acids. This fraction can be used as carbon and energy sources by soil microflora and may also have a structural function (Roldán et al. 1994). The soil under *I. crithmoides* showed the highest soluble C values in the lowest (plots I and II) and highest (plots IV and V) salt marsh zones, and there were no significant differences between both zones (Table 2). The values of water-soluble carbohydrates hardly varied among the different zones of the salt marsh. These results suggest that the biological activity associated with the rhizosphere of *I. crithmoides* should not vary spatially in the salt marsh. Microbial biomass was suggested by Powlson and Jenkinson (1981) as an index of the

Table 2. Changes in C fractions of rhizosphere soil of *I. crithmoides* along a spatial salinity gradient in La Mata salt marsh (N = 5, df = 4).

Plots	Water Soluble C (μg g^{-1})	Water Soluble CH (μg g^{-1})	Biomass C (μg g^{-1})
I	203 ± 5bc*	21 ± 1ab	277 ± 7a
II	163 ± 6ab	24 ± 2b	339 ± 9a
III	126 ± 3a	15 ± 2a	246 ± 7a
IV	259 ± 7c	29 ± 1b	268 ± 14a
V	198 ± 8bc	16 ± 1a	275 ± 11a
F-values	15.06	4.37	2.68

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

changes undergone by the organic matter of a soil. Other authors, too, such as De Luca and Keeney (1993) have found a positive relationship between soil microbial biomass and soluble C fractions. In the current study, there were no significant differences in the microbial biomass C values of the rhizosphere soil of *I. crithmoides* among the different zones of the salt marsh, which is relatively similar to the results of the soluble C fractions (Table 2).

Oxidoreductase

Dehydrogenase activity ranged from 15.4 to 225.1 mg INTF g^{-1}, with highest values for the higher marsh zones (plots IV and V) (Table 3). Dehydrogenase activity expresses the biological oxidation processes of soil microorganisms (Nannipieri 1994). As a respiratory measurement, dehydrogenase activity should be strongly representative of the size and activity of the viable microbial community. In fact, dehydrogenase has been proposed as a measure of global microbial activity in soil under aerobic conditions (García et al. 1997). This enzyme is affected by numerous environmental factors, such as soil redox potential and oxygen level (Alef and Nannipieri 1995). The substrate availability seems not to be the cause of the lowest dehydrogenase activity being in the lower marsh zones because the values for the soluble C fractions (water-soluble C and water-soluble carbohydrates) are similar in these zones and the higher salt marsh zones. The decreased dehydrogenase activity in the lower marsh zones, with higher salt concentrations, could indicate that the salinity and, probably, lower oxygen availability levels resulting from anaerobic and chemically reduced conditions around plant roots in the zone exposed to flooding (mainly plot I), inhibit soil microbial activity. However, the presumed anaerobic conditions in the lower marsh zones could be ameliorated by the high soil sand content, facilitating water circulation, and soil oxygenation.

Hydrolases

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralization of such important nutrient elements as N, P, and C (Ceccanti and García 1994). The values of urease, a hydrolase responsible for breaking down urea into ammonium, and protease-BAA, which catalyses the hydrolysis of simple peptidic substrates to inorganic N, were significantly higher in the rhizosphere soil of *I. crithmoides* plants growing in the higher salt marsh zones (Table 3). Both enzymes can be considered greatly dependent on microbial activity, indicating that the inhibition of metabolism in the rhizosphere soil of *I. crithmoides* plants grown in the lower salt marsh zones, with higher salinity, and probably lower O_2 affected the biological transformation of N.

β-glucosidase catalyses the hydrolysis of the ends of unreduced chains of β-D-glucoside to form β-D-glucose and indicates the potential for soil organic matter decomposition. β-glucosidase showed a high activity in the rhizosphere soil of *I. crithmoides* plants grown in the higher salt marsh zones in comparison with those grown in the lower salt marsh zones (Table 3). The values of β-glucosidase activity indicated that,

Table 3. Changes in soil bochemical properties and root colonisation in *I. crithmoides* along a spatial salinity gradient in La Mata salt marsh (N = 5, df = 4).

	Dehydrogenase (μg INTF g^{-1} soil)	Urease (μmol NH_3 g^{-1} h^{-1})	Protease (μmol NH_3 g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})	β-glucosidase (μmol PNP g^{-1} h^{-1})	Mycorrhizal Root (%)
I	25.0 ± 3.2a*	0.15 ± 0.02a	0.39 ± 0.01b	0.35 ± 0.02a	0.05 ± 0.01b	25.5 ± 1.4a
II	16.5 ± 1.6a	0.19 ± 0.01a	0.33 ± 0.01ab	0.44 ± 0.04ab	0.04 ± 0.01ab	40.7 ± 1.3b
III	15.4 ± 1.2a	0.17 ± 0.01a	0.23 ± 0.01a	0.56 ± 0.06ab	0.02 ± 0.01a	66.9 ± 3.1d
IV	83.1 ± 4.9b	1.02 ± 0.15b	0.56 ± 0.04b	0.68 ± 0.04b	0.10 ± 0.01c	54.4 ± 2.3c
V	225.1 ± 8.8c	0.58 ± 0.04b	1.29 ± 0.16c	1.45 ± 0.07c	0.31 ± 0.06d	41.8 ± 2.0b
F-values	37.20	14.57	13.31	11.58	16.03	40.99

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

in the salt marsh, the potential to mineralize organic matter, and hence C-cycle activity, is inversely related to salinity.

Phosphatases are enzymes with relatively broad specificity, capable of hydrolyzing various organic and inorganic phosphate esters, and are involved in the P cycle. Similar to urease, protease-BAA and β-glucosidase, the highest values of phosphatase were in the higher salt marsh zones (Table 3).

Arbuscular mycorrhizal fungi may improve plant tolerance and growth in sites affected by salinity by enhancing the supply of nutrients, such as P, and increasing photosynthetic activity and water-use efficiency (Ruíz-Lozano et al. 2000). In general, the percentage colonization by AM fungi in *I. crithmoides* roots was lower in the lower salt marsh zones, corresponding with those of higher salinity and probably lower oxygen availability levels (Table 3). These results indicate that AM fungi present in the rhizosphere of *I. crithmoides* are correlated with a gradient in soil salinity. Likewise, the reduced colonization of *I. crithmoides* in the salt marsh zones with higher concentrations of salt seems to indicate that AM fungi do not contribute greatly to ecological adaptation of this halophyte to the lower zones of the salt marsh environment studied. The presence of a considerable level of colonization in *I. crithmoides* roots has been reported also in a Portuguese salt marsh by Carvalho et al. (2001). However, these authors found that the extent of AM colonization in the roots of *I. crithmoides* showed no spatial variation within marsh zones.

Based on the results obtained, it can be concluded that soil salinity is negatively related to some parameters indicating soil microbial activity, such as dehydrogenase activity and some hydrolases (urease, protease, phosphatase, and β-glucosidase), as well as the extent of colonization by AM fungi, the presence of which can improve plant performance. However, bioactive organic matter fractions that determine soil productivity in the rhizosphere seem to be unrelated to gradients in soil salinity or other factors that vary across marsh zones.

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