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PRELIMINARY RESULTS ON ENZYMATIC ACTIVITIES IN TWO SALT MARSH SOILS DIFFERING IN HYDROMORPHIC CHARACTERISTICS AND VEGETATION COVER

Diana Bello[(1)], Chiara Ferronato[(2)], Livia Vittori Antisari[(2)], Carmen Trasar-Cepeda[(1)*],

[(1)] Departamento de Bioquímica del Suelo, IIAG-CSIC, Santiago de Compostela, Spain
[(2)] Dipartimento di Scienze Agrarie, Università di Bologna, Italy

* Corresponding author E-mail: ctrasar@iiag.csic.es

Abstract

Salt marsh soils are characterized by temporary hydric saturation and by vegetation cover that is dominated by different salt-tolerant plant species depending on factors such as duration of submersion. The composition of microbial communities is an essential component of trophic dynamics and biogeochemical processes in salt marshes and determines the activities of enzymes that catalyze the conversion of complex molecules into simpler ones. However, enzymatic activities have not yet been investigated in salt marsh soils. The aim of this study was to analyze the activities of some oxidoreductase and hydrolase enzymes in two salt marsh soils affected by different levels of water saturation and covered by vegetation dominated by different plant species [*Juncus maritimus* Lam and *Spartina maritima* (Curtis) Fernald]. The enzyme activities were measured both in air-dried (only) and in air-dried, re-moistened soil samples. The activities in soils under both types of vegetation were much lower than usually found in terrestrial ecosystems. However, in the air-dried samples, the enzyme activities were higher in the soil under *Juncus* than in the soil under *Spartina* and tended to decrease with depth, particularly in the former. The activities of all enzymes considered tended to be higher, throughout the whole soil profile, in the re-moistened than in the air-dried soils, especially in the soil under *Spartina*. Hydrolase activity was strongly and positively related to organic matter content in both air-dried and re-moistened soil samples, particularly in the latter. By contrast, oxidoreductase activity was generally only related to organic matter content in the re-moistened soil samples. Further studies, preferably with freshly collected soil samples, are required to clarify the relationship between enzymatic activities and environmental conditions.

Keywords: *salt marsh soils, soil profile, soil enzyme activities, Juncus maritimus, Spartina maritima.*

Introduction

Hydromorphic soils are affected by recurrent saturation and are characterized by poor pedogenic development. Tidal action leads to the formation of aquic

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conditions throughout the soil profile and promotes an ongoing process of erosion and re-deposition of soil organic matter and fine sediments. The topsoil of these soils is often drained during low tide and completely saturated during high tide, leading to alternation between oxic and anoxic conditions, with the consequent formation of redoximorphic features. Coastal salt marshes are dominated by herbaceous halophyte plants, which are tolerant to salts and to complete or partial submersion. The topography and morphology of salt marshes influence and promote a high level biodiversity and the vegetation patterns vary according to different factors, such as the salt concentration, water level and length of submersion (Silvestri et al., 2005).

From an ecological point of view, salt marshes are considered highly productive areas, because they often receive nutrient run-off, particulate and dissolved organic matter and plant litter (Tobias et al., 2001). Herbaceous vegetation usually produces large amounts of biomass annually, thus generating large amounts of decaying litter (Duarte et al., 2008). As in terrestrial ecosystems, microbial communities are essential drivers of trophic dynamics and biogeochemical processes in salt marsh ecosystems (Freitas et al., 2014).

The composition of the microbial community determines the potential for enzyme synthesis, and thus any modification in microbial community due to environmental factors should be reflected in the soil enzyme activities (Kandeler et al., 1996). Soil enzymes are involved in several biogeochemical cycling processes, catalyzing the conversion of multiple complex molecules into smaller ones, either by redox reactions (catalyzed by oxidoreductases) or by breakdown of organic matter (catalyzed by hydrolases).

Thus, given the contribution of soil enzymes to the degradation of soil organic matter, information about soil enzyme activities can indicate the soil degradation potential (Frankenberger and Dick, 1983). Numerous studies have investigated the influence of many different factors on soil enzyme activities in terrestrial ecosystems (Trasar-Cepeda and Gil-Sotres, 1987; Trasar-Cepeda et al., 2000a; Miguéns et al., 2007; Miralles et al., 2012), including the influence of anthropogenic activities (García and Hernández, 1997; Trasar-Cepeda et al., 2000b; Bello et al., 2008; 2013). However, studies of enzymatic activities in hydromorphic soils are almost non-existent (Duarte et al., 2008).

The Grado and Marano Lagoon, one of the largest lagoon systems in Italy, spans the boundary between the mainland and the northern Adriatic Sea. The physico-chemical properties of different hydromorphic and submerged soils in the area have recently been characterized (Gotti, 2015); however, the biochemical properties of these soils, including enzymatic activities, have not yet been investigated. Thus, the objective of this study was to measure the activities of some oxidoreductase and hydrolase enzymes in two soil profiles from the area differing in hydromorphic level and vegetation cover.

Material and methods

Two soils were selected for sampling in the Barena della Gran Chiusa salt marsh, in the central part of the Grado and Marano Lagoon system.
In one of the soils, which was located 40 cm above the seawater level and only intermittently submerged, the dominant vegetation was *Juncus maritimus* Lam: the soil (hereafter referred to as *Juncus* soil) is classified as an Udic Fine-loamy Typic Hydraquent (Soil Survey Staff 2014). The other soil, which was below seawater level and almost permanently saturated with water, was mainly covered by *Spartina maritima* (Curtis) Fernald: this soil (hereafter *Spartina* soil) is classified as an Udic Fine-loamy Typic Sulfaquent (Soil Survey Staff 2014). All pedogenic horizons of the soil profiles were collected (A1, A2, ACse, C1 and C2 in the *Spartina* soil, and O, A1, AB, AC and C1 in the *Juncus* soil).
The soil samples were analyzed to determine the activity of two oxidoreductase enzymes (catalase and dehydrogenase) and six hydrolytic enzymes (urease, benzoyl-arginine-amide (BAA)-hydrolyzing protease, arylsulphatase, alkaline phosphomonoesterase, ß-glucosidase and invertase). Dehydrogenase (DES) and catalase (CAT) activities were determined following the methods described by Leirós *et al.* (2000), and the activities of the hydrolytic enzymes phosphomonoesterase (MONO), BAA-protease (BAA), urease (URE), invertase (INV), ß-glucosidase (GLU) and arylsulphatase (ARYL)] were determined in accordance with the methods indicated by Trasar-Cepeda *et al.* (2000a), though with slight variations in the buffers used to assay the soil enzyme activities: MUB-buffer (pH 11.0) for phosphomonoesterase; phosphate buffer (pH 7.0) for urease and BAA-protease; and MUB buffer (pH 6.5) for ß-glucosidase.
The enzyme activities were determined in both air-dried samples and air-dried, remoistened soil samples (which were also incubated for 10 days at 20 ºC before the enzyme assays). The latter samples were re-moistened and incubated with the aim of re-establishing the microbial populations and their activity. All soil samples were sieved through a 2 mm screen and analyzed to determine the main physical, chemical and physico-chemical properties. The particle soil distribution was performed by pipette method (Gee and Bauder 1986) while total carbonates were quantified according to the Dietrich-Fruhling method.
The pH (pHmeter, Crison) and electrical conductivity (EC; Orion conductimeter) were measured in each sample, in a 1:2.5 (w:v) soil:distilled water suspension. Total organic carbon and total nitrogen were measured by Dumas combustion with a CHN elemental analyzer (EA 1110 Thermo Fisher, USA) after dissolution of carbonates with 2 M HCl. Total and soluble macro-element concentrations and the cation exchange capacity (CEC) were determined by Inductive Coupled Plasma-Optic Emission Spectroscopy (ICP-OES, Ametek, Germany) following the methods of Ciesielskui and Sterckeman (1997), Vittori Antisari et al. (2010) and Ferronato et al. (2013).

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Results and Discussion

Both soils were alkaline, although the pH of the *Spartina* soil was slightly higher than that of the *Juncus* soil (Table 1). In addition, the total organic carbon and total nitrogen contents were higher in the *Spartina* soil than in the *Juncus* soil, probably because of the larger amounts of poorly decomposed organic matter in the former than in the latter. The presence of shell fragments and other forms of biogenic $CaCO_3$ contributed to the relatively high $CaCO_3$ content in the A1 and A2 horizons of both soils, although this decreased with soil depth. The EC decreased only slightly with depth, highlighting the high salinity of the topsoil due to both the physical dynamics of the water and to the chelating properties of organic matter (Table 1).

Table 1. *Main properties of the different horizons of soils under* Spartina *and* Juncus

	***Spartina* soil**					***Juncus* soil**				
	A1	**A2**	**ACSe**	**C1**	**C2**	**O**	**A1**	**AB**	**AC**	**C**
OC (g kg^{-1})	**23.3**	**18.9**	**16.0**	**13.1**	**n.d.**	**43.8**	**25.4**	**21.8**	**13.1**	**20.1**
TN (g kg^{-1})	**1.90**	**1.81**	**1.44**	**1.10**	**n.d.**	**4.93**	**2.18**	**2.11**	**1.13**	**1.75**
pH water	**7.92**	**8.03**	**8.10**	**8.08**	**8.21**	**7.63**	**7.78**	**7.86**	**7.88**	**7.88**
Texture	**SIL**	**SIL**	**SIL**	**L**	**SIL**	**L**	**SIL**	**SIL**	**SICL**	**SIL**
Total P (g kg^{-1})	**0.24**	**0.27**	**0.22**	**0.18**	**0.19**	**0.50**	**0.40**	**0.33**	**0.30**	**0.23**
S (g kg^{-1})	**15.9**	**4.52**	**6.74**	**8.49**	**10.4**	**2.28**	**1.28**	**1.60**	**1.27**	**6.31**
$CaCO_3$ (g kg^{-1})	**223**	**173**	**44.7**	**35.7**	**55.8**	**107**	**131**	**150**	**179**	**143**
Ca (g kg^{-1})	**45.9**	**29.7**	**56.9**	**74.8**	**67.6**	**30.4**	**33.3**	**31.4**	**32.3**	**49.9**
Na (g kg^{-1})	**12.8**	**13.2**	**10.2**	**8.10**	**9.38**	**20.5**	**15.3**	**14.3**	**11.7**	**12.3**
CEC (cmol(+)kg^{-1})	**32.3**	**32.4**	**33.7**	**28.5**	**22.9**	**44.9**	**24.3**	**35.7**	**34.3**	**31.0**
CE (mScm^{-1})	**15.9**	**15.6**	**12.7**	**12.0**	**12.6**	**22.1**	**16.8**	**16.2**	**14.1**	**15.1**

SIL = silt-loam; **L** = loam; **SICL** = silty clay loam - **n.d.** = not determined

The total organic carbon and total nitrogen contents decreased with depth in both soils; however, in the C horizons both the total organic carbon and total nitrogen contents were slightly higher than in other terrestrial soils in coastal areas of Italy (Marinari et al., 2013). Moreover, in the *Juncus* soil, the C and N contents were higher in the C1 than in the AC horizon (Table 1), suggesting long-term effects of different sedimentary processes in these environments (Demas and Rabenhorst, 1999). The Ca content increased with depth in the *Spartina* soil, but did not vary with depth in the *Juncus* soil, except in the C1 horizon, in which it was higher than in the other horizons. In both soils, the S content decreased with depth, except in the deep layers (C1 and C2 horizons) in which a sharp increase in the content of this element was observed. In the *Spartina* soil, the S content of the C1 horizon was lower than in the surface layer (A1), although higher than in all the other soil

layers; however, in the *Juncus* soil the sulphur content was higher in the deepest horizon (C1) than in all other horizons, including the surface O horizon (Table 1).The enzyme activities were generally lower in the air-dried *Spartina* soil than in the air-dried *Juncus* soil (Table 2) and tended to decrease with depth, particularly in the *Juncus* soil. In the *Spartina* soil, the activities of all hydrolase enzymes were similar in the A1 and A2 horizons and tended to decrease with depth (Fig. 1).

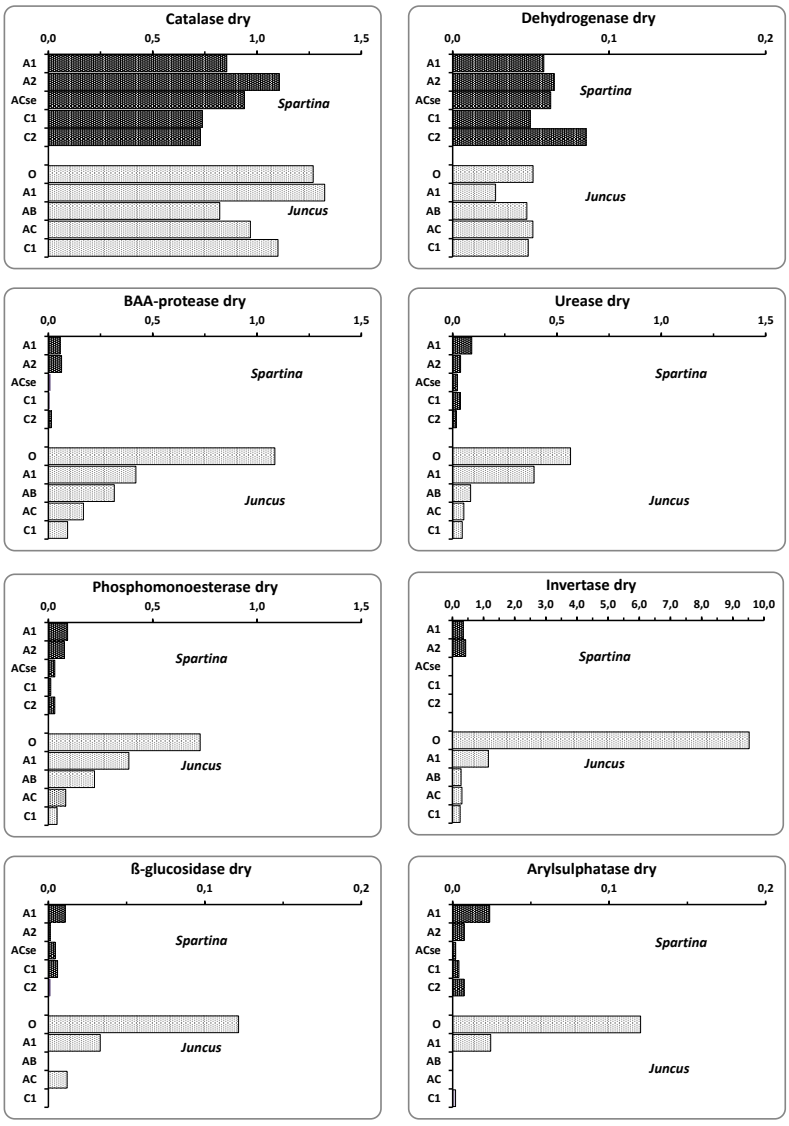

Figure 1
Values of oxidoreductase and hydrolase activities determined in air-dried soil samples of the whole profile of soils under Spartina *and* Juncus *vegetation* (For units, see Table 2).

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However, given the already low levels of activity of all the hydrolase enzymes in the A1 and A2 horizons, the decrease was not very strong, and in some cases (alkaline phosphomonoesterase, BAA-protease and invertase) the activity was almost non-existent in the deepest horizons. In the *Juncus* soil, the hydrolase enzyme activities were much higher in the O horizon than in all the other horizons, especially in the case of invertase, arylsulphatase, ß-glucosidase and BAA-protease (Fig. 1). The activities of these enzymes decreased sharply with depth, although they were relatively high in the A1 horizon - especially in the case of those activities already indicated as being particularly high in the O horizon (Fig. 1). The activities of the oxidoreductases varied in different ways from those of the hydrolases. Although the oxidoreductase activities were slightly higher in the *Juncus* soil than in *Spartina* soil, they were of the same magnitude (Table 2, Fig. 1). In both soils, the activities of catalase and particularly dehydrogenase were almost constant throughout the soil profile (Fig. 1).

Given the scarcity of studies concerning enzymatic activities in salt marsh soils (and the variety of methods used), we compared the present data on the air-dried soils with data on terrestrial ecosystems, previously obtained in our laboratory using the same enzyme assays. Thus, for purposes of comparison, we selected data from various studies in which the soils had similar characteristics to those in the present study, i.e. alkaline soils with a low organic matter content (Bello et al., 2013; Miralles et al., 2012), soils in which the enzymatic activities were very low (Miguéns et al., 2008; Miralles et al., 2012), and soils from an arid zone ("naturally air-dried" soils) (Miralles et al., 2012). In the surface layers of both *Spartina* and *Juncus* soils, the activities [both absolute and specific (i.e. expressed per carbon unit)] of all enzymes, except catalase and invertase, were generally very low in comparison with those in the above-indicated terrestrial ecosystems, especially in the *Spartina* soil. However, in the present study, the enzyme activities were determined in air-dried, rather than fresh samples. On drying soil, a large part of the enzymatic activity (basically that associated with viable microorganisms) may disappear (Ross et al., 1980; Zornoza et al., 2006), and the activities are therefore expected to be lower than when fresh samples are used (Ross, 1970). The air-drying process may have contributed to reducing the oxidoreductase and hydrolase activities; however, the fact that the enzyme activities were much lower than in soils collected from an arid zone under dry conditions (Miralles et al., 2012) suggests that the low values were caused by other factors.

In both the *Spartina* and *Juncus* soils, catalase activity in the surface layers was in the range usually found reported for terrestrial ecosystems (Miguéns et al., 2008; Bello et al., 2013). However, the activity of dehydrogenase, an enzyme only present in viable cells (Skujins, 1978; Nannipieri, 1994) and considered a direct measure of soil microbial activity (García and Hernández, 1997), was very low (Table 2). Thus, the values of these enzymes should be closely related as both are intracellular oxidoreductase enzymes involved in microbial activity.

Table 2. *Oxidoreductase and hydrolase activities in surface soil layers of air-dried and re-moistened samples of soils under* Spartina *and* Juncus

	Air-dried soils				Re-moistened, incubated soils			
	Spartina soil		*Juncus* soil		*Spartina* soil		*Juncus* soil	
	A1	A2	O	A1	A1	A2	O	A1
Catalase*	**0.86**	**1.11**	**1.27**	**1.32**	**1.05**	**0.79**	**2.08**	**1.60**
Dehydrogenase#	**0.06**	**0.06**	**0.05**	**0.03**	**0.30**	**0.14**	**0.81**	**0.27**
Urease$	**0.09**	**0.04**	**0.57**	**0.39**	**0.15**	**0.08**	**1.82**	**0.23**
BAA-protease$	**0.06**	**0.06**	**1.09**	**0.42**	**0.47**	**0.31**	**2.95**	**0.96**
Arylsulphatase&	**0.02**	**0.01**	**0.12**	**0.02**	**0.02**	**0.00**	**0.22**	**0.05**
Phosphomonoesterase&	**0.09**	**0.08**	**0.73**	**0.39**	**0.71**	**0.51**	**5.46**	**1.98**
ß-glucosidase&	**0.01**	**0.00**	**0.12**	**0.03**	**0.09**	**0.01**	**0.36**	**0.08**
Invertase^	**0.35**	**0.43**	**9.53**	**1.16**	**9.53**	**1.16**	**7.19**	**1.35**

*****mmol H_2O_2 g^{-1} h^{-1}; #µmol iodonitroterazolium formazan (INTF) g^{-1} h^{-1}; $µmol NH_3 g^{-1} h^{-1}; &µmol p-nitrophenol (PNP) g^{-1} h^{-1}; ^µmol glucose g^{-1} h^{-1}

The high level of catalase activity - in contrast to the low dehydrogenase activity- appears to suggest that the catalase activity measured is partly due to abiotic processes, because some component of the soil acts as an abiotic catalyst generating the decomposition of hydrogen peroxide, as previously observed by some authors (Skujins, 1976; Bello et al., 2008).

In both soils, the absolute values of the activity of all the investigated enzymes tended to be higher in the O, A1 and A2 horizons than in deeper horizons, especially in the soil under *Juncus*: in general, in the A1 and A2 horizons of the soil under *Spartina*, the activity was already extremely low (Fig. 1). However, when the specific activities (i.e. per unit of carbon) of the oxidoreductases were considered, the difference between soil layers was not as evident (data not shown).

The activity of all the enzymes in the organic horizons of both soils tended to be higher in the remoistened soil samples than in the air-dried samples, except for catalase in the A2 horizon and arylsulphatase in the A1 and A2 horizons of the *Spartina* soil, and for invertase activity in the O horizon and urease in the A1 horizon of the *Juncus* soil (Table 2). However, the difference in activity was very variable and not clearly related to either the soil horizon or the enzyme considered (Table 2). The catalase activity in the re-moistened soil samples tended to be lower than in air-dried soil samples throughout the whole soil profile of both soils, and it was only slightly higher in re-moistened samples of A1 horizon in the *Spartina* soil and in the upper two horizons of the *Juncus* soil (Fig. 2). By contrast, dehydrogenase activity was higher in remoistened than in air-dried samples of all horizons of both soils: although the difference depended on the soil and horizon considered, the dehydrogenase activity was 15 and 10 times higher in respectively

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the O and A1 horizons in the re-moistened samples of *Juncus* soil than in the corresponding air-dried sample (Fig. 2).

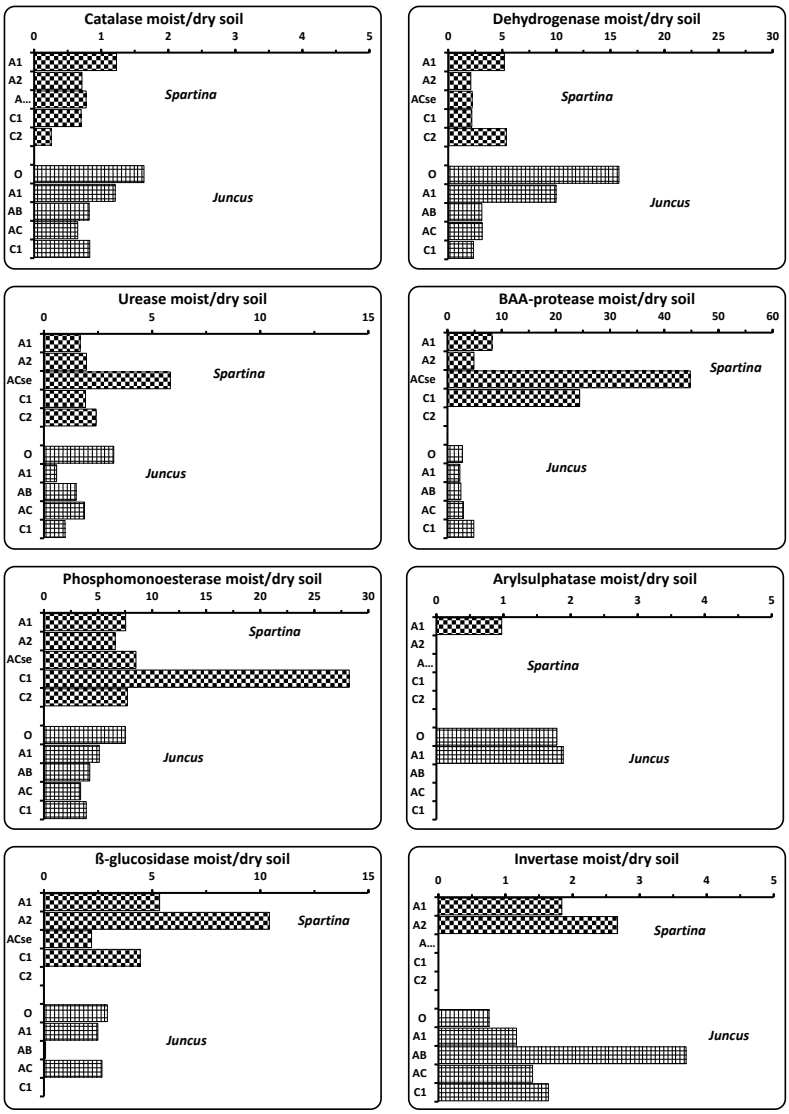

Figure 2
Ratio of enzymatic activities in re-moistened soil samples to the activity in air-dried soil samples throughout the whole profiles of soils under Spartina *and* Juncus *vegetation.*

The differences in the deepest horizons were generally smaller than in the upper horizons, except for the C2 horizon of the *Spartina* soil, in which the dehydrogenase activity was unexpectedly much higher in the re-moistened soil samples (Fig. 2). The hydrolase activities tended to be higher in remoistened samples than in air-dried samples of all horizons of the *Spartina* soil, except for

arylsulfatase and invertase in the ACSe, C1 and C2 horizons. However, although the difference was very small for some enzymes (i.e. urease), for others (i.e. BAA-protease and alkaline phosphomonoesterase) the difference was quite large, especially in the deepest horizons (Fig. 2). Differences between the *Juncus* soil and the *Spartina* soil were observed in relation to the enzyme activities measured: for some enzymes (urease, ß-glucosidase and BAA-protease) re-moistening the soil did not greatly affect the enzymatic activity, while for others (alkaline phosphomonoesterase and especially invertase, except for the O horizon) the activity was much higher after the soil samples were remoistened. In the *Juncus* soil, arylsulphatase activity was much higher in the remoistened samples than in the air-dried samples, but only in the uppermost two horizons (Fig. 2).

However, despite the differences in enzyme activities, only the catalase and dehydrogenase activities in the upper horizons reached values similar to those observed in terrestrial ecosystems (Miguéns, 2008; Miralles et al., 2012; Bello et al., 2013). The fact that dehydrogenase reached values similar to those found for terrestrial soils, determined in fresh soil samples, suggests that re-moistening the soils is a suitable method of re-establishing the activity of microbial populations. Although the catalase activity was also similar to that observed in other soils, the possible effects of some abiotic reactions on this enzyme prevents its use as an indicator of the re-establishment of the activity of microbial populations in re-moistened soils.

Considering all soil samples together, the hydrolase enzyme activities were highly correlated with total organic C and total N contents, especially in re-moistened samples (Tables 3 and 4). Regarding the oxidoreductase activities, catalase activity was correlated with C and N contents, and the correlation coefficients were higher in re-moistened samples. Dehydrogenase activity was not correlated with either the C or N content in air-dried soil samples (Table 3); however, in the remoistened soil, dehydrogenase activity was strongly correlated with both C and N (Table 4), probably due to the increased microbial activity after re-moistening the samples. In both air-dried and re-moistened samples, the catalase and all the hydrolase activities were negatively correlated with soil pH, although the correlations were not very high. In general, hydrolase activities were negatively correlated with the C/N ratio, though again the correlations were not very high (Tables 3 and 4). The differences in the deepest horizons were generally smaller than in the upper horizons, except for the C2 horizon of the *Spartina* soil, in which the dehydrogenase activity was unexpectedly much higher in the re-moistened soil samples (Fig. 2). The hydrolase activities tended to be higher in remoistened samples than in air-dried samples of all horizons of the *Spartina* soil, except for arylsulfatase and invertase in the ACSe, C1 and C2 horizons. However, although the difference was very small for some enzymes (i.e. urease), for others (i.e. BAA-protease and alkaline phosphomonoesterase) the difference was quite large, especially in the deepest horizons (Fig. 2).

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Table 3. *Correlations between different enzyme activities, and between these and soil physico - chemical and chemical properties in air-dried samples of soils under* Spartina *and* Juncus.

	CAT	DES	URE	BAA	ARYL	MONO	GLUCO	INV
pH H_2O	**-0.722***	**0.718***	**-0.806****	**-0.834****	**-0.670***	**-0.826****	**-0.722***	**-0.694***
OC			**0.900****	**0.923****	**0.941****	**0.935****	**0.906****	**0.919****
TN			**0.878****	**0.946****	**0.957****	**0.937****	**0.931****	**0.960****
C/N				**-0.765***	**-0.678***	**-0.711***	**-0.675***	**-0.722***
CE	**0.709***		**0.872****	**0.920****	**0.867****	**0.924****	**0.854****	**0.868****
Na	**0.753***		**0.872****	**0.916****	**0.818****	**0.927****	**0.822****	**0.829****
CAT	**1**		**0.738***	**0.637***		**0.676***		
DES		**1**						
URE			**1**	**0.936****	**0.884****	**0.972****	**0.922****	**0.867****
BAA				**1**	**0.911****	**0.986****	**0.947****	**0.942****
ARYL					**1**	**0.902****	**0.981****	**0.982****
MONO						**1**	**0.933****	**0.910****
GLUCO							**1**	**0.983****
INV								**1**

* $P \leq 0.05$; ** $P \leq 0.01$

Table 4. *Correlations between different enzyme activities, and between these and soil physico-chemical and chemical properties in re-moistened samples of soils under* Spartina *and* Juncus.

	CAT	DES	URE	BAA	ARYL	MONO	GLUCO	INV
pH H_2O	**-0.863****		**-0.677***	**-0.828****	**-0.744***	**-0.757***	**-0.817****	**-0.721***
OC	**0.928****	**0.943****	**0.911****	**0.943****	**0.925****	**0.951****	**0.946****	**0.933****
TN	**0.884****	**0.950****	**0.955****	**0.971****	**0.951****	**0.968****	**0.971****	**0.975****
C/N			**-0.769***	**-0.781***	**-0.710***	**-0.734***	**-0.747***	**-0.818****
CE	**0.895****	**0.705***	**0.852****	**0.912****	**0.882****	**0.907****	**0.907****	**0.902****
CEC				**0.728***	**0.673***		**0.729***	**0.741***
Na	**0.893****	**0.659***	**0.812****	**0.896****	**0.850 ****	**0.885****	**0.871****	**0.869****
CAT	**1**		**0.795****	**0.873****	**0.846****	**0.876****	**0.854****	**0.827****
DES		**1**	**0.865****	**0.959****	**0.871****	**0.848****	**0.970****	**0.827****
URE			**1**	**0.977****	**0.988****	**0.968****	**0.982****	**0.989****
BAA				**1**	**0.984****	**0.982****	**0.988****	**0.978****
ARYL					**1**	**0.985****	**0.986****	**0.980****
MONO						**1**	**0.981****	**0.977****
GLUCO							**1**	**0.979****
INV								**1**

* $P \leq 0.05$; ** $P \leq 0.01$

Differences between the *Juncus* soil and the *Spartina* soil were observed in relation to the enzyme activities measured: for some enzymes (urease, ß-glucosidase and BAA-protease) re-moistening the soil did not greatly affect the enzymatic activity, while for others (alkaline phosphomonoesterase and especially invertase, except for the O horizon) the activity was much higher after the soil samples were remoistened. In the *Juncus* soil, arylsulphatase activity was much higher in the remoistened samples than in the air-dried samples, but only in the uppermost two horizons (Fig. 2).

However, despite the differences in enzyme activities, only the catalase and dehydrogenase activities in the upper horizons reached values similar to those observed in terrestrial ecosystems (Miguéns, 2008; Miralles et al., 2012; Bello et al., 2013). The fact that dehydrogenase reached values similar to those found for terrestrial soils, determined in fresh soil samples, suggests that re-moistening the soils is a suitable method of re-establishing the activity of microbial populations. Although the catalase activity was also similar to that observed in other soils, the possible effects of some abiotic reactions on this enzyme prevents its use as an indicator of the re-establishment of the activity of microbial populations in re-moistened soils.

Considering all soil samples together, the hydrolase enzyme activities were highly correlated with total organic C and total N contents, especially in re-moistened samples (Tables 3 and 4). Regarding the oxidoreductase activities, catalase activity was correlated with C and N contents, and the correlation coefficients were higher in re-moistened samples. Dehydrogenase activity was not correlated with either the C or N content in air-dried soil samples (Table 3); however, in the remoistened soil, dehydrogenase activity was strongly correlated with both C and N (Table 4), probably due to the increased microbial activity after re-moistening the samples. In both air-dried and re-moistened samples, the catalase and all the hydrolase activities were negatively correlated with soil pH, although the correlations were not very high. In general, hydrolase activities were negatively correlated with the C/N ratio, though again the correlations were not very high (Tables 3 and 4).

This low correlation suggests that the hydrolase activities were mainly related to production by soil microorganisms and not to the activity of enzymes bound to organic colloids, a mechanism of protection of extracellular hydrolases commonly found in terrestrial ecosystems (Kiss et al., 1975; Burns 1982; Nannipieri et al., 1973; 1996). Hydrolase activities were strongly correlated with both electrical conductivity and Na content, especially in air-dried soils. The oxidoreductase activities were also strongly correlated with electrical conductivity and Na content in remoistened soils; however, in the air-dried soils dehydrogenase activity was not correlated with either of these properties (Tables 3 and 4).

The activities of all of the hydrolase enzymes were correlated in both in air-dried and in re-moistened samples, and the correlation coefficients were similar in both

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cases. The oxidoreductase activities were not correlated in either the air-dried or the re-moistened soil samples (Tables 3 and 4).

Moreover, in the air-dried samples catalase activity was correlated with that of some of the hydrolases (urease, BAA-protease and alkaline phosphomonoesterase), but dehydrogenase activity was not correlated with the activity of any of the hydrolases. However, in re-moistened samples both catalase and dehydrogenase activities were highly correlated with all the hydrolase activities.

These findings suggest that the microbial activity is well re-established by re-moistening and incubating the soils, as reflected by dehydrogenase activity but not by catalase activity (probably due to the previously indicated reason). The findings also suggest that the fraction of immobilized hydrolases in proportion to the total activity of the soils is very low and that the hydrolase activities are mainly due to enzymes being produced by proliferating microorganisms and not by immobilized enzymes. On the other hand, the lack of correlation between the oxidoreductases, together with the fact that catalase activity was correlated with hydrolase activities both in re-moistened and air-dried soils, confirms that the re-establishment of microbial activity is reflected by dehydrogenase but not by catalase activity.

When the re-moistened samples of both soils were considered independently, the findings for the *Juncus* soil (Table 5) were the same as when both soils were analyzed jointly: positive correlations between the hydrolase activities and total C and N contents, which were even higher than when all the samples were considered together, highly significant negative correlations with the soil pH and with the C/N

Table 5. *Correlations between different enzyme activities, and between these and soil physico-chemical and chemical properties in re-moistened samples of the* Juncus *soil.*

	CAT	DES	URE	BAA	ARYL	MONO	GLUCO	INV
pH H_2O	**-0.948****	**-0.979****	**-0.951***	**-0.973****	**-0.975****	**-0.995****	**-0.961****	**-0.961****
OC	**0.908****	**0.945****	**0.939****	**0.964****	**0.936****	**0.971****	**0.963****	**0.957****
TN	**0.860****	**0.962****	**0.969****	**0.984****	**0.956****	**0.973****	**0.987****	**0.982****
C/N		**-0.821***	**-0.870****	**-0.878***	**-0.815***	**-0.824***	**-0.899***	**-0.885****
CE	**0.889****	**0.966***	**0.962****	**0.985****	**0.960****	**0.987****	**0.983****	**0.977****
CAT	**1**	**0.881***	**0.829***	**0.859***				
DES		**1**	**0.992*****	**0.993****	**1.000****	**0.986****	**0.987****	**0.992****
URE			**1**	**0.993****	**0.992****	**0.969****	**0.994****	**0.998****
BAA				**1**	**0.991****	**0.989****	**0.998****	**0.998****
ARYL					**1**	**0.983****	**0.985****	**0.990****
MONO						**1**	**0.981****	**0.979****
GLUCO							**1**	**0.999****
INV								**1**

* $P \leq 0.05$; ** $P \leq 0.01$

ratio, strong correlations with electrical conductivity and highly significant correlations between the activities of all the hydrolases and also between dehydrogenase activity and all the hydrolase activities.
However, the activities of both oxidoreductases were now highly correlated, while catalase activity was only correlated with urease and BAA-protease activities. By contrast, in the *Spartina* soil, very few such correlations were observed (Table 6). This suggests that the close correlations between all properties in both salt marsh soils were mainly due to the soil under *Juncus* i.e. the soil least affected by water saturation. The reason for the lack of correlations between the enzyme activities and any of the soil properties in the frequently saturated *Spartina* soil is not clear, although it could be due to a difference in the aeration conditions of the sample or to some of the environmental conditions not considered in this study. Further investigation is required to clarify this finding.

Table 6. *Correlations between different enzyme activities, and between these and soil physico-chemical and chemical properties in re-moistened samples of the* Spartina *soil.*

	CAT	**DES**	**URE**	**BAA**	**ARYL**	**MONO**	**GLUCO**	**INV**
pH H_2O	**-0.947****					**-0.930***		
OC	**0.985****							
CAT	**1**							
DES		**1**						
URE			**1**	**0.932***				
BAA				**1**			**0.949***	
ARYL					**1**			
MONO						**1**		
GLUCO							**1**	
INV								**1**

* $P \leq 0.05$; ** $P \leq 0.01$

Conclusions

The study findings showed that the enzymatic activities in the salt-marsh soils considered are very low, but generally higher in the less frequently saturated soil (under *Juncus*) than in the permanently saturated soil (under *Spartina*).
The findings also suggest that in air-dried hydromorphic soils, re-moistening the soils to saturation and then incubating the soils for a short time is a good method of re-establishing the activity of soil microorganisms. However, the increased activity of all enzymes, especially the oxidoreductases, also suggest that fresh soils should be used in preference to dried soil for investigating soil enzyme activity, in accordance with what has been suggested by diverse authors in previous studies. Further research is needed to clarify the relationships between enzymatic activities

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and the environmental conditions affecting the different soil horizons in salt marsh soils.

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