Ecological Restoration of Salt Marshes in the Gulf of Cádiz

PhD Thesis

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RESTAURACIÓN ECOLÓGICA DE MARISMAS COSTERAS EN EL GOLFO DE CÁDIZ

Tesis presentada por Guillermo Curado Ferrera para optar al grado de Doctor en Biología

Sevilla, Enero de 2012

UNIVERSIDAD DE SEVILLA

Department of Plant Biology and Ecology
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Sevilla, Enero de 2012

To my parents

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CHAPTER 1

General introduction

CHAPTER 1.- General introduction.

According to the Society for Ecological Restoration International (SERI, 2004), ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed. Today, the practice of ecological restoration is receiving immense attention, because it offers hope of recovery from much of the environmental damage inflicted by misuse or mismanagement of the earth's natural resources, especially by technologically advanced societies (Economist, 2002; Malakoff, 2004). In addition, ecological restoration allows the testing of ecological theories (Cairns & Heckman, 1996). Thus, restoration of degraded tropical forests (Lamb *et al*., 2005), grasslands (Toeroek *et al*., 2011), rivers (Hughes *et al*., 2005) or wetlands (Zedler *et al*., 2000) is becoming increasingly common practice.

Coastal areas around the world are highly affected by degradation (Mitsch, 2010), so many efforts have been aimed at restoring coastal wetlands. Wetland creation and restoration are frequently used to replace ecological functions and values lost when natural wetlands are degraded or totally destroyed (Craft *et al*., 1999). In this context, plant and animal invasive species play a very important role since in many cases they have been the cause of salt marsh degradation (Talley *et al*., 2001; Gratton & Denno, 2005), and may become significant management problems in restoration projects (D'Antonio & Meyerson, 2002).

Currently, wetland losses are restricted by law in some countries. When losses are inevitable, they must be compensated for, at least in some parts of the world. For example, compensation for wetland losses is a particular tradition in the U.S.A. (Zedler, 2004), and is now also a “hot topic” in Europe, due to the Habitats Directive (92/43/EEC) and Birds Directive (79/409/EEC) (Pethick, 2002). Hence, endangered European and North American salt marshes have been subjected to increasing restoration programs (e.g. Scheffer *et al*., 2001; Lillebø *et al*., 2005; Elliott *et al*., 2007), and transplanting marsh vegetation is a common practice (Broome *et al*., 1988).

Cordgrasses (genus *Spartina*) are one of the most popular biotools in soft engineering salt marsh restoration projects (Craft *et al*., 1999), with some *Spartina* species extensively used for coastal protection, phytoremediation, habitat creation or restoration, amongst other uses (Fang *et al*., 2004; Czakó *et al*., 2006; Castillo & Figueroa, 2009). For example, Ranwell (1967) reported that *Spartina townsendii* H. & J. Groves was transplanted extensively in Europe, particularly during the 1920s and 1930s, to reduce channel silting, for coastal erosion protection and to reclaim land for agriculture. *Spartina anglica* Hubb. has been planted around the coast of South East England, and throughout the world, to help stabilize sediments and reduce wave erosion (Bakker *et al*., 2002; Paramor & Hughes, 2007). Estuarine marshes in the south-western Netherlands have therefore largely increased since the 1920s, when *S. anglica* was locally planted to enhance sedimentation (Verhoeven, 1938). Cordgrasses can also be used for ecological engineering after oil spills; *Spartina alterniflora* Loisel. was proved to be relatively tolerant to fuel oil spill (Lin *et al*., 2002). Nevertheless, special care must be taken when selecting *Spartina* plants in natural ecosystems for transplantation. The effect of parental growth form on ramets introduced to new sites has broad implications for ecological restoration, although in some cases environmental differences are more important than parental height in determining growth (Trnka & Zedler, 2000). In addition, facilitation mechanisms can operate in *Spartina*-created marshes, contributing to enhance species' composition. For example, Egerova *et al*. (2003) described how *S. alterniflora* favored the survival and growth of *Baccharis halimifolia* L. in a created Louisiana salt marsh with respect to the growing out of plants.

A properly planned restoration project attempts to fulfill clearly stated goals that reflect important attributes of the reference ecosystem (SERI, 2004). Then, monitoring of the results and, if necessary, gradual readjustment of the governing factors, together form an essential part of restoration projects (de Jonge & de Jong, 2002). Environmental monitoring of restored or created coastal wetlands has increased in recent years (eg. Dionne *et al*., 1998; Craft *et al*., 1999; Anastasiou & Brooks, 2003; Wolters *et al*., 2005; Gallego-Fernández & García-Novo, 2007; Zedler *et al*., 2008; Ballantine & Schneider, 2009). For example, the success of the development of restored marshes after restoring tidal flow has been widely evaluated in Europe (e.g. Eertman *et al*., 2002; Wolters *et al*., 2005; Gallego-Fernández & García-Novo, 2007).

Vegetation monitoring is essential to assess the success of restoration (Steyer & Stewart, 1992; Ruiz-Jaen & Mitchell, 2005) since primary productivity is an important function and indicator of success for salt marsh creation and restoration projects (Turner *et al*., 2004; Edwards & Mills, 2005). In addition, other information parameters about the quality of restored ecosystem can be used, such as the microphytobenthic community (Janousek *et al*., 2007), sediment properties (Zedler & Lindig-Cisneros, 2002), invertebrate and fish populations (Warren *et al*., 2002) and bird community (Konisky *et al*., 2006). The compromise between different areas of knowledge such as ornithology, ichthyology, soil sciences or the study of plant and invertebrate communities, promote the understanding of interactions that relate restored marshes. In addition, a comparison with degraded and preserved areas allows us to determine the maturity and evolution of the restored ecosystem.

Incorporating scientific approaches into restoration efforts with rigorously designed scientific experiments that identify cause-effect relationships for the development of restored salt marshes could substantially improve the design, implementation, and monitoring of restoration projects (Callaway, 2005). Until now, most of the salt marsh restoration projects have used *S. alterniflora* and *S. anglica* as biotools. However, little is known about restoration of European salt marshes using Small Cordgrass (*Spartina maritima* (Curtis) Fernald), the only native cordgrass in many European estuaries that was introduced in South Africa (Pierce, 1982). *Spartina maritima* produces very few fertile seeds, so its propagation is mainly vegetative through rhizome fragments (Castillo *et al*., 2010). Due to its narrow distribution, *S. maritima* has been included on some European red lists (England—Cooper, 1993; Cantabrian Coast of Spain—Bueno, 1997; south-west coast of Spain—Cabezudo *et al*., 2005). So far, only Castillo *et al*. (2008) studied salt marsh restoration using *S. maritima*, working in small-size plots of created *S. maritima* marshes, but recently (since January 2007) an extensive restoration project (8.37 ha), based on plantations of *S. maritima*, *Sarcocornia perennis*

(Miller) A.J.Scott ssp. *perennis* and *Zostera noltii* Hornemann, was carried out in the Odiel Marshes in the joint estuary of Odiel and Tinto rivers (south-west Iberian Peninsula) (Castillo & Figueroa, 2009). This project offers a great opportunity to study the development of *S. maritima* restored salt marshes. In the same estuary, the invasion of the highly acidic and metal-polluted Tinto River by *Spartina densiflora* Brongn. is also a very good opportunity to study cordgrass tolerance to extreme environmental conditions.

The overall objective of this PhD thesis was to contribute to the general knowledge about European salt marsh restoration using *S. maritima*, providing an extensive monitoring for assessing the evolution of *S. maritima* restored salt marshes. Our specific goals were:

1.- To compile available information about ecological restoration and environmental monitoring of coastal wetlands.

2.- To analyze the tolerance of the invasive *Spartina densiflora* Brongn. to abiotic sediment conditions (low pH and high metal loads) along the Tinto River, in terms of germination and establishment, in order to clarify whether invasion of this alien species will expand inland from its present distribution.

3.- To assess vegetation changes and vegetation zonation in *S. maritima* restored marshes compared with *S. maritima* preserved and degraded marshes.

4.- To quantify the concentration and stock of nitrogen and carbon in the sediments of restored marshes colonized by *S. maritima* and in the tissues of the dominant halophytes (*S. maritima* and *S. perennis*).

5.- To determine decomposition rates of *S. maritima* (leaves, stems and roots) in restored salt marshes.

6.- To analyze the sedimentary abiotic environment (elevation, pH, redox potential and conductivity) and to quantify the concentration and stock of nine metals (Al, As, Cd, Cr, Cu, Fe, Ni, Pb and Zn) in the sediments colonized by *S. maritima* and in the tissues of the dominant halophytes (*S. maritima* and *S. perennis*), as part of the integral monitoring of restored wetlands in the Odiel Marshes.

7.- To quantify vertical sediment dynamics along the tidal gradient in *S. maritima* restored marshes in comparison with adjacent non-restored marshes and preserved marshes.

8.- To monitor the macroinvertebrate community in restored marshes using *S. maritima,* in comparison with non-restored and preserved marshes.

9.- To monitor the bird community in restored marshes using *S. maritima*, in comparison with non-restored marshes.

10.- To understand the beliefs, perceptions and behaviors of the local population of Huelva city in relation to salt marshes and salt marsh restoration.

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CHAPTER 2

Study sites

CHAPTER 2.- Study sites.

We described in this chapter the sites where our work was carried out: Tinto River and its salt marshes, and Odiel Marshes, both located at Southwest Iberian Peninsula. They are under a Mediterranean climate with Atlantic influence. The estuary has mild, wet winters (January mean temperature of 11 ºC; frosts do not occur in most years). The summers are warm and sunny (August mean temperature of 25 ºC; the maximum temperature rarely exceeds 40 ºC because of the Atlantic influence). Rainfall is extremely sparse between June and September and during this period evapotranspiration leads to extreme hypersalinity on the higher parts of the marsh (Castellanos *et al*., 1994).

Tinto River

Tinto River is protected as ‘Protected Landscape’ (Paisaje Protegido) included in the European Natura 2000 Network (3.125 ha). Tinto river banks and marshes are highly polluted with heavy metals coming from two sources: long-term mining activities carried out landward in the Iberian Pyrite Belt (van Geen *et al*., 1997; Elbaz-Poulichet *et al*., 1999; Leblanc *et al*., 2000; Morillo *et al*., 2002) and industrial activities situated at the joint estuary of the Odiel and Tinto rivers (Elbaz-Poulichet *et al*., 2000). Heavy metal pollution is accompanied by very low pH, which is a product of pyrite oxidation and the metabolism of specific bacteria (López-Archilla *et al*., 1993). A pH gradient has been recorded from landward areas with very acidic pH (ca. 2) to the mouth of the estuary where the Tinto River joins the Odiel River. There, the pH is close to neutrality due to the buffering capacity of dissolved salts from seawater and dilution of the polluted waters (Galán *et al*., 1999; Elbaz-Poulichet *et al*., 2001; Grande *et al*., 2003).

This acidity gradient, together with changes in salinity, is responsible for a marked vegetation zonation along the banks along the channel of the Tinto River. The typical plant zonation of salt marshes in the Gulf of Cadiz is present close to the mouth of the estuary, with species such as *Spartina maritima* (Curtis) Fernald, *Sarcocornia perennis* (Mill.) Scott ssp. *perennis* and *Salicornia ramosissima* J. Woods occurring in low marshes; *Sarcocornia fruticosa* (L.) Scott, *Sarcocornia perennis x fruticosa* (Figueroa *et al*., 2003), *Atriplex portulacoides* L., *Suaeda maritima* L. (Dumort) and *S. densiflora* Brong. (an invasive species from South America) occurring in middle marshes, and *Arthrocnemum macrostachyum* (Moric.) Moris and *Suaeda vera* Gmelin occurring in high marshes. In contrast, only a few marsh plants, such as *S. densiflora*, *Typha dominguensis* (Pers.) Steudel, *Phragmites australis* (Cav.) Trin. and *Scirpus maritimus* L., colonize more acidic salt marshes. Other plant species, such as some *Juncus*, *Scirpus* and *Tamarix*, *Cynodom dactylon* L. (Pers.) or *Chaetopogon fasciculatus* (Link) Hayek sp., colonize acidic banks that are free from tidal influence.

Our work (Chapter 3) was carried out along the Tinto river at five different sites from its mouth to 28.9 km landward (lat 37º 06’ – 37º 29’ N, long 6º 36’ –6º 56’ W) (Fig. 2.1): The first site (T1) was situated at the mouth of the Tinto River, where it joins the Odiel River, and coincides with a salt marsh restoration project with neutral pH (Castillo & Figueroa, 2009). Sites 1 and 2 were located inside the Tinto estuary, and sites 3, 4 and 5 had no tidal influence.

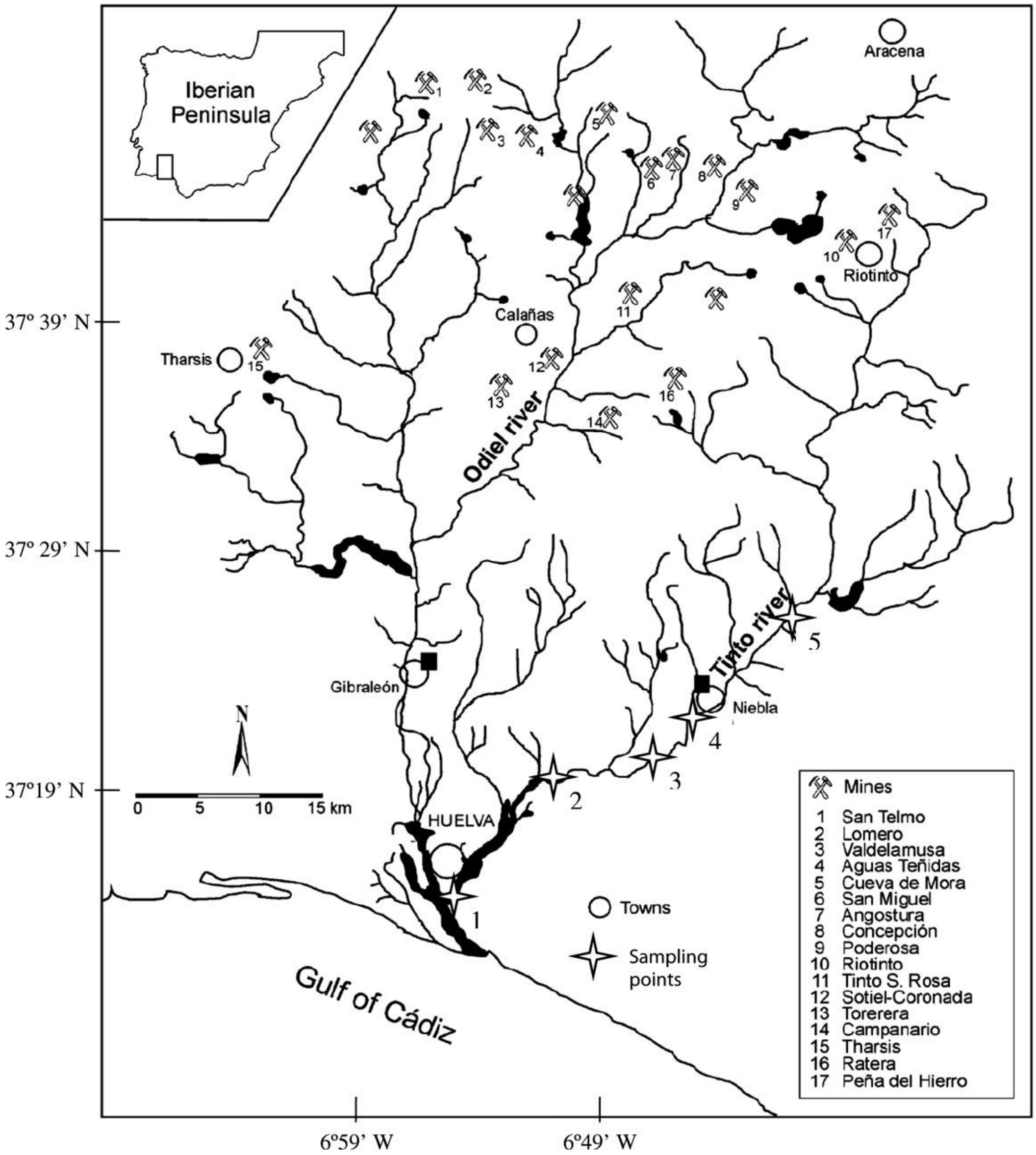

Figure 2.1. Sketch map of the Tinto and Odiel Rivers, showing the sites of sampling points (1–5), towns and main mine locations. Adapted from Nieto *et al*. (2007).

Odiel Marshes

Odiel Marshes (7.158 ha) are located in the joint estuary of Odiel and Tinto rivers (lat 37º 08' – 37º 20' N, long 6º 45' –7º 02' W). It is protected as Natural Park (Paraje Natural) and as a Biosphere Reserve and are a site of international importance for migratory waders through the East Atlantic flyway (Garrido-Guil, 1996; Sánchez *et al.*, 2006). It can be visited through a network of open-access hiking and biking trails that includes the restored salt marshes. The semidiurnal tides have a mean range of 2.10 m and a mean spring tidal range of 2.97 m, representing 0.40-3.37 m above Spanish Hydrographic Zero (SHZ). Mean sea level is +1.85 m relative to SHZ. The vegetation of the marsh complex is diverse and heterogeneous. The typical plant zonation at lower marsh areas tend to be dominated by *Zostera noltii* Hornem. at lower elevations, followed in elevation gradient for *S. maritima,* but they can also be colonized by *S. densiflora*, *Salicornia europaea* agg. and *S. perennis*. Higher on the marsh the vegetation tends to be dominated by long-lived, shrubby species. At intermediate levels, *S. perennis*, *A. portulacoides* and *S. densiflora* can become dominants. On the highest parts of the marsh, *Arthrocnemum macrostachyum* (*Moric*.) Moris, *Limoniastrum monopetalum* (L.*)* Boiss, *Suaeda vera* J. F. Gmelin, *Limonium ferulaceum* (L.) Chaz. and *Limonium diffusum* (Pourret*)* O. Kuntze are prominent. The banks of creeks and channels in mature marsh typically support stands of *S. densiflora*, *A. portulacoides* and *Inula crithmoides* (L.). Numerous other species occur after disturbance, where sand has blown on to the surface of the marsh and where the influence of tides is very small (Castellanos *et al.*, 1994).

Specifically, our works were conducted in three *S. maritima* low salt marsh areas (Fig. 2.2): (1) Restored salt marshes (RM) located next to the Chemical Pole and the city of Huelva, on the left bank of the "Canal del Padre Santo", the main channel of the estuary. This area was restored from November 2006 to January 2007 using mainly *S. maritima* plantations (8.37 ha; current relative cover 62 ± 6% in *Spartina* prairies). *Spartina maritima* clumps coming from natural populations were planted manually at a density of 1 clump m^{-2} (ca. 20 shoots $clump^{-1}$). *Sarcocornia perennis* ssp. *perennis* and *A. portulacoides* were also planted at some middle marshes (1.78 ha). In addition, the invasive cordgrass *S. densiflora* was eliminated however some clumps remained beside to isolated individuals of *Suaeda maritima* at higher elevations (Castillo & Figueroa 2009). Z*ostera noltii* was transplanted also from natural populations in 6 experimental plots. No attempt was made to remove local sediment adhering to the roots. Five sediments blocks (20 cm x 20 cm x 10 cm deep) were transplanted in each experimental plot of 1 m^2, one at each corner and one at the center.

(2) Adjacent non-restored salt marshes (NRM) invaded by *S. densiflora* (relative cover ca. 20%), with small and isolated *S. maritima* clumps and extensive intertidal mudflats on the right bank of the “Canal del Padre Santo”, which were similar to the restored marshes prior to their restoration. These marshes are highly affected by erosion (Castillo *et al*., 2002).

(3) Preserved marshes (PM) dominated by *S. maritima* (ca. 50%) and *Z. noltii* (ca. 10%), with isolated clumps of *S. perennis* at the higher elevations.

RM and NRM were a combination of successional and non-successional marshes located at a channel edge while PM were successional marshes located at the coastal lagoon since no well-conserved marshes of *S. maritima* remained at channel banks in Odiel Marshes.

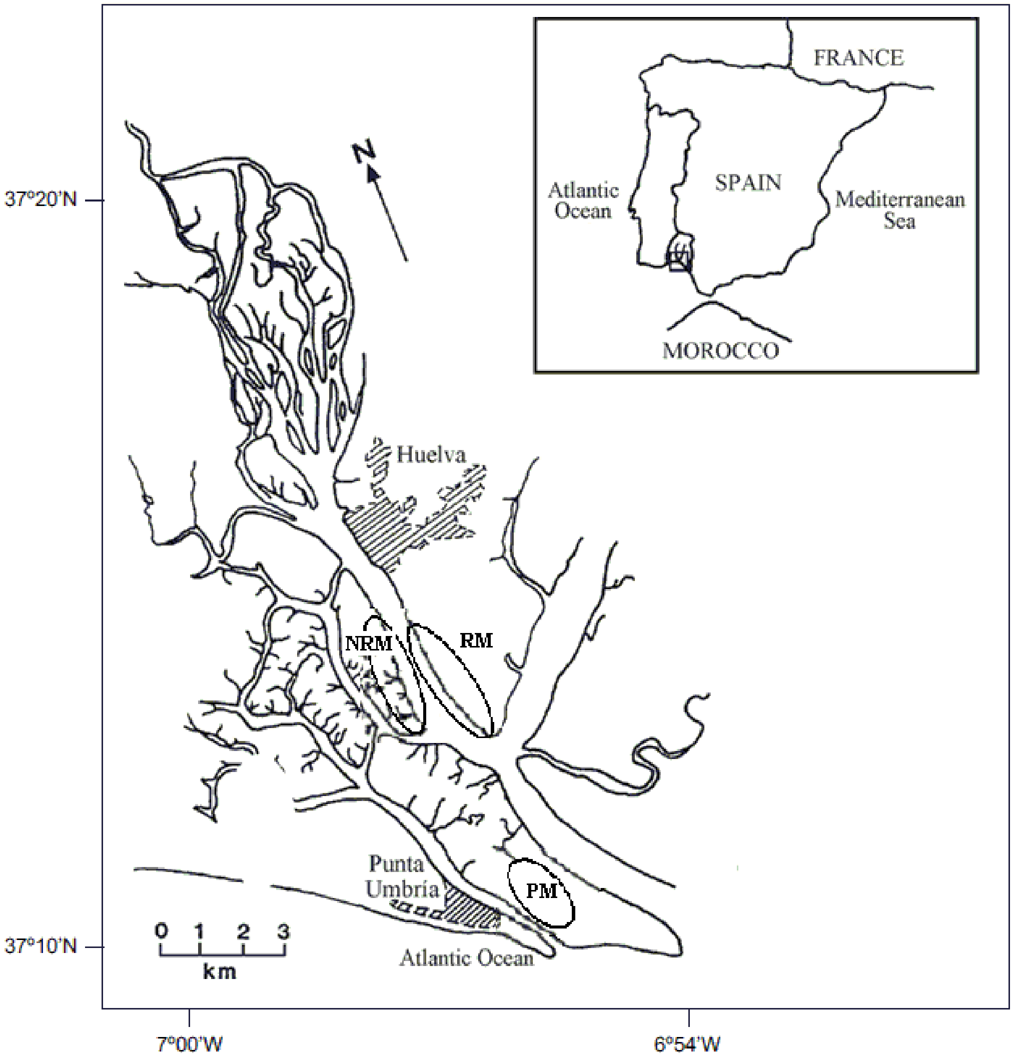

Figure 2.2. Map of the Odiel Marshes and their situation in the Iberian Peninsula. General location of sampled marshes being RM = restored marshes, NRM = non-restored marshes and PM = preserved marshes.

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CHAPTER 3

Germination and establishment of the invasive cordgrass *Spartina densiflora* in acidic and metal polluted sediments of the Tinto River

CAPÍTULO 3.- Germinación y establecimiento de la especie invasora *Spartina densiflora* en sedimentos del Río Tinto ácidos y contaminados por metales.

RESUMEN

La contaminación por metales pesados es común en los ríos vecinos de zonas mineras. En estos ambientes contaminados, la supervivencia de especies exóticas con alta tolerancia a los metales puede ser favorecida. El Río Tinto (suroeste de la Península Ibérica) es un excelente laboratorio natural para el estudio de las respuestas de las plantas a sedimentos ácidos contaminados por metales. Este capítulo analiza la tolerancia de la especie exótica *Spartina densiflora* a bajo pH y altos niveles de metales en el Río Tinto. El principal objetivo de este estudio fue determinar si esta especie exótica puede invadir tierra adentro a lo largo de las orillas del Río Tinto. Las semillas de *S. densiflora* pudieron germinar en sedimentos aeróbicos contaminados por metales pesados incluso a pH 2. Sin embargo, estas condiciones disminuyen la germinación final, alterando la dinámica de germinación, disminuyendo el crecimiento aéreo y subterráneo y previniendo su establecimiento.

CHAPTER 3.- Germination and establishment of the invasive cordgrass *Spartina densiflora* in acidic and metal polluted sediments of the Tinto River.

ABSTRACT

Heavy metal pollution is common in rivers in the vicinity of mining areas. In these polluted environments, the survival of alien species with a high tolerance to metals may be favored. The Tinto River (southwest Iberian Peninsula) is an excellent natural laboratory for the study of plants' responses to acidic and metal polluted sediments. This work analyzes the tolerance of the alien species *Spartina densiflora* to low pH and high metal loads in the Tinto River. The main aim of this chapter was to determine if this alien species can invade landward along the banks of the Tinto River. *Spartina densiflora* seeds were able to germinate in heavy metal polluted aerobic sediments even at pH 2. However, these conditions decreased *S. densiflora* final germination, altered germination dynamics, decreased aerial and subterranean growth rates, and prevented its establishment.

3.1. INTRODUCTION

Heavy metal pollution of rivers and salt marshes from pyrite mines is increasing in the extent and number of affected areas (Handa & Jefferies, 2000). Polluted waters also tend to contain highly acidic sediments due to the oxidation of pyrite (Fyson, 2000). In these conditions, pH may determine plant zonation (Batty *et al*., 2005) as it does in other flooded ecosystems, such as bogs (Asada, 2002). Acidity increases the bioavailability of many metals, including Al, Fe and Zn (Otero & Macías, 2002), which may be toxic for plants and affect their distributions (Heckman, 1990). In addition, invasion by alien plant species is very common in salt marshes and on river banks (Wang *et al*., 2006; Schnitzler *et al*., 2007). Exotic species that are highly tolerant to metals may be favored in these polluted areas, where they may have serious environmental impacts (Lavoie *et al*., 2005).

The banks and salt marshes of the Tinto River (Southwest Iberian Peninsula) are an excellent natural laboratory in which to study plants' responses to acidic and metal polluted sediments because there is a marked gradient of pH and metal pollution from its mouth (pH ca. 7) to more inland areas close to the mining sector (pH ca. 2) (Grande *et al*., 2003). The Tinto River salt marshes are being actively invaded by *S. densiflora* Brongn., a South American cordgrass that has colonized Morocco and the Gulf of Cadiz in the SW Iberian Peninsula, as well as the west coast of North America from San Francisco Bay to British Columbia (Bortolus, 2006). *Spartina densiflora* is a facultative halophyte that is able to invade diverse habitats, including river banks, low salt marshes and salt pans (Nieva *et al*., 2001, 2005; Castillo & Figueroa, 2008). Presently, *S. densiflora* occupies Tinto River salt marshes where most native halophytes are absent. However, this alien species has not frequently invaded unvegetated river banks inland of the estuary. The absence of *S. densiflora* in more inland areas may be related to dispersion limitations, despite its production of viable seeds (Kittelson & Boyd, 1997), or to stressful abiotic conditions that limit colonization.

This chapter aims to analyze the tolerance of *S. densiflora* to abiotic sediment conditions (low pH and high metal loads) along the Tinto River, in terms of germination and establishment, in order to clarify whether invasion of this alien species will expand inland from its present distribution.

3.2. MATERIALS AND METHODS

3.2.1. Germination and establishment experiments.

These experiments were carried out at the greenhouse facilities of the University of Seville from October 2007 to July 2008. Sediments for the greenhouse experiments were collected on October 2007 at five different sites distributed along the Tinto River channel, from its mouth to 28.9 km landward (see Chapter 2 for description of the study site). Plant cover for every site was recorded using a semi-quantitative scale (0–5 following Domin–Krajina cover-abundance scale; Table 3.1). Ripe seeds of *S. densiflora* collected in October 2007 from Site 1 were added to Tinto River sediments originating from the five sites located along the Tinto River (where each site corresponds to a different treatment, T1–5). Seeds were buried at a depth of 0.3 cm in the sediments in 21 cm x 21 cm sealed plastic pots (9.5 cm deep). Vertical sediment distribution in the field was maintained in the experimental pots. Pots were watered daily with 500 ml of tap water. Four to six replicates of 50 seeds were used for every treatment.

Over the course of the experiment, the mean monthly air temperature in the greenhouse varied between 17.9 ± 0.1 ºC on December 2007 and 24.2 ± 0.1 ºC in May 2008. The minimum monthly temperature was between 7.5 ºC and 17.5 ºC, and the maximum monthly temperature varied between 25.5 ºC and 42.7 ºC. Mean monthly air relative humidity ranged from 56.1 ± 0.4% and 66.1 ± 0.2%.

Site/treatment	Coordinates	Distance	Plant species (relative cover)
1	37º13´14´´ N – 6º57´40´´ O	0	Ap (1), Am (1), Sd (1), Sma (5), Smr (1) Spp (1), Spxf (1), Sr (1), Sv (1)
2	37º31'28'' N – 6º81'26'' O	1.7	Pa (1), Sd (3), us (3)
3	37º31'48'' N – 6º74'89'' O	10.0	Ca (1), Cd (2), Ec (1), Ja (1), Jb (1), Pa (2), Pl (1), Po (1), Pr (1), Sh (1), Td (1), Ts (1), us (4)
4	37º34'26'' N – 6º71'11'' O	15.1	Cd (1), Cf (1), Ec (1), Ja (1), Lm (1), Pl (2), Sh (2), Sm (1), Td (3), Ts (1), us (4)
5	37º25'23'' N – 6º36'39'' O	28.9	Ch (1), Jar (1), Jm (1), No (1), Pa (3), Pr (1), Sh (1), Sm (1), Td (1), us (3)

Table 3.1. Geographic coordinates, distance from the river mouth along the river channel (km), plant species and their relative cover (1 = 1-20 %; 2 = 21-40 %; 3 = 41-60 %; 4 = 61-80 %; 5 = 81-100 %) for the five sites where sediments were collected for the greenhouse treatments. Species legend: Am, *Arthrocnemum macrostachyum*; Ap, *Atriplex portulacoides*; Ca, *Chenopodium album*; Cd, *Cynodom dactylon*; Cf, *Chaetopogon fasciculatus*; Ec, *Eucaliptus camaldulensis*; Ja, *Juncus acutifolium*; Jar, *Juncus articulatus*; Jb, *Juncus bufonius*, Jm, *Juncus maritimus*; Lm, *Lolium multiflorum*; Pa, *Phragmites australis*; Pl, *Polygonum lapathifolium*; Po, *Portulaca oleracea*; Pr, *Panicum repens*; Sd, *Spartina densiflora*; Sh, *Scirpus holoschoenus*; Sm, *Scirpus maritimus*; Sma, *Spartina maritima*; Smr, *Suaeda maritima*; Spp, *Sarcocornia perennis ssp. perennis*; Spxf, *Sarcocornia perennisxfruticosa*; Sr, *Salicornia ramosissima*; Sv, *Suaeda vera*; Td, *Typha dominguensis*; Ts, *Tamarix* sp.; us, unvegetated sediments.

3.2.2. Abiotic environment.

Sediment texture (as % of sand, silt and clay) was recorded for every treatment using a hydrometer method (Gee & Bauder, 1986). pH of the interstitial water of the sediments was recorded (n = 6-9; pH/redox *Crison* with the electrode *M-506*) after adding distilled water to the sediment (1:1, v/v). Redox potential of the sediment between 0-5 cm deep (n = 10-15) was determined with a portable meter and electrode system (Crison pH/mV p-506). Sediment salinity of the interstitial water between 0-5 cm deep (n = 5-6) was measured as electrical conductivity (conductivity meter, Crison-522) in the laboratory.

The sediment concentrations of Al, As, Cd, Cr, Cu, Fe, Ni, Pb and Zn were recorded (n = 5). Sediments samples were dried to constant weight at 80° C during 48 hours and they were pulverized using a grinder (*Cyclotec, Foss Tecator AB,* Höganäs, Sweden). Then, samples were sieved through an 80 µm light and digested with microwaves (Anton Paar, modelo multiwave 3000, Austria). Every sample was digested in 6 ml HNO_3 and 25 ml ultrapure water. The product was measured by inductively coupled plasma (ICP) spectroscopy (Horiba Jobin Yvon, Última 2, France). The lowest detection hold is showed when the concentration was below the detection limit. Every sediment characteristic was recorded between 0-5 cm deep.

3.2.3. Germination of *S. densiflora*.

Germinated seeds were counted daily and were considered to have germinated when the radicle emerged. Five germination parameters were determined: (1) final germination percentage; (2) number of days to first germination; (3) number of days to final germination; (4) mean daily germination (MDG) where MDG = final germination percentage/number of days to final germination; (5) germination rate (GR, a measure of germination speed, with lower values indicating faster germination). Germination rates were calculated as follows:

$$Gr= (n_1t_1) + (n_2t_2) + \ldots + (n_xt_x) /x^n,$$

where, n_1 is the number of germinants on the first day of germination, t_1 the number of days from initial sowing to first germination and X^n the total number of germinated seeds (Osborne *et al.*, 1993).

3.2.4. Establishment and growth of *S. densiflora* seedling.

Survival of *S. densiflora* seedlings was recorded daily; plants were considered dead when no green leaves and tillers remained. Aerial growth of seedlings was recorded in January

2008 as the increase in maximum height between two consecutive measurements and the production of tillers from the beginning of the experiment. Mean aerial growth rate (mm day^{-1}) was calculated as the ratio between the final maximum height and the number of days since germination.

Subterranean growth was recorded in January 2008 as the production of roots from the beginning of the experiment and root length. Mean subterranean growth rate (mm day^{-1}) was calculated as the ratio between the final maximum length for the longest root and the number of days since germination.

3.2.5. Statistical analysis.

Analyses were carried out using SPSS release 12.0 (SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM). Pearson correlation coefficients and regressions were calculated between abiotic variables and responses of *S. densiflora*. Data were tested for normality with the Kolmogorov– Smirnov test and for homogeneity of variance with the Levene test ($P > 0.05$). When homogeneity of variance between groups was not found, data were transformed using the following functions: ln (x), 1/x and √x. Student's *t*-test for independent samples was applied to compare the two treatments. If homogeneity of variance was not achieved by data transformation, then the means were compared using Mann–Whitney *U*-test. Variations in abiotic parameters and in seedling development between more than two treatments were compared by one-way analysis of variance. Tukey's t-test between means was calculated only if *F*-test was significant at the 0.05 level of probability. If homogeneity of variance was not achieved by data transformation, then the means were compared by Kruskal–Wallis H-test.

3.3. RESULTS

3.3.1. Abiotic environment.

Sediments from the three sites located closest to the river mouth (T1–3) had sandy textures while the other two sites (T4–5) had finer textures. Sediment redox potential was positive for every treatment, varying between +171 ± 25 mV for T1 and ca. +500 mV for T3 and T4 (Kruskal–Wallis, $\chi^2 = 47.597$, $P < 0.0001$, df = 4). Electrical conductivity of the sediment interstitial water ranged between 7 mS cm^{-1} for T1 and T2 (sediments from inside the Tinto River estuary) to 3.7 ± 0.3 mS cm^{-1} for T3 (from a site without tidal influence) (Anova, $F = 12.361$, $P < 0.0001$, df = 26). Sediment pH varied significantly between 7.5 ± 0.0 for T1 and 2.5 ± 0.1 for T4 (Kruskal–Wallis, $\chi^2 = 30.83$, $P < 0.0001$, df = 4). Thus, sediment pH was higher for T1 and T2 than for the other treatments (*U*-test, $P < 0.001$) (Table 3.2).

High concentrations of the following heavy metals were recorded: Cu (109–504 µg g^{-1}), Pb (32–7575 µg g^{-1}), Al (577– 2476 µg g^{-1}), Zn (770–3118 µg g^{-1}), and Fe (333–8698 µg g^{-1}). The highest total heavy metal concentrations were recorded for T5, the most inland site, for Zn (3118 ± 131 µg g^{-1}), Pb (7575 ± 232 µg g^{-1}), Al (2476 ± 49 µg g^{-1}), Fe (8698 ± 89 µg g^{-1}) and As (3872 ± 107 µg g^{-1}). T1 had lower heavy metal concentrations than the other sites for Cu (109 ± 4 µg g^{-1}), Pb (32 ± 3 µg g^{-1}), Al (577 ± 23 µg g^{-1}), Fe (333 ± 11 µg g^{-1}) and As (25 ± 2 µg g^{-1}) (Table 3.3).

3.3.2. Germination of *S. densiflora*.

The number of days to first germination decreased markedly in more acidic sediments ($r = 0.904$, $P < 0.05$, $n = 5$), from 16 ± 2 days in neutral sediments (T1) to about 7 days in acidic sediments (Anova, $F = 16.72$, $P < 0.0001$; HSD-test, $P < 0.05$) (Fig. 1; Table 4). After the onset of germination, T1 plants showed a high germination rate (32.5 ± 1.6 day) until reaching a final peak germination percentage (90 ± 3%).

Treatment	**Texture**	**Redox potencial*****	**Conductivity*****	**pH*****
1	98 / 1 / 1	171 ± 25^{a}	6.8 ± 0.5^{a,b}	7.5 ± 0.0^{a}
2	89 / 7 / 4	258 ± 12^{b}	7.2 ± 0.3^{b}	4.0 ± 0.1^{b}
3	87 / 9 / 4	510 ± 15^{c,d}	3.7 ± 0.3^{c}	2.9 ± 0.1^{c}
4	61 / 36 / 3	501 ± 6^{c}	5.1 ± 0.3^{c}	2.5 ± 0.1^{c}
5	25 / 69 / 6	475 ± 4^{d}	5.3 ± 0.3^{a,c}	2.8 ± 0.1^{c}

Table 3.2. Texture (percentage of sand / loam / clay), redox potential (mV), electrical conductivity (mS cm^{-1}) and pH for five different treatments based on sediments collected along Tinto river (SW Iberian Peninsula). Anova or Kruskal-Wallis test between treatments: *** P < 0.001. Different letters indicate significant differences between treatments (Tukey´s test or Man-Whitney *U*-Test).

	Treatment				
(µg g^{-1})	1	2	3	4	5
Zn***	770 ± 121^{a}	1062 ± 114^{a}	778 ± 41^{a}	1233 ± 114^{a}	3118 ± 131^{b}
Cu**	109 ± 4^{a}	413 ± 36^{b}	289 ± 55^{b}	276 ± 38^{b}	504 ± 24^{c}
Cd	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	1.0 ± 0.0
Pb**	32 ± 3^{a}	1085 ± 179^{b}	811 ± 87^{b}	1195 ± 92^{b}	7575 ± 232^{c}
Cr**	13 ± 1^{a}	35 ± 5^{b}	35 ± 7^{b}	40 ± 3^{b}	61 ± 1^{c}
Ni**	≤ 0.1^{a}	6 ± 0^{a}	6 ± 0^{a}	11 ± 1^{a}	15 ± 1^{b}
Al**	577 ± 23^{a}	1254 ± 77^{b}	1714 ± 391^{b,c,d}	1856 ± 132^{c}	2476 ± 49^{d}
Fe***	333 ± 11^{a}	4659 ± 565^{b}	3587 ± 166^{b}	3820 ± 193^{b}	8698 ± 89^{c}
As**	25 ± 2^{a}	967 ± 129^{b}	906 ± 96^{b}	766 ± 36^{b}	3872 ± 107^{c}

Table 3.3. Heavy metal concentration (µg g^{-1} dry weight; n = 5) for five different treatments based on sediments collected along Tinto river (SW Iberian Peninsula). Anova or Kruskal-Wallis test between treatments: * P < 0.05, ** P < 0.01, *** P < 0.001. Different letters indicate significant differences between treatments (Tukey´s test or Man-Whitney *U*-Test).

The germination dynamics for T2 were similar to those of T3 and T4 (Fig. 3.1), although this treatment had a higher pH (Table 3.2). Germination in the T5 group, which had a similar pH to T3 and T4 (Table 3.2) but higher heavy metal concentrations (Table 3.3), began earlier but it slowed from an initial 20% to a final germination rate of 58 ± 4%. This was reflected in a lower mean daily germination (0.5 ± 0.0 seedlings day^{-1}), the highest germination rate (45.1 ± 7.3 day) and more days to final germination (112 ± 2 days) (Fig. 3.1; Table 3.4).

Final germination decreased with pH ($r = 0.95$, $P < 0.01$, $n = 5$), ranging from 90 ± 3% for pH 7.5 (T1) to 58 ± 4% for pH 2.8 (T5), and was independent of sediment redox potential or conductivity (Fig. 3.1, Table 3.4). In addition, final germination also decreased with higher concentrations of Al ($r = -0.840$, $P < 0.005$, $n = 5$) and Cr ($r = -0.931$, $P < 0.05$, $n = 5$).

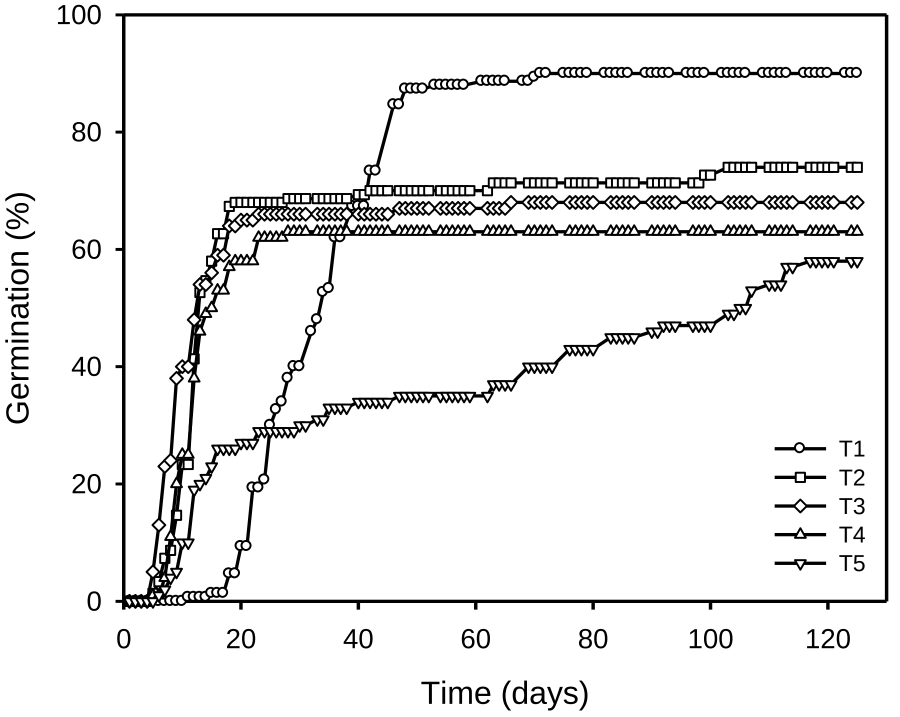

Figure 3.1. Mean germination (n = 4–6) of seeds of *Spartina densiflora* during 127 days exposed to five treatments (T) composed of sediments collected along the Tinto River.

Treatment	**1**	**2**	**3**	**4**	**5**
n	6	6	4	4	4
Final germination	90 ± 3^{a}	74 ± 3^{b}	$67 \pm 16^{b,c}$	63 ± 3^{c}	58 ± 4^{c}
Days to first germination	16 ± 2^{a}	6 ± 0^{b}	6 ± 1^{b}	8 ± 1^{b}	7 ± 0^{b}
Days to last germination	55 ± 5^{a}	57 ± 16^{a}	38 ± 11^{a}	23 ± 2^{a}	112 ± 2^{b}
Germination rate	32.5 ± 1.6^{a}	17.0 ± 2.4^{b}	11.0 ± 1.2^{b}	12.5 ± 0.3^{b}	45.1 ± 7.3^{a}
Mean daily germination	$1.8 \pm 0.1^{a,b}$	$2.3 \pm 0.8^{a,b}$	3.4 ± 0.8^{b}	3.0 ± 0.1^{b}	0.5 ± 0.0^{a}
Seedling growth	1.73 ± 0.09^{a}	1.70 ± 0.05^{a}	0.87 ± 0.06^{b}	0.51 ± 0.03^{c}	0.85 ± 0.07^{b}
Seedling survivorship	93 ± 4^{a}	95 ± 2^{a}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}

Table 3.4. Final germination (%), days to first and last germination, germination rate, mean daily germination (seedling day^{-1}), seedling aerial growth (mm day^{-1}) and seedling survivorship (%) for *Spartina densiflora* at five different treatments based on sediments collected along Tinto river (SW Iberian Peninsula). Different coefficients indicate significant differences between treatments (test de Kruskal-Wallis and Mann-Whitney *U*-test) (n = 4-6).

3.3.3. Survivorship and growth of *S. densiflora* seedlings.

No seedlings survived in with pH lower than 3 (T3, T4 and T5) while the survivorship for T1 (pH 7) and T2 (pH 4) were both close to 95% (Fig. 3.2, Table 3.4). Mortality of seedlings in groups T4 and T5 was faster than in T3, increasing markedly 20–30 days after the beginning of the experiment (Fig. 3.3).

Mean aerial growth rate of *S. densiflora* seedlings was between 0.51–0.87 mm day^{-1} in sediments with pH of less than 4 (T3–5), increasing to 1.73 mm day^{-1} with higher pH (T1–2) (Table 3.4). Seedlings in T1 with neutral pH developed more roots than those in T2 with pH ca. 4 by 100 days after the beginning of the experiment (4.5 ± 0.2 roots *vs* 2.9 ± 0.2 roots; $t = -4.66$, $P < 0.0001$). In addition, roots grew faster in neutral than in acidic sediments (1.25 ± 0.06 mm day^{-1} *vs* 0.35 ± 0.02 mm day^{-1}; $U = 3341.00$, $P < 0.0001$).

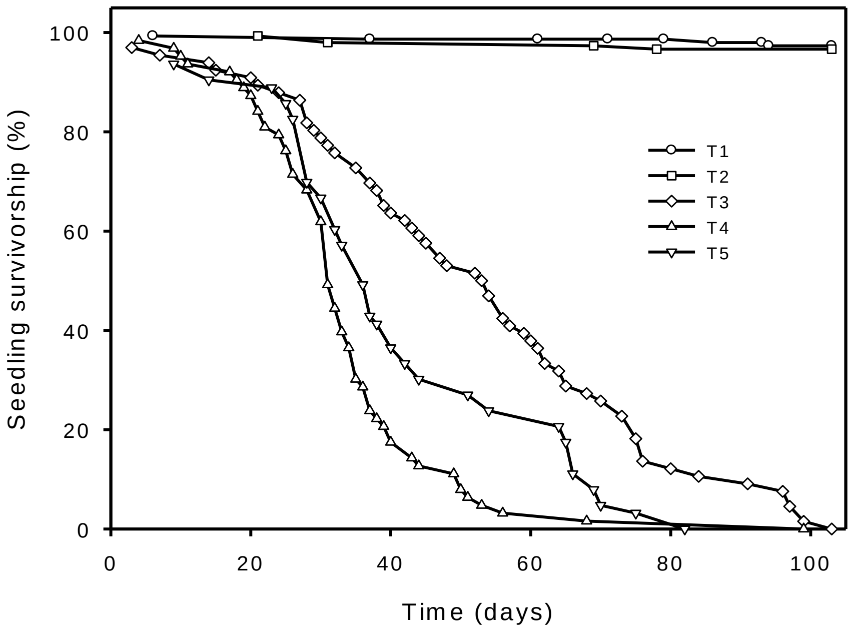

Figure 3.2. Survivorship (n = 4-6) of *Spartina densiflora* seedlings during 112 days since the beginning of the experiment exposed to five treatments (T) based on sediments collected along Tinto river (SW Iberian Peninsula).

3.4. DISCUSSION

The banks of the Tinto River, one of the most heavy metal polluted rivers in the world, show many areas devoid of vegetation. The invasive cordgrass *S. densiflora* has colonized some of these areas in the estuary, but not more inland sites along the river. In this study, we analyzed the germination and establishment of this alien cordgrass in acidic and heavy metal polluted sediments collected along the Tinto River, from its mouth up to the mountains where polluted waters drain from pyrite mines.

Total metal concentrations in the sediments recorded in our study were very high and in the range recorded previously along the Tinto River (Nieto *et al*., 2007; Fernandez-Caliani *et al*., 2009). Heavy metal bioavailability along the Tinto River varies depending on sediment abiotic conditions such as pH, salinity and redox potential (Morillo *et al*., 2002). In this context, pH and heavy metals are closely related, as pyrite oxidation induces highly acidic conditions that increase the bioavailability of metals such as Al, Cd, Cu, Fe, Pb, Ni and Zn (Gambrell, 1994; Bertolin *et al*., 1997; Yu *et al*., 2000; Kashem & Singh, 2001; Otero & Macías, 2002). In contrast, other metals such as Cr show a very low bioavailability in acidic sediments (Liang & Wong, 2003).

Spartina densiflora seeds were able to germinate in aerobic sediments polluted with high levels of heavy metals even when the pH was as low as 2. However, these conditions, which represent the more inland areas along the Tinto River, lowered *S. densiflora* final germination and finally deterred its establishment.

Mean final germination decreased linearly with low concentrations of Cr (13–61 $\mu g\ g^{-1}$) and high concentration of Al (500– 2500 $\mu g\ g^{-1}$). In addition, germination was slowest in the most polluted sediments coming from the most inland sites along the Tinto River. These results indicate that high heavy metal bioavailability, especially of aluminum, may inhibit or alter the germination dynamics of *S. densiflora*. Other studies have shown that high concentrations of heavy metals inhibited the germination of different species (Peralta-Videa *et al*., 2001; Munzuroglu & Geckil, 2002; Roem *et al*., 2002; Jadia & Fulekar, 2008; Mahmood *et al*., 2007; Ozdener & Kutbay, 2009). However, this is the first study to demonstrate this inhibition for a cordgrass species. In spite of the high heavy metal loads recorded, *S. densiflora* seeds were able to germinate even in very acidic conditions (pH ca. 2), probably due to the role of the seed coat as a barrier to the entrance of metals during the hydratation process (Munzuroglu & Geckil, 2002).

On the other hand, acidic sediments accelerated germination, probably due to corrosion of the seed coat, facilitating its hydratation. However, it is unlikely that germination dynamics were directly altered by pH since the germination process is very tolerant to acidification (Kim *et al*., 2008; Chauhan & Johnson, 2009a,b; Chauhan & Johnson, 2009c; Leyer & Pross, 2009; Oliva *et al*., 2009; Wing *et al*., 2009).

Aerial and subterranean growth rate of *S. densiflora* seedlings decreased in more acidic sediments highly polluted with heavy metals. Furthermore, seedlings grown in more acidic (pH < 3) and polluted sediments died only a few months after germination, resulting in high mortality rates at more polluted and acidic sites. Adult individuals of *S. densiflora* are able to accumulate high concentrations of heavy metals in their leaves when they grow on neutral and extremely polluted sediments (Luque *et al*., 1999; Cambrollé *et al*., 2008). However, the seedlings capacity to tolerate accumulated heavy metals (Verkleij & Schat, 1990) was overcome when they were grown in acidic sediments, where very high bioavailable metal loads would lead to gradually increasing toxicity. Mateos-Naranjo *et al*. (2008a,b) reported that adult *S. densiflora* showed increasing stress levels at neutral pH with Cu and Zn concentrations even lower than those recorded in our study, and seedlings were more sensitive to metals pollution than adult plants (Peralta-Videa *et al*., 2004). On the other pH extreme, *S. densiflora* has been described to be absent in alkaline soil (pH >9) (Carnevale *et al*., 1987).

As in this study, previous reports have shown lower tolerance to heavy metal pollution in acidic environments for the establishment of seedlings, as opposed to during germination (De Graaf *et al*., 1997; Rader *et al*., 1997; Roem *et al*., 2002; Ozdener & Kutbay, 2009). *Spartina densiflora* seedlings were able to establish themselves on highly heavy metal polluted sediments with pH 4, where they showed similar population dynamics (survivorship ca. 90%) and aerial growth rates compared to non-acidic conditions. Previous studies on cordgrass have shown that *Spartina patens* (Ait.) Muhl. was able to grow in acid sediments (pH 4–5) (Anastasiou & Brooks, 2003) and that *Spartina alterniflora* Loisel. died following the acidification (pH ca. 3–4) of salt marshes after removing the tidal influence (Anisfeld & Benoit, 1997; Portnoy & Valiela, 1997).

Root development was clearly affected in acidic sediments even when aerial growth was not, which could be related to high loads of heavy metals, such as aluminum, in the sediment interstitial water (De Graaf *et al*., 1997). The inhibition of root growth may affect *S. densiflora* seedlings in many ways, for example limiting their water and nutrient uptake capacity or their anchoring capacity to resist river runoff, currents and waves.

From these results, we conclude that *S. densiflora* can invade salt marshes along the Tinto River, where it finds a very low level of interspecific competition due to the absence of most of the native halophytes, where the pH of sediments is higher than 4. However, it would not be able to not colonize (by seed) the barren riverbanks in more landward areas out the estuary where the establishment of seedlings is prevented. More work is still necessary to determine if these landward areas could be colonized by *S. densiflora* rhizome fragments.

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CHAPTER 4

Plant zonation in restored, non-restored and preserved *Spartina maritima* salt marshes

CAPÍTULO 4.- Zonación vegetal en marismas de *Spartina maritima* restauradas, no restauradas y conservadas.

RESUMEN

Este capítulo analiza la zonación de la vegetación y el medio abiótico (elevación del sedimento, pH, potencial rédox y conductividad) 2.5 años tras la restauración de marismas costeras mediante plantaciones de *Spartina maritima, Sarcocornia perennis* y *Zostera noltii* en las Marismas de Odiel (suroeste de la Península Ibérica) en comparación con marismas bajas conservadas y otras degradadas. Las marismas restauradas presentaron mayor biodiversidad (10 especies) que las degradas no restauradas (6 especies) y las conservadas (3 especies) debido a las condiciones abióticas mas suavizadas (mayores potenciales rédox que las marismas conservadas) y mayor diversidad de hábitats (parches arenosos en las zonas más elevadas). El menor potencial rédox de las marismas conservadas, incluso con elevaciones topográficas similares a las otras marismas, parece tener relación con su localización en un lago costero con poca capacidad de drenaje, estrechando el rango de distribución de las plantas a lo largo del gradiente mareal. Los trasplantes de *Z. noltii* incrementaron su cobertura 74 ± 19% cada mes por debajo del límite bajo de distribución de *S. maritima* en las marismas restauradas. Los pastos marinos parecen expandirse activamente por semillas probablemente producidas dentro de las marismas restauradas. Las marismas restauradas usando *S. maritima* (plantadas a una densidad de 1 clon m^{-2}) alcanzó una cobertura relativa similar que las marismas conservadas (ca. 50%) 2.5 años después de los trasplantes. *Spartina maritima* mostró mayor altura de tallos en las marismas conservadas (40 ± 2 cm) que en las restauradas (34 ± 1 cm). La cobertura de *S. perennis* fue similar en las tres áreas estudiadas estando concentradas en las mayores elevaciones del gradiente mareal. Nuestros resultados muestran el éxito de la restauración de marismas bajas europeas mediante plantaciones de *S. maritima*, *S. perennis* y *Z. noltii* ya que son capaces de reproducir tras 2.5 años de la restauración el típico patrón de zonación vegetal en las marismas del Golfo de Cádiz.

CHAPTER 4.- Plant zonation in restored, non-restored and preserved *Spartina maritima* salt marshes.

ABSTRACT

This chapter analyzes vegetation zonation and abiotic environment (sediment elevation, pH, redox potential and conductivity) 2.5 years after salt marsh restoration using *Spartina maritima*, *Sarcocornia perennis* and *Zostera noltii* plantations in the Odiel Marshes (south-western Iberian Peninsula) in comparison with *S. maritima* preserved and degraded low marshes. Restored marshes presented higher species richness (10 spp.) than non-restored (6 spp.) and preserved (3 spp.) marshes, due to milder abiotic conditions (higher redox potentials than preserved marshes) and higher habitat diversity (sandy patches at higher elevations). The lower redox potentials recorded for preserved marshes, even with similar elevations than restored and non-restored marshes, appeared to be related to their location in a coastal lagoon with a low drainage capacity, narrowing the plant distribution range along the tidal gradient. *Zostera noltii* transplants increased in cover by 74 ± 19% per month below the lower *S.maritima* distribution limit in restored marshes. The seagrass seemed to be expanding actively by seeds, probably produced within the restored marshes. Marshes restored using *S. maritima* (planted at a density of 1 clump m^{-2}) achieved a similar relative cover to preserved marshes (ca. 50%) 2.5 years after transplanting. *Spartina maritima* showed higher tiller height in preserved (40 ± 2 cm) than in restored marshes (34 ± 1 cm). *Sarcocornia perennis* cover was similar in the three areas, being concentrated at higher elevations in the tidal gradient. Our results show the success of restoring European low salt marshes using *S. maritima*, *S. perennis* and *Z. noltii* plantations since they are able to reproduce, 2.5 years after restoration, the typical zonation pattern of the Gulf of Cadiz.

4.1. INTRODUCTION

Salt marshes fulfill many functions, such as biodiversity support, water quality improvement, flooding control, or carbon sequestration. However, they have been affected by anthropogenic degradation worldwide, so there is now a need for salt marsh ecological restoration to replace the ecological functions and values lost when natural wetlands are degraded or destroyed (Zedler & Kercher, 2005; Mitsch, 2010). Cordgrasses (genus *Spartina*) are one of the most popular biotools in soft engineering salt marsh restoration projects (Craft *et al*., 1999).

Vegetation monitoring is essential to assess the success of restoration (Steyer & Stewart, 1992; Ruiz-Jaen & Mitchell, 2005). Different studies have examined vegetation changes after introduction of Smooth cordgrass, *Spartina alterniflora* Loisel., to plantations along the East Coast of North America (Broome *et al*., 1988; Craft *et al*., 1999; Travis *et al*., 2010) and China (e.g. Chung, 1993, 2006), but only a single study on pilot transplants has documented vegetation changes after European salt marsh restoration using Small cordgrass, *S. maritima* (Curtis) Fernald, (Castillo *et al*., 2008a). *Spartina maritima* is the only native cordgrass in many European estuaries (Mobberley, 1956), and is included on some European red lists (England—Cooper, 1993; Cantabrian Coast of Spain—Bueno, 1997; south-west coast of Spain—Cabezudo *et al*., 2005). An innovative and extensive (8.37 ha) restoration project was carried out from November 2006 to January 2007 based on planting *S. maritima*, perennial glassworth, *Sarcocornia perennis* (Mill.) A. J. Scott spp. *perennis*, and the seagrass *Zostera noltii* Hornemann (Castillo & Figueroa, 2009a).

Zostera transplanting has been widely discussed (e.g. Phillips, 1974; Dennison & Alberte, 1986; Zimmerman *et al*., 1995) and some works have analyzed its success (Orth, 1999; Van Katwijk & Hermus, 2000; Martins *et al*., 2005), but *Z. noltii* transplants have never been monitored in the Gulf of Cadiz. In some Portuguese estuaries the area occupied by *Z. noltii* decreased drastically in only one decade (Martins *et al*., 2005) and it has also been included as “vulnerable” on the red list of Andalusian plants (Cabezudo *et al*., 2005).

This chapter assessed vegetation changes 2.5 years after salt marsh restoration using *S. maritima, S. perennis* and *Z. noltii* plantations in the Odiel Marshes (south-western Iberian Peninsula). To achieve this, abiotic environment (sediment elevation, pH, redox potential and conductivity) and vegetation zonation of *S. maritima* restored marshes were compared with *S. maritima* preserved and degraded marshes.

4.2. MATERIALS AND METHODS

4.2.1. Study site.

This work was carried out in the three studied marshes of the Odiel Marshes showed in Chapter 2.

4.2.2. Abiotic environment.

Elevation relative to Spanish Hydrographic Zero (SHZ) was surveyed *in situ* to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore); reference points were determined in relation to measurements of tidal extremes (Ranwell *et al*., 1964). Sediment abiotic environmental characteristics were recorded from April to June 2009 every 10 m along altitudinal transects perpendicular to the tidal line, from the lower distribution limit of *S. maritima* to the upper distribution limit of salt marsh vegetation (between 40 and 60 m in length). Nine transects were set up in restored marshes (RM) and non-restored marshes (NRM), and six transects in preserved marshes (PM) due to the homogeneity of this area. Every sediment abiotic characteristic was recorded between 0 and 5 cm deep (except redox potential, which was also recorded at depths of between 5 and 10 cm) apart on each transect (n = 30-71). Sediment redox potential was determined in the field with a portable meter and an electrode system (Crison pH/mV p-506), with every sample being the mean of three sub-samples. The pH of the interstitial water of the sediments was recorded (pH/redox Crison with the electrode M-506) after adding distilled water to the sediment (1:1, v/v). Finally, sediment salinity of the interstitial water was measured as conductivity (conductivity meter, Crison-522) in the laboratory after adding distilled water to the sediment (1:2, v/v).

4.2.3. Analysis of vegetation.

Vegetation cover was measured from April to June 2009 on the same transects along which the abiotic environment had been sampled. We annotated plant species that appeared every 2 m, as well as their maximum height. Maximum height (measured as the entire extent of the longest stem) and standing height (maximum height of the plant above the ground without extending its stems) were measured for *S. perennis*. On the other hand, the number and the area occupied by *Z. noltii* patches colonizing the RM were recorded in the field in May 2010.

4.2.4. Statistical analysis.

Analyses were carried out using Sigma-Plot for Windows version 11.0. Deviations were calculated as the standard error (SEM). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene’s test ($P > 0.05$). The cover of the same species in different salt marsh areas was compared using a one-way Anova (analysis of variance) with Tukey’s Honestly Significant Difference (HSD) test as pos-hoc analysis. When homogeneity of variance or normality was not achieved, means were compared using a Kruskal–Wallis non-parametric Anova, with Bonferroni-Dunn’s test as pos-hoc analysis. A student *t*-test was used to compare *S. densiflora* height between RM and NRM. A Pearson correlation coefficient (r) was used to analyze relationships between abiotic and biotic variables.

4.3. RESULTS

4.3.1. Environmental matrix.

Elevation was similar (ca. +2.45 m SHZ) at each marsh area (Kruskal–Wallis, $P > 0.05$). PM showed lower sediment redox potential at surface and depth than RM and NRM (Surface: Kruskal–Wallis, $\chi^2 = 80{,}198$, $P < 0.001$, df = 2; Depth: Kruskal–Wallis, $\chi^2 = 77{,}919$, $P < 0.001$, df = 2). Redox potential at the surface was higher in NRM than in RM (Dunn's test, $P < 0.05$). Redox potential increased with elevation at the three *S. maritima* areas (Pearson correlation, $P < 0.05$). Sediment pH was higher for PM than for the other two marsh areas (Kruskal–Wallis, $\chi^2 = 41{,}116$, $P < 0.001$, df = 2; Dunn's test, $P < 0.05$). Conductivity increased with elevation in NRM (Pearson correlation, $r = 0.324$, $P < 0.05$), being higher than in PM, with RM showing intermediate values (Kruskal–Wallis, $\chi^2 = 22{,}153$, $P < 0.001$, df = 2; Dunn's test, $P < 0.05$) (Table 4.1).

	RM		**NRM**		**PM**	
Abiotic characteristics	*Mean ± SEM*	*max-min*	*Mean ± SEM*	*max-min*	*Mean ± SEM*	*max-min*
Elevation	2.40 ± 0.06^{a}	3.39 / 1.50	2.46 ± 0.06^{a}	3.39 / 1.72	2.56 ± 0.04^{a}	3.05 / 2.29
Redox (0-5 cm)	+16 ± 6^{a}	+194 / -180	-27 ± 6^{b}	+218 / -130	-82 ± 5^{c}	+45 / -210
Redox (5-10 cm)	-32 ± 8^{a}	+215 / -245	-65 ± 7^{a}	+225 / -201	-132 ± 6^{b}	+63 / -285
Conductivity	15.1 ± 0.7^{ab}	35.5 / 7.2	17.1 ± 0.3^{a}	21.1 / 12.7	15.4 ± 0.4^{b}	18.0 /12.4
pH	7.1 ± 0.0^{a}	8.0 / 6.6	7.2 ± 0.1^{a}	8.0 / 6.8	7.4 ± 0.0^{b}	7.7 / 7.1

Table 4.1. Elevation over Spanish Hydrographic Zero (m over SHZ), sediment redox potential at surface and depth (mV), conductivity (mS cm^{-1}) and pH in three *Spartina maritima* marsh areas (RM, restored mashes; NRM, non-restored marshes; PM, preserved marshes) in Odiel Marshes. Mean ± Standard Error Mean (SEM) and maximum and minimum values are presented. Different letters indicate significant differences between marsh areas (RM: n = 71; NRM: n = 42; PM: n = 30).

4.3.2. Analysis of vegetation.

RM showed the highest species richness (10 spp.) followed by NRM (6 spp.) and PM (3 spp.). Most of the surface of NRM was occupied by bare sediment (69 ± 2%), this being more abundant than at the other two marsh areas (ca. 33%) (Anova, $F = 21.423$, $P < 0.001$, df = 23; Tukey`s HSD test, $P < 0.05$) (Figs. 4.1 and 4.2).

Zostera noltii was much more abundant in PM (10 ± 1%) than in the other two *S. maritima* areas, where only a few isolated clumps were observed (Kruskal–Wallis, $\chi^2 = 11.120$, $P < 0.01$, df = 2; Dunn’s test, $P < 0.05$). In PM, *Z. noltii* colonized bare patches within *S. maritima* prairies (Figs. 4.1 and 4.2). At elevations lower than the lower distribution limit of *S. maritima* in RM (+1.6 m SHZ), *Z. noltii* increased its occupied area from 1.2 m^2 and 30 clumps (in six experimental plots) immediately after the restoration, to 333 m^2 and 253 clumps 40 months after transplanting, when the size of the *Z. noltii* patches was 1.32 ± 0.31 m^2. Each pilot transplant (20 cm quadrants) colonized 295 ± 74 cm^2 $month^{-1}$, increasing its area by 74 ± 19% per month.

Relative cover of *S. maritima* was similar in PM and RM (50 ± 3% and 42 ± 2%, respectively) and higher than in NRM (4 ± 2%) (Kruskal–Wallis, $\chi^2 = 16\ 676$, $P < 0.001$, df = 2; Dunn’s test, $P < 0.05$). In RM, *S. maritima* cover was lower where *S. perennis* and *S. densiflora* were more abundant (Pearson correlation, $r = -0.475$, $P < 0.001$; $r = -0.310$, $P < 0.05$, respectively), decreasing at higher elevations (Pearson correlation, $r = -0.295$, $P < 0.05$), with higher sediment redox potential (Pearson correlation, $r =-0.426$, $P < 0.01$) and higher conductivity (Pearson correlation, $r = -0.299$, $P < 0.059$). In contrast, *S. maritima* cover in NRM was greater at higher elevations (Pearson correlation, $r = 0.537$, $P < 0.01$), with higher redox potential (Pearson correlation, $r = 0.541$, $P < 0.01$) and it was independent of every abiotic factor in PM (Figs. 4.1 and 4.2). Tiller height of *S. maritima* in PM (40 ± 2 cm) was higher than in RM (34 ± 1 cm), with NRM showing intermediate values (35 ± 3 cm) (Anova, $F = 4.366$, $P < 0.05$, df = 195; Tukey’s HSD test, $P < 0.05$) (Fig. 4.3).

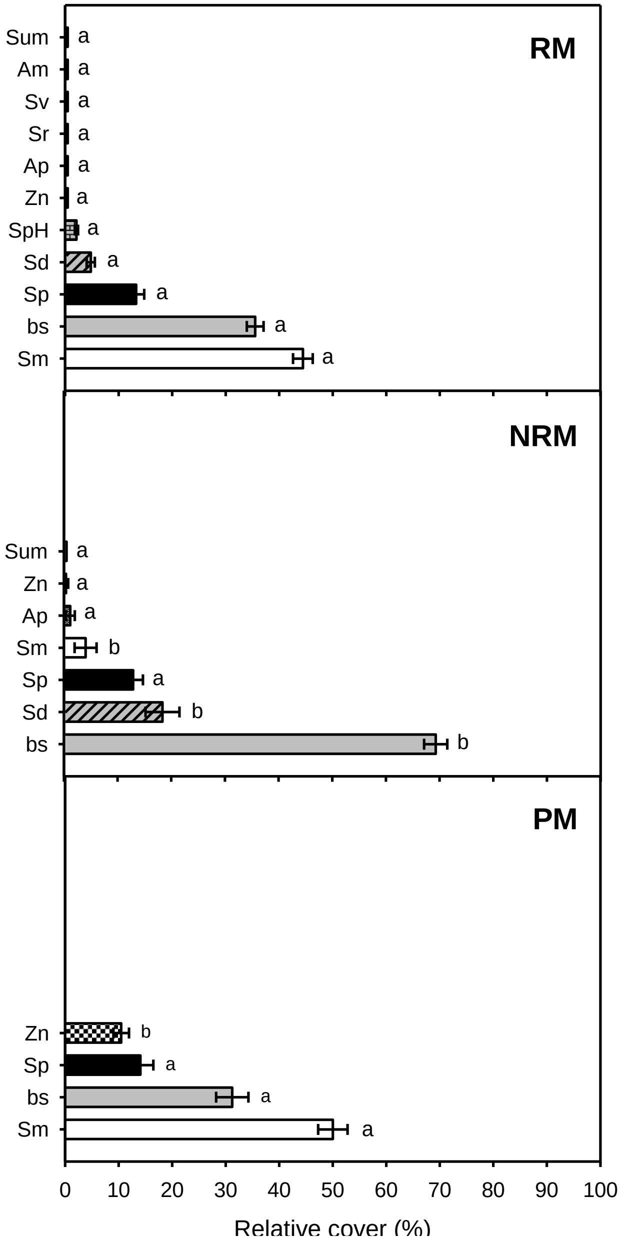

Figure 4.1. Relative coverage (%) of plant species in three *Spartina maritima* salt marsh areas in Odiel Marshes (SW Iberian Peninsula). Different letters indicate significant differences between the same species of different marshes (Anova, $P < 0.05$; Tukey's HSD test, $P < 0.05$ or Kruskal–Wallis, $P < 0.05$; Dunn's test, $P < 0.05$). Sm = *Spartina maritima*, bs = bare soil, Sp = *Sarcocornia perennis*, Sd = *Spartina densiflora*, SpH = *Sarcocornia perennis* x *fruticosa*, Zn = *Zostera noltii*, Ap = *Atriplex portulacoides*, Sr = *Salicornia ramosissima*, Sv = *Suaeda vera*, Am = *Arthrocnemum macrostachyum* and Sum. = *Suaeda maritima*.

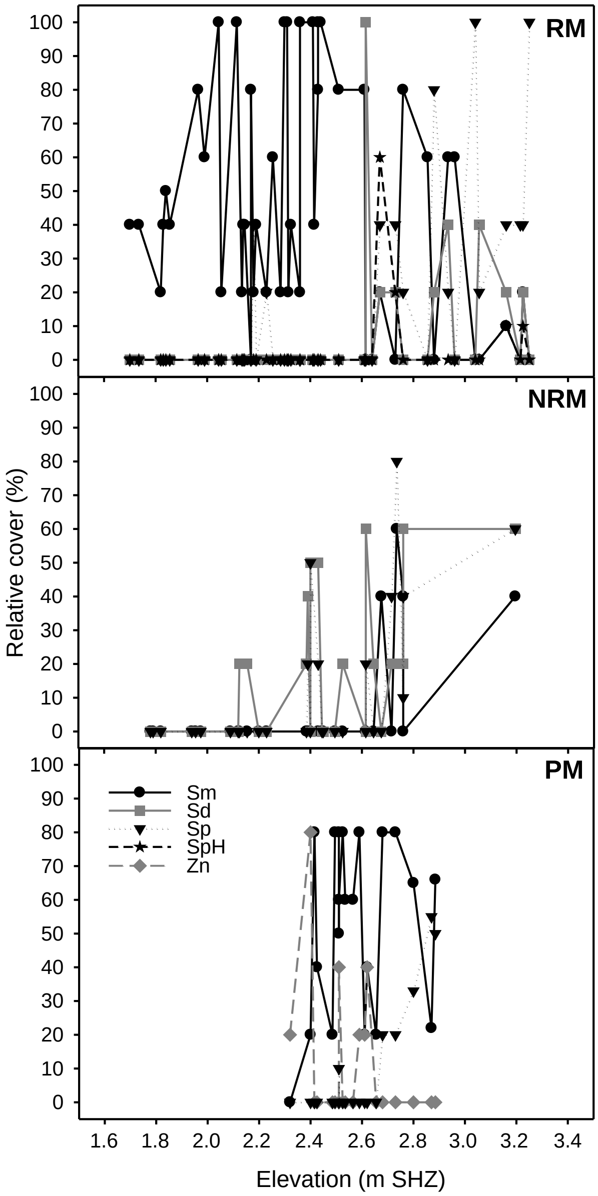

Figure 4.2. Relative cover (%) of more abundant halophytes along the intertidal gradient in three *Spartina maritima* marsh areas in Odiel Marshes (SW Iberian Peninsula). Sm = *Spartina maritima*, Sp = *Sarcocornia perennis*, Sd = *Spartina densiflora*, SpH = *Sarcocornia perennis* x *fruticosa*, Zn = *Zostera noltii*.

Sarcocornia perennis cover was similar in the three studied marshes (13–14%) (Kruskal–Wallis, P > 0.05), increasing with elevation and sediment redox potential at the three *S. maritima* areas (Pearson correlation, P < 0.01) (Figs. 4.1 and 4.2). Standing height of *S. perennis* clumps did not differ between marsh areas (Anova, P > 0.05), but the maximum length of extended stems was higher in RM than NRM (36 ± 3 cm and 22 ± 3 cm, respectively) (Kruskal–Wallis, $\chi^2 = 7.479$, P < 0.05, df = 2; Dunn's test, P < 0.05) (Fig. 4.3).

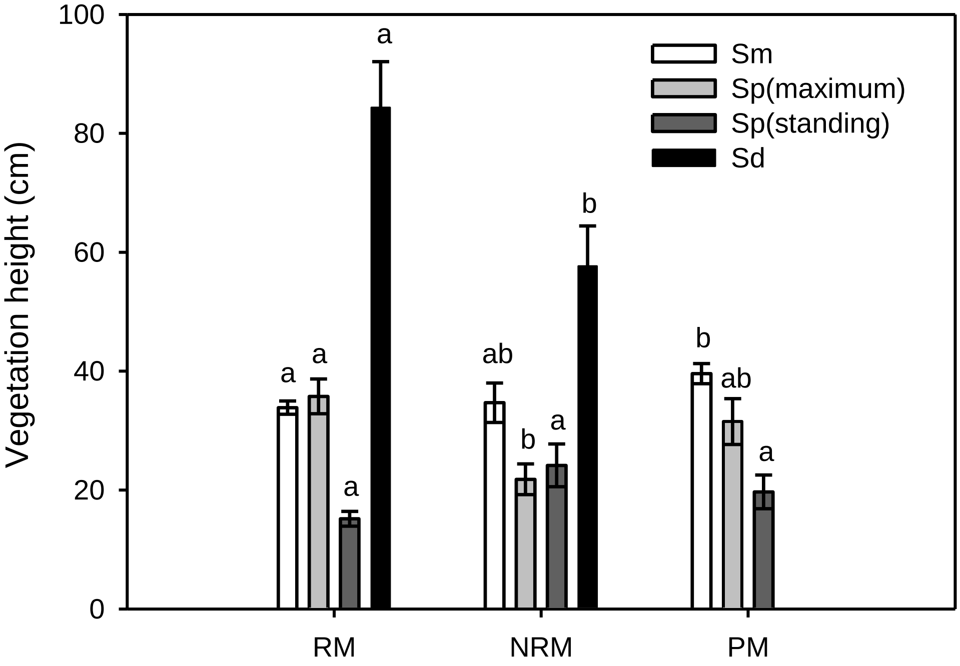

Figure 4.3. Maximum vegetation height of the dominants halophytes in three *Spartina maritima* marsh areas in Odiel Marshes (SW Iberian Peninsula). Different letters indicate significant differences between the same species of different marshes (Anova, P < 0.05; Tukey's HSD test, P < 0.05 or Kruskal–Wallis, P < 0.05; Dunn's test, P < 0.05). Sm = *Spartina maritima*, Sp (maximum) = *Sarcocornia perennis* with extended stems, Sp (standing) = *Sarcocornia perennis* without extended stems, Sd = *Spartina densiflora*.

Spartina densiflora presented its maximum cover in NRM (18 ± 3%), being very scarce in RM (5 ± 1%) and totally absent in PM (Anova, $F = 11.927$, P < 0.001, df = 23; Tukey`s HSD test, P < 0.05). *Spartina densiflora* cover increased at higher elevations, with higher sediment redox potential at RM and NRM (Pearson correlation, P < 0.05).

Thus, *S. densiflora* cover was usually lower than 30% at elevations below +2.6 m SHZ (Fig 4.2). The tallest *S. densiflora* tillers (84 ± 8 cm) were recorded in RM ($t = 2.605$, $P < 0.05$, df = 36) (Fig. 4.3).

The hybrid *Sarcocornia perennis* x *fruticosa* (Figueroa *et al*., 2003) colonized only RM. *Atriplex portulacoides* L. and *Suaeda maritima* (L.) Dumort. were recorded in RM and NRM with a relative cover lower than 1%, and *Arthrocnemum macrostachyum* (Moric.) Moris., *Salicornia ramosissima* J. Woods and *Suaeda vera* Forsskal ex J.F. Gmelin colonized higher elevations only in RM (Fig. 4.1).

4.4. DISCUSSION

Restored *S. maritima* salt marshes presented higher species richness (10 spp.) than NRM (6 spp.) and PM (3 spp.), due to milder abiotic conditions and higher habitat diversity. Native halophytes such as the hybrid *S. perennis x fruticosa*, *A. portulacoides* and *Suaeda maritima*, and the invasive cordgrass *S. densiflora*, were colonizing both RM and NRM with more oxygenated sediments (higher redox potential) than PM. These species show a low tolerance to anoxic sediments (Sánchez *et al*., 1998; Tessier *et al*., 2000; Figueroa *et al*., 2003; Mateos-Naranjo *et al*., 2008). The lower redox potentials recorded for PM in comparison with PM and NPM, even with similar elevations over SHZ, seemed to be related with their physiographic location, since PM was located in a coastal lagoon with a lower drainage capacity than the channel banks in which RM and NRM were situated. This provoked a narrower plant distribution range along the tidal gradient in PM than in NRM, and especially in RM. On the other hand, high marsh halophytes (*A. macrostachyum* and *S. vera*) were also colonizing RM in sandy patches at the highest elevations.

Zostera noltii transplants increased their cover by 74 ± 19% per month below the lower *S. maritima* distribution limit in RM. Little is known about the use of *Z. noltii* in salt marsh restoration projects (Martins *et al*., 2005; Katwijk *et al*., 2009). Much lower growth rates than in our study were recorded for *Zostera marina* L. plantations on the northeast coast of the United States (Orth *et al*., 1999; Leschen *et al*., 2010). In addition to its vegetative expansion by rhizomes, *Z. noltii* was expanding actively by seeds, probably produced within RM since, prior to planting, *Z. noltii* was not colonizing the restored area and its seeds dispersed only up to a few meters under the influence of currents, becoming rapidly incorporated into the sediment (Orth *et al*., 1994). Therefore, our positive results with *Z. noltii* transplants in the Gulf of Cadiz should be considered for future restoration projects using this red list seagrass.

RM using *S. maritima* (planted at a density of 1 clump m^{-2}) achieved similar relative cover to PM (ca. 50%) 2.5 yr after transplanting. This result is in accordance with a pilot study that recorded *S. maritima* plantations achieving relative cover of about 50% after ca. 3 yr in the Odiel Marshes (Castillo *et al*., 2008a). On the west coast of North America, *Spartina foliosa* Trin. and *Salicornia virginica* L. covered more than 50% of diked low salt marshes 4 yr after reopening them to tidal influence (Williams & Orr, 2002), and *S. alterniflora* plantations in China (with clumps spaced 50 cm apart from each other) coalesced after 1 yr (Chung, 1993). On the other hand, *S. maritima* reached higher tiller height in PM (40 ± 2 cm) than in RM (34 ± 1 cm), which could be related to a lower sediment redox potential in PM both at the surface and at depth, since *S. maritima* grows taller in more anoxic sediments (Castillo *et al*., 2005).

Sarcocornia perennis cover was similar in the three *S. maritima* areas, being concentrated at higher elevations in the tidal gradient than were the zones less affected by erosion in NRM (Castillo *et al*., 2002). In spite of this, *S. perennis* showed shorter stems, a sign of plant vigor (Neckles *et al*., 1999), in NRM than in RM and PM, being always within the range reported by Davy *et al*. (2006). *Sarcocornia perennis* was absent from low elevations with low redox potential, as described by Castellanos *et al*. (1994). *Spartina maritima* acts as a sediment trap (Moeller *et al*., 1996; Castillo *et al*., 2002) facilitating the colonization and the growth of *S. perennis* (Castellanos *et al*., 1994), a process recorded at RM and PM. In addition, the perennial glasswort facilitates the colonization of the hybrid *S. perennis x fruticosa*, explaining its presence at higher elevation in RM (Figueroa *et al*., 2003).

The cover of *S. densiflora* in RM was lower than in NRM since it was eliminated manually from 2.00 ha during restoration (Castillo & Figueroa, 2009a) and it was absent from PM, probably due to its anoxic sediments. *Spartina densiflora* shows lower tolerance to low marsh abiotic environment than *S. maritima* (Castillo *et al*., 2000), which was reflected in lower coverage and shorter tillers in more anoxic sediments. *Spartina densiflora* establishes biennial populations with low density of clumps with short tillers in low marshes (Nieva *et al*., 2005; Castillo & Figueroa, 2009b): our results agreed with this. Furthermore, healthy prairies of *S. maritima* seem to limit *S. densiflora* establishment in low marshes (Castillo *et al*., 2008b), an inter-specific competition process that seemed to be operating in RM.

Our results showed the success, from the point of view of vegetation development, of the restoration methodology described by Castillo & Figueroa (2009a) for European low salt marshes. Thus, *Z. noltii*, *S. maritima* and *S. perennis* plantations developed correctly 2.5 yr after restoration, creating the typical zonation pattern of the Gulf of Cadiz.

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CHAPTER 5

Nitrogen sequestration capacity of restored European salt marshes using *Spartina maritima* and *Sarcocornia perennis*

CAPÍTULO 5.- Capacidad de secuestro de nitrógeno por marismas europeas restauradas empleando *Spartina maritima* y *Sarcocornia perennis*.

RESUMEN

Este capítulo cuantifica la reserva y la tasa de acumulación de nitrógeno en sedimentos y en las halófitas predominantes (*Spartina maritima*, *Spartina densiflora* y *Sarcocornia perennis*) en marismas costeras restauradas en el estuario conjunto del los ríos Tinto y Odiel, donde se han documentados altos niveles de eutrofización. El contenido de N en el sedimento fue similar en superficie (0-2 cm: 1.6 ± 0.3 mg N g^{-1} PS) y en profundidad (2-20 cm: 2.1 ± 0.2 mg N g^{-1} PS). *Spartina densiflora* mostró el mayor contenido de nitrógeno en cada órgano, excepto para las hojas de *S. maritima*, que mostró valores similares (30 ± 1 y 31 ± 1 mg N g^{-1} PS, respectivamente). *Spartina maritima* y *S. perennis* concentraron más nitrógeno en sus órganos fotosintéticos (31 ± 1 mg N g^{-1} PS y 26 ± 1 mg N g^{-1} PS, respectivamente) que en los otros órganos. La reserva de nitrógeno en el sedimento fue de 329.0 g N m^{-2} en las áreas colonizadas por *S. maritima* y 373.1 g N m^{-2} en las áreas colonizadas por *S. perennis* en los primeros 20 cm. 2.8 t N y 0.1 t N fueron acumuladas anualmente por sedimentación en las áreas de *S. maritima* (8.37 ha) y en las áreas de *S. perennis* (1.78 ha). Los tallos de *S. maritima* acumularon más nitrógeno (23 ± 4 g N m^{-2}) que los otros órganos, siendo las raíces las que mostraron la menor acumulación (4 ± 1 g N m^{-2}). Veintiocho meses después de los trasplantes, *S. maritima* colonizó 8.37 ha con una cobertura relativa del 62 ± 6%, acumulando cerca de 2.5 t N. Los tallos fotosintéticos de *Sarcocornia* acumularon más nitrógeno (22 ± 1 g N m^{-2}) que los tallos no fotosintéticos y una cantidad similar a las raíces. *Sarcocornia* ocupó 1.78 ha con una cobertura relativa del 69 ± 13%, acumulando 0.7 t N. La reserva total de nitrógeno en 10.15 ha de las marismas restauradas colonizadas por *S. maritima* y *S. perennis* incluyendo sus sedimentos en los 20 primeros centímetros de profundidad fue de 37.3 t N (3.7 t N ha^{-1}; 367 g m^{-2}), con el 7.3 % secuestrado en la hierba salada y el 2.1% en la sosa de las salinas.

CHAPTER 5.- Nitrogen sequestration capacity of restored European salt marshes using *Spartina maritima* and *Sarcocornia perennis*.

ABSTRACT

Salt marshes have been identified as strategically located nutrient sinks, fighting eutrophication in estuaries and coastal waters. This study quantifies the nitrogen-stock and the nitrogen accumulation rate in the sediments and in the dominant halophytes (*Spartina maritima*, *Spartina densiflora* and *Sarcocornia perennis*) in restored salt marshes in the joint estuary of the Odiel and Tinto rivers, where high levels of eutrophication have been documented. Sedimentary N content was similar at the surface (0–2 cm: 1.6 ± 0.3 mg N g^{-1} DW) and at depth (2–20 cm: 2.1 ± 0.2 mg N g^{-1} DW). *Spartina densiflora* showed the highest nitrogen content for every organ, except for the leaves of *S. maritima* that presented similar values (30 ± 1 and 31 ± 1 mg N g^{-1} DW, respectively). *Spartina maritima* and *S. perennis* concentrated more nitrogen in their photosynthetic organs (31 ± 1 mg N g^{-1} DW and 26 ± 1 mg N g^{-1} DW, respectively) than in the other organs. Sediment nitrogen-stock was 329.0 g N m^{-2} for *S. maritima* areas and 373.1 g N m^{-2} for *S. perennis* areas in the first 20 cm of sediment. 2.8 t N and 0.1 t N were accumulated annually by sedimentation in *S. maritima* areas (8.37 ha) and in *S. perennis* areas (1.78 ha). *Spartina maritima* stems accumulated more nitrogen (23 ± 4 g N m^{-2}) than its other organs, with the roots having the lowest N accumulation (4 ± 1 g N m^{-2}). Twenty-eight months after transplanting, *S. maritima* colonized 8.37 ha with a relative cover of 62 ± 6%, accumulating ca. 2.5 t N. *Sarcocornia* photosynthetic stems accumulated more nitrogen (22 ± 1 g N m^{-2}) than non-photosynthetic stems, and a similar quantity to roots. *Sarcocornia* occupied 1.78 ha with a relative cover of 69 ± 13%, accumulating 0.7 t N. Total nitrogen-stock in 10.15 ha of the restored salt marshes colonized by *S. maritima* and *S. perennis*, including their sediments in the first 20 cm deep, was 37.3 t N (3.7 t N ha^{-1}; 367 g m^{-2}), with 7.3% stored in the small cordgrass and 2.1% in the perennial glasswort.

5.1. INTRODUCTION

Eutrophication is an important conservation problem in coastal ecosystems worldwide, altering habitats, energy fluxes, trophic networks and community composition (de Jonge *et al*., 2002; Bertness *et al*., 2007; Caffrey *et al*., 2007). In this context, salt marshes have been identified as strategically located nutrient sinks at the mouth of rivers, fighting eutrophication in estuaries and coastal waters (Adam, 1990; Romero *et al*., 1999; Castro *et al*., 2009).

Cordgrasses (*Spartina* Genus, Poaceae) are one of the most extended group of halophytes in salt marshes around the world. *Spartina alterniflora* Loisel. is very efficient in its uptake of nitrogen, its marshes reducing water eutrophication in its North American native distribution range (Valiela & Teal, 1974; Patrick & DeLaune, 1976; White & Howes, 1994; McFarlin *et al*., 2008) and as an invasive species in China (Wan *et al*., 2009; Zhang *et al*., 2009). In European salt marshes, it has also been described that natural populations of the native small cordgrass, *Spartina maritima* (Curtis) Fernald, fights eutrophication through N accumulation (Caçador *et al*., 2007; Sousa *et al*., 2008; Castro *et al*., 2009). However, no study has analyzed the capacity of *S. maritima* plantations to accumulate nitrogen in restored and expanding marshes.

This study quantifies the nitrogen-stock and the nitrogen accumulation rate in the sediments and in the dominant halophytes (*S. maritima* (Poaceae) and *Sarcocornia perennis* (Mill.) Scott spp. *perennis* (Chenopodiaceae)) in restored salt marshes in the joint estuary of the Odiel and Tinto rivers in the south-west Iberian Peninsula (Castillo & Figueroa, 2009), where high levels of eutrophication have previously been documented (Elbaz-Poulichet *et al*., 1999). With this aim, biomass accumulation, sedimentation rate and nitrogen content for sediments and the dominant native halophytes *S. maritima* and *S. perennis* ssp. *perennis* (Davy *et al*., 2006) and the invasive *Spartina densiflora* Brongn., were recorded.

5.2. MATERIALS AND METHODS

5.2.1. Study site.

Our work was carried out in a restored salt marsh area (Chapter 2).

5.2.2. Sediment and vegetation sampling.

Sediment elevation relative to Spanish Hydrographic Zero (SHZ), redox potential, pH, electrical conductivity of the interstitial water of the sediments, organic matter content and bulk dry density were recorded in *S. maritima* (n = 20) and *S. perennis* (n = 6) areas in May–June 2009. Each sediment characteristic was recorded between 0 and 10 cm deep, except for redox potential which was sampled at the surface (0–2 cm) and at depth (2–10 cm). Elevation was surveyed to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore); reference points were determined in relation to measurements of tidal extremes (Ranwell *et al*. 1964). Redox potential of the sediment was determined *in situ* with a portable meter and electrode system (pH/mV *Crison* p-506). pH was recorded in the laboratory (pH/rédox *Crison* with the electrode *M-506*) after adding distilled water to the sediment (1:1, v/v). Electrical conductivity was recorded together with pH (conductivity meter, *Crison*-522) (1:2, v/v). Sediment organic content was analyzed in triplicate sub-samples by the loss-on-ignition method after 4 h at 450 ºC. Sediment bulk dry density was recorded by weighing (DW) the volume of sediments in a 5 x 5 cm cylindrical core. In addition, sedimentation rate was determined by markers consisting of an iron structure with two vertical posts (ca. 1.5 m tall and 1 cm diameter) inserted in the sediment to a depth of around 1 m in *S. maritima* and *S. perennis* areas and supporting a horizontal crossbar (ca. 0.5 m long). The distance from the middle point of the crossbar to the sediment surface was measured quarterly from March 2009 to March 2010 (n = 9). This method prevented measurement errors due to the erosive vortex generated at the base of the vertical markers.

Plant material (*S. maritima*, *S. densiflora* and *S. perennis*) and sediment samples (between 0–2 cm and 2–20 cm deep) for N content analysis were collected in October 2009 along two 10-m long rows established parallel to the tidal line between +2.16 and +2.67 m SHZ (n = 10; 5 equidistant sampling points per row). Superficial sediments (0–2 cm deep) were collected using nylon horizons set up six months earlier to ensure they were recently deposited.

Plant cover was recorded in May–June 2009 by contact every 2 m along nine 60 to 80-m long transects established perpendicular to the tidal line from the lower distribution limit of *S. maritima* to the upper border of the marsh (n = 9). The relative cover for each taxon was calculated in relation to the area that it colonized. *Spartina maritima* and *S. perennis* above- and below-ground biomass (BGB and AGB, respectively) were recorded in totally colonized 10-cm quadrant plots in October 2009 (n = 10).

5.2.3. Nitrogen content analysis.

Once in the laboratory, biomass was washed carefully, separated into photosynthetic organs (leaves for cordgrasses and stems for *Sarcocornia*), non-photosynthetic stems, roots and rhizomes (only for cordgrasses). Sediments and plant samples were dried to constant dry weight (DW) at 80 °C for 48 hours. Them, plant material for biomass recording was weighed (*Pinnacle* P-403, Denver Instrument Company, Denver, USA) and sediment and plant samples for N content determination were pulverized using a grinder (*Cyclotec, Foss Tecator AB,* Höganäs, Sweden) and sieved through an 80 µm light. Total N content was determined (in mg g^{-1} DW) for undigested samples using an elemental analyzer (*Leco* CHNS-932, Spain). The value for each sample corresponded to the mean of three replicated measurements.

5.2.4. Nitrogen accumulation rate and stock.

Sediment nitrogen-stock was calculated for each of two depths (0–2 cm and 2–20 cm) as the product of the mass of sediment (obtained as the product of the bulk density and the total volume (area x depth)) and the sediment nitrogen content. Nitrogen-stock was calculated for every taxon and organ as the product of their mean biomass density (biomass x occupied area x relative cover) and its nitrogen content. Annual accumulated N in *S. maritima* biomass was calculated taking into consideration both net annual standing above- and below-ground productivity (NAPP or NBPP), and its N contents. Annual accumulation of N in the sediments was calculated multiplying the annual sedimentation rate and the sediment N content in the upper 2 cm. NAPP and NBPP were calculated as the total AGB or BGB, respectively, divided by years since transplantation. Sampling plots were located in areas with bare sediments adjacent to clumps just after transplanting, to ensure that all the standing biomass was effectively produced *in situ* after restoration plantings (Castillo *et al*., 2008 a). No evidence of herbivory was observed during the study.

5.2.5. Statistical analysis.

Analyses were carried out using SPSS release 14.0 (SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene's test ($P > 0.05$). When homogeneity of variance between groups was not found, data were transformed using the following functions: ln(x), 1/x and √x. A Student's *t*-test for independent samples was applied to compare two means. If homogeneity of variance or normality were not achieved by data transformation, then means were compared using a Mann–Whitney *U*-test. Variation in N content between organs was compared by one-way Anova (analysis of variance) or by a Kruskal–Wallis non parametric Anova. Tukey's HSD test between means was calculated only if an *F*-test was significant ($P < 0.05$).

5.3. RESULTS

5.3.1. Sediment characterization

Sediment elevation was +2.28 ± 0.06 m SHZ in *S. maritima* areas and +2.92 ± 0.07 m SHZ in *S. perennis* areas (t = -5.123, P < 0.001, df = 24). pH was always close to neutrality and electrical conductivity varied between ca. 8 and 35 mS cm^{-1}, both being sediment characteristics higher in *S. perennis* than in *S. maritima* areas (pH: t = -2.300, P < 0.05, df = 24; conductivity: U = 9.000, P < 0.01). Sediment redox potential at the surface varied between -5 ± 18 mV in *Spartina* areas and +118 ± 11 mV in *Sarcocornia* areas (*t*-test, P > 0.05). Redox potential at depth was also significantly lower in *S. maritima* than in *S. perennis* areas (t = -3.508, P < 0.01, df = 24). Sediment bulk dry density was ca. 0.85 g cm^{-3}, organic content varied between 4.6 ± 0.7% for *Spartina* areas and 6.1 ± 1.8% for *Sarcocornia* areas (*t*-test, P > 0.05). The sedimentation rate was 2.6 ± 0.3 cm year^{-1} in *Spartina* areas and 0.2 ± 1.3 cm year^{-1} in *Sarcocornia* areas (Table 5.1).

	Spartina maritima **areas**		***Sarcocornia perennis*** **areas**	
Abiotic variables	mean ± SEM	max-min	mean ± SEM	max-min
Elevation relative to SHZ	2.28 ± 0.06a	2.72 / 1.78	2.92 ± 0.07b	3.15 / 2.67
Redox potential (0-2cm)	-5 ± 18a	164 / -117	118 ± 11a	152 / 73
Redox potential (2-20cm)	-44 ± 20a	172 / -171	90 ± 29b	182 / -35
Conductivity	15.2 ± 1.5a	33.7 / 8.3	19.9 ± 4.2b	35.5 / 11.2
Organic matter content	4.6 ± 0.7a	9.18 / 0.31	6.1 ± 1.8a	13.10 / 1.7
pH	7.1 ± 0.1a	7.7 / 6.6	7.3 ± 0.1b	7.9 / 7.1
Bulk density	0.80 ± 0.06a	1.43 / 0.43	0.91 ± 0.16a	1.26 / 0.25
Sedimentation rate	2.6 ± 0.3a	4.1 / 1.6	0.2 ± 1.3a	2.4 / -3.5

Table 5.1. Elevation above Spanish Hydrographic Zero (m), sediment redox potential (mV), sediment conductivity (mS cm^{-1}), pH, sediment organic content (%), bulk density (g cm^{-3}) and sedimentation rate (cm year^{-1}) in the vegetated area of restored salt mash in the Odiel Marshes, south-west Iberian Peninsula (n = 5–20). Different letters indicate significant differences between areas colonized by different species.

5.3.2. Nitrogen content in sediments and vegetation.

Sediment N content was similar at the surface (0-2 cm: 1.6 ± 0.3 mg N g^{-1}) and at depth (2-20 cm: 2.1 ± 0.2 mg N g^{-1}) (*U*-test, $P > 0.05$), varying between 0.8–3.1 mg N g^{-1}.

Spartina densiflora showed the highest N content for each organ, except for the leaves of *S. maritima*, which presented similar values (photosynthetic organs: Kruskal–Wallis test, $\chi^2 = 6.139$, $P < 0.05$, df = 2; stems: Anova, $F = 39.324$, $P < 0.001$, df = 29; roots: Anova, $F = 4.683$, $P < 0.05$, df = 27; *Spartina* rhizomes: $t = -7.202$, $P < 0.001$, df = 17). Nitrogen content in photosynthetic organs and non-photosynthetic stems was higher for *S. maritima* than for *S. perennis* (Tukey's test, $P < 0.05$) (Table 5.2).

Spartina maritima and *S. perennis* concentrated more N in their photosynthetic organs (31 ± 1 mg N g^{-1} and 26 ± 2 mg N g^{-1}, respectively) than in the other organs (*S. maritima*: Anova, $F = 19.415$, $P < 0.001$, df = 39; *S. perennis*: Kruskal–Wallis test, $\chi^2 = 9.502$, $P < 0.01$, df = 2). In contrast, *S. densiflora* showed its highest N content in its rhizomes (36 ± 1 mg N g^{-1}) (Anova, $F = 19.302$, $P < 0.001$, df = 37) (Table 5.2).

5.3.3. Nitrogen accumulation and stock.

Sediment nitrogen-stock of the total vegetated area was 2.7 t N between 0–2 cm deep, and 31.4 t N between 2–20 cm deep, corresponding to 329.0 g N m^{-2} in *S. maritima* areas and 373.1 g N m^{-2} in *S. perennis* areas in the first 20 cm of sediment. 2176 m^3 of sediments were accumulated annually in areas colonized by *S. maritima* (8.37 ha) and 36 m^3 in areas colonized by *S. perennis* (1.78 ha), representing an annual accumulation of 2.8 t N and 0.1 t N, respectively.

	Species	mg N g^{-1}
Photosynthetic organs	Sm	$31 \pm 1^{a/a}$
	Sd	$30 \pm 1^{a/ab}$
	Spp	$26 \pm 2^{a/b}$
Stems	Sm	$24 \pm 1^{b/a}$
	Sd	$31 \pm 1^{a/b}$
	Spp	$18 \pm 1^{b/c}$
Roots	Sm	$19 \pm 1^{c/a}$
	Sd	$23 \pm 1^{b/b}$
	Spp	$19 \pm 1^{b/a}$
Rhizomes	Sm	$22 \pm 1^{bc/a}$
	Sd	$36 \pm 1^{c/b}$

Table 5.2. Nitrogen content for photosynthetic organs (leaves or stems), non-photosynthetic stems, roots and rhizomes of *Spartina maritima* (Sm), *Spartina densiflora* (Sd) and *Sarcocornia perennis* (Sp) in a restored salt marsh in the Odiel Marshes. Different letters indicate significant differences between organs for the same species (first coefficient) and between species for the same organ (second coefficient) ($P < 0.05$).

Total biomass of *S. maritima* in totally colonized areas 28 months after restoration was ca. 2 kg m^{-2}, accumulating more biomass in aerial shoots (935 ± 145 g DW m^{-2}) than in rhizomes (424 ± 60 g DW m^{-2}), leaves (356 ± 53 g DW m^{-2}) or roots (192 ± 44 g DW m^{-2}) (Kruskal–Wallis, $\chi^2 = 24.905$, $P < 0.05$). Relating these biomass data with the respective N contents, stems of *S. maritima* accumulated more N (23 ± 4 g N m^{-2}) than its other organs, with the lowest N accumulation in the roots (4 ± 1 g N m^{-2}) (Kruskal–Wallis, $\chi^2= 28.143$, $P < 0.001$, df = 3). Twenty-eight months after transplanting, the small cordgrass colonized 8.37 ha with a relative cover of 62 ± 6%, accumulating ca. 2.5 t N; ca. 50% of this N was stored in stems. Small cordgrass prairies showed a NAPP of 553 ± 83 g DW m^{-2} yr^{-1} and a NBPP of 264 ± 42 g DW m^{-2} yr^{-1}, corresponding both together with N accumulation of ca. 20 g N m^{-2} yr^{-1} (Table 5.3).

Total biomass of *S. perennis* was ca. 3 kg m^{-2}. Perennial glasswort accumulated similar AGB to *S. maritima* (Sp: 1576 ± 102 g m^{-2}; Sm: 1290 ± 194 g m^{-2}; *t*-test, $P > 0.05$) and more BGB (Sp: 1234 ± 227 g m^{-2}; Sm: 616 ± 98 g m^{-2}; $U = 18.000$, $P < 0.05$). No significant differences were recorded in biomass accumulation between organs of

Sarcocornia, varying between 748 ± 93 g DW m^{-2} for non-photosynthetic stems and 1285 ± 235 g DW m^{-2} for roots (Kruskal–Wallis, $P > 0.05$). Relating these biomass data with its N contents, *Sarcocornia* photosynthetic stems accumulated more N (22 ± 1 g N m^{-2}) than non-photosynthetic stems, and a similar quantity to roots (Kruskal–Wallis, $\chi^2 = 6.305$, $P < 0.05$, df = 2). *Sarcocornia* occupied 1.78 ha, with a relative cover of 69 ± 13%, accumulating 0.7 t N; ca. 80% of this nitrogen-stock was stored in roots and photosynthetic stems (Table 5.3).

	Biomass	**N-stock**	**Total N-stock**
Spartina maritima			
Leaves	356 ± 53^{a}	11 ± 2^{a}	601 ± 89^{a}
Tillers	935 ± 145^{b}	23 ± 4^{b}	1231 ±190^{b}
Rhizomes	424 ± 60^{a}	9 ± 1^{a}	504 ±71^{a}
Roots	192 ± 44^{c}	4 ± 1^{c}	196± 45^{c}
Total	**1906 ± 283**	**47 ± 7**	**2532 ± 374**
Sarcocornia perennis			
Photosinthetic stems	838 ± 46^{a}	22 ± 1^{ab}	268 ± 15^{ab}
Non-photosinthetic stems	739 ± 85^{a}	13 ± 2^{b}	163 ± 19^{b}
Roots	1234 ± 227^{a}	23 ± 4^{a}	288 ± 53^{a}
Total	**2811 ± 301**	**59 ± 6**	**719 ± 72**

Table 5.3. Biomass (dry weight), nitrogen-stock (g m^{-2}) and total nitrogen-stock (kg) (with 62% relative cover for *Spartina* and 69% for *Sarcocornia*, 28 months after transplanting) for *Spartina maritima* in 8.37 ha and for *Sarcocornia perennis* in 1.78 ha at a restored salt marsh area in the Odiel Marshes. Different letters indicate significant differences between organs for the same species (first coefficient) and between species for the same organ (second coefficient) ($P < 0.05$).

Twenty-eight months after transplanting, total nitrogen-stock in 10.15 ha of the restored salt marshes colonized by *S. maritima* and *S. perennis*, including their sediments in the first 20 cm depth, was 37.3 t N (3.7 t N ha^{-1}; 367 g m^{-2}), with 7.3% stored in the small cordgrass and 2.1% in the perennial glasswort. 2.9 t N were being added annually by deposition of sediments and ca. 1.0 t N was sequestrated by *Spartina* colonization.

5.4. DISCUSSION

This study shows the capacity of European restored expanding salt marshes that have been planted with small cordgrass and perennial glasswort, to reduce eutrophication through N sequestration.

Sediment N content was within the range reported previously for bare sediments in Portuguese *S. maritima* marshes (1.8 mg N g^{-1}) (Castro *et al*., 2009). Instead, higher N contents have been recorded in sediments colonized by the small cordgrass (between ca. 3–7 mg N g^{-1}) (Caçador *et al*., 2007; Castro *et al*., 2009). In this sense, sediment organic content was ca. 5%, while values ca. 11% have been recorded previously for other *S. maritima* marshes (Lillebø *et al*., 2006; Castillo *et al*. 2008a). However, our sediment N contents were higher than those obtained for Dutch marshes of *Zostera marina* L. (ca. 1 mg N g^{-1} following van Lent & Verschuure, 1994) and comparable to those reported for *S. alterniflora* marshes in USA (Tyler *et al*., 2003). Sediment nitrogen-stock in *S. maritima* and *S. perennis* restored marshes (329.0 g N m^{-2} and 373.1 g N m^{-2}, respectively) was similar to that recorded for *Spartina patens* (Aiton) Muhl. and *S. alterniflora* in USA marshes (337-379 g N m^2) (Elsey-Quirk *et al*., 2011) and lower than those reported for *Sarcocornia* marshes (1.02-1.23 kg N m^2) in the Gulf of Cádiz (Palomo & Niell, 2009).

Spartina maritima AGB values in fully colonized patches 28 months after transplanting (ca. 1300 g DW m^{-2}) were similar to those recorded previously for natural populations in the Iberian Peninsula (Benito & Onaindia, 1991; Cartaxana & Catarino, 1997; Caçador *et al*. 1999; Castillo *et al*., 2008a.b; Castro *et al*., 2009), as expected since created populations develop similar AGB to natural populations in about two years (Castillo *et al*. 2008a). Nitrogen content in *S. maritima* leaves and shoots were higher than those recorded in Portuguese natural populations in the same season (leaves: ca. 30 mg N g^{-1} *vs* ca. 20 mg N g^{-1}; shoots: ca. 24 mg N g^{-1} *vs* ca. 14 mg N g^{-1}; Caçador *et al*., 2007; Sousa *et al*., 2008; Castro *et al*., 2009) and than those recorded for *S. alterniflora* in USA marshes (10 mg N g^{-1} following Patrick & DeLaune (1976) and McFarlin *et al*. (2008)), and in Chinese marshes (12 mg N g^{-1} following Liao *et al*. (2007)). Nitrogen standing stock for *S. alterniflora* AGB in marshes with similar AGB, but much lower N content than found in our study, was 9 g N m^{-2} (Darby & Turner, 2008), lower than our results (34 g N m^{-2}). *Spartina patens*, and even *Phragmites australis* (Cav.) Trin. ex Steudel, in coastal marshes of the Eastern United States also showed lower nitrogen standing stock (11 g N m^{-2}) than *S. maritma* restored marshes (Windham & Ehrenfeld, 2003). Elsey-Quirk *et al*. (2011) describe an above-ground nitrogen-stock in halophytes such as *Juncus roemerianus* Scheele, *S. patens* and *S. alterniflora* of 16.0, 8.7 and 5.4 g N m^{-2}, respectively, also lower than our results. Hence, *S. maritima* AGB in the Odiel Marshes captured large amounts of N compared with other halophytes.

Small cordgrass plantations need between 2.5–4.0 yr, depending on the sedimentation dynamic, to develop similar BGB to natural populations (Castillo *et al*., 2008a). Thus, *S. maritima* BGB (ca. 620 g m^{-2}) in the restored marshes (2.5 yr old) was much lower than that recorded in matured populations (ca. 2500–4800 g m^{-2}) (Cartaxana & Catarino, 1997; Castillo *et al*., 2008a,b; Caçador *et al*., 2009). Nitrogen content in roots and rhizomes of *S. maritima* (19 and 22 mg N g^{-1}, respectively) was slightly higher than that reported for Portuguese natural marshes (between ca. 12–16 mg N g^{-1}) (Caçador *et al*., 2007) and much higher than those reported for *Spartina foliosa* Trin. in created and natural marshes in California (ca. 8 and 11 mg N g^{-1}, respectively), with lower sediment N contents (ca. 0.8–1 mg N g^{-1}) (Boyer *et al*., 2000) and *S. alterniflora* invaded marshes in China (6.1 mg N g^{-1}) (Liao *et al*., 2007). Darby & Turner (2008) described a higher BGB nitrogen-stock for *S. alterniflora* marshes than in our restored marshes (67.9 g N m^{-2} and 13 g N m^{-2}, respectively), due to much higher BGB. However, *S. patens* and *P. australis* marshes in USA showed similar, and even lower, BGB nitrogen-stock than *S. maritima* restored marshes (maximum values of ca. 8.0 g N m^{-2} and 9.5 g N m^{-2}, respectively) (Windham & Ehrenfeld, 2003). Elsey-Quirk *et al*. (2011) described BGB nitrogen-stock of *J. roemerianus*, *S. patens* and *S. alterniflora* of ca. 10 g N m^{-2}, similar to our results. Liao *et al*. (2007) reported a mean annual total nitrogen-stock for *S.*

alterniflora stands of 46.49 g N m^{-2}, very similar to our results for *S. maritima* total N stock (47 ± 7 g N m^{-2}).

Based on our results, it is not possible to identify the environmental factors determining the recorded interpopulation differences in N content. On one hand, N bioavailability in the sediments is a key factor determining N acquisition by halophytes (Darby & Turner, 2008). On the other hand, N content depends on the age of the population, since it has been reported that young populations of *S. maritima* show higher N contents in their AGB than more mature populations (Caçador *et al*., 2007).

Spartina maritima NAPP (ca. 550 g DW m^{-2} yr^{-1}) was within the wide ranges recorded in natural populations (120–2800 g DW m^{-2} yr^{-1} following Sousa *et al*., 2010a,b) and in created marshes (131–590 g DW m^{-2} yr^{-1} following Castillo *et al*., 2008a). *Spartina maritima* NBPP (ca. 260 g DW m^{-2} year^{-1}) was lower than in natural populations (ca. 700–3500 g DW m^{-2} yr^{-1} following Sousa *et al*, 2010a,b) and close to the minimum for created populations (ca. 370–3600 g DW m^{-2} year^{-1} following Castillo *et al*., 2008a), which was consistent with the relatively low accretion rate recorded in *Spartina* areas (ca. 2.6 ± 0.3 cm year^{-1}) (Castillo *et al*., 2008a). The recorded NAPP and NBPP of *S. maritima* corresponded to a N accumulation of ca. 15 g N m^{-2} yr^{-1} and ca. 6 g N m^{-2} yr^{-1}, respectively, with relatively low sediment N content (ca. 2 mg N g^{-1}). Sousa *et al*. (2008, 2010b) described N catches by *S. maritima* in the AGB that varied between ca. 2 g N m^{-2} yr^{-1} for matured marshes with ca. 6 mg N g^{-1} in sediment, and ca. 48 g N m^{-2} yr^{-1} with a sediment N content of ca. 3 mg N g^{-1}. The below-ground nitrogen catches in Portuguese estuaries varied between 6.0 g N m^{-2} yr^{-1} in young marshes and 45.2 g N m^{-2} yr^{-1} in mature marshes (Sousa *et al*., 2008). Elsey-Quirk *et al*. (2011) also described a similar AGB nitrogen accumulation of ca. 11 g N m^{-2} for *S. patens* and *S. alterniflora* marshes, but a higher BGB nitrogen accumulation (between 17.5 and 22.5 g N m^{-2}).

Sarcocornia perennis AGB (ca. 1580 g m^{-2}) was within the range recorded previously for natural populations in the Iberian Peninsula in the same season (between ca. 600–2000 g m^{-2} following Castellanos *et al*. 1994; Figueroa *et al*. 2003; Davy *et al*., 2006; Caçador *et al*., 2009). In contrast, *S. perennis* BGB (ca. 1290 g m^{-2}) was close to the lower values recorded for Iberian natural populations (ca. 1400–4900 g m^{-2}) (Castellanos *et al*. 1994; Figueroa *et al*. 2003; Caçador *et al*., 2009). Nitrogen content in all studied tissues was higher than those reported for *Sarcocornia perennis* ssp. *alpini* (Lag.) Castroviejo in Southern Spain (Palomo & Niell, 2009). *Sarcocornia* AGB nitrogen-stock in restored marshes (35 g N m^{-2}) was within the range described by Palomo & Niell (2009) in the Gulf of Cádiz (between 25.2 and 59.7 g N m^{-2}), while BGB nitrogen-stock in our restored marshes was much higher (23 g N m^{-2}) than values reported for *Sarcocornia perennis* ssp. *alpini* (between 1.56 and 3.11 g N m^{-2}).

The remaining few clumps of invasive *S. densiflora* in the restored marshes concentrated more N in their tissues than *S. perennis* and *S. maritima,* especially in shoots and rhizomes. Thus, *S. densiflora* could be used for to reduce eutrophication in South America, where it is native (Bortolus, 2006). N content for *S. densiflora* leaves in restored marshes (30 ± 1 mg N g^{-1}) was much higher than that recorded by Alberti *et al*. (2011) in Argentina (ca. 11 mg N g^{-1}). These differences could be influenced by the high heavy metal pollution in the Odiel Marshes (van Geen *et al*. 1997; Leblanc *et al*. 2000), which produces a strong oxidative stress for this cordgrass (Martínez-Domínguez *et al*., 2008). Thus, *S. densiflora* in the Odiel Marshes may accumulate large quantities of enzymes to combat oxidative stress, which would be reflected in high N contents in its photosynthetic tissues.

Sediment nitrogen-stock (34.1 t N in the top 20 cm of 10.15 ha) was more than 10 times that stored in plant biomass (3.2 t N). Although the sediment both captures and retains N, the effect of cleaning estuarine waters is performed mostly by the biota, through bacterial denitrification and N uptake by vegetation (Adam, 1990; White & Howes, 1994; Alongi, 1998; Dollhopf *et al*., 2005). A proportion of the N contained in the AGB of the expanding small cordgrass plantations will be accumulated gradually within the restored marshes as dead matter in their sediments, and a further proportion will be exported in the form of dead leaves and shoots, mainly after the second year

(Castillo *et al*., 2008a) since its shoots are semelparous and their mean shoot life span is about two years (Castellanos *et al*., 1998).

Our results showed that the restored marshes, 2.5 yr after planting, are reducing eutrophication, retaining 37.3 t N in 10.15 ha in the top 20 cm of sediments and vegetation. However, they have not yet developed their full capacity for N storage in sediments and BGB, even in totally colonized areas, and in AGB, as the colonization of bare sediments by halophytes is still progressing. The relatively low N content recorded in sediments colonized by *S. maritima*, and its low BGB, seemed to be related to the youth of the restored marshes (ca. 2.5 yr old), which may therefore have the capacity to sequestrate much more N below ground during their maturation.

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CHAPTER 6

Carbon-stock in restored salt marshes using small cordgrass

CAPÍTULO 6.- Reserva de carbono en marismas restauradas usando la hierba salada.

RESUMEN

Las marismas costeras se encuentran entre los ecosistemas más afectados por el cambio climático. Al mismo tiempo, los sedimentos y la vegetación marismeña pueden actuar como sumidero de carbono reduciendo la concentración de CO_2 atmosférico. En este contexto, los proyectos de restauración o creación de marismas deberían incluir la mitigación del cambio climático como uno de sus objetivos específicos. El objetivo de este capítulo fue cuantificar las reservas y la tasa de secuestro de carbono, tanto en el sedimento como en la vegetación, en marismas europeas restauradas con *Spartina maritima*. La acumulación de biomasa, la tasa de sedimentación y el contenido en C de los sedimentos y en las halófitas nativas dominantes *S. maritima* y *Sarcocornia perennis* ssp. *perennis* fueron registrados. El contenido de C del sedimento 28 meses tras los trasplantes fue de 11 mg C g^{-1} PS en la superficie (0-2 cm) y 16 mg C g^{-1} PS en profundidad (2-20 cm). La reserva de C en los primeros 20 cm de sedimento fue de 2.5 kg C m^{-2} en las areas con *Spartina* y 2.8 kg C m^{-2} en las áreas con *Sarcocornia*. El contenido de C para *S. maritima* fue ca. 400 mg C g^{-1} PS en la biomasa aérea y ca. 380 mg C g^{-1} PS en la subterránea. Las raíces de *S. maritima* presentaron el menor contenido de C (361 mg C g^{-1} PS) respecto a otros órganos. La biomasa áerea de *S. maritima* en parches completamente colonizados 28 meses después de los trasplantes fue 1290 ± 194 g m^{-2} PS y 1577 ± 102 g m^{-2} PS para *S. perennis*. Por tanto, la acumulación de C en la biomasa de *S. maritima* fue 533 g C m^{-2} y 386 g C m^{-2} para *S. perennis*. La biomasa subterránea de *S. maritima* fue de 616 ± 98 g m^{-2} PS y 1285 ± 235g m^{-2} PS para *S. perennis* que condujeron a una acumulación de 242 g C m^{-2} para *S. maritima* y 312 g C m^{-2} para *S. perennis*. Las reservas de C en marismas restauradas con *S. maritima* eran aún bajas 28 meses después de los trasplantes (30.2 t C ha^{-1}) mostrando una capacidad potencial de secuestrar más C en la biomasa aérea por expansión de la cobertura de las halófitas nativas y especialmente en los sedimentos y en la biomasa subterránea.

<u>CHAPTER 6</u>.- Carbon-stock in restored salt marshes using small cordgrass

ABSTRACT

Coastal marshes are among the ecosystems that are more greatly affected by global climate change and, at the same time, salt marsh vegetation and sediments may act as carbon sinks, reducing atmospheric CO_2 concentration. In this context, salt marsh creation and restoration projects should include climate change mitigation as one of their specific goals. The aim of this study was to quantify the carbon-stock and the carbon sequestration rate, both in sediments and vegetation, in European restored salt marshes using *Spartina maritima*. Biomass accumulation, sedimentation rate and C content for sediments and the dominant native halophytes, *S. maritima* and *Sarcocornia perennis* ssp. *perennis*, were recorded. Sediment C content 28 months after transplanting was 11 mg C g^{-1} DW at the surface (0-2 cm) and 16 mg C g^{-1} DW at depth (2-20 cm). Carbon-stock in the top 20 cm of sediment was 2.5 kg C m^{-2} for *Spartina* areas and 2.8 kg C m^{-2} for *Sarcocornia* areas. The C content for *S. maritima* was ca. 400 mg C g^{-1} DW in above-ground biomass and ca. 380 mg C g^{-1} DW in below-ground biomass. The root system of *S. maritima* was the organ that showed the lowest C content (361 mg C g^{-1} DW). *Spartina maritima* above-ground biomass in patches fully colonized 28 months after transplanting was 1290 ± 194 g DW m^{-2} and 1577 ± 102 g DW m^{-2} for *S. perennis*. Thus, above-ground biomass C accumulation in *S. maritima* biomass was 533 g C m^{-2} and 386 g C m^{-2} for *S. perennis*. *Spartina maritima* below-ground biomass was 616 ± 98 g DW m^{-2} and 1285 ± 235g DW m^{-2} for *S. perennis*, leading to 242 g C m^{-2} for *S. maritima* and 312 g C m^{-2} for *S. perennis*. In general, our results showed that the carbon-stock in *S. maritima* restored marshes is still low 28 months after planting (30.2 t C ha^{-1}), showing potential capacity to sequestrate more C in above-ground biomass by expanding cover of halophytes, and especially in sediments and below-ground biomass.

6.1. INTRODUCTION

Warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice and rising global average sea level (IPCC, 2007). This reality not been successfully addressed by international agreements.

Coastal marshes are among those ecosystems that are more greatly affected by global climate change. For example, sea-level rise is causing salt marsh losses (Hartig *et al.*, 2002; Morris *et al.*, 2002; Nicholls, 2004) and provoking changes in vegetation zonation through salinity alterations (Watson & Byrne, 2009). But, at the same time, vegetation and sediments of salt marshes may act as carbon sinks, reducing atmospheric carbon dioxide concentration (Caçador *et al.*, 2004; Sousa *et al.*, 2010a,b). Despite the fact that salt marshes are highly productive systems, there is an important lack of data about the potential of salt marshes for carbon storage (Chmura *et al.*, 2003).

In this context, salt marsh creation and restoration projects should include global climate change mitigation as one of their specific goals (Crooks *et al.*, 2011). This was the case for an innovative restoration project carried out in the Odiel Marshes (south-west Iberian Peninsula) from November 2006 to January 2007 using plantations of small cordgrass, *Spartina maritima* (Curtis) Fernald, accompanied by *Sarcocornia perennis* (Mill.) A. J. Scott. ssp *perennis*. This project included carbon sequestration as a specific goal (Castillo & Figueroa, 2009a), since *S. maritima* and *S. perennis* contribute effectively to the stabilization and carbon enrichment of salt marsh sediments (Figueroa *et al.*, 2003; Castillo *et al.*, 2008; Sousa *et al.*, 2010b) at the same time that they develop high biomass densities (Castillo *et al.*, 2008; Sousa *et al.*, 2008; Caçador *et al.*, 2009).

The aim of this study was to quantify the carbon-stock and carbon sequestration rate, both in sediments and vegetation, in European restored salt marshes using *S. maritima*. With this aim, biomass accumulation, sedimentation rate and carbon content for sediments and the dominant native halophytes *S. maritima* and *S. perennis* ssp. *perennis* (Davy *et al*., 2006) and the invasive *Spartina densiflora* Brongn. were recorded.

6.2. MATERIALS AND METHODS

6.2.1. Study site.

Our work was carried out in a restored salt marsh area (Chapter 2).

6.2.2. Sediment and vegetation sampling.

Sediment samples for carbon content analysis were collected in October 2009 in vegetated areas between 0–2 cm and 2–20 cm deep, where roots were concentrated, in two 10-m long rows established parallel to the tidal line at ca. 2.16 m over Spanish Hydrographic Zero (SHZ) (n = 10; 5 equidistant sampling points per row). Sediment bulk dry density was recorded by weighing (DW) the volume of sediments in a 5 x 5 cm cylindrical core in *S. maritima* and *S. perennis* areas (n = 20 and 6, respectively). Sedimentation rate (n = 9) was determined by markers that consisted of an iron goal (two upright poles joined by a crossbar) approximately 1.5 m tall, 0.5 m wide and with poles of 1 cm in diameter, inserted to a depth of around 1 m in vegetated areas. The distance from the middle of the crossbar to the sediment surface was measured quarterly from March 2009 to March 2010. This prevents measurement errors due to the erosive vortex generated in the base of the markers.

Plant material for carbon content analysis was collected together with the sediment samples at +2.16 m SHZ for *S. maritima* and between +2.40 and +2.67 m SHZ for *S. perennis* ssp. *perennis* and *S. densiflora* (n = 10 for each species). Previous studies have reported that *S. maritima* carbon concentration does not change seasonally (Cartaxana & Catarino, 1997; Caçador *et al*., 2004).

Plant cover was recorded by contact every 2 m along nine 60 to 80-m long transects, established perpendicular to the tidal line, from the lower distribution limit of *S. maritima* (ca. +1.5 m SHZ) to the upper border of the marsh. The relative cover for each taxon was calculated in relation to the area that it colonized. The area occupied by each species was determined by the software ArcGIS 9 (ESRI, 2008) after recording the distribution of each taxon in the field using a GPS (model eTrex Vista Garmin). Above- and below-ground biomass (AGB and BGB, respectively) was recorded in October in plots totally colonized (monospecific cover of 100%) by *S. maritima* and *S. perennis* (10 cm quadrants; n = 10 quadrants per species). Once in the laboratory, biomass was washed carefully, separated into photosynthetic organs (leaves for cordgrasses and stems for *S. perennis*), non-photosynthetic stems, roots and rhizomes and dried to constant weight at 80 °C over 48 hours. Net annual standing above- and below-ground productivity (NAPP and NBPP) for *S. maritima* were calculated as the total AGB or BGB, respectively, divided by years since transplantation. Sampling plots were located in areas with bare sediments adjacent to clumps just after transplanting, to ensure that all the standing biomass was effectively produced in situ after restoration plantings (Castillo *et al*., 2008). No evidence of herbivory was observed during the study.

6.2.3. Carbon content analysis.

Plant samples were separated into photosynthetic organs, non-photosynthetic stems, roots and rhizomes. Sediments and plants samples were dried to constant weight at 80 °C over 48 hours, pulverized using a grinder (*Cyclotec, Foss Tecator AB,* Höganäs, Sweden) and sieved through an 80-µm mesh. Total carbon content was determined (in mg g^{-1} DW) for undigested samples using an elemental analyzer (Leco CHNS-932, Spain). The value for each sample corresponded to the mean of three replicated measurements.

6.2.4. Carbon accumulation rate and stock.

Sediment carbon-stock was calculated for each of two depths (0–2 cm and 2–20 cm) as the product of the mass of sediment (obtained as the product of the bulk density and the total volume (area x sediment depth)) and the sediment carbon content. Annual accumulation of carbon in the sediments was calculated as the product of the mean annual sedimentation rate and the sediment C content in the upper 2 cm deposited on nylon horizons. Soil carbon density was calculated as the product of sediment carbon content and sediment dry bulk density.

Spartina maritima and *S. perennis* carbon-stocks were calculated for each organ as the product of their mean biomass density (biomass x occupied area x relative cover) and carbon content. Carbon accumulation rate for *S. maritima* was calculated as the quotient of the carbon-stock accumulated in plant tissues at the time since clumps were planted (in years).

6.2.5. Statistical analysis.

Analyses were carried out using SPSS release 12.0 (SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene test ($P > 0.05$). When homogeneity of variance between groups was not found, data were transformed using the following functions: ln(x), 1/x and √x. Student’s *t*-test for independent samples or a Mann–Whitney *U*-test were applied to compare two means. One-way analysis of variance (Anova) was applied to compare more than two means. A Tukey HSD test between two means was calculated only if an *F*-test was significant ($P < 0.05$). If homogeneity of variance was not achieved by data transformation, then the means were compared by Kruskal–Wallis *H* test.

6.3. RESULTS

6.3.1. Carbon content in sediments and vegetation.

Sediment carbon content was lower at surface (0–2 cm) than at depth (2–20 cm) (surface: 11 ± 1 mg C g^{-1}; depth: 16 ± 1 mg C g^{-1}; $t = -2.266$, df = 18, $P < 0.05$). Minimums were 5 mg C g^{-1} at the surface and 9 mg C g^{-1} at depth, and maximums were 19 mg C g^{-1} at the surface and 24 mg C g^{-1} at depth. The sediment characterization was shown in Chapter 5 (nitrogen sequestration).

Spartina maritima showed a higher carbon content than *S. densiflora* and *S. perennis* for every organ (Anova or Kruskal–Wallis, $P < 0.001$), and *S. perennis* showed a lower carbon content than both cordgrasses for every organ (Table 6.1). Every species showed significant differences in carbon content between organs (Anova or Kruskal–Wallis, $P < 0.001$). The highest carbon contents were recorded in leaves and stems for both cordgrasses (between 378 ± 2 mg C g^{-1} for *S. densiflora* leaves and 419 ± 1 mg C g^{-1} for *S. maritima* leaves) and the lowest in their roots (288 ± 9 mg C g^{-1} for *S. densiflora* and 361 ± 4 mg C g^{-1} for *S. maritima*). *Sarcocornia perennis* showed its lowest carbon content in photosynthetic stems (199 ± 5 mg C g^{-1}) (Table 6.1).

6.3.2. Carbon accumulation and stock.

Sediment bulk dry density was similar in *S. maritima* areas (0.80 ± 0.06 g DW cm^{-3}) and in *S. perennis* areas (0.91 ± 0.16 g DW cm^{-3}) (*t*-test, $P > 0.05$). The total volume of sediment colonized by vegetation was 2030 m^3 between 0–2 cm and 18270 m^3 between 2–20 cm, accumulating 18.3 t C in superficial sediments and 239.5 t C at depth, corresponding to 2480 g C m^{-2} in *S. maritima* areas and 2821g C m^{-2} in *S. perennis* areas in the first 20 cm of sediment. Sediment carbon density was 0.009 g cm^{-3} at the surface and 0.013 g cm^{-3} at depth in *Spartina* areas, and 0.010 g cm^{-3} at the surface and 0.015 g cm^{-3} at depth in *Sarcocornia* areas.

Organ	Species	C content (mg g^{-1})
Photosynthetic organ	Sm	419 ± 1$^{a/a}$
	Sd	378 ± 2$^{a/b}$
	Sp	199 ± 5$^{a/c}$
Non-photosynthetic stems	Sm	415 ± 1$^{a/a}$
	Sd	382 ± 3$^{a/b}$
	Sp	296 ± 5$^{b/c}$
Roots	Sm	361 ± 4$^{b/a}$
	Sd	288 ± 9$^{b/b}$
	Sp	253 ± 6$^{c/c}$
Rhizomes	Sm	408 ± 1$^{c/a}$
	Sd	365 ± 3$^{c/b}$

Table 6.1. Carbon content for photosynthetic organs (leaves or stems), non-photosynthetic stems, roots and rhizomes of *Spartina maritima* (Sm), *Spartina densiflora* (Sd) and *Sarcocornia perennis* (Sp) in restored salt marshes. Different letters indicate significant differences between organs for the same species (first coefficient; Anova, $P < 0.05$) and for the same organ between species (second coefficient; t-test, $P < 0.05$).

The sedimentation rate was 2.6 ± 0.3 cm year^{-1} in *Spartina* areas and 0.2 ± 1.3 cm year^{-1} in *Sarcocornia* areas (*t*-test, $P > 0.05$). Thus, 2176 m^{3} of sediment per year was accumulated in the area colonized by *S. maritima* (8.37 ha) and 36 m^{3} in the area colonized by *S. perennis* (1.78 ha). This represented an annual accumulation of 19.1 t C and 0.3 t C, respectively.

Total biomass accumulated by *S. maritima* in monospecific stands formed after restoration was ca. 2 kg DW m^{-2}, accumulating more biomass in aerial stems (935 ± 145 g DW m^{-2}) than in rhizomes (424 ± 60 g DW m^{-2}), leaves (356 ± 53 g DW m^{-2}) or roots (192 ± 44 g DW m^{-2}) (Kruskal–Wallis, $\chi^2 = 24.905$, $P < 0.05$, df = 3). Relating these biomass data with the respective carbon contents, *S. maritima* stems accumulated more carbon (388 ± 60 g C m^{-2}) than the other organs (Kruskal–Wallis, $\chi^2 = 25.835$, $P < 0.01$, df = 3). Twenty eight months after transplanting, *S. maritima* colonized 8.37 ha with a relative cover of 62 ± 6 %, accumulating close to 40.2 t C; 50% of this carbon-stock was stored in stems (Table 6.2). *Spartina maritima* prairies showed a NBPP of 264 ± 42 g

DW m^{-2} yr^{-1} and a NAPP of 553 ± 83 g DW m^{-2} yr^{-1}, which corresponded to 104 g C m^{-2} yr^{-1} in BGB and 228 g C m^{-2} yr^{-1} in AGB.

Total biomass accumulated by *S. perennis* in monospecific stands was ca. 3 kg DW m^{-2}. *S. perennis* accumulated similar AGB to *S. maritima* (Sp: 1577 ± 102 g DW m^{-2}; Sm: 1290 ± 194 g DW m^{-2}; *t*-test, $P > 0.05$) and more BGB (Sp: 1234 ± 235 g DW m^{-2}, Sm: 616 ± 98 g DW m^{-2}; $U = 18.000$, $P < 0.05$). No significant differences were recorded in the accumulation of biomass between organs for *S. perennis*, varying between 739 ± 85 g DW m^{-2} of non-photosynthetic stems and 1234 ± 227 g DW m^{-2} of roots (Kruskal–Wallis, $P > 0.05$). Relating these biomass data with the respective carbon contents, *S. perennis* roots accumulated more carbon (312 ± 57 g C m^{-2}) than photosynthetic stems (Kruskal–Wallis, $\chi^2 = 5.988$, $P < 0.05$, df = 2; *U*-test = 19.000, $P < 0.05$) (Table 6.2). *Sarcocornia perennis* occupied 1.78 ha of the restored marshes with a relative cover of 69 ± 13 %, accumulating close to 8.6 t C; 45% of this carbon-stock was stored in roots (Table 6.2).

Total carbon-stock in the 10.15 ha of restored salt marshes colonized by *S. maritima* and *S. perennis*, including their sediments, was 306.6 t C (30.2 t C ha^{-1}); ca. 13% was stored in *S. maritima* tissues and ca. 3% in *S. perennis*. This carbon-stock was increasing annually 19.4 t C from sedimentation and 17.2 t C due to *S. maritima* expansion.

	Biomass	Carbon-stock	Total carbon
Spartina maritima			
Leaves	356 ± 53^{a}	145 ± 23^{a}	7520 ± 1197^{a}
Stems	935 ± 145^{b}	388 ± 60^{b}	20131 ± 3115^{b}
Rhizomes	424 ± 60^{a}	173 ± 25^{a}	8980 ± 1270^{a}
Roots	192 ± 44^{c}	69 ± 16^{c}	3593 ± 826^{c}
Total	**1907 ± 283**	**775 ± 117**	**40224 ± 6055**
Sarcocornia perennis			
Photosinthetic stems	838 ± 46^{a}	167 ± 9^{a}	2048 ± 113^{a}
Non-photosinthetic stems	739 ± 85^{a}	219 ± 25^{ab}	2685 ± 311^{ab}
Roots	1234 ± 227^{a}	312 ± 57^{b}	3835 ± 706^{b}
Total	**2811 ± 301**	**698 ± 77**	**8568 ± 948**

Table 6.2. Biomass (dry weight), carbon-stock density and total carbon-stock in restored salt marshes using *Spartina maritima* (8.37 ha with a relative cover of 62 %) and *Sarcocornia perennis* (1.78 ha and 69% of relative cover), 28 months after transplanting. Different coefficients indicate significant differences between organs (Anova, $P < 0.05$).

6.4. DISCUSSION

Sediment carbon content in the studied restored marshes was still low 28 months after transplanting (11 mg C g^{-1} at surface and 16 mg C g^{-1} in depth) in comparison with mature natural marshes of *S. maritima* (22–75 mg C g^{-1} following Cartaxana & Catarino, 1997; Caçador *et al*., 2004; Lillebø *et al*., 2006), *Atriplex portulacoides* L. marshes (ca. 65 mg C g^{-1}) (Mendoça *et al*. 2004) and similar to the values recorded in young marshes of *Spartina anglica* C. E. Hubbard in the Netherlands (Hemminga *et al*., 1998). We recorded a carbon budget of 2.5 kg C m^{-2} in *Spartina* areas and 2.8 kg C m^{-2} in *Sarcocornia* areas in the top 20 cm of sediment, which was lower than those reported for *S. maritima* Portuguese marshes (9–22 kg C m^{-2} in the top 50 cm following Caçador *et al*., 2004), for *Spartina alterniflora* Loisel. invaded marshes in China (ca. 21 kg C m^{-2} in the top 100 cm of sediments following Liao *et al*., 2007) and for USA natural marshes colonized by *Spartina patens* (Aiton) Muhl. and *S. alterniflora* in the first 22.5 cm of depth (7.5–8.5 kg C m^{-2}) (Elsey-Quirk *et al*., 2011). Sediment carbon density recorded in our study was also lower (between 0.009 g cm^3 and 0.015 g cm^3) than the average soil carbon density of salt marshes along the Western and Eastern Atlantic and Pacific coasts of North America, Indian Ocean, Mediterranean Sea and Gulf of Mexico (0.039 ± 0.003 g cm^{-3}) (Chmura *et al*., 2003). In view of these results, it seems that the studied restored marshes can still sequester much more carbon in their sediments 28 months after plantations. In this context, Craft *et al.* (1988) reported that sediment carbon content in restored marshes changed more slowly than the development of *Spartina*. Cornell *et al.* (2007) suggested that, with a proper construction, most major carbon fluxes can be established in created salt marshes with *S. alterniflora* in less than five years.

The carbon content for *S. maritima* AGB (ca. 400 mg C g^{-1}) was similar to that reported for Portuguese populations (400-450 mg C g^{-1} following Cartaxana & Catarino, 1997; Castro *et al*., 2009) and for other cordgrasses, such as *Spartina patens* (Ait.) Muhl. and *S. alterniflora* (Foote & Reynolds, 1997; Hou *et al*., 2010). Our carbon content data for *S. maritima* BGB (ca. 380 mg C g^{-1}) also coincided with those reported for *S. alterniflora* (Hou *et al*., 2010). The root system of *S. maritima* was the organ that showed the lowest carbon content (361 mg C g^{-1}), coinciding with Cartaxana & Catarino (1997); however, their value was much lower (179 mg C g^{-1}). In general, *S. densiflora* had lower carbon content than the other mentioned cordgrasses such as *S. anglica*, probably due to its high silica content (de Bakker *et al*., 1999). Palomo & Niell (2009) recorded higher carbon content for *Sarcocornia perennis* ssp. *alpinii* (Lag.) Castroviejo (ca. 280-440 mg C g^{-1}) than that recorded in our study for the closely related *S. perennis* ssp. *perennis* (200–300 mg C g^{-1}). In both subspecies, the lowest carbon content was recorded for photosynthetic stems.

High carbon contents are related to fairly rigid walls which limit the amount of water influx during hypoosmotic stress, which would be beneficial in relatively stable saline environments (Touchette, 2007), such as lower elevations in the tidal gradient colonized by *S. maritima* (Adam, 1990, Castillo & Figueroa, 2009b). In contrast, *S. densiflora* and *S. perennis* inhabit higher elevations (Nieva *et al*., 2003; Castillo *et al*., 2005; Davy *et al*., 2006; Redondo-Gómez *et al*., 2007) where salinities fluctuate considerably over short intervals and high carbon contents could promote flaccid cells with no turgor pressure during hyperosmotic conditions (Touchette, 2007).

Spartina maritima AGB levels in fully colonized patches of the restored salt marshes 28 months after transplanting (1290 ± 194 g DW m^{-2}) were similar to those recorded previously for natural populations in the Iberian Peninsula (Benito & Onaindia, 1991; Cartaxana & Catarino, 1997; Castillo *et al*., 2008; Castro *et al*., 2009) and for created populations (Castillo *et al*. 2008). Created populations of *S. maritima* develop similar AGB to natural populations within ca. 2 yr after transplanting (Castillo *et al*. 2008). On the other hand, *S. perennis* AGB values (1577 ± 102 g DW m^{-2}) were higher than those previously recorded for natural populations in the Iberian Peninsula in the same season (ca. 600 g DW m^{-2} following Caçador *et al*., 2009) and *Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J. Scott in Australian marshes (ca. 200–600 g DW m^{-2} following Clarke & Jacoby, 1995), and similar to the biomass of *Sarcocornia fruticosa* (L.) A. J. Scott in Mediterranean wetlands (ca. 1600 g DW m^{-2} following Curco *et al*., 2002). AGB carbon accumulation in *S. maritima* biomass in the studied restored marshes was 533 g C m^{-2} and 386 g C m^{-2} for *S. perennis*, similar to those recorded in USA salt marshes for *S. patens* (ca. 500 g C m^{-2}) (Elsey-Quirk *et al*., 2011). In the same marshes, *S. alterniflora* presented a lower carbon accumulation (ca. 200 g C m^{-2}) than *S. maritima* and *S. perennis* in our restored marshes. However, the shrub *Baccharis halimifolia* L. Groundseltree (1140 g C m^{-2}) and *Juncus roemerianus* Scheele (817 g C m^{-2}) (Elsey-Quirk *et al*., 2011) showed higher carbon accumulation in their AGB than those recorded for small cordgrass and the perennial glasswort in the restored marshes.

Spartina maritima NAPP (553 ± 83 g DW m^{-2} yr^{-1}) was within the range recorded by Sousa *et al*. (2010a,b) in Portuguese natural marshes (120 - 2800 g DW m^{-2} yr^{-1}) and by Castillo *et al*. (2008) in created marshes in the Odiel estuary (131–590 g DW m^{-2} yr^{-1}). Carbon accumulation rate of *S. maritima* AGB in the restored marshes (228 g C m^{-2} yr^{-1}) was within the range reported for natural Portuguese marshes (ca. 50–1800 g C m^{-2} yr^{-1}) (Sousa *et al*, 2010a,b) . Nevertheless, *S. maritima* carbon production in the restored marshes was higher than that recorded for other halophytes, such as *Aster* *tripolium* L. (60 g C m^{-2} yr^{-1}), *Salicornia* spp. (10 g C m^{-2} yr^{-1}), *Suaeda maritima* (L.) Dumort. and *Puccinellia maritima* (Huds.) Parl. (210 g C m^{-2} yr^{-1}) in European marshes (Bouchard & Lefeuvre, 2000).

Spartina maritima BGB values (616 ± 98 g DW m^{-2}) were much lower than those recorded in matured natural populations (ca. 2500–4800 g DW m^{-2}) (Cartaxana & Catarino, 1997; Castillo *et al*., 2008; Caçador *et al*., 2009). Therefore, the analyzed restored marshes had not still raised its maximum BGB even in totally colonized areas, in agreement with Castillo *et al*. (2008), who reported that *S. maritima* transplants need between 2.5 and 4.0 yr, depending on the sedimentation dynamic, to develop a similar BGB to natural marshes. *Sarcocornia perennis* BGB (1285 ± 235g DW m^{-2}) was lower than that reported in the Tagus estuary (ca. 3900 g DW m^{-2}). Thus, *S. perennis*, like *S. maritima*, would still be able to increase its BGB in the restored marshes. The BGB carbon accumulation for *S. maritima* in the studied restored marshes was 242 g C m^{-2} and 312 g C m^{-2} for *S. perennis*, this being lower than values recorded for *S. patens* (366 g C m^{-2}), *J. roemerianus* (591g C m^{-2}), *B. halimifolia* (ca. 450 g C m^{-2}) and *S. alterniflora* (ca. 450 g C m^{-2}) in USA salt marshes (Elsey-Quirk *et al*., 2011).

The NBPP of *S. maritima* (264 ± 42 g DW m^{-2} yr^{-1}) was close to the minimum values reported for created populations in the Odiel Marshes (366–3598 g DW m^{-2} yr^{-1}) (Castillo *et al*., 2008) and lower than those recorded in natural *S. maritima* populations (700–3500 g DW m^{-2} yr^{-1}) with similar carbon contents, resulting in lower carbon accumulation rates in BGB (104 g C m^{-2} yr^{-1} vs. 239 – 1008 g C m^{-2} yr^{-1}) (Sousa *et al*, 2010a,b).

Carbon accumulation for the studied *S. maritima* restored marshes (0.7–0.8 kg C m^{-2}) was lower than that reported for invaded *S. alterniflora* Chinese marshes (2.95 kg C m^{-2}), due to higher biomass accumulation with similar carbon content (Liao *et al*., 2007). In the same estuary, *Phragmites australis* (Cav.) Trin. ex Steud. marshes also accumulated more carbon (2.21 kg C m^{-2}) than *S. maritima* restored marshes, which accumulated more carbon than *Scirpus mariqueter* Tang et Wang (0.42 kg C m^{-2}) (Liao *et al*., 2007). *Juncus roemerianus* and the tall shrub *B. halimifolia* also accumulated more carbon (ca. 1.40 kg C m^{-2} and ca. 1.50 kg C m^{-2}, respectively; excluding fine roots that could not be identified by species) in USA salt marshes than our restored marshes, whereas *S. patens* (ca. 0.85 kg C m^{-2}), and the short-form of *S. alterniflora* (0.65 kg C m^{-2}), showed similar values to *S. maritima* restored marshes (Elsey-Quirk *et al*., 2011).

In general, our results show that the carbon-stock in *S. maritima* restored marshes is still low 28 months after planting (30.2 t C ha^{-1}), when compared with natural marshes (e.g. 156–166 t C ha^{-1} for *S. patens* and *S. alterniflora*-dominated areas following Elsey-Quirk *et al*., 2011; 209 t C ha^{-1} for *S. alterniflora* areas in the first 100 cm of sediments, following Liao *et al*., 2007). Thus, analyzed restored salt marshes showed a potential capacity to sequestrate more carbon in AGB by expanding halophyte coverage, especially in sediments and BGB.

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CHAPTER 7

Metal stock in restored salt marshes using *Spartina maritima* in a very polluted estuary

CAPÍTULO 7.- Reservorio de metales en marismas restauradas mediante plantaciones de *Spartina maritima* en un estuario muy contaminado.

RESUMEN

Este capítulo analiza el medio abiótico de los sedimentos, así como la concentración y acumulación de metales en la vegetación y sedimentos en marismas restauradas mediante plantaciones de *Spartina maritima* en uno de los estuarios más contaminados del mundo, el estuario conjunto de los ríos Tinto y Odiel (suroeste de la Península Ibérica). Hierro, aluminio, cobre y zinc fueron los metales más concentrados tanto en los sedimentos superficiales como en los rizosedimentos. Cada metal, excepto el níquel, mostró mayor concentración en la zona de las raíces que en la superficie, con valores tan altos como ca. 70 g Fe kg PS $^{-1}$. La mayoría de los metales estuvieron más concentrados en los tejidos de *Sarcocornia perennis* que en los de *S. maritima*, acumulando ca. hasta 15 veces más contenido en metales por metro cuadrado. Las mayores concentraciones de metales fueron recogidas en las raíces de ambas halófitas, denotando gran capacidad de inmovilización de metales en la biomasa subterránea. De esta manera, la concentraciones medias máximas de metales fueron registradas para el hierro y el aluminio en las raíces de *S. perennis* (Fe = 25832.9 ± 2223.1 mg kg PS $^{-1}$; Al = 13781.2 ± 1283.5 mg kg PS $^{-1}$). Todos los tejidos de *Sarcocornia* acumularon más aluminio, hierro y cadmio que los sedimentos superficiales. Los rizosedimentos mostraron mayor concentración para cada metal que los tejidos de las plantas, excepto para el níquel que fue más concentrado en los tejidos de ambas especies. La biomasa foliar y radical de *S. maritima* presentó mayor acumulación total de metales que tallos y rizomas. Las raíces de *Sarcocornia* (con una biomasa de 1234 ± 227 g PS m^{-2}) acumularon más metales que sus tejidos aéreos. En vista a nuestros resultados, *S. perennis* y *S. martima* son bioherramientas útiles para proyectos de fitorremediación en marismas europeas contaminadas a bajas elevaciones topográficas en el gradiente mareal.

CHAPTER 7.- Metal stock in restored salt marshes using *Spartina maritima* in a very polluted estuary.

ABSTRACT

This chapter analyzed the sedimentary abiotic environment, and vegetation and sediment metal concentrations and stocks, in restored salt marshes using plantations of *Spartina maritima* in one of the globally most polluted estuaries, the joint estuary of the Odiel and Tinto rivers (southwest Iberian Peninsula). Iron, aluminum, copper and zinc were the most concentrated metals in both superficial and rhizo-sediments. Every metal, except nickel, showed higher concentration in the root zone than at the surface, with values as high as ca.70 g Fe kg DW $^{-1}$. Most metals were more concentrated in *Sarcocornia perennis* than in *S. maritima* tissues, accumulating ca. 15 times more metals per square meter. The highest metal concentrations were recorded in the roots of both halophytes, denoting high capacities for metal immobilization in the subterranean biomass. Thus, maximum mean metal concentrations were recorded for iron and aluminum in *S. perennis* roots (Fe = 25832.9 ± 2223.1 mg kg DW $^{-1}$; Al = 13781.2 ± 1283.5 mg kg DW $^{-1}$). All tissues of *Sarcocornia* accumulated more aluminum, iron and cadmium than superficial sediments, and concentrations of aluminum and iron in *S. maritima* leaves and roots were higher than in superficial sediments. Rhizosediments showed higher concentrations of every metal than in plant tissues, except for nickel, which was more concentrated in the tissues of both species. The biomass of *S. maritima* in leaves and in roots showed higher total metal stocks than stems and rhizomes. *Sarcocornia* roots (with a biomass of 1234 ± 227 g DW m^{-2}) accumulated more metals than its aerial tissues. Our results show *S. perennis* and *S. maritima* to be useful biotools for phytoremediation projects in European polluted salt marshes at low elevations in the tidal gradient.

7.1. INTRODUCTION

Coastal marshes are very vulnerable to metal contamination since they are located at river mouths (Beeftink, 1977; Williams *et al*., 1994a,b), especially in the vicinity of mining and industrial areas (Curado *et al*., 2010). In this context, some marsh plants such as *S. alterniflora* Loisel., *Phragmites australis* (Cav.) Trin. ex Steud., *Sarcocornia perennis* (Miller) A.J. Scott and *Juncus maritimus* Lam. can be used in restoration projects for phytoremediation in polluted estuaries (Weis & Weis, 2004; Czakó *et al*., 2006; Duarte *et al*., 2010; Marques *et al*., 2011).

Once a restoration project has been implemented, good monitoring is essential to improving the restoration methodology for future applications, and to solving of unexpected problems during its evolution (England *et al*., 2008). Nevertheless, only a handful of studies have monitored the results of phytoremediation efforts in constructed wetlands. For example, Imfeld *et al*. (2009) discussed some of the key characteristics of constructed wetlands control of the removal of organic chemicals, and Vymazal *et al*. (2010) showed that concentrations of heavy metals in the sediments of constructed wetland used to treat municipal wastewater were low, and comparable with those found in unpolluted natural wetlands.

In the Odiel Marshes, globally one of the most metal-polluted salt marshes (Pérez *et al*., 1991; Ruiz, 2001), an innovative restoration project was carried out from November 2006 to January 2007 using plantations of small cordgrass, *Spartina maritima* (Curtis) Fernald. This project included phytostabilization of metal-polluted sediments as a specific goal (Castillo & Figueroa, 2009), since *S. maritima* and the accompanying species *Sarcocornia perennis* (Mill.) A. J. Scott. ssp *perennis* contribute effectively to the stabilization of metals in salt marsh sediments (Luque, 1996; Reboreda & Caçador 2007; Cambrollé *et al*., 2008; Reboreda *et al*. 2008; Caçador *et al*., 2009; Castillo & Figueroa, 2009; Duarte *et al*., 2010).

The aim of this chapter was to analyze the sedimentary abiotic environment (elevation, pH, redox potential and conductivity) and to quantify the concentration and stock of nine metals (Al, As, Cd, Cr, Cu, Fe, Ni, Pb and Zn) in the sediments colonized by *S. maritima* and in the tissues of the dominant halophytes (*S. maritima* and *S. perennis*) as part of the integral monitoring of restored salt marshes in the Odiel Marshes (Castillo & Figueroa, 2009), 28 months after plantation. This is the first study that analyzes a wide spectrum of metals (nine metals) in *S. perennis* tissues. We hypothesized that expanding plantations of *S. maritima* and *S. perennis* growing on very polluted sediments in restored marshes in the Odiel Marshes would accumulate high metal loads in their tissues, as well as in colonized sediments.

7.2. MATERIALS AND METHODS

7.2.1. Study site.

Our work was carried out in a restored salt marsh area (Chapter 2). The area is very polluted with metals from two sources: industrial activities developed in the estuary and long-term mining activities carried out landward on the Iberian Pyrite Belt (van Geen *et al*., 1997; Leblanc *et al*., 2000).

7.2.2. Abiotic environment.

The abiotic environment was recorded in *S. maritima* (n = 20) and *S. perennis* (n = 6) areas in May–July 2009. Elevation relative to Spanish Hydrographic Zero (SHZ) was surveyed to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore); reference points were determined in relation to measurements of tidal extremes (Ranwell *et al*., 1964). Every sediment characteristic was recorded between 0 and 10 cm deep, except for the redox potential, which was sampled at the surface (0–2 cm) and at depth (2–20 cm). pH of the interstitial water of the sediments was recorded (pH/redox *Crison* with the electrode *M-506*) after adding distilled water to the sediment (1:1, v/v). Sediment salinity of the interstitial water was measured as electrical conductivity (conductivity meter, Crison-522) immediately after pH (1:2, v/v). Redox potential of the sediment was determined with a portable meter and electrode system (Crison pH/mV p-506). Sediment bulk dry density was recorded by weighing (DW) the volume of sediments in a cylindrical core of 5 x 5 cm. Sedimentation rate was determined by markers consisting in an iron structure with two vertical posts (ca. 1.5 m tall and 1 cm diameter) inserted in the sediment to a depth of approximately 1 m in *S. maritima* areas and supporting a horizontal crossbar (ca. 0.5 m long). The distance from the middle point of the crossbar to the sediment surface was measured quarterly from March 2009 to March 2010 (n = 9).

7.2.3. Metal analysis.

The concentration of Al, As, Cd, Cr, Cu, Fe, Ni, Pb and Zn were recorded for: (1) superficial sediments (0–2 cm deep) collected in *S. maritima* areas on nylon horizons between April and October 2008 (Salgueiro & Caçador, 2007); (2) sediments between 2–20 cm deep colonized by *S. maritima* roots—both were collected along two 10 –m long rows established parallel to the tidal line (n = 10; 5 equidistant sampling points per row) between +2.16 and +2.67 m SHZ in July 2009; and (3) photosynthetic organs (leaves or stems), non-photosynthetic stems, rhizomes and roots of the two dominant halophytes *S. maritima* and *S. perennis* in July 2009 (n = 10).

Samples were dried to constant weight at 80° C for 48 h, pulverized using a grinder (*Cyclotec, Foss Tecator AB*, Höganäs, Sweden), sieved through an 80 µm screen. Samples were digested in 6 ml HNO_3 and 25 ml ultrapure water using microwaves (Anton Paar, multiwave 3000, Austria). The product was measured by inductively coupled plasma (ICP) spectroscopy (Horiba Jobin Yvon, Última 2, France).

The lowest detection hold showed when the concentration was below the detection limit. For the sediment samples that showed metal concentrations under the detection limit, mean concentration was calculated considering these samples with a value of 0.1 mg kg^{-1} DW for Zn, Cu, Cd, Cr, Ni, Al, Fe; 0.3 mg kg^{-1} DW for As and 0.6 mg kg^{-1} DW for Pb in soil. For vegetation, these values were 0.05 mg kg^{-1} DW for Zn, Cu, Cd, Cr, Ni, Al, Fe; 0.15 mg kg^{-1} DW for As and 0.3 mg kg^{-1} DW for Pb.

7.2.4. Metal stock.

Metal stocks in *S. maritima* sediments were calculated as the product of each metal concentration (in mg kg^{-1} DW) and the mass of sediment at the surface (0–2 cm deep) and at depth (2–20 cm deep). The mass of sediment was calculated as the product of the volume and its bulk dry density.

Metal stocks in *S. maritima* and *S. perennis* tissues were calculated multiplying each metal concentration (in mg g^{-1} DW) by the biomass of every organ (in g DW m^{-2}). *Spartina maritima* and *S. perennis* biomass were recorded in October in totally colonized 10-cm quadrant plots (n = 10) (monospecific cover of 100%). In the laboratory, biomass was washed carefully, plant structures were separated and dried to constant weight at 80 °C for 48 h. Net annual standing above- and below-ground productivity (NAPP and NBPP) for *S. maritima* prairies were calculated as the total AGB or BGB, respectively, divided by years since transplantation (Castillo *et al*., 2008a). No evidence of herbivory was observed during the study.

7.2.5. Statistical analysis.

Analyses were carried out using SPSS release 14.0 (SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene test ($P > 0.05$). When no homogeneity of variance between groups was found, data were transformed using the following functions: ln(x), 1/x and √x. Student's *t*-test for independent samples was applied to compare two means. If homogeneity of variance was not achieved by data transformation, then means were compared using Mann–Whitney *U*-test. Variations in metal loads between organs of the same halophyte were compared by one-way Anova (analysis of variance). Tukey's test between means was calculated only if the *F*-test was significant ($P < 0.05$). If homogeneity of variance was not achieved by data transformation, then the means were compared by a Kruskal–Wallis non parametric Anova.

7.3. RESULTS AND DISCUSSION

7.3.1. Abiotic environment.

Sediment surface was at a mean elevation of +2.28 ± 0.06 m SHZ in areas colonized by *S. maritima* and +2.92 ± 0.07 m SHZ in *S. perennis* areas ($t = -5.123$, $P < 0.001$, df = 24). pH was always close to neutrality (Sm: 7.1 ± 0.1, Sp: 7.3 ± 0.1; $t = -2.300$, $P < 0.05$, df = 24) and conductivity was higher in *S. perennis* than in *S. maritima* areas (Sm: 15.2 ± 1.5 mS cm^{-1}, Sp: 19.9 ± 4.2 mS cm^{-1}; $U = 9.000$, $P < 0.01$). For superficial sediments (0-2 cm), redox potential varied between -5 ± 18 mV in *Spartina* areas and +118 ± 11 mV in *Sarcocornia* areas (*t*-test, $P > 0.05$). At depth (2-20 cm), redox potential was significantly lower in *S. maritima* (-44 ± 20 mV) than in *S. perennis* areas (+90 ± 29 mV) ($t = -3.508$, $P < 0.01$, df = 24). Sediment bulk dry density was 0.80 ± 0.06 g cm^{-3} in *S. maritima* areas and 0.91 ± 0.16 in *S. perennis* areas (*t*-test, $P > 0.05$). Sedimentation rate was +2.6 ± 0.3 cm yr^{-1} in areas colonized by *Spartina* and +0.2 ± 1.3 cm yr^{-1} in *Sarcocornia* areas.

7.3.2. Metal concentrations.

Iron, aluminum, copper and zinc were the most concentrated metals in both superficial sediments and rhizosediments. Every metal, except nickel, showed higher concentration in the root zone than at the surface, with values as high as ca. 70 g Fe kg DW^{-1} (Al, Cu and Fe: *U*-test, $P < 0.001$; Cd, Cr, Pb, Zn and As: *t*-test, $P < 0.01$, df = 8) (Table 7.1), which could be related with transport and precipitation of metals in the rhizosphere (Caçador *et al.*, 1996a). Previous studies have recorded higher metal concentrations in sediments colonized by roots of *S. maritima* than in sediments without roots (Caçador *et al.*, 1996a, b; Reboreda & Caçador, 2007; Cambrollé *et al.*, 2008; Reboreda *et al.* 2008). Reported metal concentrations were in accordance with those recorded previously in sediments from the Odiel Marshes (e.g. Luque *et al.* 1998; Santos Bermejo *et al.*, 2002; Cambrollé *et al*. 2008, 2011; Sánchez-Moyano *et al.*, 2010).

Metal	***Surface (0-2cm)***	***Depth (2-20 cm)***	**TOTAL**
Al	568.4 ± 102.5^{a}	43375.6 ± 4065.2^{b}	**43944.0 ± 4087.1**
As	138.3 ± 34.4^{a}	340.4 ± 51.8^{b}	**478.7 ±78.6**
Cd	0.4 ± 0.1^{a}	19.5 ± 1.8^{b}	**19.9 ± 1.8**
Cr	18.0 ± 4.9^{a}	68.0 ± 2.9^{b}	**86.0 ± 4.7**
Cu	405.2 ± 114.3^{a}	3085.5 ± 293.0^{b}	**3490.7 ± 294.4**
Fe	808.0 ± 179.7^{a}	69138.7 ± 6509.0^{b}	**69946.7 ± 6572.0**
Ni	10.5 ± 4.2^{a}	21.4 ± 2.18^{a}	**31.9 ± 4.6**
Pb	120.3 ± 30.9^{a}	512.6 ± 61.3^{b}	**632.9 ± 81.2**
Zn	467.8 ± 105.4^{a}	1831.4 ± 179.8^{b}	**2299.2 ± 232.7**
TOTAL	**2536.7 ± 565.8^{a}**	**118393.0 ± 10816.7^{b}**	**120929.7 ± 10996.0**

Table 7.1. Metal concentration (n = 10; mg kg DW^{-1}) in superficial sediments (0–2 cm deep) and sediment colonized by *Spartina maritima* roots (2–20 cm deep) in restored salt marshes in the Odiel Marshes (south-west Iberian Peninsula). Different coefficients indicate significant differences between depths (*t*-test or *U*-test, P < 0.01).

Previous works with halophytes have described that the metal concentration in sediments was not reflected in their tissues; only zinc concentrations in plant material reflected levels within the sediment (William *et al.*, 1994b). In contrast, we recorded lower zinc concentration in plant tissues than in sediments, except for *S. perennis* roots (Tables 7.1 and 7.2). Nickel was more accumulated in all plant tissues than in the sediment (Tables 7.1 and 7.2), but not hyperaccumulated, according to Brooks *et al.* (1977). Hyperaccumulation thresholds in the aerial plant tissues have been established as 1000 mg kg $^{-1}$ for copper, chrome, nickel, lead, arsenic and aluminum, 10000 mg kg $^{-1}$ for zinc, and 100 mg kg $^{-1}$ for cadmium (Brooks *et al.*, 1977; Baker & Brooks, 1989;

Jansen *et al*., 2002; Robinson *et al*., 2006). For iron, it was not possible to find any general threshold of hyperaccumulation (Branquinho *et al*., 2007). Following this, both halophytes only hyperaccumulated aluminum in aerial tissues and iron was accumulated above 1000 mg kg $^{-1}$, reaching a value of ca. 0.35% DW in *Sarcocornia* AGB and ca. 2.6% DW in roots (Table 7.2).

In anoxic (low redox potential) and neutral sediments with salinities as high as those of the studied restored marshes, zinc, chrome and cadmium would be the most bioavailable metals (Tingzong-Guo *et al*., 1997; López-González *et al*., 2005). However, cadmium and chrome did not reach high concentrations in plant tissues (Table 7.2), probably because their total sediment concentrations were low. Iron and aluminum were the most concentrated metals in the sediments and in the plant tissues.

Sarcocornia perennis showed a higher capacity to concentrate metals in its tissues than *S. maritima* (Table 7.2), as has been previously recorded for this species (Duarte et. al., 2010) and for other species of *Sarcocornia* (Santos-Echeandía *et al*., 2010). *Sarcocornia perennis* shows a lower root turnover than *S. maritima* (Duarte *et al*., 2010), accumulating metals in its roots during longer periods which, together with higher BGB and higher metal concentrations, make *S. perennis* a better biotool than *S. maritima* for phytoextraction projects.

Maximum mean metal concentrations were recorded for iron and aluminum in *S. perennis* roots (Fe = 25832.9 ± 2223.1 mg kg DW $^{-1}$; Al = 13781.2 ± 1283.5 mg kg DW^{-1}). Thus, the highest metal concentrations were recorded in the roots of both halophytes (Table 7.2), denoting high capacities for metal immobilization in the subterranean biomass to protect photosynthetic tissues (Fitzgerald *et al*., 2003; Duarte *et al*., 2010). Species of *Spartina*, such as *Spartina patens* (Aiton) Muhl and *Spartina densiflora* Brongn. also accumulated metals in their roots without significant translocation into their shoots (Suntornvongsagul *et al*., 2007; Cambrollé *et al*., 2008).

		Spartina maritima		
Metal	**Leaves**	**Stems**	**Rhizomes**	**Roots**
Al	1356.4 ± 130.7 a/a	236.5 ± 33.7 b/a	297.8 ± 38.4 b/a	1334.9 ± 109.6 a/a
As	5.7 ± 0.4 a/ a	1.2 ± 0.1 b/a	3.2 ± 0.3 c/a	29.0 ± 7.1 d/a
Cd	0.4 ± 0.0 a/a	0.7 ± 0.2 ab/a	1.0 ± 0.2 b/a	2.4 ± 0.5 c/a
Cr	3.7 ± 0.3 a/a	1.1 ± 0.1 b/a	1.3 ± 0.1 b/a	3.9 ± 0.6 a/a
Cu	83.0 ± 5.0 a/a	35.9 ± 4.3 b/a	74.1 ± 9.9 a/a	348.3 ± 58.2 c/a
Fe	1513.2 ± 136.7 a/a	270.0 ± 22.3 b/a	635.9 ± 83.1c/a	4160.2 ± 945.3 d/a
Ni	99.6 ± 9.1 a/a	118.6 ± 11.5 ab/a	199.4 ± 0.6 b/a	245.4 ± 48.9 c/a
Pb	4.5 ± 0.7 a/a	*	0.4 ± 0.1 c/a	6.0 ± 2.4 b/a
Zn	102.1 ± 9.4 a/a	32.5 ± 10.9 b/a	48.2 ± 13.9 b/a	193.1 ± 54.1 c/a
TOTAL	**3168.5 ± 274.2 a/a**	**696.5 ± 57.7 b/a**	**1261.0 ± 103.5 ab/a**	**6323.1 ± 967.7 c/a**

		Sarcocornia perennis	
Metal	**Phot. stems**	**Non-phot. stems**	**Roots**
Al	2320.5 ± 247.3 a/b	2783.6 ± 340.9 a/b	13781.2 ± 1283.5 b/b
As	19.2 ± 2.0 a/b	24.4 ± 2.9 a/b	213.4 ± 39.8 b/b
Cd	2.4 ± 0.3 a/b	2.5 ± 0.1 a/b	5.6 ± 0.6 b/a
Cr	9.8 ± 0.8 a/b	11.8 ± 1.0 a/b	36.5 ± 2.4 b/b
Cu	166.6 ± 18.4 a/b	231.6 ± 28.3 a/b	1786.4 ± 199.2 b/b
Fe	3138.0 ± 344.7 a/b	3784.0 ± 476.6 a/b	25832.9 ± 2223.1 b/b
Ni	99.2 ± 12.5 a/a	285.6 ± 78.8 b/b	1712.5 ± 334.7 c/b
Pb	28.1 ± 2.4 a/b	33.7 ± 3.9 a/b	161.6 ± 10.3 b/b
Zn	192.8 ± 16.5 a/b	214.1 ± 24.4 a/b	1222.1 ± 100.3 b/b
TOTAL	**5979.5 ± 625.6 a/b**	**7371.4 ± 919.9 a/b**	**44752.1 ± 1944.0 b/b**

Table 7.2. Metal concentration (n = 10; mg kg DW^{-1}) in photosynthetic organs (leaves or stems), non-photosynthetic stems, rhizome and roots of *Spartina maritima* and *Sarcocornia perennis* in restored salt marshes in the Odiel Marshes. Different coefficients indicate significant differences between organs for the same species (first coefficient; analysis of variance) and between species for the same organ (second coefficient; *t*-test or *U*-test, $P < 0.01$). (* measurements under the detection threshold).

Aluminum and chrome in *S. maritima* showed similar concentrations in roots and leaves (Al: *t*-test, $P > 0.05$; Cr: *U*-test, $P > 0.05$) (Table 7.2). Reported metal concentrations were in accordance with those recorded previously for *S. maritima* in the Odiel Marshes (Cambrollé *et al*., 2008) and Tagus estuary, except for lead and copper (Caçador *et al*., 1996a; Reboreda *et al*., 2008; Duarte *et al*., 2010). In Portuguese marshes in the same season, lead concentration was higher and copper concentration was lower in *S. maritima* roots than in our study. *Sarcocornia perennis* showed higher metal concentrations in our study than in the Tagus marshes, except that lead showed a higher concentration in Portuguese sediments. Cadmium had a similar concentration in *S. perennis* in both locations (Caçador *et al*., 2009; Duarte *et al*., 2010).

All tissues of *Sarcocornia* accumulated more aluminum, iron and cadmium than superficial sediments (*t*-test or *U*-test, $P < 0.001$). Only the roots of *S. perennis* accumulated more chrome, copper and zinc than superficial sediments (Cr: $t = -3.274$, $P < 0.01$, df = 17; Cu: $t = -5.943$, $P < 0.001$, df = 17; Zn: $t = -5.040$, $P < 0.001$, df = 17).

Aluminum and iron in *S. maritima* were accumulated at higher concentrations than in superficial sediments both in leaves (*U*-test, $P < 0.001$) and roots (*t*-test and *U*-test, $P < 0.001$). Cadmium was more concentrated in rhizomes and roots of *S. maritima* than in superficial sediments (rhizomes: *t*-test = -2.733, $P < 0.05$, df = 18; roots: *U*-test = 14.000, $P < 0.01$). *Spartina maritima* roots had a metal load three times higher than in superficial sediments (however Zn, Pb and As were less concentrated in *Spartina* roots than in the sediments). This difference was eighteen times higher for the roots of *S. perennis*.

Rhizosediments showed higher concentrations of every metal than in plant tissues, except for nickel, which was more concentrated in both species' tissues (*t*-test or *U*-test, $P < 0.001$); up to eighty times more in *Sarcocornia* roots than in rhizosediments (Tables 7.1 and 7.2).

7.3.3. Metal stocks.

Sediment metal stock in the first 20 cm deep was ca. 1470 t. Iron was the most abundant metal (ca. 857 t), followed by aluminum (ca. 538 t), copper (ca. 39 t) and zinc (ca. 24 t). 2176 m^3 of sediments were deposited annually in *Spartina* areas (8.37 ha), which represented 1.3 times the pool of metals in the first 2 cm (Table 7.3).

Biomass of *S. maritima* in leaves (356 ± 53 g DW m^{-2}) and in roots (192 ± 44 g

DW m^{-2}) showed higher total metal stocks than stems (935 ± 145 g DW m^{-2}) and rhizomes (424 ± 60 g DW m^{-2}) (Kruskal–Wallis, $\chi^2 = 22.515$, $P < 0.001$, df = 3) (Table 7.4). Iron and aluminum were the most accumulated metals in *S. maritima* tissues and cadmium, lead and chrome the least (Tables 7.2, 7.3 and 7.4). About 2.5 yr after transplanting, *S. maritima* prairies, with relative cover of 62 ± 6% in 8.37 ha of restored marshes, accumulated 182.8 ± 12.0 kg of metals, corresponding to 152.7 kg of iron and aluminum (Table 7.3). The recorded values of BGB for *S. maritima* (ca. 0.63 kg DW m^{-2}) were lower than those reported previously for natural populations in the Tajo estuary (3.60 ± 0.15 kg DW m^{-2} by Rebordea & Caçador, 2007) and in the Odiel Marshes (from 4.82 ± 0.59 to 7.46 ± 1.35 kg DW m^{-2} by Castillo *et al.*, 2008a,b). These differences seemed to be related to the slower development of BGB in relation to AGB in transplanted populations of *S. maritima* (Castillo *et al.*, 2008a). Thus, even more metals would be captured by roots during the maturation of the restored marshes.

	Sediments colonized by *S. maritima*		**TOTAL in sediments**	***S. maritima***
Metal	(0-2 cm)	(2-20 cm)	(0-20 cm)	
Al	761.2 ± 137.2^a	522797.3 ± 48996.7^b	523558.5 ± 49024.6	56.3 ± 4.7
As	185.2 ± 46.1^a	4102.3 ± 623.9^b	4287.5 ± 654.6	0.5 ± 0.1
Cd	0.5 ± 0.2^a	235.3 ± 21.8^b	235.7 ± 21.8	0.1 ± 0.0
Cr	24.0 ± 6.5^a	819.2 ± 35.2^b	843.3 ± 33.4	0.2 ± 0.0
Cu	542.6 ± 153.1^a	37188.8 ± 3532.0^b	37731.4 ± 3507.2	8.4 ± 0.9
Fe	1082.0 ± 240.7^a	833315.4 ± 78451.9^b	834397.4 ± 78533.6	96.4 ± 11.3
Ni	14.1 ± 5.7^a	257.9 ± 26.3^b	272.0 ± 26.5	14.4 ± 1.0
Pb	161.1 ± 41.3^a	6178.7 ± 738.7^b	6339.8 ± 760.1	0.2 ± 0.0
Zn	626.5 ± 141.1^a	22073.2 ± 2166.8^b	22699.7 ± 2210.8	6.3 ± 1.3
TOTAL	**3397.1 ± 757.5^a**	**1426968.1 ± 130371.6^b**	**1430365.2 ± 130595.8**	**182.8 ± 12.0**

Table 7.3. Metal stock (kg) in the first twenty centimeter of sediment and in *Spartina maritima* biomass for restored salt marshes 28 months after transplanting (8.37 ha with a *S. maritima* relative cover of 62 %) in the Odiel Marshes. Different coefficients indicate significant differences between surface and depth ($P < 0.05$).

During their expansion, *S. maritima* prairies accumulated annually 43% of its own total metal stock accumulated during 28 months, showing a NBPP of 264 ± 42 g

DW m^{-2} yr^{-1} and a NAPP of 553 ± 83 g DW m^{-2} yr^{-1}. The total recorded metal pool in *S. maritima* areas 28 months after transplanting, including their sediments in the first 20 cm deep, was 1430.5 t, corresponding only by 0.013% to vegetation; ca. 4.4 t metals was added annually by sedimentation and ca. 0.1 t metals yr^{-1} was sequestered by *S. maritima* expansion.

Spartina maritima

Metal	**Leaves**	**Stems**	**Rhizomes**	**Roots**	**TOTAL**
Al	0.4826 ± 0.0465a/a	0.2210 ± 0.0314bc/a	0.1263 ± 0.0163b/a	0.2559 ± 0.0210c/a	**1.0858 ± 0.0898**
As	0.0020 ± 0.0001a/a	0.0009 ± 0.0001b/a	0.0014 ± 0.0001b/a	0.0056 ± 0.0013ab/a	**0.0099 ± 0.0015**
Cd	0.0001 ± 0.0000a/a	0.0005 ± 0.0001ab/a	0.0004 ± 0.0000ab/a	0.0005 ± 0.0001b/a	**0.0015 ± 0.0002**
Cr	0.0013 ± 0.0001a/a	0.0011 ± 0.0001ab/a	0.0006 ± 0.0000c/a	0.0007 ± 0.0001bc/a	**0.0037 ± 0.0003**
Cu	0.0295 ± 0.0018a/a	0.0336 ± 0.0040a/a	0.0314 ± 0.0042a/a	0.0668 ± 0.0112b/a	**0.1613 ± 0.0168**
Fe	0.5384 ± 0.0486ab/a	0.2523 ± 0.0208a/a	0.2696 ± 0.3523a/a	0.7975 ± 0.1812b/a	**1.8579 ± 0.2174**
Ni	0.0354 ± 0.0032a/a	0.1108 ± 0.0107b/a	0.0845 ± 0.0084b/a	0.0470 ± 0.0094a/a	**0.2778 ± 0.0189**
Pb	0.0016 ± 0.0002a/a	*	0.0001 ± 0.0000b/a	0.0011 ± 0.0005a/a	**0.0028 ± 0.0005**
Zn	0.0363 ± 0.0033a/a	0.0273 ± 0.0100a/a	0.0205 ± 0.0059a/a	0.0370 ± 0.0104a/a	**0.1211 ± 0.0242**
TOTAL	**1.1274 ± 0.0976a/a**	**0.6478 ± 0.0563b/a**	**0.5348 ± 0.0439b/a**	**1.2121 ± 0.1885a/a**	**3.5219 ± 0.2314**

Sarcocornia perennis

Metal	**Phot. stems**	**Non-phot. stems**		**Roots**	**TOTAL**
Al	1.9446 ± 0.2072a/b	2.0560 ± 0.2518a/b		17.7074 ± 1.7384b/b	**21.6177± 1.9400**
As	0.0161 ± 0.0016a/b	0.0180 ± 0.0021a/b		0.1788 ± 0.0351b/b	**0.2119 ± 0.0355**
Cd	0.0020 ± 0.0002a/b	0.0015 ± 0.0003a/b		0.0041 ± 0.0007b/b	**0.0070 ± 0.0012**
Cr	0.0082 ± 0.0007a/b	0.0087 ± 0.0007a/b		0.03061 ± 0.0021b/ b	**0.0477 ± 0.0026**
Cu	0.1396 ± 0.0155a/b	0.1711 ± 0.0209a/b		1.4970 ± 0.1760b/b	**1.8012 ± 0.1818**
Fe	2.6296 ± 0.2889a/b	2.7949 ± 0.3521a/b		21.6480 ± 1.9637b/b	**26.9575± 1.9908**
Ni	0.0748 ± 0.0132a/b	0.2110 ± 0.0583a/a		1.4350 ± 0.2956b/b	**1.6836 ± 0.3154**
Pb	0.0235 ± 0.0020a/b	0.0249 ± 0.0029a/b		0.1354 ± 0.0091b/b	**0.1827 ± 0.0100**
Zn	0.1616 ± 0.0138a/b	0.1581 ± 0.0180a/b		1.0241 ± 0.0886b/b	**1.3405 ± 0.1021**
TOTAL	**4.9994 ± 0.5242a/b**	**5.4443 ± 0.6795a/b**		**43.6605 ± 1.9810b/b**	**53.8496± 2.5376**

Table 7.4. Metals accumulated in above- and below-ground biomass (g m^{-2}) separated by organs of *Spartina maritima* and *Sarcocornia perennis* (n = 10) in restored salt marshes in the Odiel Marshes. Different coefficients indicate significant differences between organs for the same species (first coefficient; analysis of variance) and between species for the same organ (second coefficient; *t*-test or *U*-test, P < 0.01). * measurements under the detection threshold.

In *Sarcocornia* areas, with relative cover of 69 ± 13% in 1.78 ha, roots (with a biomass of 1234 ± 227 g DW m^{-2}) accumulated more metals than in AGB (1576 ± 102 g DW m^{-2}) (Kruskal–Wallis, $\chi^2 = 18.069$, $P < 0.001$, df = 2). 81% of the total metal pool in

S. perennis was stored in its roots. Iron and aluminum were the most accumulated metals by the perennial glasswort (ca. 2.6% and 1.4% DW, respectively) and cadmium, lead and chrome the least, similar to the small cordgrass. The perennial glasswort, with similar AGB and higher BGB than the small cordgrass, accumulated ca. 15 times more metals per square meter (Tables 7.3 and 7.4).

Our results showed that *S. perennis* and *S. maritima* are useful biotools for phytoremediation projects in European polluted salt marshes at low elevations in the tidal gradient. In phytoextraction, sediments adhered to the roots should be extracted, together with the plants, since they are rich in metals, as proposed by Almeida *et al*. (2004) for *Juncus maritimus* Lam. Nevertheless, phytoextraction is not an option in the Odiel Marshes since sediments continuously receive metal loads coming from the Iberian Pyrite Belt along the Odiel and Tinto rivers (Nieto *et al*., 2007).

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CHAPTER 8

Spartina maritima decomposition in Odiel Marshes

CAPÍTULO 8.- Descomposición de *Spartina maritima* en las Marismas del Odiel.

RESUMEN

Este trabajo cuantifica por primera vez la tasa de descomposición (k day^{-1}) y el peso seco remanente en hojas, tallos y rizomas de *Spartina maritima* en el estuario conjunto de los ríos Tinto y Odiel (suroeste de la Península Ibérica). Las hojas mostraron menor peso seco remanente que tallos y rizomas. El peso seco remanente de las hojas de *S. maritima* tras un año fue del 27 ± 10 %. El peso seco remanente de los tallos y los rizomas fueron 59 ± 11 % y 57 ± 2 %, respectivamente. La mayor pérdida de peso para las hojas fue recogida al inicio del proceso de descomposición (33 ± 4 % en los primeros tres meses correspondiendo con primavera) y para los tallos y rizomas durante los segundos tres meses correspondientes a verano (21 ± 6 % y 17 ± 5 %, respectivamente). Las hojas de *S. maritima* mostraron mayor *k* que los tallos y rizomas durante todo el estudio, siendo estadísticamente significativo en primavera y otoño. La *k* anual para las hojas fue 0.0043 ± 0.0013 dias^{-1} y cerca de 0.0015 dias^{-1} para tallos y rizomas. Por tanto, concluimos que el ciclado de los nutrientes de *S. maritima* es muy activo, especialmente en hojas, en las Marismas del Odiel.

CHAPTER 8.- *Spartina maritima* decomposition in Odiel Marshes.

ABSTRACT

This work quantifies for the first time the decomposition rate (k day^{-1}) and the remaining weight in leaves, stems and rhizomes of *Spartina maritima* in the joint estuary of the Odiel and Tinto rivers (south-west Iberian Peninsula). Leaves showed a lower remaining dry weight than stems and rhizomes. The remaining dry weight of *S. maritima* leaves was 27 ± 10 % after one year. The remaining dry weight for stems and rhizomes was 59 ± 11 % and 57 ± 2 %, respectively. The highest weight loss for leaves was recorded at the beginning of the decomposition process (33 ± 4 % for the first three months, corresponding to spring) and for stems and rhizomes during the second three months, corresponding to summer (21 ± 6 % and 17 ± 5 %, respectively). During the whole study, *S. maritima* leaves showed a higher value of *k* than stems and rhizomes, with statistically significant results during spring and autumn. The annual *k* value for leaves was 0.0043 ± 0.0013 days^{-1} and ca. 0.0015 days^{-1} for stems and rhizomes. We conclude that nutrient recycling of *S. maritima* is very active, especially on leaves, in the Odiel Marshes.

8.1. INTRODUCTION

Plant decomposition is a key process in salt marshes, influencing the formation of soil particulate organic matter and releasing inorganic nutrients for plants (Adam, 1990). Decomposition is influenced by many environmental factors, such as sediment texture (De Boer, 2000), water availability (Menéndez & Sanmartí, 2007), salinity (Hemminga *et al*., 1991) and the decomposer community (Pozo & Colino, 1992; Alkemade *et al*., 1993).

Decomposition has been studied for *Spartina alterniflora* Loisel and *Spartina patens* (Ait.) Muhl. in North American estuaries and in invasive Chinese populations (White *et al*., 1978; Frasco & Good, 1982; Valiela *et al*., 1985; Foote & Reynolds,1997; Windham, 2001; Liao *et al*., 2008), and for *Spartina anglica* C.E. Hubbard and *Spartina maritima* (Curtis) Fernald in European marshes (Buth & Wolf, 1985; Hemminga *et al*., 1991; Pozo & Colino, 1992; Lillebø *et al*., 1999; Castro & Freitas, 2000; Caçador *et al*., 2007; Sousa *et al*., 2008).

This is the first study of the decomposition dynamic for *S. maritima* tissues (leaves, stems and rhizomes) in the Odiel Marshes in the joint estuary of the Odiel and Tinto rivers (southwest Iberian Peninsula). This is globally the most metal-polluted estuary (Elbaz-Poulichet *et al*., 2001; Grande *et al*., 2003), where *S. maritima* accumulates high heavy metals loads in its tissues (Luque *et al*., 1999).

8.2. MATERIALS AND METHODS

Our work was carried out in a restored salt marsh area (Chapter 2) from March 2010 to March 2011. 2 g per organ of *S. maritima* (leaves, stems and rhizomes) were dried to a constant weight (DW) at 80 °C over 48 h (n = 10). To determine the decomposition rate of *S. maritima*, 2 g per organ were placed into bags (10 cm x 20 cm) with a light mesh of

1 mm (Castro & Freitas, 2000). 12 litter bags (4 bags per organ) were placed on the sediment surface in 6 salt marshes (n = 6) colonized by *S. maritima* and *Sarcocornia perennis* (Mill.) A.J. Scott ssp. *perennis*. One bag per organ was collected from each marsh every 3 months from March 2010 to March 2011. After collection, plant material was carefully cleaned, dried to constant weight and weighed. The remaining dry weight in percentage was calculated, as was decomposition rate (*k*) using the Olson equation (Olson, 1963):

$$\text{Ln (final weight (DW) / initial weight (DW))} = -k * \text{time (days)}.$$

Abiotic environment was characterized during March and April 2009 at the same points where *S. maritima* samples were placed for decomposition. Elevation relative to Spanish Hydrographic Zero (SHZ) was surveyed to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore). Electrical conductivity and pH of the interstitial water of the sediment were measured in the laboratory with a conductivity meter (Crison-522) and a pH meter (pH/rédox *Crison* with electrode *M-506*), respectively (n = 6). Conductivity and pH were recorded after adding distilled water to the sediment (1:2 and 1:1 v/v, water/sediment, respectively). Sediment redox potential was determined in the field in triplicate sub-samples with a portable meter and electrode system (Crison pH/mV p-506) (n = 6). Each sediment characteristic was recorded for depths between 0–5 cm during spring.

Analyses were carried out using SPSS for Windows, release 17.0 (SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene test ($P > 0.05$). Variations in decomposition rate and remaining dry weight between organs were compared using a one-way analysis of variance (Anova). A Tukey *t*-test between means was calculated only if the *F*-test was significant ($P > 0.05$).

8.3. RESULTS AND DISCUSSION

Decomposition samples were placed at a mean topographical elevation of +2.85 ± 0.08 m SHZ on sediments with a pH of 7.0 ± 0.1, a conductivity of 17 ± 3 mS cm^{-1} and a redox potential of +81 ± 38 mV.

Leaves showed lower remaining dry weight than stems and rhizomes during the whole study (Anova, $P < 0.05$), but these differences were not significant in summer due to a high data dispersion. The remaining dry weight of *S. maritima* leaves was 27 ± 10 % after 1 yr, being within the range of those reported previously for *Spartina* leaves (40 % and 12 % after 1 yr for *S. maritima*, following Pozo & Colino (1992) and Castro & Freitas (2000), respectively, and 12% for *S. alterniflora* in nine months, following Liao *et al*. (2008)). The remaining dry weight for stems and rhizomes recorded in this study (59 ± 11 % and 57 ± 2 %, respectively, after 1 yr) was also within the range reported previously for *Spartina cynosuroides* (L.) Roth., *S. alterniflora* and *S. maritima* (Hackney, 1980; Pozo & Colino, 1992; Liao *et al*., 2008; Sousa *et al*., 2008) (Fig. 8.1).

The highest weight loss for leaves was recorded at the beginning of the decomposition process (33 ± 4 % for the first three months, corresponding to spring) and for stems and rhizomes during the second three months, corresponding to summer (21 ± 6 % and 17 ± 5 %, respectively) (Fig. 8.1). Faster decomposition could be related to a higher C:N ratio at the ignition of decomposition (Sousa *et al*., 2008) and with warmer conditions during spring and summer in the Mediterranean climate (Pozo & Colino, 1992; Castro & Freitas, 2000).

S. maritima leaves showed higher k than stems and rhizomes during the whole study, being statistically significant during spring and autumn (Anova, $P < 0.05$). Annual k for leaves was 0.0043 ± 0.0013 $days^{-1}$, being within the range recorded by Castro & Freitas (2000) for *S. maritima* (0.0050 $days^{-1}$) and Liao *et al*. (2008) for *S. alterniflora* (0.0063 $days^{-1}$). Annual k for stems and rhizomes (ca. 0.0015 $days^{-1}$) was lower than those recorded previously (0.0024-0.0043 days^{-1}, following Liao *et al.*, 2008 and Sousa *et al.*, 2008) (Table 8.1).

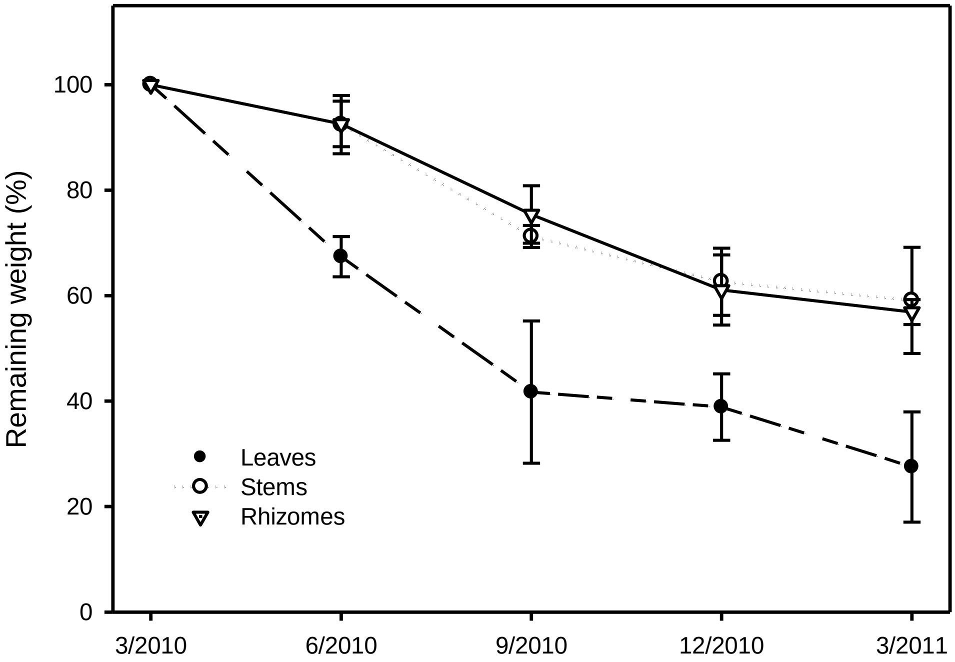

Figure 8.1. Litter mass remaining in different plants structures of *Spartina maritima* during one year in restored salt marshes in the joint estuary of the Odiel and Tinto rivers (n = 6). Asterisks indicate significant differences between leaves, and stems and rhizomes (Anova, $P < 0.05$).

This is the first study quantifying decomposition of *S. maritima* in the Odiel Marshes. Our results showed that decomposition of *S. maritima* tissues is similar to that recorded for Small Cordgrass and other *Spartina* species in other estuaries, even at the upper distribution limit of low salt marshes with less tidal influence and with very high loads of metals in plant tissues and sediments (see Chapter 7). We conclude that nutrient recycling of *S. maritima* is very active, especially on leaves, in the Odiel Marshes.

	Organ	**Decomposition rate (day^{-1})**
0-3 months (spring)	*Leaves*	0.0048 ± 0.0007^{a}
	Stems	0.0010 ± 0.0007^{b}
	Rhizomes	0.0013 ± 0.0004^{b}
3-6 months (summer)	*Leaves*	0.0060 ± 0.0020^{a}
	Stems	0.0018 ± 0.0002^{a}
	Rhizomes	0.0016 ± 0.0004^{a}
6-9 months (autumn)	*Leaves*	0.0043 ± 0.0008^{a}
	Stems	0.0021 ± 0.0004^{b}
	Rhizomes	0.0022 ± 0.0004^{b}
9-12 months (winter)	*Leaves*	0.0043 ± 0.0013^{a}
	Stems	0.0015 ± 0.0005^{a}
	Rhizomes	0.0014 ± 0.0001^{a}

Table 8.1. Decomposition rate (day^{-1}) in leaves, stems and rhizomes of *Spartina maritima* in the Odiel Marshes during one year. The letters indicate significant differences between organs for the same period (Anova, $P < 0.05$).

8.4. REFERENCES

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CHAPTER 9

Vertical sediment dynamic in *Spartina maritima* restored, non-restored and preserved marshes

CAPÍTULO 9.- Dinámica vertical del sedimento en marismas de *Spartina maritima* restauradas, no restauradas y conservadas.

RESUMEN

Los proyectos de restauración utilizando especies del género *Spartina* son una alternativa relativamente barata y viable para mejorar la calidad ambiental mientras se estabilizan los sedimentos. Este capítulo cuantifica la dinámica vertical del sedimento a lo largo del gradiente mareal en marismas bajas en un extenso proyecto de restauración de marismas costeras 26 meses después de las plantaciones de *Spartina maritima*. Estas marismas se compararon con unas marismas adyacentes no restauradas y otras conservadas en las Marismas del Odiel (suroeste de la Península Ibérica). La dinámica vertical sedimentaria fue recogida usando unos marcadores que consistían en una estructura de hierro con dos postes verticales y un travesaño horizontal, midiéndose la distancia desde el punto medio del travesaño hasta la superficie del sedimento periódicamente desde marzo de 2009 hasta marzo de 2011. La dinámica de sedimentación fue independiente de la elevación de la marisma dependiendo principalmente de la cobertura de las plantas. La erosión fue menor, dominando sedimentación en la mayoría de los lugares, en la zona de *S. marítima* (tasa media de acreción entre +10 y +27 mm año^{-1}; erosión media acumulada entre +22 y +51 mm después de 2 años) que en planicies lodosas intermareales (-14 a +12 mm año^{-1}; -36 a +37 mm después de 2 años). En las zonas más elevadas colonizadas por *Sarcocornia perennis*, la dinámica sedimentaria cambió marcadamente dependiendo de las marismas (-10 a 14 mm año^{-1}; -31 a +29 mm después de 2 años). Las extensas plantaciones de *S. maritima*, con cobertura relativa de 62%, se comportaron de manera similar a las marismas naturales conservadas después de 2 años, siendo una herramienta útil para estabilizar áreas erosivas en marismas europeas dado que reducen la erosión e incrementan la acreción.

CHAPTER 9.- Vertical sediment dynamic in *Spartina maritima* restored, non-restored and preserved marshes.

ABSTRACT

Salt marsh restoration projects using cordgrasses are relatively cheap and viable alternatives to improve the environmental quality while stabilizing sediments. This chapter quantifies the vertical sediment dynamic along the tidal gradient in low marshes in an extensive salt marsh restoration project, 26 months after planting *Spartina maritima*, in comparison with adjacent non-restored and preserved marshes in the Odiel Marshes (southwest Iberian Peninsula). The vertical sediment dynamic was recorded using markers consisting of an iron structure with two vertical posts and a horizontal crossbar, measuring the distance from the middle point of the crossbar to the sediment surface periodically between March 2009 and March 2011. The sediment dynamic was independent of the marsh elevation, depending mainly on plant cover. Erosion, dominating sedimentation in many locations, was lower in the *S. maritima* zone (mean accretion rate between +10 and +27 mm yr^{-1}; mean accumulated erosion between +22 and +51 mm after 2 yr) than in bare intertidal mudflats (-14 to +12 mm yr^{-1}; -36 to +37 mm after 2 yr). At higher elevations colonized by *Sarcocornia perennis*, sediment dynamics changed markedly, depending on marsh type (-10 to 14 mm yr^{-1}; -31 to +29 mm after 2 yr). Extensive salt marsh plantations using *S. maritima* behaved in a similar way to natural preserved marshes after ca. 2 yr with a relative cover of 62%, this being a useful tool to stabilize eroding areas in European marshes, since they reduced erosion and increased accretion.

9.1. INTRODUCTION

Wetland losses are a global problem, with 50% of the original wetlands that existed before human intervention now lost (Mitsch, 2010). Erosion and insufficient sediment supply cause great loss of salt marsh surface (Adam, 2002; Ravens *et al*., 2009). Thus, horizontal erosion of channel banks leads to high salt marsh losses, for example, 17000 m^2 yr^{-1} in the Odiel Marshes (Castillo *et al*., 2002). Vertical erosion is also a serious problem for the conservation of coastal marshes, especially in low marshes where the lost of a few centimeters in topographic elevation means life or death for salt marsh plant species (Castillo *et al*., 2000a; Castillo & Figueroa, 2009a) and erosive processes can result in irreversible changes (Day *et al*., 2011).

In this context, salt marsh restoration projects using cordgrasses, *Spartina* spp., are relatively cheap and viable alternatives to help improve the environmental quality while stabilizing sediments (Ranwell, 1967; Broome *et al*., 1988).

Different studies have described how smooth cordgrass *Spartina alterniflora* Loisel plantations increase accretion and reduce erosion (e.g. Chung *et al*., 2004; Utomo *et al*., 2010), but the effects of plantations using the only native cordgrass in many European estuaries, the small cordgrass *Spartina maritima* (Curtis) Fernald, have only been studied in pilot restoration plots (Castillo *et al*., 2008).

This work describes and quantifies the vertical sediment dynamic along the tidal gradient in low marshes where *S. maritima* is present in an extensive salt marsh restoration project 26 months after *S. maritima* has been planted (Castillo & Figueroa, 2009b), in comparison with adjacent non-restored marshes and preserved marshes in the Odiel Marshes (southwest Iberian Peninsula). We hypothesized that *S. maritima* plantations will reduce erosion, favoring accretion.

9.2. MATERIALS AND METHODS

9.2.1. Study sites.

This work was carried out in the three studied marshes of Odiel Marshes showed in Chapter 2.

9.2.2. Vertical sediment dynamic.

The vertical sediment dynamic was recorded using markers consisting of an iron structure with two vertical posts (ca. 1.5 m tall and 1 cm diameter) inserted in the sediment to a depth of around 1 m, and supporting a horizontal crossbar (ca. 0.5 m long) (n = 9 markers per elevation in restored marshes (RM) and non-restored marshes (NRM) and n = 6 markers per elevation in preserved marshes (PM)). The distance from the middle point of the crossbar to the sediment surface was measured periodically between March 2009 and March 2011. This method prevents measurement errors caused by an erosive vortex generated in the base of vertical structures. Initially, all the markers were placed in areas where the sediment was completely flat, without any evident signs of erosion (Fig. 9.1). At every low marsh area, altitudinal transects, separated from each other by at least 100 m, were established perpendicular to the tidal line, and erosion markers were placed randomly at each of the three altitudinal cover bands (mudflat, *Spartina* and *Sarcocornia* zones). The vertical erosion/accretion rate between consecutive measurements was calculated as the difference between the initial and final distance (in mm) divided by the number of years. Positive values indicated sedimentation and negative values indicated erosion. Elevation above Spanish Hydrographic Zero (SHZ) for every marker was surveyed *in situ* to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore); reference points were determined in relation to measurements of tidal extremes (Ranwell *et al.*, 1964).

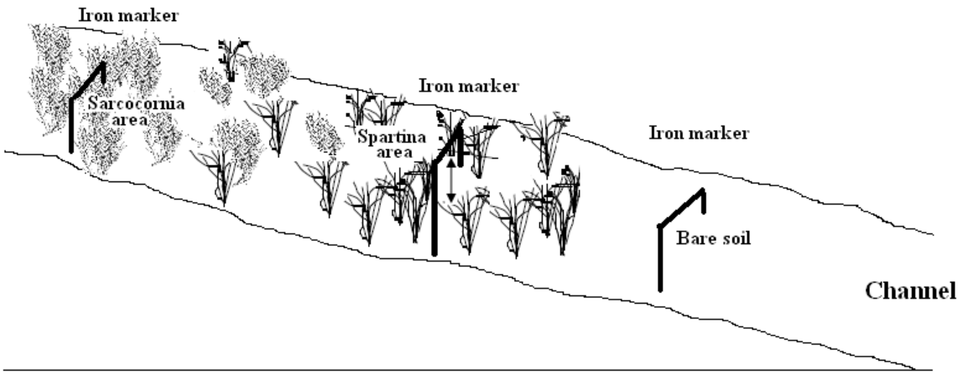

Figure 9.1. Diagram showing the placement of markers along the intertidal gradient in low marshes.

9.2.3. Statistical analysis.

Analyses were performed with SigmaPlot 11.0 for Windows. Outlier values were detected (using a standard residual method) and removed from the data series before analysis. Data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test). The sediment dynamic between elevations and marsh areas were compared by one-way analysis of variance (Anova) or Kruskal–Wallis test (non-normal data). Tukey or Dunn's test was used as post-hoc analysis. Spearman's coefficient was used to analyze the relationship between erosion/accretion rate and elevation. Deviations were calculated as the standard error of the mean (SEM).

9.3. RESULTS

In general, erosion/accretion rate was independent of elevation, varying between -68 ± 42 mm yr^{-1} at the *Sarcocornia* zone in PM in March 2011, and +74 ± 68 mm yr^{-1} at intertidal mudflats at PM in October 2010 (Table 9.1, Figs. 9.2 and 9.3).

In RM, the *Spartina* band showed higher total mean erosion/accretion rates (+27 ± 11 mm yr^{-1}) than intertidal mudflats (-10 ± 15 mm yr^{-1}), with the *Sarcocornia* band showing intermediate and positive values (+14 ± 12 mm yr^{-1}) (Kruskal-Wallis, χ^2 = 8.192, P < 0.05, df = 2; Dunn's test, P < 0.05) (Table 9.1). These erosion/accretion rates were driven to higher but non-significant mean accumulated sedimentation after 2 yr in the *S. maritima* zone (+51 ± 27 mm) (Kruskal–Wallis, P > 0.05; Fig. 9.2a). The accretion rate in the *Sarcocornia* zone was significantly higher than intertidal mudflats in September 2009 (Anova, *F* = 4,658, P < 0.05, d.f. = 26; Tukey test, P < 0.05) (Fig. 9.2a). In general, markers at *Spartina* and *Sarcocornia* zones recorded net sedimentation, except for a single marker in the *Spartina* zone located where an intertidal pond was emerging, and two markers at the highest elevations (Figs. 9.2a and 9.3).

Erosion/accretion rates in NRM were similar at every elevation (ranging between -10 ± 13 mm yr^{-1} in the *Sarcocornia* zone and-14 ± 8 mm yr^{-1} in intertidal mudflats) (Kruskal–Wallis, P > 0.05) (Table 9.1). Erosion dominated at every zone, varying between -29 ± 14 mm at the *S. densiflora* zone and -36 ± 16 mm at intertidal mudflats after 2 yr (Kruskal–Wallis, P > 0.05). The erosion/accretion rate was similar for every zone on every sampling day (Fig. 9.2b), with most markers recording net erosion rates (Fig 9.3). An erosive event affected the *Sarcocornia* zones in the RM and NRM during autumn to winter 2009 (Fig. 9.2a,b).

PM also showed similar mean erosion/accretion rates at every elevation (Kruskal–Wallis, P > 0.05), varying between -10 ± 10 mm yr^{-1} at the *Sarcocornia* zone and +12 ± 13 mm yr^{-1} at intertidal mudflats (Table 9.1). Accumulated sedimentation after 2 yr ranged between +37 ± 33 mm at intertidal mudflats and -31 ± 26 mm at the *Sarcocornia* zone (Kruskal–Wallis, P > 0.05) (Table 9.2, Fig. 9.2c). Accretion dominated at the *S. maritima* zone during the whole study, whereas higher and lower elevations were exposed temporally to sedimentary and erosive periods (Fig. 9.3).

	RM	**NRM**	**PM**
Intertidal mudflat			
Cover	Bare sediment	Bare sediment	Bare sediment, *Sm* and *Zn*
Maximum accretion rate (mm yr^{-1})	+43 ± 53^{a,a}	+16 ± 18^{a,a}	+74 ± 68^{a,a}
Maximum erosion rate (mm yr^{-1})	-43 ± 18^{a,a}	-43 ± 53^{a,a}	-29 ± 23^{a,a}
Mean accretion/erosion rate (mm yr^{-1})	-10 ± 15^{ab,a}	-14 ± 8^{a,a}	+12 ± 13^{b,a}
***Spartina* zone**			
Cover	*Sm*	Bare sediment and *Sd*	*Sm*
Maximum accretion rate (mm yr^{-1})	+69 ± 44^{a,a}	+13 ± 12^{a,a}	+37 ± 17^{a,a}
Maximum erosion rate (mm yr^{-1})	-	-33 ± 33^{a,a}	-25 ± 31^{a,a}
Mean accretion/erosion rate (mm yr^{-1})	+27 ± 11^{a,b}	-12 ± 6^{b,a}	+10 ± 7^{ab,a}
***Sarcocornia* zone**			
Cover	*Sm* and *Sp*	*Sm* and *Sp*	Bare sediment and *Sp*
Maximum accretion rate (mm yr^{-1})	+50 ± 24^{a,a}	+46 ± 56^{a,a}	+26 ± 12^{a,a}
Maximum erosion rate (mm yr^{-1})	-26 ± 20^{a,a}	-53 ± 45^{a,a}	-68 ± 42^{a,a}
Mean accretion/erosion rate (mm yr^{-1})	14 ± 12^{a,ab}	-10 ± 13^{a,a}	-10 ± 10^{a,a}

Table 9.1. Sediment cover, mean maximum accretion/erosion rate (mm year^{-1}) and mean total erosion/accretion rate (mm yr^{-1}) in three altitudinal zones in the three studied marsh areas. The first coefficient indicates significant differences between marshes at the same altitudinal zone, and the second one indicates significant differences between altitudinal zones for the same marshes (Anova, $P < 0.05$). Abbreviations: RM = restored marshes, NRM = non-restored marshes and PM = preserved marshes; *Sm = Spartina maritima*, *Sd = Spartina densiflora*, *Sp = Sarcocornia perennis* and *Zn = Zostera noltii.*

The erosion/accretion rate was higher in PM than in NRM in intertidal mudflats (Kruskal–Wallis, $\chi^2 = 7.137$, $P < 0.05$, df = 2; Dunn’s test, $P < 0.05$), and was higher in RM than in NRM at the *Spartina* zone (Kruskal–Wallis, $\chi^2 = 7.794$, $P < 0.05$, df = 2; Dunn’s test, $P < 0.05$) and did not show significant differences between areas in the *Sarcocornia* zone (Anova, $P > 0.05$) (Table 9.1; Fig. 9.2).

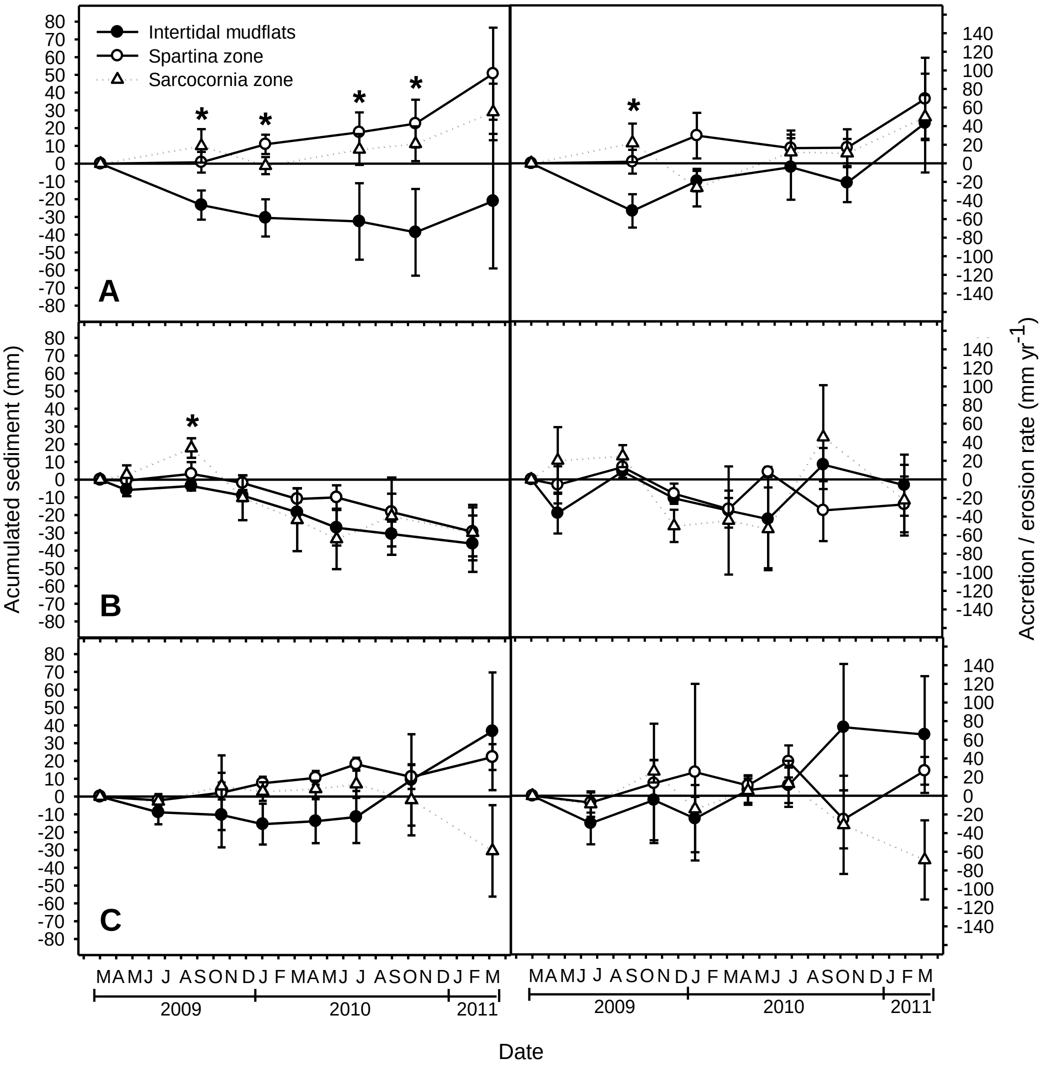

Figure 9.2. Mean values of erosion/accretion rates in mm year^{-1} (right) and accumulated values of erosion/accretion (left) in three marshes at different elevation. The sampling dates are shown on the abscissa axis. A = restored marshes, B = non-restored marshes and C = preserved marshes. * indicates significant differences between different altitudinal zones.

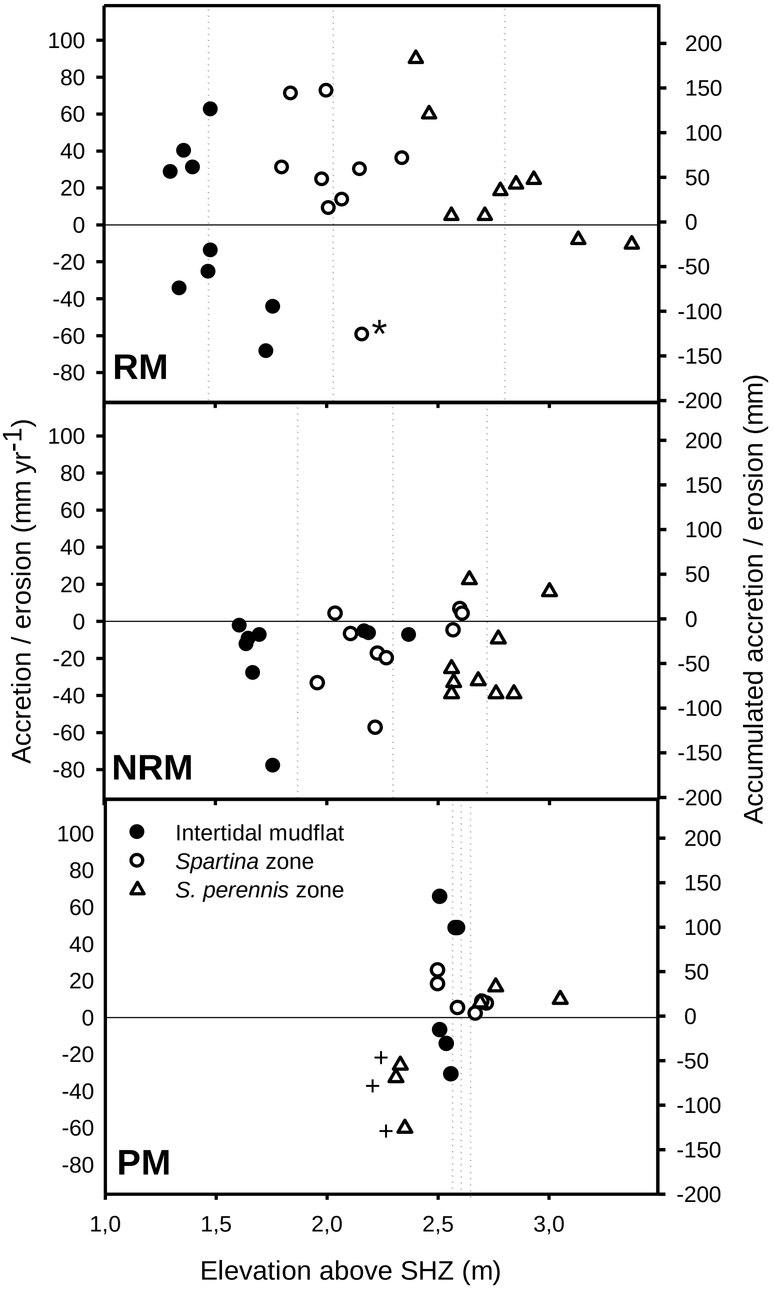

Figure 9.3. Total erosion/accretion in mm year^{-1} and accumulated values of erosion/accretion after 2 yr in three marshes at different elevations. The sampling elevation is shown on the abscissa axis. RM = restored marshes, NRM = non-restored marshes and PM = preserved marshes. Dotted lines indicate average elevation for each altitudinal zone (intertidal mudflat, *Spartina* zone and *S. perennis* zone). * Intertidal ponds forming in *S. maritima* prairie. + Bare patches at *Sarcocornia* zone.

9.4. DISCUSSION

The present work is the first study monitoring the sediment dynamic in extensive created salt marshes using the native European *S. maritima*. Results verified our hypothesis, showing that *S. maritima* plantations promote sedimentation and protect from erosion.

Sediment dynamic was independent of the marsh elevation, depending mainly on plant cover. Thus, erosion was lower, dominating sedimentation in many locations, in *S. maritima* and *S. perennis* zones than in bare intertidal mudflats in RM, as has previously been described for *S. maritima* natural marshes (Castellanos *et al*., 1998; Salgueiro & Caçador, 2007). In PM, sedimentation also dominated in the *S. maritima* zone during the whole study; however, differences at lower and higher elevations were not marked. In contrast, NRM where the low marsh was colonized by isolated clumps of *S. densiflora* did not present any spatial differentiation in sediment dynamic in relation to other altitudinal zones, with erosion dominating at every altitude.

Our results of vertical erosion at bare intertidal mudflats in RM and NRM (mean erosion rate between -14 ± 8 mm yr^{-1} in NRM and -10 ± 15 mm yr^{-1} in RM) were within the range of those recorded previously in the Odiel Marshes (Castillo *et al*., 2002) and in North American *S. alterniflora* marshes (Meyer *et al*., 1997). Agreeing with the results of our study, Gleason *et al*. (1979) in a laboratory experiment reported a net loss of sediment from bare sediments in *S. alterniflora* marshes, which seemed to be related with a higher exposition of mudflats to currents, tidal fluxes and, in our case, with the frequent boat traffic in the navigation channel where RM and NRM were located (Castillo *et al*., 2000b). The reduction of erosion due to plant colonization is only a local effect, operating within a static vegetation patch, but friction exerted by the vegetation seems to increase erosion in bare areas such as drainage channels (Temmerman *et al*., 2007). In addition, intertidal sediment stability depends upon the biological properties of the surface sediments. For example, biostabilization by cyanobacteria, diatoms and algae in *S. maritima* marshes has been described (Friend *et al*., 2003). In our study, accretion dominated in intertidal mudflats in PM (+12 ± 13 mm yr^{-1}; +37 ± 33 mm after 2 yr) that were covered by algae and isolated clumps of *Z. noltii* and *S. maritima*.

Accretion rates recorded at *S. maritima* zones (from +10 to +27 mm yr^{-1}) were in the range of those reported in pilots of created *S. maritima* marshes (Castillo *et al*., 2008), in *Spartina anglica* C.E. Hubbard wetlands (Lee & Partridge, 1983) and in S. alterniflora marshes (Nyman *et al*., 1993; Anisfeld *et al*., 1999; Erwin *et al*., 2006). *Spartina maritima* accumulates high below- and above-ground biomass, stabilizing sediments and promoting sedimentation (Castellanos *et al*., 1994; Castillo *et al*., 2008; Castillo & Figueroa, 2009a). It has been pointed out that *S. maritima* acts more to protect from erosion during storms than to enhance sedimentation during normal conditions (Neumeier & Ciavola, 2004). Our results showed both: (1) *S. maritima* prairies protected from erosion during storms, for example, during autumn and winter 2009 when *Sarcocornia* zones but not *Spartina* zones recorded high erosion rates in the RM and NRM, coinciding with unusually high rainfalls (AEMET, 2010), and in PM where sediment dynamic was more stable in the *Spartina* zone than in lower and higher elevations; and (2) *S. maritima* increased overall sedimentation, as recorded in RM and PM.

Sarcocornia perennis also has the ability of to trap and stabilize sediments (Castellanos *et al*., 1994; Figueroa *et al*., 2003). Nevertheless, *S. perennis* zones showed lower accretion/erosion rates than *S. maritima* zones in RM and PM (mean erosion/accretion rate between +14 and -10 mm yr^{-1}), which seemed to be related with decreasing sediment deposition when moving away from the lower distribution limit of vegetation at creek edges (Salgueiro & Caçador, 2007), colonized by dense *S. maritima* prairies in the RM and PM. Thus, sediment dynamic was very similar at *Sarcocornia* and *Spartina* zones in NRM where *S. densiflora* cover was low (ca. 20%).

This study showed that extensive salt marsh restoration projects using small cordgrass plantations behave in a similar way to natural populations of *S. maritima* after ca. 2 yr with relative cover of ca. 62%, being useful tools to stabilize eroding areas in

European marshes, since they reduce erosion and increase accretion.

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CHAPTER 10

Macroinvertebrate community in restored salt marshes using small cordgrass, *Spartina maritima*, in comparison with non-restored and preserved areas

CAPÍTULO 10.- Comunidad de macroinvertebrados en marismas restauradas con *Spartina maritima* en comparación con marismas no restauradas y áreas preservadas

RESUMEN

Este capítulo analizó la matriz ambiental y la comunidad de macroinvertebrados en marismas europeas restauradas usando *Spartina maritima* y *Zostera noltii* 3 años después de las plantaciones en comparación con un área no restauradas y otra preservada. El contenido edáfico en materia orgánica, densidad seca aparente, pH y el potencial rédox fueron las variables que mejor explicaban la distribución de los macroinvertebrados. Las marismas restauradas alcanzaron valores similares de diversidad ecológica e incluso mayores de riqueza específica que las marismas preservadas, aunque con diferencias en la composición de especies. Las marismas no restauradas e invadidas por *Spartina densiflora* mostraron la menor diversidad. La abundancia total y biomasa de macroinvertebrados no difirieron entre las marismas restauradas y preservadas, siendo en ambas mayores que en las marismas no restauradas. Las mayores diferencias en la composición de taxones se dieron entre las marismas preservadas y las no restauradas, con las marismas restauradas ocupando una posición intermedia. Nuestros resultados muestran que la restauración de marismas costeras usando *S. maritima* incrementa la complejidad y la abundancia de la comunidad bentónica de macroinvertebrados.

CHAPTER 10.- Macroinvertebrate community in restored salt marshes using small cordgrass, *Spartina maritima*, in comparison with non-restored and preserved areas

ABSTRACT

This chapter analysed the environmental matrix and macroinvertebrate community in European restored marshes using *Spartina maritima* and *Zostera noltii* 3 years after plantation in comparison with a non-restored area and a preserved one. Sediment organic matter content, bulk dry density, pH and redox potential are the variables that best explain macroinvertebrate distribution. Restored marshes achieved similar ecological diversity values and even higher specific richness than preserved marshes, although with differences in species composition. Non-restored marshes invaded by *Spartina densiflora* showed the lowest diversity. Restored and preserved marshes did not differ in total abundance or in total biomass, both being higher than in non-restored marshes.The macroinvertebrate communities in preserved marshes and non-restored marshes presents the largest differences in taxa composition, with restored marshes occupying an intermediate position. Our results show that salt marsh restoration using *Spartina maritima* increased the complexity and abundance ofthe benthic macroinvertebrate community.

10.1. INTRODUCTION

Salt marshes are among the most impacted ecosystems in the world (Gedan *et al*., 2009) with their ecological engineering being very important (Mitsch, 2010). In this sense, monitoring the progression of marsh restoration projects is a key point to improve and to project restoration practices (Konisky *et al*.,2006).

Ecological restoration of salt marshes may enhance the macroinvertebrate community (Warren *et al*., 2002) since estuarine mudflats support a high biomass of macroinvertebrates with relatively low diversity (Cardoso *et al*., 2008), providing vegetated areas with a wide range of microhabitats that may increase the diversity of macroinvertebrates (Netto & Lana, 1997).Thus, the study of benthic invertebrates is used to assess the development following salt marsh restoration, which has been studied mainly in *Spartina alterniflora* Loisel marshes (Peck *et al*., 1994; Swamy *et al*., 2002; Zedler &Lindig-Cisneros, 2000; Craft & Sacco, 2003). Neverthless, little is known about the development of ecological engineering projects in European salt marshes using small cordgrass (*Spartina maritima* (Curtis) Fernald) (Castillo & Figueroa, 2009).

This chapter aimed to monitor the environmental matrix and macroinvertebrate community in restored marshes using *S. maritima* and *Zostera noltii* Hornemann, 2 years after plantation (Castillo & Figueroa 2009) in comparison with a non-restored areas and a preserved one in Odiel Marshes (southwest Iberian Peninsula). Comparisons between restored and reference preserved marshes have proven to be an appropriate methodology to assess the success of restoration projects (Armitage *et al*., 2007). Our study provides scientific data on the macroinvertebrate community of the Odiel Marshes, where they are scarcely found (Sánchez-Moyano *et al*., 2010a, 2010b).

10.2. MATERIALS AND METHODS

10.2.1. Macroinvertebrates sampling and laboratory analysis.

This work was carried out in the three studied marshes of Odiel Marshes showed in Chapter 2. The sampling was conducted during September (mean temperature, 23.7 ºC; number of daylight hours = 246) and October 2009 (mean temperature, 21.7 ºC; number of daylight hours = 249) (INE, 2009). Benthic macrofauna samples to 5cm depth were taken by hand in plots of 20 cm x 25 cm (0.05 m^2). Samples were placed every 20 m along transects orthogonal to the tidal line between +1.8 and +3.0 m above Spanish Hydrographic Zero (SHZ) (9 transects and 26 plots in RM, 9 transects and 17 plots in NRM, and 6 transects and 15 in CM). Collected sediments were processed in the laboratory.

Sediment samples were sieved (1-mm mesh), fixed with 70% ethanol and stained with Rose Bengal. Macroinvertebrates were separated and cleaned carefully, and analysed under a Leica binocular magnifying glass (model WildM3C). Samples were stored in small plastic boats with 70% alcohol until proceeding with the identification of the specimens and quantification of the biomass. To record their biomass, samples were dried to constant dry weight (DW) at 80° C during 48 hours. Ash-free dry weight (AFDW) was recorded by the loss-on-ignition method after 4 h at 500 ºC (10h for those species with shells, following Garnerd & Thomas (1987) and modified by Pagola-Carte *et al.*(2002)).

10.2.2. Environmental matrix.

Below-ground plant biomass (BGB) in macroinvertebrate samples was separated carefully, cleaned with tap water and dried to constant weight at 80° C during 48 hr. Elevation relative to SHZ, sediment redox potential, interstitial water pH and electrical conductivity, sediment organic content, bulk dry density and sediment water content were recorded in the area adjacent to every macroinvertebrate plot together with macroinvertebrate samples. Every sediment characteristic was recorded between 0–5 cm deep. Elevation was surveyed *in situ* to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore); reference points were determined in relation to measurements of tidal extremes (Ranwell *et al*. 1964). Sediment redox potential was determined in the field with a portable meter and electrode system (Crison pH/mV p-506), with every sample being the mean of three sub-samples. pH (pH / redox Crison with the electrode *M-506*) and electrical conductivity (conductivity meter, Crison-522) was recorded in the laboratory after adding distilled water to the sediment with 1:1, v/v and 1:2, v/v, respectively. Sediment organic content was analysed in triplicate sub-samples by the loss-on-ignition method after 4 h at 450 ºC. Sediment bulk dry density was recorded by weighing the sedimentscollected in a cylindrical core of 5 x 5 cm after drying them at 80 ºC during 48 h (DW). Sediment water content (%) was recorded as the difference between fresh and dry weights of ca. 100–150 g (FW) samples.

10.2.3. Macroinvertebrate community.

Fauna was sorted, identified to the lowest possible taxon and counted. Some unidentified taxa within polychaete group,called sp. 1and sp. 2, were included in the inventory.The samples of each transect were grouped for analyses.The structure of the benthic community at each site was calculated in terms of total number of species (S_{total}), mean species number per sample (S_{mean}), mean number of individuals per sample (N_{mean}), species abundance (ind. m^{-2}), total abundance (ind. m^{-2}), species biomass(g m^{-2}) and total biomass(g m^{-2}). Ecological diversity was calculated following the Shannon-Weaver index (H’) (Shannon & Weaver, 1949). We also calculated maximum diversity (H_{max}), evenness (J) and Simpson index of dominance (D) (Simpson, 1949) using the PRIMER v 5.2.8 software package.

10.2.4. Statistical analysis.

Pearson correlation coefficient for normal data and Spearman coefficient for non-normal data were used to analyse relationships between environmental variables and macroinvertebrate abundance and biomass. Marsh areas were compared by one-way Anova or Kruskal-Wallis test. B-Tukey test was used as post-hoc analysis (SPSS release 17.0, SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM).

ABC (Abundance Biomass Comparison) curves, MDS (non-metric multidimensional scaling), SIMPER (percentage of similarity analysis), PCA (Principal Components Analysis) and BIOENV analysis were carried out using the software package PRIMER v 5.2.8. ABC curves is a graphical representation of both cumulative percentage of abundances and biomass and it was employed to detect community perturbation (Warwick, 1986).The W statistic was calculated according to Clarke (1990). MDS establishes affinities between sampled areas and the taxa abundance. Data was standardised using the fourth root. We verified the analysis validity using the Kruskal stress coefficient. SIMPER, based on the similarity matrix obtained from the Bray-Curtis index, calculated the contribution of each taxa to the dissimilarity between groups of stations (Clarke, 1993). PCA examined the influence of environmental variables in the studied stations, and BIOENV analysis compared the rank similarity matrix of species abundance with the abiotic variables (Clarke & Ainsworth, 1993).

10.3. RESULTS

10.3.1. Environmental matrix.

Elevation above SHZ did not differ between marsh areas (Anova, $P > 0.05$). PM showed lower sediment redox potential (Kruskal-Wallis, $\chi^2 = 17.978$, $P < 0.001$, $df = 2$), lower conductivity (Kruskal-Wallis, $\chi^2 = 10.198$, $P < 0.01$, df = 2) and higher pH (Anova, $F = 9.535$, $P < 0.001$, df = 57) than RM and NRM. Organic matter content was the highest in NRM (Kruskal-Wallis, $\chi^2 = 29.608$, $P < 0.001$, df = 2) reaching values of up 16.7%. Sediment water content was lower in PM than in NRM (Kruskal-Wallis, $\chi^2 = 17.175$, $P < 0.001$, df = 2; $U = 2.000$, $P < 0.001$) and similar to RM, while bulk dry density was the highest in the PM (Kruskal-Wallis, $\chi^2 = 16.850$, $P < 0.001$, df= 2) (Table 10.1).

BGB of *S. maritima* was the lowest in NRM, showing similar values in RM and PM (Kruskal-Wallis, $\chi^2=15.213$, $P < 0.001$, df = 2). *Spartina densiflora* BGB was only sampled in NRM and *Z. noltii* in PM. *Sarcocornia perennis* BGB was not sampled in PM and it did not differ between RM and NRM (*U*-test, $P > 0.05$) (Table 10.1).

The variance explained by the two principal axes of PCA analysis was 54.0% (eigenvalues 3.93 and 2.01, respectively). The principal axis discriminated the transects mainly for sediment water content (-0.461), bulk dry density (-0.458), organic matter content (-0.408) and pH (+0.370), as stated by their eigen vectors. The second axis was mainly influenced by redox potential (+0.514) and by specific BGB (*S.maritima* (+0.376), *Z.nolti* (-0.375) and *S. perennis* (+0.349)) (Fig. 10.1).

10.3.2. Macroinvertebrate community.

37 species were recorded in all sampled marshes. The PM had 8 species that were not present in the other areas, RM presented 6 exclusive species and NRM had only 2 exclusive species (Table 10.2 and 10.3). MDS analysis clearly separated PM and NRM according to their species composition, while RM appeared in intermediate position (Fig. 10.2). Generally, all exclusive species were recorded in low abundances, except for the phylum *Phoronidea*, the bivalve *Abra tenuis*, the polichaete *Alkmaria romijni* and the crustacean *Corophium multisetosum* that were abundant in PM (Table 10.2 and 10.3).

	RM		NRM		PM	
Abiotic variables	*Mean ± SEM*	*max-min*	*Mean ± SEM*	*max-min*	*Mean ± SEM*	*max-min*
Elevation relative to SHZ	2.42 ± 0.08^{a}	3.15 / 1.77	2.58 ± 0.08^{a}	3.39 / 2.22	2.57 ± 0.05^{a}	3.05 / 2.29
Redox potential	24 ± 17^{a}	164 / -117	23 ± 20^{a}	193 / -81	-86 ± 15^{b}	37 / -155
Conductivity	16.3 ± 1.5^{ab}	35.5 / 8.3	17.5a ± 0.5^{a}	21.1 / 12.7	15.4 ± 0.4^{b}	18.0 /12.4
Organic matter content	5.0 ± 0.66^{a}	13.1 / 0.3	11.4 ± 0.6^{b}	16.7 / 8.1	4.6 ± 0.3^{a}	6.9 /2.7
pH	7.1 ± 0.0^{a}	7.9 / 6.6	7.2 ± 0.1^{a}	8.0 / 6.8	7.4 ± 0.0^{b}	7.7 / 7.3
Water content of sediment	44 ± 3^{ab}	70 / 22	51 ± 1^{a}	58 / 43	36 ± 1^{b}	45 / 27
Bulk dry density	0.8 ± 0.1^{a}	1.4 / 0.2	0.7 ± 0.0^{a}	0.9 / 0.6	1.1 ± 0.0^{b}	1.3 / 0.8
Sm BGB	177 ± 46^{a}	451 / 37	8 ± 8^{b}	71 / 0	73 ± 21^{a}	174 /28
Sd BGB	0 ± 0^{a}	0 / 0	16 ± 6^{b}	41 / 0	0 ± 0^{a}	0 / 0
Sp BGB	154 ± 51^{a}	375 / 0	85 ± 34^{a}	240 / 0	0 ± 0^{b}	0 / 0
Zn BGB	0 ± 0^{a}	0 / 0	0 ± 0^{a}	0 / 0	16 ± 6^{b}	33 / 0

Table 10.1. Elevation above Spanish Hydrographic Zero (m), sediment redox potential (mV), sediment conductivity (mS cm^{-1}), sediment organic content (%), pH, sediment water content (%), sediment bulk dry density (g cm^{-3}) and belowgound biomass (BGB, g m^{-2}) in the first 5 cm deep in the three marsh areas (RM, restored mash; NRM, non-restored marsh; PM, preserved marsh) in the Odiel Marshes. Sm, *Spartina maritima*; Sd, *Spartina densiflora*; Sp, *Sarcocornia perennis*; Zn, *Zostera noltii*. Different letters indicate significant differences between areas (n = 15–26). Mean ± Standard Error Mean and maximum and minimum values are presented.

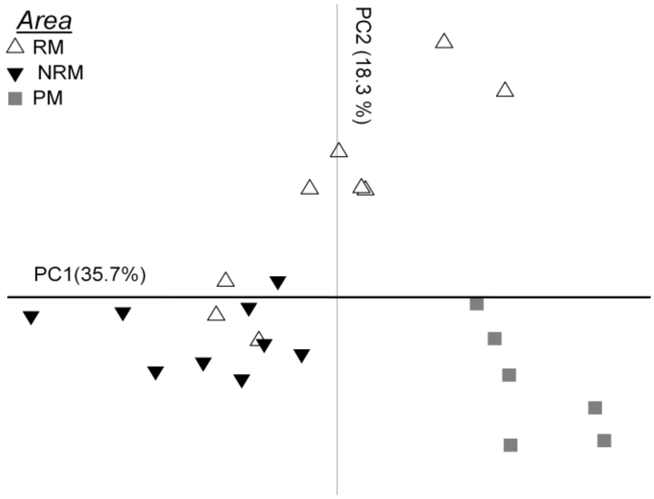

Figure 10.1. PCA analysis plot for all transects from environmental variables within sediments. The percentage of variability explained by the two principal axes is given. RM, restored marshes; NRM, non-restored marshes; PM, preserved marshes.

	Restored marsh			Non-restored marsh		
	Abundance	*DW*	*AFDW*	*Abundance*	*DW*	*AFDW*
ANNELIDA						
Polychaeta						
Alkmaria romijni	0 ± 0	-	-	0 ± 0	-	-
Capitella capitata	106 ± 46	0.004 ± 0.001	0.004 ± 0.001	0 ± 0	-	-
Hediste diversicolor	126 ± 38	0.964 ± 0.247	0.865 ± 0.230	40 ± 4	0.227 ± 0.027	0.207± 0.074
Melinna palmata	0 ± 0	-	-	0 ± 0	-	-
Nephtys hombergii	2 ± 1	0.001 ± 0.001	0.000 ± 0.000	0 ± 0	-	-
Polychaete sp.1	1 ± 1	0.009 ± 0.009	0.003 ± 0.003	0 ± 0	-	-
Polychaete sp.2	0 ± 0	-	-	0 ± 0	-	-
Scolelepis cantabra cf.	9 ± 5	0.001 ± 0.001	0.001 ± 0.001	0 ± 0*	-	-
Oligochaeta	50 ± 38	0.001 ± 0.001	0.001 ± 0.001	134 ± 28	0.005 ± 0.001	0.004 ± 0.003
ARACHNIDA						
Araneae	0 ± 0	-	-	1 ± 0	0.001 ± 0.000	0.001 ± 0.000
Acarina	3 ± 2	0.000 ± 0.000	0.000 ± 0.000	0 ± 0	-	-
BRYOZOA						
Bugula neritina	0 ± 0	-	-	0 ± 0	-	-
COLLEMBOLA						
Collembola	4 ± 2	0.000 ± 0.000	0.000 ± 0.000	1 ± 0	0.000 ± 0.000	0.000 ± 0.000
Anurida maritima	0 ± 0*	-	-	1 ± 0	0.000 ± 0.000	0.000 ± 0.000
CRUSTACEA						
Isopoda						
Eurydice affinis	1 ± 1	0.000 ± 0.000	0.000 ± 0.000	49 ± 11	0.009 ± 0.002	0.006 ± 0.003
Lekanesphaera hookeri	14 ± 3	0.034 ± 0.004	0.016 ± 0.002	7 ± 2	0.021 ± 0.007	0.008 ± 0.007
Cyathura carinata	449 ± 68	0.105 ± 0.015	0.060 ± 0.008	167 ± 12	0.038 ± 0.004	0.027 ± 0.009
Paragnathia formica	16 ± 16	0.003 ± 0.003	0.003 ± 0.003	81 ± 19	0.023 ± 0.006	0.020 ± 0.015
Decapoda						
Juvenile decapoda	3 ± 2	0.000 ± 0.000	0.000 ± 0.000	0 ± 0	-	-
Carcinus maenas	7 ± 3	0.354 ± 0.246	0.143 ± 0.099	1 ± 0	0.009 ± 0.003	0.004 ± 0.004
Uca tangeri	2 ± 1	0.044 ± 0.035	0.019 ± 0.016	3 ± 1	0.006 ± 0.003	0.005 ± 0.005
Palaemonidae	1 ± 1	0.000 ± 0.000	0.000 ± 0.000	0 ± 0	-	-
Amphipoda						
Melita palmata	10 ± 7	0.003 ± 0.002	0.002 ± 0.002	0 ± 0	-	-
Orchestia stephenseni	0 ± 0	-	-	1 ± 0	0.001 ± 0.000	0.000 ± 0.000
Corophium multisetosum	0 ± 0	-	-	0 ± 0	-	-
Cirripedia						
Balanus sp.	5 ± 3	0.009 ± 0.006	0.002 ± 0.001	7 ± 1	0.046 ± 0.014	0.001 ± 0.001
INSECTA						
Diptera						
Dolichopodidae	40 ± 14	0.011 ± 0.005	0.010 ± 0.004	29 ± 2	0.008 ± 0.001	0.005 ± 0.002
Diptera larva	1 ± 1	0.000 ± 0.000	0.000 ± 0.000	7 ± 1	0.000 ± 0.000	0.000 ± 0.000
Chironomus sp.	48 ± 18	0.001 ± 0.000	0.000 ± 0.000	0 ± 0*	-	-
Hemiptera						
Hemiptero Sord. homoptero	4 ± 4	0.000 ± 0.000	0,00 ± 0,00	0 ± 0	-	-

Cicadellidae	2 ± 2	0.000 ± 0.000	0,00 ± 0,00	0 ± 0	-	-
MOLLUSCA						
Opisthobranchia	3 ± 3	0.000 ± 0.000	0.000 ± 0.000	0 ± 0	-	-
Bivalvia						
Abra tenuis	0 ± 0	-	-	0 ± 0	-	-
Cerastoderma edule	3 ± 1	0.002 ± 0.001	0.000 ± 0.000	3 ± 1	0.008 ± 0.002	0.001 ± 0.000
Gastropoda						
Hydrobia ulvae	0 ± 0	-	-	0 ± 0	-	-
NEMERTEA	0 ± 0	-	-	0 ± 0	-	-
PHORONIDEA	0 ± 0	-	-	0 ± 0	-	-
Total	**910 ± 121^{a}**	**1.546 ± 0.456^{a}**	**1.133 ± 0.278^{a}**	**532 ± 95^{b}**	**0.402 ± 0.034^{b}**	**0.291 ± 0.079^{b}**

Table 10.2. Mean ± SEM of abundance (ind. m^{-2}) of macrofauna, dry biomass (DW) and ash-free dry biomass (AFDW) (gm^{-2}) of all observed taxa in restored and non-restored low marsh areas in Odiel Marsh. Values with the symbol (-) means absent. *Scolelepis cantabra* cf. (cf. = to confirm). * indicates that the species was present in the area with an abundance < 0.01 indv. m^{-2}. Different letters indicate significant differences between marshes.

	Preserved marsh		
	Abundance	*DW*	*AFDW*
ANNELIDA			
Polychaeta			
Alkmaria romijni	48 ± 37	0.006 ± 0.003	0.002 ± 0.002
Capitella capitata	30 ± 12	0.015 ± 0.008	0.010 ± 0.005
Hediste diversicolor	4 ± 3	0.008 ± 0.007	0.006 ± 0.006
Melinna palmata	2 ± 2	0.001 ± 0.001	0.001 ± 0.001
Nephtys hombergii	13 ± 9	0.020 ± 0.011	0.010 ± 0.007
Polychaete sp.1	0 ± 0	-	-
Polychaete sp.2	2 ± 2	0.002 ± 0.001	0.001 ± 0.001
Scolelepis cantabra cf.	0 ± 0	-	-
Oligochaeta	0 ± 0	-	-
ARACHNIDA			
Araneae	0 ± 0	-	-
Acarina	0 ± 0	-	-
BRYOZOA			
Bugula neritina	1 ± 1	0.022 ± 0.019	0.006 ± 0.006
COLLEMBOLA			
Collembola	9 ± 5	0.001 ± 0.000	0.000 ± 0.000
Anurida maritima	0 ± 0	-	-
CRUSTACEA			
Isopoda			
Eurydice affinis	0 ± 0	-	-
Lekanesphaera hookeri	9 ± 3	0.022 ± 0.013	0.006 ± 0.005

Cyathura carinata	522 ± 89	0.377 ± 0.064	0.184 ± 0.037
Paragnathia formica	32 ± 28	0.009 ± 0.007	0.006 ± 0.006
Decapoda			
Juvenile decapoda	0 ± 0	-	-
Carcinus maeñas	6 ± 4	0.218 ± 0.046	0.070 ± 0.056
Uca tangeri	2 ± 2	0.069 ± 0.057	0.025 ± 0.025
Palaemonidae	0 ± 0	-	-
Amphipoda			
Melita palmata	5 ± 3	0.003 ± 0.002	0.001 ± 0.001
Orchestia stephenseni	0 ± 0	-	-
Corophium multisetosum	37 ± 16	0.017 ± 0.007	0.010 ± 0.005
Cirripedia			
Balanus sp.	6 ± 3	0.061 ± 0.039	0.005 ± 0.003
INSECTA			
Diptera			
Dolichopodidae	45 ± 14	0.014 ± 0.004	0.007 ± 0.003
Diptera larva	0 ± 0	-	-
Chironomus sp.	0 ± 0*	-	-
Hemiptera			
Hemiptero Sord. homoptero	0 ± 0	-	-
Cicadellidae	0 ± 0	-	-
MOLLUSCA			
Opisthobranchia	0 ± 0	-	-
Bivalvia			-
Abra tenuis	79 ± 14	0.203 ± 0.050	0.022 ± 0.019
Cerastoderma edule	2 ± 2	0.306 ± 0.248	0.018 ± 0.016
Gastropoda			
Hydrobia ulvae	5 ± 5	0.015 ± 0.013	0.001 ± 0.001
NEMERTEA	3 ± 2	0.004 ± 0.002	0.002 ± 0.001
PHORONIDEA	73 ± 49	0.459 ± 0.258	0.025 ± 0.019
Total	**935 ± 90**^{a}	**1.855 ± 0.621**^{a}	**0.419 ± 0.103**^{ab}

Table 10.3. Mean ± SEM of abundance (ind. m^{-2}) of macrofauna, dry biomass (DW) and ash-free dry biomass (AFDW) (gm^{-2}) of all observed taxa in preserved low marsh areas in Odiel Marsh. Values with the symbol (-) means absent. *Scolelepis cantabra* cf. (cf. = to confirm). * indicates that the species was present in the area with an abundance < 0.01 indv. m^{-2}. Different letters indicate significant differences between marshes.

SIMPER analysis obtained the best discriminating taxa between marsh areas, showing the most differences between PM and NRM (Average dissimilarity = 65.82%) with *A. tenuis, Capitella capitata, C. multisetosum* and *Hediste diversicolor* as the species that contributed mainly to the dissimilarity. For RM and NRM (Average dissimilarity = 58.08%) were *C. capitata*, *Chironomus* sp., *Oligochaeta* and *Lekanesphaera hookeri*. RM and PM showed a slightly lower dissimilarity (Average dissimilarity = 57.22%) with *A. tenuis*, *H. diversicolor, Chironomus* sp. and *C. multisetosum* as the most important species to separate both marshes (Table 10.4, Fig. 10.2).

Taxa	**Av. abund**		**Av.diss**	**Ratio**	**Contrib(%)**	**Cum.(%)**
Dissimilarity between areas						
	RM	NRM	(Average dissimilarity = 58.08)			
Capitella capitata	2.44	0.00	5.84	1.60	10.05	10.05
Chironomus sp.	2.06	0.00	5.18	1.57	8.93	18.97
Oligochaeta	0.84	1.61	4.58	0.87	7.89	26.86
Lekanesphaera hookeri	1.90	0.49	4.18	2.07	7.19	34.05
Hediste diversicolor	3.08	2.15	3.12	1.20	5.37	39.42
Paragnathia formica	0.38	1.20	3.05	0.75	5.24	44.66
Eurydice affinis	0.18	1.11	3.00	0.78	5.16	49.82
	RM	PM	(Average dissimilarity = 57.22)			
Abra tenuis	0.00	2.93	5.83	5.71	10.19	10.19
Hediste diversicolor	3.08	0.62	4.96	1.96	8.66	18.85
Chironomus sp.	2.06	0.00	4.03	1.63	7.05	25.90
Corophium multisetosum	0.00	1.77	3.51	1.35	6.13	32.03
Alkmaria romijni	0.00	1.63	3.24	1.18	5.67	37.70
Capitella capitata	2.44	1.96	2.95	1.20	5.16	42.86
Phoronidea	0.00	1.27	2.30	0.69	4.02	46.88
	NRM	PM	(Average dissimilarity = 65.82)			
Abra tenuis	0.00	2.93	7.21	5.24	10.95	10.95
Capitella capitata	0.00	1.96	4.68	1.87	7.12	18.06
Corophium multisetosum	0.00	1.77	4.34	1.34	6.59	24.65
Hediste diversicolor	2.15	0.62	4.23	1.53	6.43	31.08
Alkmaria romijni	0.00	1.63	4.01	1.18	6.09	37.17
Oligochaeta	1.61	0.00	3.90	0.76	5.93	43.10
Paragnathia formica	1.20	0.96	3.63	0.94	5.51	48.61

Table 10.4. Zonal variation of the average abundance (Av. abund), average dissimilarity (Av. diss), ratio (Dissimilarity/SD), contribution to dissimilarity (%) and contribution to dissimilarity accumulated (%) of the most relevant taxa in restored marshes (RM), non-restored marshes (NRM) and preserved marshes (PM) in Odiel Marhes. Taxa are listed in decreasing order according to its contribution to the Av. diss between areas.

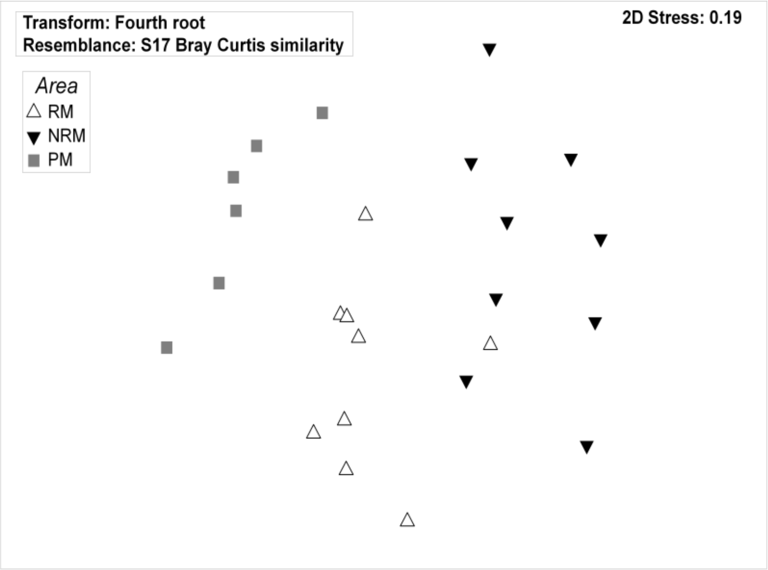

Figure 10.2. MDS ordination using Bray-Curtis similarities on taxa abundance. RM, restored marshes; NRM, non-restored marshes; PM, preserved marshes.

S_{total} was higher in RM (S = 26) than in NRM and PM (S = 18-23, respectively). S_{mean} was higher for RM and PM than for NRM (Anova, $F = 7.440$, $P < 0.01$, df = 58). N_{mean} was maximum at PM ($N_{mean} = 47.5 \pm 6.5$ ind. sample^{-1}) and minimum at NRM ($N_{mean} = 26.6 \pm 4.9$ ind. sample^{-1}) (Kruskal-Wallis, $\chi^2 = 6.628$, $P < 0.05$, df = 2); RM showed N_{mean} values more similar to PM (Table 10.5).

Location	**S_{total}**	**S_{mean}**	**N_{mean}**
Restored marshes	26	5.6 ± 0.4^a	45.5 ± 6.2^{ab}
Non-restored marshes	18	3.6 ± 0.5^b	26.6 ± 4.9^a
Preserved marshes	23	6.1 ± 0.5^a	47.5 ± 6.5^b

Table 10.5. Total species richness (S_{total}), mean species richness per sample (S_{mean}) and mean individuals number per sample (N_{mean}) in the three salt marsh areas in the Odiel Marshes. Different letters indicate significant differences between areas.

RM and PM showed higher H' and H_{max} than NRM (Anova, $P < 0.05$; Tukey B, $P < 0.05$). J and D were similar for every marsh area (Anova, $P > 0.05$) with high relative J values and low D values (Fig. 10.3).

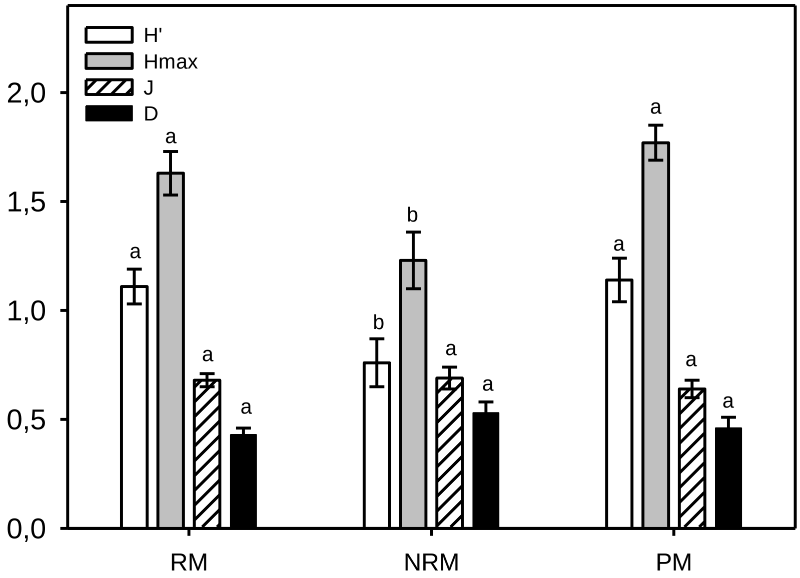

Figure 10.3. Ecological diversity (H'), maximum ecological diversity (H_{max}), dominance (D) and evenness (J) of the more abundant benthic macroinvertebrates in restored marshes (RM), non-restored marshes (NRM) and preserved marshes (PM). Different letters indicate significant differences between marshes.

Crustaceans and annelids were the major groups in all studied marshes. The most abundant species were present in all areas (except *Oligochaeta*, which did not appear in PM and *C. capitata* in NRM).The crustacean isopod *Cyathura carinata* was the most abundant taxon, its lowest abundance being in NRM and its highest in PM (522 ± 89 ind. m^{-2}) (Anova, $F = 9.052$, $P < 0.01$, df = 23; Tukey B, $P < 0.05$). RM and PM showed higher total macroinvertebrate abundance than NRM (Anova, $F = 4.660$, $P < 0.05$, df = 23; Tukey B, $P < 0.05$). *H. diversicolor* and *C. capitata* were abundant in RM, *Oligochaeta*, *Paragnathia formica* in NRM, and *A. tenuis* and *Phoronidea* in PM. *H. diversicolor* abundance in PM was lower than for the other two areas (Kruskal-Wallis, $\chi^2 = 11\ 835$, $P < 0.01$, df = 2; *U*-test, $P < 0.05$) (Table 10.2 and 10.3, Fig. 10.4).

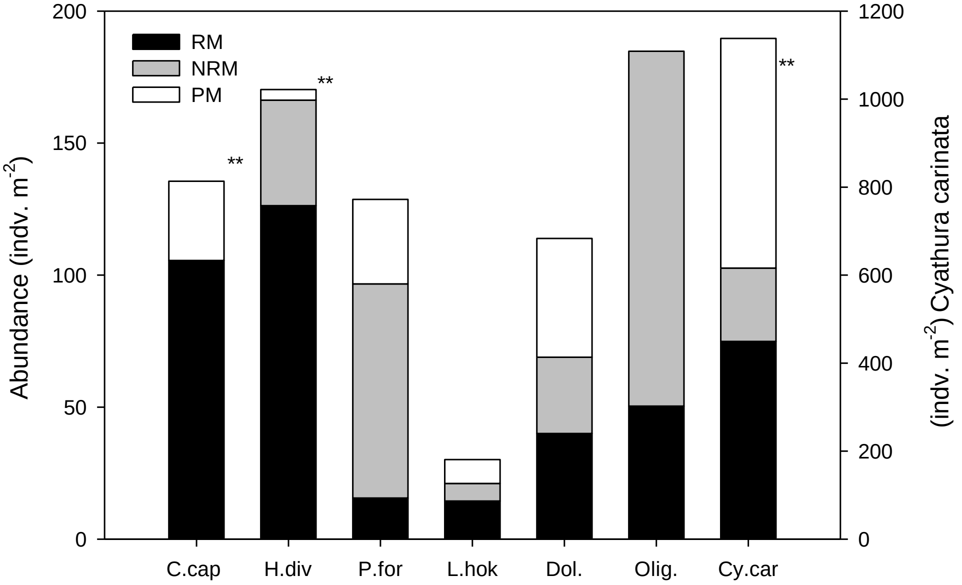

Figure 10.4. Abundance (ind. m^{-2}) of the most abundant macroinvertebrate species in restored marshes (RM), non-restored marshes (NRM) and preserved marshes (PM). C.cap, *Capitella capitata*; H.div, *Hediste diversicolor*; P.for, *Paragnathia formica*; L.hok, *Lekanesphaera hookeri*; Dol., *Dolichopodidae*; Olig., *Oligochaeta*; Cy.car, *Cyathura carinata*. The right axis (ind.m^{-2}) refers only to *C. carinata*. Asterisk (*) indicates significant differences between areas (Anova or Kruskal-Wallis, P < 0.01).

Macroinvertebrate community biomass was higher in PM and RM (1.855 ± 0.621 g DWm^{-2} and 1.546 ± 0.456 g DWm^{-2}, respectively) than in NRM (0.402 ± 0.034 $gDWm^{-2}$) (Kruskal-Wallis, $\chi2 = 8,728$, $P < 0.05$, df = 2). RM showed higher AFDW than NRM, while PM showed intermediate values (Kruskal-Wallis, $\chi^2 = 6,219$, $P < 0.05$, df = 2) (Table 10.2 and 10.3). *Hediste diversicolor* showed the highest biomass and AFDW values in RM (0.964 ± 0.247 g DW m^{-2} and 0.865 ± 0.230 g m^{-2}, respectively) together with *Carcinus maenas* (0.354 ± 0.246 g DW m^{-2} and 0.143 ± 0.099 DW g m^{-2}, respectively) (Table 10.2, Fig. 10.4).

According to criteria proposed by Warwick (1986) for detecting the severity of disturbance in a macrobenthic community, the average ABC curve in RM, where the biomass curve was above that for abundance, indicates undisturbed conditions. For NRM the crossing of abundance and biomass curves suggests a moderate state of disturbance. In the case of PM, the ABC curve indicated disturbed conditions since the abundance curve was always above the biomass curve, showing the lowest W statistic value (W = -0.07) (Fig. 10.5).

10.3.3. Environment-macroinvertebrates.

The results of BIOENV analysis evaluating the relationship between environmental variables and macrofauna presence indicated that the best correlations always occurred with sediment pH, redox potential, organic matter content and bulk dry density (maximum correlations of 0.441). Individually, organic matter content (0.398) and sediment bulk dry density (0.334) were the variables that better explained the macroinvertebrates distribution. pH and redox potential presented lower correlations (0.261 and 0.181, respectively).

Total macroinvertebrate abundance decreased with increasing organic matter content (Spearman, $r = -0.304$, $P < 0.05$, $n = 58$) and total AFDW increased with *S. maritima* BGB (Pearson, $r = 0.367$, $P < 0.01$, $n = 58$).

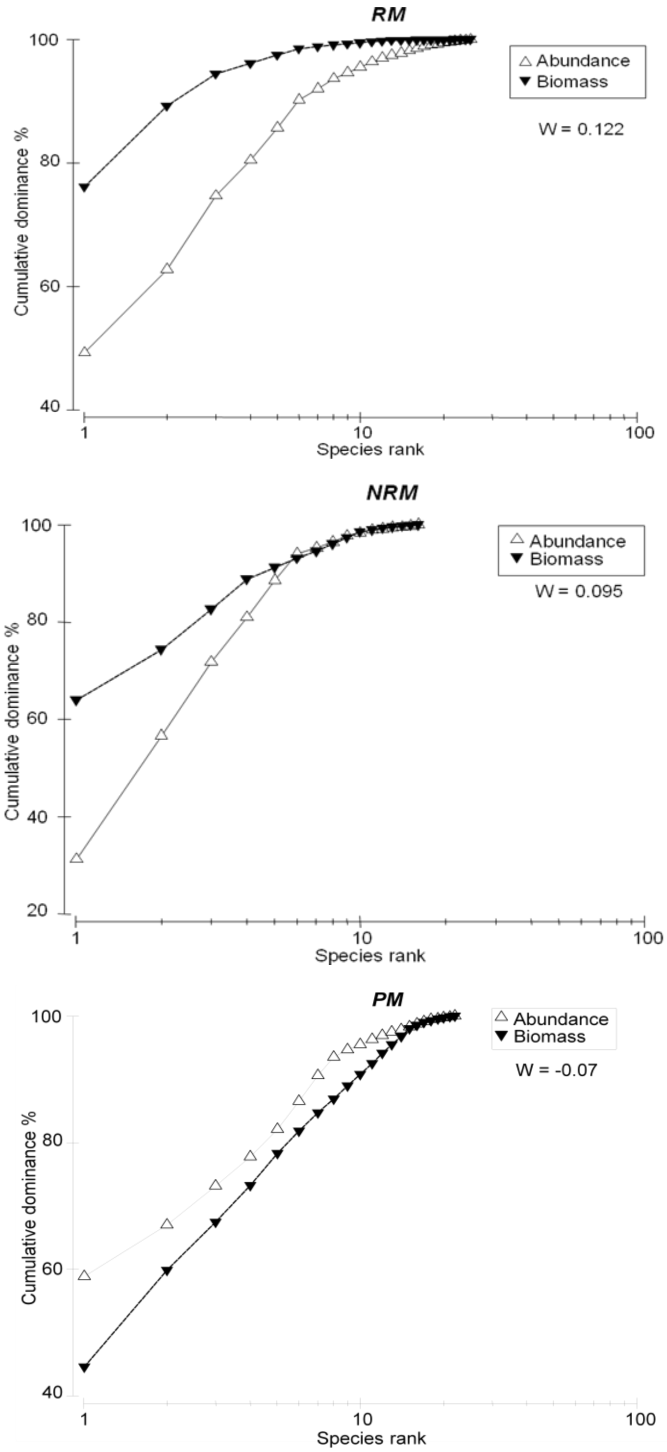

Figure 10.5. Average abundance biomass comparison (ABC) curves at restored marshes (RM), non-restored marshes (NRM) and preserved marshes (PM).

10.4. DISCUSSION

Our results showed that the restoration of European salt marshes by planting the native small cordgrass *S. maritima* after eliminating the invasive *S. densiflora* increases species richness, ecological diversity, density and biomass of the benthonic macroinvertebrate community. Restored areas 3 years after restoration showed an intermediated state according with their similarity (MDS analysis) between non-restored and preserved areas. In *S. alterniflora* created marshes in North America, it has been described that full recovery relative to reference marshes, if it is ever achieved, may require more than two decades (Swamy *et al*., 2002). However, macroinvertebrate density and species richness equivalent to reference marshes is achieved within 8 years (Craft & Sacco, 2003).

PM presented the most different macrobenthos community, influenced mainly (according to BIOENV analysis) by higher sediment bulk dry density (1.1 g cm^{-3}), higher pH (7.4) and lower redox potential (-86 mV) than RM and NRM. These marsh areas also showed important differences in their macroinvertebrate communities with similar sediment environmental conditions, except for lower organic matter content, higher *S. maritima* BGB and lower *S. densiflora* BGB in RM. As in our study, Tavares *et al*. (2009) and Zhou *et al*.(2009) found a negative correlation between sediment organic matter content and macrobenthos diversity and abundance and, to the contrary, Sacco *et al*., (1994) reported that high organic matter content may be a possible cause for high infaunal abundances at the North American Atlantic Coast.

D and J were similar for every marsh area, whereas RM and PM showed higher H’ and H_{max} than NRM due to higher species richness. As in our study, diversity and dominance did not differ between created and natural North American marshes (Ferguson & Rakocinski, 2008), however, species richness was higher. H’ in RM and PM was similar to that reported for *S. maritima* marshes in the Tagus estuary during the same season (Salgado *et al*., 2007).

Macroinvertebrate abundance 3 years after restoration in RM was reaching similar values to PM, as described for *S. alterniflora* North American marshes (Peck *et al*., 1994; Havens *et al*., 1995; Swamy *et al*., 2002; Craft & Sacco, 2003; Warren *et al*., 2002). Total macrobenthos abundances (532 ± 95 indv. m^{-2} in NRM, 910 ± 121 indv. m^{-2} in RM and 935 ± 90 indv. m^{-2} in PM) were similar to those found for pioneer *S. maritima* marshes (Salgado etal., 2007) and slightly lower than those found in invaded Chinese *S. alterniflora* marshes (Chenetal., 2009). *Cyathura carinata*, a typical species in eutrophic marshes (Ferreira *et al*., 2004; Marques *et al*., 1994), showed much higher abundances (167-522 indv. m^{-2}) in Odiel Marshes highly polluted with nitrates (Elbaz-Poulichet *et al.* 1999) than in less contaminated Portuguese marshes (10–65 indv. m^{-2} following Salgado *et al*., (2007) and Cardoso *et al*., (2008)). The rest of the macroinvertabrate community reported by Salgado *et al*.(2007) for Atlantic Portugueses marshes was similar in species composition and abundances to our study, except for *Oligochaeta* that presented a higher density in the Tagus estuary, which may be related to the higher organic matter content (Finogenova, 1996).

Comparing abundance and biomass curves is a method sensitive to various kinds of disturbance, both natural (physical and biological) and pollution-induced, to the macrobenthos community (Warwick *et al*., 1987) however, this method is not exempt of controversy (Beukema, 1988; Craeymeersc, 1991). In our study, ABC curves pointed to PM as the most disturbed area, and RM the least. The three studied areas present similar and very high pollution levels (López-González *et al*., 2005), so the detected alteration levels seemed to be related to physical disturbances, high erosion rates in NRM and bait capture in PM, where the low density of *H. diversicolor* was probably due to the high extraction activity carried out in this area since this polychaete is used as fresh bait for fishing.

Total AFDW increased with *S. maritima* BGB, which according to the PCA analysis marked important differences between areas. The crustaceans *C. maenas*, *C. carinata*, and *Uca tangerii*,among others,contributed considerably to the total biomass of *S. maritima* marshes in RM and PM, together with *H. diversicolor* in RM. These species, with a relative abundance of 64% in RM and 58% in PM, represented 96% and 69% of the total biomass, respectively. Previous studies have recorded higher abundances and biomasses of macroinvertebrates in *Spartina* and *Zostera* marshes than in mudflats (Almeida *et al*., 2008; Spruzen *et al*., 2008; Zhou *et al*., 2009; Tang &Kristensen, 2010). Differences between biomass and AFDW in RM and PM seemed to mainly be related to weight loss of inorganic matter adhered to the coriaceous tubes of the phylum *Phoronidea* and shells of *Cerastoderma edule* and *A. tenuis*.

In conclusion, our results have showed that the restoration of European salt marshes has a positive effect on the ecological diversity of these impacted ecosystems in relatively short periods of time. Therefore, restoration plans at a larger scale, together with the eradication of invasive species, could be implemented along the European coast as a tool against the degradation of these environments.

10.5. REFERENCES

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CHAPTER 11

The avian community in *Spartina maritima* restored and non-restored salt marshes

CAPÍTULO 11.- Comunidad de aves en marismas de *Spartina maritima* restauradas y no restauradas.

RESUMEN

La creación y restauración de marismas degradadas es una práctica de conservación clave para la conservación de aves limícolas y playeras. El objetivo principal de este capítulo fue evaluar la evolución de la comunidad de aves en marismas bajas restauradas con *Spartina maritima* dos años después de las plantaciones en comparación con marismas **adyacentes** no restauradas e invadidas por *Spartina densiflora* en las Marismas del Odiel (suroeste de la Península Ibérica). Los censos de aves se realizaron desde octubre de 2008 hasta septiembre de 2009 en parcelas rectangulares de 2.30 ha durante las pleamares y en 5.80 ha durante las bajamares en tres marismas de las áreas restauradas y no restauradas. Se registraron un total de 44 especies de aves, incluyendo 20 de limícolas (34 especies en las marismas restauradas y 40 en las no restauradas). La mayoría de las especies pertenecieron a las familias Charadriidae, Scolopacidae, Laridae y Sternidae. Ocho especies protegidas fueron registradas en las marismas restauradas. La diversidad ecológica (H’) de la comunidad de aves varió entre 1.13 y 1.77. En los periodos cercanos a pleamares, las marismas estudiadas no difirieron en ningún índice de diversidad ecológica. Generalmente, los vuelvepiedras (*Arenaria interpres*) y chorlitejos (*Charadrius* spp.) presentaron mayores densidades en las marismas restauradas. Durante los periodos cercanos a las bajamares, las marismas restauradas presentaron mayor H’ y equitatividad (J) y menor dominancia (D) que las marismas no restauradas en invierno, primavera y verano. Ambas áreas mostraron mayor diversidad máxima (H_{max}) en otoño e invierno que en primavera y verano. Por tanto, este estudio muestra los beneficios para la comunidad de aves de los proyectos de restauración ecológica basados en plantaciones de la especie nativa *S. maritima*. Nuestros resultados cumplen con las expectativas de que las aves marismeñas colonizan rápidamente los ecosistemas restaurados.

CHAPTER 11.- The avian community in *Spartina maritima* restored and non-restored salt marshes.

ABSTRACT

Creation and restoration of degraded salt marshes is a key conservation practice for shorebird biodiversity conservation. The main goal of this study was to assess the evolution of the avian community in *Spartina maritima* restored low salt marshes two years after planting, in comparison with adjacent degraded and non-restored marshes invaded by *Spartina densiflora* in the Odiel Marshes (southwest Iberian Peninsula). Bird censuses were conducted from October 2008 to September 2009 in rectangular plots of 2.30 ha during high tides and 5.80 ha during low tides in three marshes in restored and non-restored marshes. A total of 44 bird species, including 20 shorebird species, were recorded (34 spp. in restored marshes and 40 spp. in non-restored marshes). Most species belonged to Charadriidae, Scolopacidae, Laridae and Sterninae. Eight threatened bird species were recorded in restored marshes. H' (Shannon–Weaver index) of the avian community varied between 1.13 and 1.77. In high tide periods, restored and non-restored marshes did not differ in any ecological diversity index. Generally, Ruddy Turnstone (*Arenaria interpres*) and *Charadrius* spp. presented higher densities in restored than in non-restored marshes. During low tides, restored marshes showed higher H' and J (evenness index) and lower D (Simpson dominance index) than non-restored marshes in winter, spring and summer. Both marsh areas showed higher H_{max} (the maximum achievable diversity if all species were equally represented) in autumn and winter than in spring and summer. This study showed the benefits to the bird community of an ecological restoration project based on plantations of *S. maritima*. Our results met expectations that marsh birds colonize restored ecosystems quickly.

11.1. INTRODUCTION

Salt marsh habitats are recognized for their importance for many bird species, both migratory and resident, being habitats for feeding, resting and breeding (Howe, 1987; Ferns, 1992; Hughes, 2004; Laegdsgaard, 2006). For example, many shorebird species, with extremely high energy requirements and very high feed rates (Nagy, 2001), feed on invertebrates living in sediments that, in turn, feed on detritus produced by marsh vegetation (Brown & Atkinson, 1996) or their vegetative organs (Chung, 1993; Rowcliffe *et al*., 1995). Other birds nest only on certain salt marsh plants (Post & Greenlaw, 1994).

Salt marshes are shrinking and degrading due to human pressure, and are also threatened by climate change (Hughes, 2004). Shorebirds are a good indicator of environmental health (Beintema, 1983). Thus, the destruction and alteration of habitat is the greatest threat for marsh birds (e.g. Howe *et al*., 1989; Yalden, 1992; Goss-Custard *et al*., 1995; Weber *et al*., 1999; Figuerola & Amat, 2003; Rosa *et al*., 2003). In the context of salt marsh degradation and destruction, creation and restoration of degraded areas is a key conservation practice for bird biodiversity conservation. In fact, some salt marsh restoration projects are deliberately planned to favor determinate bird species as the main objective (Zedler, 1993).

Monitoring is essential to assess the success of salt marsh restoration projects, and the avian community should be taken into account to assess the evolution of restored marshes: the complexity achieved for the marsh bird community may be related to the success of the project (Lewis & Casagrande, 1997). Although there are many studies monitoring the bird community in wetlands when the tidal influence has been restored (e.g. Brawley *et al*., 1998; Warren *et al*., 2002; Konisky *et al*., 2006; Gallego-Fernández & García-Novo, 2007; Raposa, 2009), a handful of studies analyze bird responses to *Spartina* plantations (Zedler, 1993; Melvin & Webb, 1998; Havens *et al*., 2002).

The main goal of this study was to assess the evolution of the avian community in restored low salt marshes developed through an innovative project based mainly in *Spartina maritima* (Curtis) Fernald and *Zostera noltii* Hornem. plantations (Castillo & Figueroa, 2009) two years after planting, in comparison with adjacent degraded and non-restored marshes in the Odiel Marshes (southwest Iberian Peninsula). We hypothesized that salt marsh restoration with native halophytes would increase bird community complexity (species richness, ecological diversity and density) in comparison with degraded salt marshes invaded by *Spartina densiflora* Brong.

11.2. MATERIALS AND METHODS

11.2.1. Study sites.

This study was carried out in *S. maritima* restored and non-restored marshes in the Odiel Marshes as shown in Chapter 2.

11.2.2. Bird censuses.

Bird censuses were conducted in rectangular plots of 2.30 ha during high tides (between the lower distribution limit of *Spartina* spp. band (+1.5 m above Spanish Hydrographic Zero (SHZ) and the upper distribution limit of salt marshes) and 5.80 ha during low tides (between the average elevation in low tide during low tide samplings (+0.8 m SHZ) and the upper distribution limit of salt marshes (ca. +3.4 m SHZ)) in three marshes in each of both areas, restored marshes (RM) and non-restored marshes (NRM) (n = 9-15 per season, tidal level and marsh area; 3 marshes x 3-5 sampling days per season and tidal level).

Censuses were carried out on clear mornings when low or high tide occurred between 9:00 and 10:00 a.m., during rising tides close to high tide level and ebbing tides close to low tide level (Dias *et al*., 2006). Observations were recorded for 20 min at each sampling point using 8X binoculars and a 20–60X spotting scope, from a distance greater than 50 m to minimize disturbances. Censuses were performed from October 2008 to September 2009 once a week for each sampling point. Each sampling point was visited four to five times per season (autumn (October-November 2008), winter (December 2008-February 2009), spring (March-May 2009) and summer (June-August 2009)), at both low and high tides (Havens *et al*., 1995; Neckles *et al*., 2002). Every marsh area was sampled during each sampling day, except during summer when just four points were visited on each day due to high temperatures that reduced bird activities. Every day the sampling was started in a different marsh area to reduce effects related to daily changes in bird distribution. Preliminary sampling showed that all bird species may be recorded with the application of this sampling time and this number of visiting days. All observed bird species and the number of individuals of each species were recorded. The degree of legal protection of bird species according to the International Union for Conservation of Nature and Natural Resources (I.U.C.N.) was consulted at Andalusian, Spanish and International levels (Franco Ruíz & Rodríguez de los Santos, 2001; Madroño *et al*., 2004; IUCN, 2011)

11.2.3. Data processing and statistical analysis.

Ecological diversity was calculated using the Shannon–Weaver index (H´), based on the inventoried data and abundance of each species (Shannon & Weaver, 1949), which is sensitive to changes in rare taxa, where $H' = -\Sigma (p_i) (\ln p_i)$ and pi is the proportion of the shorebird community belonging to the *i*th species (Magurran, 1988; Krebs, 1994). Evenness (J) and Simpson dominance index (D) (Simpson, 1949) of the bird community were also calculated as $J = H'/ H_{max}$ and $D = \Sigma p_i^2$, H_{max} being the maximum achievable diversity if all species were equally represented ($H_{max} = \text{Ln } S$; S = species number).

Bird species density was determined as the mean of partial densities (n = 4–5), for every sampling, for the most abundant species and genus (*Arenaria interpres*, *Pluvialis squatarola*, *Calidris sp*., *Charadrius sp*., *Larus sp*., *Limosa sp*., *Numenius sp*., and *Tringa sp*.). Relative abundance of a particular bird species was calculated as the percentage with respect to the total bird population in a given marsh. Measurements of relative abundance were represented in a table and in rank-abundance diagrams. Deviation from the mean was calculated as standard error (SEM).

Statistical analyses were carried out with SigmaPlot version 11.0. Data were tested for normality using Kolmogorov–Smirnov's test, and for homogeneity of variance with Levene's test ($P < 0.05$). Mean comparisons of H´, H_{max}, J, D and density between marsh areas for the same season and tidal level were compared using either a Student *t*-test or a Mann–Witney *U* test for non-normal dates. Seasonal changes for a tidal level within a marsh area were compared using One-way Analysis of Variance (Anova), with Tukey's test as pos-hoc analysis. A Kruskal–Wallis test was used for non-normal dates, with Bonferroni–Dunn's test as pos-hoc analysis.

11.3. RESULTS

A total of 44 bird species, including 20 shorebird species, were recorded in the two marsh areas (34 spp. in RM and 40 spp. in NRM). The number of shorebird species was relatively similar between marsh areas and through the year, except in summer when it was lower (Table 11.1). Most species belonged to Charadriidae, Scolopacidae, Laridae and Sterninae. Three species were recorded only in RM, and 8 species appeared very rarely and only in NRM (Table 11.2). Sixteen threatened bird species were recorded. Osprey (*Pandion haliaetus*), Common Kingfisher (*Alcedo atthis*), European Shag (*Phalacrocorax aristotelis*), Kentish Plover (*Charadrius alexandrinus*), Western Marsh-harrier (*Circus aeruginosus*), Eurasian Curlew (*Numenius arquata*), Black-tailed Godwit (*Limosa limosa*) and Eurasian Spoonbill (*Platalea leucorodia*) all fed in RM.

Tidal level	**Marshes**	**Autumn**	**Winter**	**Spring**	**Summer**
High	RM	11 / 19	12 / 20	13 / 23	-
	NRM	13 / 25	14 / 23	11 / 27	-
	Total	15 / 28	14 / 28	14 / 30	-
Low	RM	13 / 22	14 / 24	11 / 25	8 / 18
	NRM	15 / 28	16 / 29	13 / 29	9 / 19
	Total	16 / 30	16 / 32	15 / 32	13 / 25

Table 11.1. Number of bird species (shorebird / total bird species) in autumn 2008 and winter, spring and summer 2009 in *Spartina maritima* restored marshes (RM), non-restored marshes (NRM) and in total during high and low tide level in the Odiel Marshes (south-west Iberian Peninsula).

In high tide periods, RM and NRM did not differ according to any ecological diversity index (*t*-test or *U* test, $P < 0.05$). Nevertheless, NRM showed higher species richness than RM for the whole year, including very rare species (Tables 11,1 and 11.2; Figs. 11.1 and 11.2).

Generally, Ruddy Turnstone (*A. interpres*) and *Charadrius* spp. presented higher densities in RM than in NRM ($U = 305.500$, $P < 0.001$; $U = 327.500$, $P < 0.01$, respectively), while the Grey Plover (*P. squatarola*) showed the opposite distribution pattern ($U = 267.500$, $P < 0.001$) (Table 11.3). Maximum H’ values were recorded in RM during high tides (autumn: H’ = 1.75 ± 0.07).

Species	Common name	Restored Marshes	Non-Restored Marshes
Acrocephalus scirpaceus	Eurasian Reed-warbler	-	-/-; -/-; -/-; 0.2
Actitis hypoleucos	Common Sandpiper	4.3/10.6; 1.1/11.2; 6.6/10.2; 2.5	2.3/-; 0.1/0.6; -/-; -
Alcedo atthis	Kingfisher	*/0.4; -/*; -/-; -	-
Anas platyrhynchos	Mallard	*/- ; 0.8/-; 0.7/1.1; 0.4	0.3/0.9; -/-; 0.5/0.2; 3.4
Anthus pratensis	Meadow Pipit	*/-; 0.6/-; -/-; -	-
Ardea cinerea	Grey Heron	1.8/0.7; -/0.3; 3.6/2.7; 1.4	0.4/0.6; 0.3/1.6; 0.5/0.9; 0.5
Arenaria interpres	Ruddy Turnstone	13.7/25.4; 5.2/13.4; 18.2/13.4; 0.7	0.9/8.2; 0.1/0.7; 0.8/1.9; -
Buteo buteo	Eurasian Buzzard	-	0.1/; -/-; -/0.2; -
Calidris alba	Sanderling	5.8/2.8; 2.0/8.8; 5.0/9.1; -	0.3/; 0.4/0.9; 0.4/0.5; -
Calidris alpina	Dunlin	3.0/0.4; 7.1/4.9; 3.9/6.5; -	57.8/4.7; 55.8/21.4; 43.5/29.4; 0.9
Calidris canutus	Red Knot	-/-; -/-; -/*; -	*/-; 0.4/-; 0.4/-; 0.2
Calidris ferruginea	Curlew Sandpiper	0.5/-; -/0.1; -/-; -	*/-; */0.1; -/-; -
Charadrius alexandrinus	Kentish Plover	-/*; 0.2/-; -/1.1; -	0.7/0.3; 0.3/0.7; 0.8/0.2; 0.5
Charadrius hiaticula	Common Ringed Plover	34.2/20.4; 14.7/17.0; 7.3/20.4; -	6.6/6.2; 3.5/2.4; 5.2/4.9; 10.6
Circus aeruginosus	Western Marsh-Harrier	0.4/-; -/-; -/-; -	0.2/0.9; -/0.3; 0.9/0.2; -
Circus pygargus	Montagu’s Harrier	-	-/-; -/-; -/0.2; -
Cisticola juncidis	Zitting Cisticola	*/-; 0.4/-; 0.7/0.5; 0.7	*/-; */0.1; 0.1/1.4; *
Egretta garzetta	Little Egret	1.8/3.2; 0.7/1.5; 5.0/1.6; 3.2	0.3/0.6; 0.2/0.3; 1.6/1.9; 0.8
Himantopus himantopus	Black-Winged Stilt	-	*/1.5; 0.1/-; 0.1/-; 1.2
Larus audouinii	Audouin’s Gull	-	-/-; -/0.1; -/-; -
Larus cachinnans	Yellow-legged Gull	4.1/3.9; 4.7/1.2; 7.9/9.7; 13.0	0.8/0.9; 1/1; 11.9/3.0; 11.8
Larus fuscus	Lesser Black-backed Gull	2.5/-; 4.9/0.9; 2.3/-; 1.1	1.2/0.9; 0.3/-; 0.1/-; 2.2
Larus genei	Slender-billed Gull	-	-/-; -/-; 0.3/-; -
Larus ridibundus	Black-headed Gull	8.9/1.4; 25.6/20.1; 6.3/1.6; 14.1	2.6/2.1; 13.6/8.1; 3.9/0.9; 6.2
Limosa lapponica	Bar-tailed Godwit	0.3/4.2; 1.3/0.3; -/-; 0.7	3.0/3.2; 3.1/ 21.7; -/9.3; -
Limosa limosa	Black-tailed Godwit	1.8/2.8; 0.1/-; 0.3/0.5; 43.3	6.2/7.6; 5.2/10.2; 0.9/-; 43.4
Motacilla flava	Yellow Wagtail	-/-; -/-; 1.0/-; 4.7	-/-; -/-; 0.1/0.5; 1.2
Numenius arquata	Eurasian Curley	1.0/1.8; 0.8/1.5; 2.0/0.5; 1.8	0.1/0.3; 0.2/0.1; 2.5/1.9; 1.8
Numenius phaeopus	Whimbrel	2.0/5.3; 1.9/3.3; 4.6/7.0; 5.1	0.8/2.6; 0.4/2.5; 1.8/4.0; 1.5
Pandion haliaetus	Osprey	*/*; */-; -/-; -	0.3/1.2 ; -/*; -/-; -
Phalacrocorax aristotelis	European Shag	-/-; -/-; 0.3/-; -	-/-; -/-; 0.1/-; -
Phalacrocorax carbo	Great Cormorant	2.0/2.8; 17.9/2.1; 6.9/3.8; 1.1	1.2/5.9; 3.3/1.8; 4.4/0.9; -
Phylloscopus collybita	Common Chiffchaff	1.0/-; 0.1/1.8; -/- ;-	-
Platalea leucorodia	Eurasian Spoonbill	*/*; */*; 3.0/0.5; *	*/-; */-; 1.6/0.2; 0.5
Pluvialis squatarola	Grey Plover	1.3/1.4; 2/0.9; 3.0/0.5; -	5.7/11.7; 7.7/1.2; 8.4/21.0; -
Podiceps nigricollis	Black-necked Grebe	-/-; -/*; -/-; -	0.2/0.6; 0.5/13.6; -/-; -
Recurvirostra avosetta	Pied Avocet	-	*/-; */-; -/-; -
Sterna albifrons	Little Tern	-/-; -/-; 1.0/1.6; 0.7	-/-; -/-; 5.4/8.2; 0.5
Hydroprogne caspia	Caspian Tern	0.3/-; */-; -/-; -	0.4/0.6; 0.2/5.4; -/0.2; -
Thalasseus sandvicensis	Sandwich Tern	0.8/-; 0.1/-; -/3.8; -	0.3/0.6; 0.2/-; 0.6/3.7; -
Tringa nebularia	Common Greenshank	0.8/3.9; 1.2/1.2; 3.0/3.2; 1.1	0.1/0.9; 0.1/4.8; 0.8/2.8; -
Tringa ochropus	Green Sandpiper	-/-; -/-; 0.3/-; -	-
Tringa totanus	Common Redshank	8.4/8.5; 6.6/8.2; 7.2/0.5; 4.3	6.6/32.0; 3.1/0.1; 2.3/0.9; 12.8
Vanellus vanellus	Nothern Lapwing	-	0.5/1.5; -/-; -/-; -

Table 11.2. Relative abundance (%) of bird species in *Spartina maritima* restored and non-restored areas in the Odiel Marshes (south-west Iberian Peninsula). The values correspond to: autumn low tide/autumn high tide; winter low tide/winter high tide; spring low tide/spring high tide; summer low tide. ‘*’ indicates species that appeared in the sampling areas but not during censuses. ‘-‘ indicates that the species was not present.

Low tide

Season	Area	*Arenaria*	*Calidris*	*Charadrius*	*Larus*	*Limosa*	*Numenius*	*Pluvialis*	*Tringa*
Au	RM	$0.7 \pm 0.2^{a,a}$	$0.6 \pm 0.3^{a,ab}$	$1.8 \pm 0.4^{a,a}$	$1.2 \pm 0.4^{a,a}$	$0.1 \pm 0.1^{a,a}$	$0.3 \pm 0.1^{a,a}$	$0.2 \pm 0.1^{a,a}$	$0.7 \pm 0.2^{a,ab}$
	NRM	$0.2 \pm 0.1^{b,a}$	$6.2 \pm 2.6^{b,ab}$	$1.6 \pm 0.5^{a,a}$	$1.1 \pm 0.4^{a,ab}$	$2.6 \pm 1.4^{b,a}$	$0.2 \pm 0.1^{a,a}$	$1.3 \pm 0.3^{b,ab}$	$1.3 \pm 0.3^{a,a}$
Wi	RM	$0.5 \pm 0.2^{a,a}$	$1.4 \pm 0.6^{a,a}$	$1.7 \pm 0.5^{a,a}$	$4.5 \pm 1.1^{a,b}$	$0.3 \pm 0.2^{a,a}$	$0.4 \pm 0.1^{a,a}$	$0.3 \pm 0.1^{a,a}$	$1.0 \pm 0.2^{a,a}$
	NRM	$0.0 \pm 0.0^{b,a}$	$10.1 \pm 2.9^{b,b}$	$1.5 \pm 0.4^{a,a}$	$5.1 \pm 3.9^{a,a}$	$3.4 \pm 1.6^{a,a}$	$0.2 \pm 0.1^{a,a}$	$3.2 \pm 1.5^{b,a}$	$1.2 \pm 0.5^{a,ab}$
Sp	RM	$0.9 \pm 0.3^{a,a}$	$0.4 \pm 0.2^{a,ab}$	$0.4 \pm 0.2^{a,b}$	$1.0 \pm 0.4^{a,a}$	$0.0 \pm 0.0^{a,a}$	$0.3 \pm 0.1^{a,a}$	$0.2 \pm 0.1^{a,a}$	$0.5 \pm 0.2^{a,ab}$
	NRM	$0.0 \pm 0.0^{b,a}$	$2.1 \pm 1.1^{a,ac}$	$0.7 \pm 0.3^{a,b}$	$0.5 \pm 0.3^{a,b}$	$0.1 \pm 0.1^{a,b}$	$0.3 \pm 0.1^{a,a}$	$0.7 \pm 0.4^{a,ab}$	$0.3 \pm 0.1^{a,b}$
Su	RM	$0.0 \pm 0.0^{a,b}$	$0^{a,c}$	$0^{a,c}$	$1.4 \pm 0.3^{a,a}$	$1.6 \pm 0.4^{a,b}$	$0.4 \pm 0.1^{a,a}$	$0^{a,b}$	$0.3 \pm 0.1^{a,b}$
	NRM	$0^{a,a}$	$0.1 \pm 0.1^{a,c}$	$0.9 \pm 0.7^{a,b}$	$1.8 \pm 0.8^{a,ab}$	$3.6 \pm 1.1^{a,a}$	$0.2 \pm 0.1^{a,a}$	$0^{a,b}$	$1.0 \pm 0.5^{a,ab}$

High tide

Season	Area	*Arenaria*	*Calidris.*	*Charadrius*	*Larus*	*Limosa*	*Numenius*	*Pluvialis*	*Tringa*
Au	RM	$2.6 \pm 0.7^{a,a}$	$0.5 \pm 0.5^{a,a}$	$2.7 \pm 0.7^{a,a}$	$1.3 \pm 0.3^{a,a}$	$0.3 \pm 0.1^{a,a}$	$1.1 \pm 0.4^{a,a}$	$0.2 \pm 0.1^{a,a}$	$1.4 \pm 0.6^{a,a}$
	NRM	$1.0 \pm 0.7^{b,a}$	$0.8 \pm 0.5^{a,a}$	$0.8 \pm 0.6^{b,a}$	$0.7 \pm 0.1^{a,a}$	$1.3 \pm 1.1^{a,a}$	$0.4 \pm 0.2^{b,a}$	$2.7 \pm 1.4^{b,a}$	$2.8 \pm 1.4^{a,ab}$
Wi	RM	$2.0 \pm 0.5^{a,a}$	$2.7 \pm 1.3^{a,a}$	$3.9 \pm 1.5^{a,a}$	$5.5 \pm 3.0^{a,a}$	$0.1 \pm 0.1^{a,a}$	$1.1 \pm 0.3^{a,a}$	$0.3 \pm 0.3^{a,a}$	$2.2 \pm 0.8^{a,a}$
	NRM	$0.2 \pm 0.2^{b,a}$	$7.3 \pm 3.5^{a,a}$	$1.0 \pm 0.6^{a,a}$	$3.1 \pm 2.0^{a,a}$	$6.9 \pm 6.2^{a,a}$	$1.2 \pm 0.6^{a,a}$	$1.6 \pm 0.8^{a,a}$	$3.9 \pm 0.9^{a,a}$
Sp	RM	$0.7 \pm 0.3^{a,a}$	$1.5 \pm 0.8^{a,a}$	$5.9 \pm 4.7^{a,a}$	$0.9 \pm 0.3^{a,a}$	$0.1 \pm 0.1^{a,a}$	$0.5 \pm 0.2^{a,a}$	$0.1 \pm 0.1^{a,a}$	$0.3 \pm 0.1^{a,b}$
	NRM	$0.3 \pm 0.2^{a,a}$	$4.4 \pm 2.5^{a,a}$	$0.8 \pm 0.3^{a,a}$	$0.6 \pm 0.2^{a,a}$	$1.3 \pm 1.3^{a,a}$	$0.9 \pm 0.2^{a,a}$	$3.1 \pm 1.6^{b,a}$	$0.6 \pm 0.3^{a,b}$

Table 11.3. Shorebird and gull density (individuals ha^{-1}) of more abundant species and genus in low and high tides in *Spartina maritima* restored and non-restored marshes in autumn (Au), winter (Wi), spring (Sp) and summer (Su). 0.0 ± 0.0 indicates density lower than 0.05 individuals ha^{-1}. '0' indicates that no individuals were recorded. Different letters indicate significant differences between marsh areas (first letter; *t*-test or *U*-test, $P < 0.05$) and season (second letter; Anova or Kruskal–Wallis test, $P < 0.05$).

In RM, Godwits (*Limosa* spp.) showed their highest densities in summer (Kruskal-Wallis, $\chi^2 = 16.906$, df = 3, $P < 0.001$; Dunn's test, $P < 0.05$) when *A. interpres*, *Calidris* spp., *Charadrius* spp. and *P. squatarola* showed their lowest densities (Anova or Kruskal–Wallis, $P < 0.05$). In NRM, *Calidris* spp. and *P. squatarola* also presented their lowest densities in summer (Kruskal–Wallis, $P < 0.05$) and Godwits (*Limosa* spp.) in spring (Kruskal–Wallis, $\chi^2 = 12.568$, df = 3, $P < 0.01$; Dunn's test, $P < 0.05$).

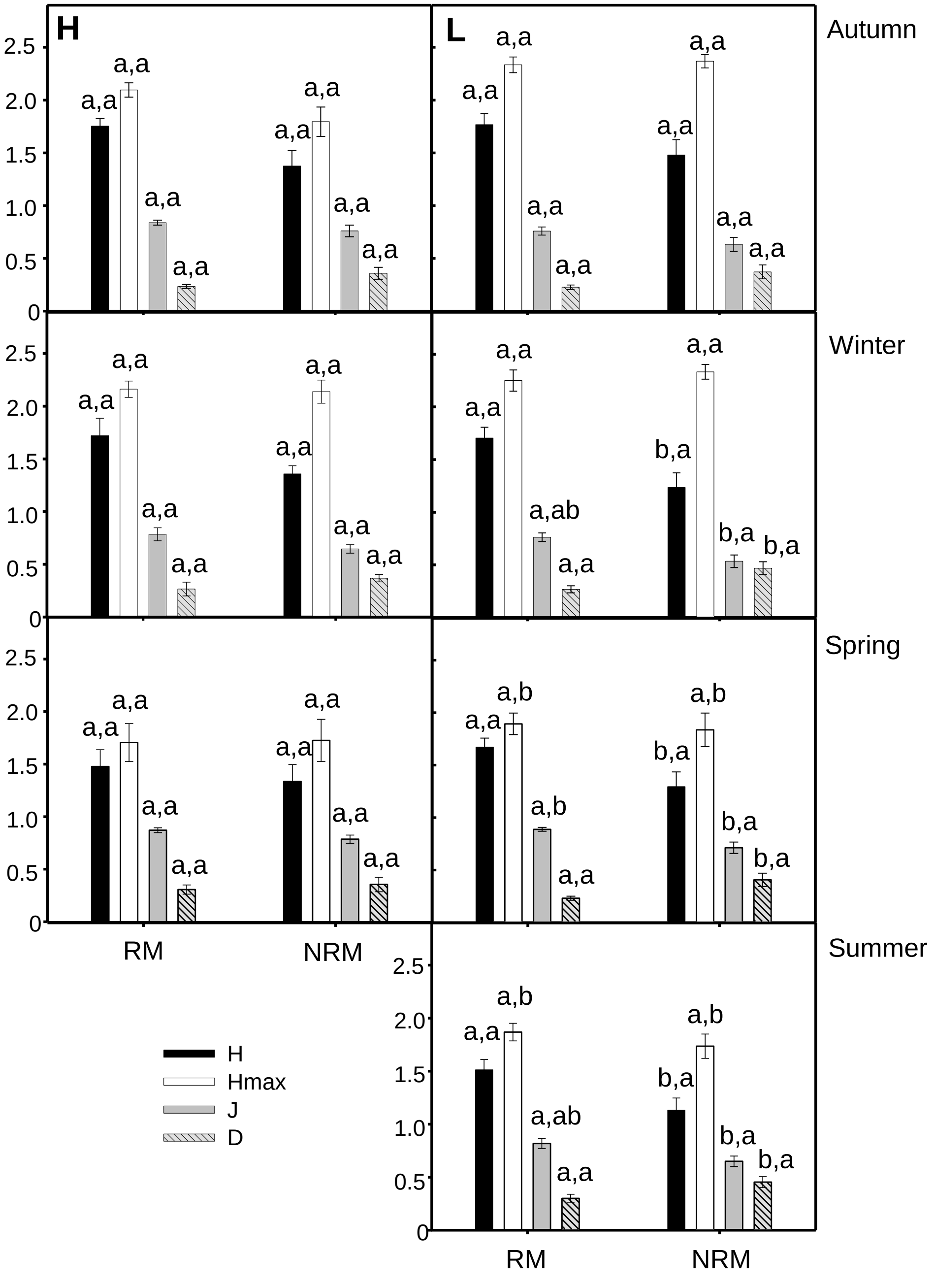

Figure 11.1. Ecological diversity (black bar), maximum diversity (white bar), evenness (gray bar) and dominance (striped bar) for the bird community at high tide (H) and low tide (L) in autumn, winter, spring and summer in restored and non-restored *Spartina maritima* marshes (RM and NRM, respectively) in the Odiel Marshes (SW Iberian Peninsula). Different letters indicate significant differences between marsh areas (first coefficient) and between seasons (second coefficient).

During low tides, RM showed higher H' and J and lower D than NRM in winter, spring and summer (*t*-test or *U* test, $P < 0.05$) (Fig. 11.1). These differences were mainly due to the high relative abundance of Dunlin (*Calidris alpina*), Gulls (*Larus spp.*) and Grey Plover (*Pluvialis squatarola*) in NRM, representing ca. 70% of the total individuals in winter and spring. In summer, RM showed higher H', J and lower D than NRM, although both marsh areas presented similar relative abundance for the most abundant species (*Limosa limosa*, relative abundance ca. 44%) (Table 11.2; Figs. 11.1 and 11.2). In general, Godwits (*Limosa* spp.), Sandpipers (*Calidris* spp.) and Grey Plover presented their maximum densities in NRM (*U* test, $P < 0.05$), and the Ruddy Turnstone (*A. interpres*) in RM ($U = 702.500$, $P < 0.001$).

During high tide periods, RM and NRM did not show seasonal differences according to any diversity index (Anova, $P > 0.05$) (Fig. 11.1). Common Redshank (*T. totanus*) presented its lowest density in spring (Kruskal–Wallis, $P < 0.001$; Dunn's test, $P < 0.05$) and the highest density of Ruddy Turnstone (*Arenaria interpres*) was recorded during autumn and winter in RM (2.6 ± 0.7 ind ha^{-1} and 2.0 ± 0.5 ind ha^{-1}, respectively) (Table 11.3).

During low tides, RM and NRM showed higher H_{max} in autumn and winter than in spring and summer (Anova, $F = 6.582$, df = 50, $P < 0.001$; Tukey's test, $P < 0.05$; Kruskal–Wallis, $\chi^2 = 22.638$, df = 3, $P < 0.001$; Dunn's test, $P < 0.05$, respectively) (Fig. 11.1). In addition, J was lower in autumn than in spring in RM (Kruskal–Wallis, $\chi^2 = 9.537$, df = 3, $P < 0.05$; Dunn's test, $P < 0.05$). Analyzing both marsh areas together, autumn showed higher H' than summer, while winter and spring presented intermediate values (Kruskal–Wallis, $\chi^2 = 7.560$, df = 3, $P < 0.05$; Dunn's test, $P < 0.05$). In fact, maximum H' during low tides was recorded in autumn in RM (1.77 ± 0.10) (Fig. 11.1). *Charadrius* spp. showed their highest densities in RM and NRM during autumn and winter (ca. 2 ind ha^{-1}), with the same being true for *Calidris* spp. in NRM (Kruskal–Wallis, $\chi^2 = 17.375$, df = 3, $P < 0.001$; Dunn's test, $P < 0.05$) (Tables 11.4 and 11.5).

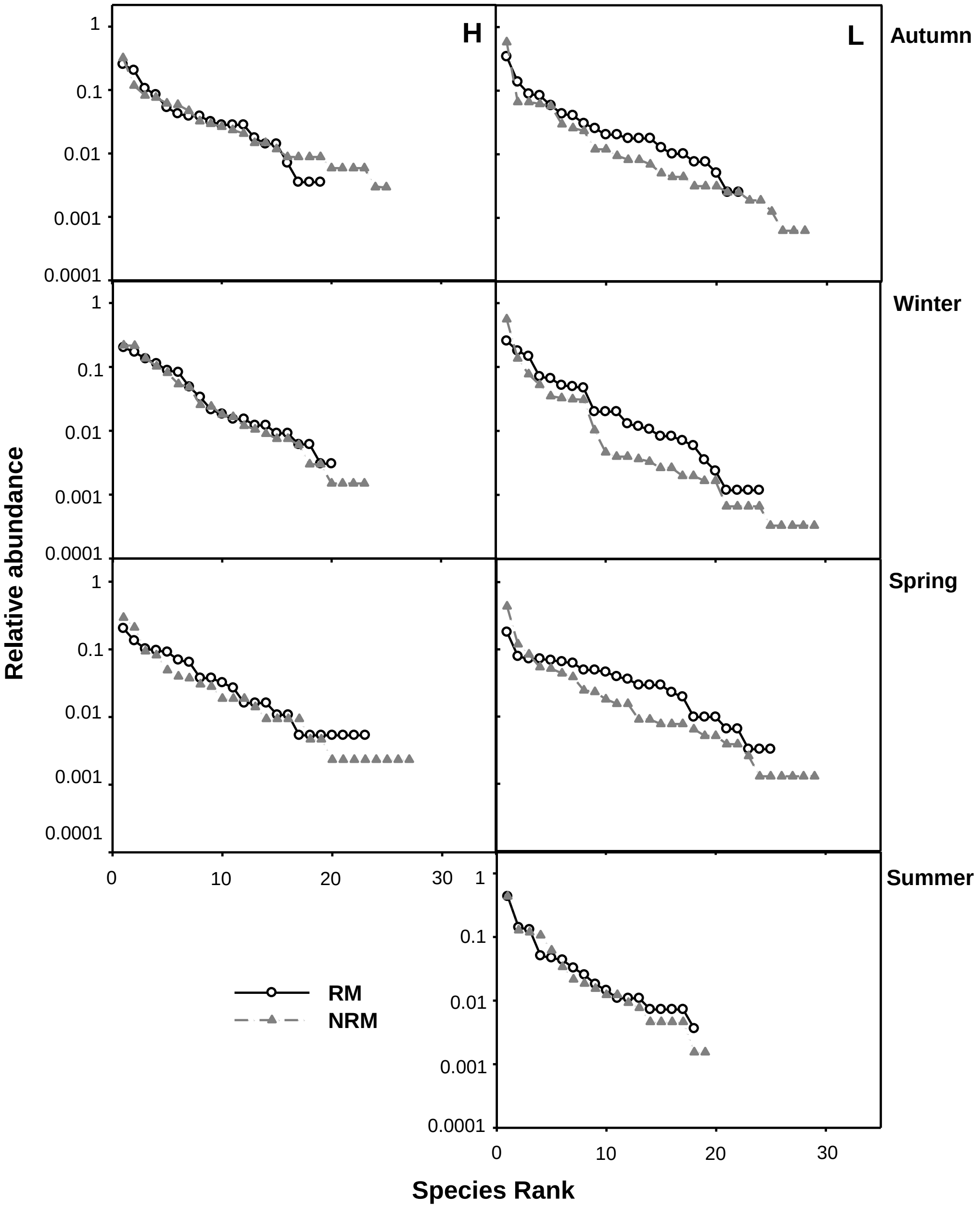

Figure 11.2. Rank-abundance diagrams (logarithmic scale) for the bird community at high tide (H) and low tide (L) in autumn, winter, spring and summer in restored and non-restored *Spartina maritima* marshes (RM and NRM, respectively) in the Odiel Marshes (southwest Iberian Peninsula).

11.4. DISCUSSION

This study shows the benefits to the bird community of an ecological restoration project based on plantations of *S. maritima* and *Z. noltii* after the elimination of invasive *S. densiflora* in low marsh areas of the Odiel Marshes.

The values of H' of the avian community described in this work 2 years after restoration (between 1.13 and 1.77) were higher than those found in other restored salt marshes in the U.S.A. between 3 and 15 years after restoration (H' between 0.26 and 0.92) (Melvin & Webb, 1998; Armitage *et al*., 2007), and lower than those recorded in North American mature restored marshes (12 years after restoring; H' between 1.83 and 2.12) (Havens *et al*., 2002). On the other hand, reported bird densities were very dynamic and variable in time and space (Brusati *et al*., 2001), being within the range of those reported, for example, for *Calidris* spp. by Warren *et al*. (2002).

RM showed higher H' than NRM in winter, spring and summer during low tides. The availability of food seems to be the main environmental factor in determining the suitability of a particular habitat for marsh bird species (Evans *et al*., 1984; Weller, 1994; Desholm, 2000; Ma *et al*., 2007). *Spartina maritima* and *Z. noltii* act as marsh structuring halophytes (Castellanos *et al*., 1994; Figueroa *et al*., 2003; Bouma *et al*., 2009), increasing habitat diversity, providing organic matter and stabilizing sediments (Salgueiro & Caçador, 2007; Widdows *et al*., 2008). Thus, plantations increased environmental heterogeneity in RM, adding higher diversity of birds' foraging habitats (Weller & Spatcher, 1965), since microhabitats are home for different invertebrates and fish species in low marshes (Nienhuis & Groenendijk, 1986; Cardoso *et al*., 2007; MacKenzie & Dionne, 2008; Parker *et al*., 2008). In addition, some birds such as geese feed directly on leaves and rhizomes of *Spartina* (Chung, 1993) and of *Zostera* (Inger *et al*., 2006; Moore & Black, 2006). However, since most shorebirds feed in intertidal mudflats without vegetation where invertebrates are more abundant (Davis & Moss, 1984; Rosa *et al*., 2003), we must take into account that the secondary production of non-vegetated areas depends on adjacent vegetated marshes from which they receive organic matter feeding saprovore invertebrates (Valiela *et al*., 2000). In addition, some macroinvertebrate groups are mainly associated with areas with vegetation (Arocena, 2007). Therefore, some bird species such as Common Redshank (*Tringa totanus*) like to feed on the edges of vegetated areas (Ferns, 1992). This species presented generally higher relative abundance in RM than in NRM. On the other hand, Ruddy Turnstone (*Arenaria interpres*) also showed high densities in RM, which may be related to large numbers of shell fragments offering a wide range of the macroinvertebrates on which Ruddy Turnstone feeds (Whitfield, 1990).

NRM, colonized by invasive *S. densiflora* and containing extensive tidal mudflats, offered a more homogeneous environment, specifically favoring a few dominant bird species which exploited available resources very efficiently, increasing D, and decreasing J and H'. For example, Dunlin (*Calidris alpina*), the most abundant migrant shorebird in the Atlantic East (Smith & Piersma, 1989), prefers intertidal mudflats without vegetation for feeding (Goss-Custard & Moser, 1988), and in our study was much more abundant in NRM than in RM. Previous works have linked low bird diversity and species richness with plant invasions in marshes (Benoit & Askins, 1999; Gan *et al*., 2009) and it has been shown that the bird community recovered after removal of invasive species (Patten & O'Casey, 2007).

The absence of some occasional species recorded in NRM from RM may be related to high exposure to human impacts such as noise and the presence of pedestrians and vehicles. Many of the rarest bird species are very elusive in the vicinity of humans, and the presence of urban areas and others infrastructures can adversely affect some marsh birds (De Boer, 2002; Rosa *et al*., 2003; Armitage *et al*., 2007). In addition, the proximity to NRM of different ecosystems such as pastureland, extensive medium-high marshes or salt pans, none of which occurred close to RM since that space was occupied by infrastructures, may influence the presence of rare species such as *Buteo buteo* and *Circus pygargus* that hunt for prey on neighboring pasturelands, *Himantopus himantopus* which is typical of salt pans or *Acrocephalus scirpaceus* which is associated with patches of Common Reed (*Phragmites australis* (Cav.) Trin. ex Steud.).

Our results met expectations that marsh birds colonize restored ecosystems quickly (Hemesath & Dinsmore, 1993; Brawley *et al*., 1998; Passell, 2000; Gallego-Fernández & García-Novo, 2007; Raposa, 2009). Restored marshes using *S. maritima* and *Z. noltii* showed higher ecological diversity than non-restored marshes only two years after planting, which seemed to be related to the rapid growth of *Z. noltii* and *S. maritima* transplants. The lineal expansion of *S. maritima* rhizomes on bare sediments has been calculated to be 1.1 ± 0.0 cm month^{-1} in the restored area (Castillo & Figueroa, 2009), and the spread of *Z. noltii* by seeds and rhizomes was very active (G. Curado personal observation).

The highest species richness, H' and H_{max} were recorded during migrating (autumn and spring) and wintering periods, according to previous studies covering Northern Hemisphere wetlands (Weller, 1994; Lopes *et al*., 2005). Seasonal differences in individual densities recorded in this work for *A.interpres* spp. and *Tringa* spp. during high tides, and for *A. interpres*, *Calidris* spp., *Charadrius* spp., *Limosa* spp. and *P. squatarola* during low tides, corresponded to those described by Garrido-Guil, (1996) in the Odiel Marshes.

11.5. REFERENCES

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CHAPTER 12

Public perception about natural and restored salt marshes in the city of Huelva (Spain)

CAPÍTULO 12.- Percepción pública sobre marismas naturales y restauradas en la ciudad de Huelva (España).

RESUMEN

Solo unos pocos proyectos de restauración ecológica incorporan la percepción pública en su monitoreo, sin embargo, la participación de la gente local es un proceso clave para alcanzar beneficios sociales y detectar problemas en el uso público de ecosistemas restaurados. Este capítulo analiza, a través de una encuesta, la percepción y uso de los ciudadanos de las marismas costeras en la ciudad de Huelva (España), específicamente sobre un proyecto de restauración de marismas ubicadas junto a su polo químico. La mayoría de los ciudadanos de Huelva reconocieron los beneficios de las marismas costeras (75%) incrementando dicha percepción con el nivel educativo. La mayor parte de los encuestados mostraron un bajo nivel de conocimiento acerca de las funciones y servicios prestados por las marismas costeras. Los encuestados con más de 60 años y bajo nivel educativo fueron los que menos conocían el proyecto de restauración estudiado. 77% de los encuestados pensaban que las marismas no eran fuentes de enfermedades, 74% dijeron que las marismas poseen especies protegidas y un 80% decían que era un bonito ecosistema. Respecto a las preferencias paisajísticas, la mayoría de los encuestados prefirieron las marismas nativas de Quenopodiáceas y el paisaje de la nativa *Spartina maritima* fue el menos elegido. El proyecto de restauración estudiado está basado principalmente en la plantación de *S. maritima* que fue el paisaje menos elegido, sin embargo, esta *Spartina* nativa facilita el desarrollo de la sucesión ecológica hacia marismas de Quenopodiáceas. El proyecto de restauración estudiado incrementó su número de visitantes en 27% debido a la mejora del acceso a las marismas y la mejora paisajística. En vista a nuestros resultados, deberían llevarse a cabo campañas de educación ambiental en la ciudad de Huelva para incrementar el conocimiento de los ciudadanos sobre marismas costeras, ecosistema amenazado que prácticamente rodea la ciudad.

CHAPTER 12.- Public perception about natural and restored salt marshes in the city of Huelva (Spain).

ABSTRACT

Only a few restoration projects incorporate public perception in their monitoring, however, participation of local people is a key process if social benefits are to be achieved and problems of public use in restored ecosystems detected. This chapter analyzes, through a survey, citizens' perception and usage of coastal marshes in Huelva city (Spain), specifically a salt marsh restoration project next to the Chemical Pole. Most of Huelva's citizens recognized the benefits of coastal marshes (75%), a perception which increased with level of education. The majority of the respondents showed a low level of knowledge about the ecological services and functions of salt marshes. Respondents over 60 years with low educational level were those less aware about the studied restoration project. 77% of respondents thought that marshes were not a source of diseases, 74% said that marshes had protected species and 80% said that they are a beautiful ecosystem. Regarding landscape preferences, most of the respondents preferred the native Chenopodiaceae salt marsh, and the native *Spartina maritima* landscape was chosen less often. The studied restoration project was based mainly in *S. maritima* plantations, which was the less favored landscape, but this native cordgrass facilitates the development of ecological succession, resulting in Chenopodiaceae salt marshes. Visitor numbers to the studied restoration project increased by 27% due to improved access to the marshes and to an enhanced landscape. In light of our results, environmental education campaigns should be carried out in Huelva City to increase its citizens' knowledge of salt marshes, a threatened ecosystem that virtually surrounds the city.

12.1. INTRODUCTION

Ecological restoration is becoming a common practice to improve the ecological quality of many degraded ecosystems (Mitsch, 2010). Once a restoration project has been carried out, good monitoring is essential to improving the restoration methodology for future applications and to solving unexpected problems (England *et al*., 2008). During the monitoring, participation of local people is key to achieving social benefits; to detecting problems of public use; and to improving management in restored ecosystems with the aim of increasing population acceptability (Casagrande, 1997; Webler & Tuler, 2001; Sharp *et al*., 2011; Shindler *et al*., 2011). Local residents attached importance to public consultation and expected to be consulted about restoration works (Tunstall *et al*., 2000). Nevertheless, only a few restoration projects incorporate public perception into their monitoring.

Salt marshes are one of the most impacted ecosystems globally, so restoration projects are being carried out to compensate for their degradation and loss (Broome *et al*., 1988; Mitsch & Gosselink, 2000; Gedan *et al*., 2009) and a few of these projects incorporate public perception and use in their evaluation process (Casagrande, 1996; Burger, 2003; Myatt-Bell *et al*., 2002, Myatt *et al*., 2003a, b).

This study analyzes, through a survey, citizens' perception and usage of natural and restored coastal marshes in Huelva city (Andalusia, Spain), specifically of a salt marsh restoration project next to the Chemical Pole (Castillo *et al*., 2009). The main goals were to understand the beliefs, perceptions and behavior of the local population in respect to the salt marshes, and to compare natural and restored marshes from the viewpoint of the local population.

12.2. MATERIALS AND METHODS

12.2.1. Study site.

The focus of this study was Huelva City, a middle-size industrial town, and the adjacent Odiel Marshes (lat. 37º 08'– 37º 20' N, long. 6º 45'– 7º 02' W; 7.158 ha), specifically 8.37 ha of restored salt marshes located in the city of Huelva (Andalusia, Spain) (see Chapter 2).

12.4.2.2. Survey instrument.

A two-page questionnaire with 10 questions was developed as a quantitative survey instrument (Appendix 1), primarily using a qualitative exploration of the thematic dimensions (Myers & Oetzel, 2003) of the public perception and usage of Odiel Marshes. In order to understand the beliefs, perceptions and behavior of the local population with respect to the salt marshes, questions 1 and 4 indicated for us perception of value. Question 4 also elicited information about physical perception. Questions 2, 3, 7, 8, and 9 gave information about the use or utility of the Odiel Marshes for the respondent, and questions 2, 4, 5, 6, 7 gave us further knowledge about the beliefs and behavior of the local population. In order to compare natural and restored marshes from the viewpoint of the local population, we used the questions (3–9) (2–6,7).The last question (10) incorporated three salt marsh landscape photographs representing different salt marsh communities (native *S. maritima* prairie, invasive *S. densiflora* prairie and Chenopodiaceae salt marsh), to evaluate visual public preference (Brown *et al.*, 1988; Misgav, 2000). Sex, age and education level were also included in the questionnaire (Appendix 1). Three levels of education were established: high (university degree), medium (secondary school diploma) and low (middle school, elementary school or none) (Lazzeri *et al.*, 2011). The questionnaire used multiple-choice questions with a variable number of response categories according to the targets measured. We use some recommendations of Haladyna & Downing (1989) about the format. The definitive survey was conducted after a specific pilot study.

12.2.3. Survey organisation and participants.

The survey was carried out by means of a street questionnaire on the central street 'Concepción' in Huelva City. This location was selected because it encompasses a wide section of the population. We based the selection on expert knowledge about both the city and its neighborhoods. In both zone and unit selections, we took into account recommendations adapted from Jaenson *et al*. (1992). The survey population comprised those living in Huelva City that were ≥ 20 years of age. A total of 1773 people were asked by trained interviewers to participate in the survey, with a response rate of 22.2% (a total of 394 completed questionnaires were ultimately obtained). The total number of Huelva City inhabitants older than 20 years was 108957 (Statistical Institute of Andalusia, 2001), and the minimum statistically significant sampling number was 383 people with a population variance of 0.25, an error probability of 0.05 and a precision error of 0.05 (calculated using the free software 'Muestreo Aleatorio Simple' by Manzano-Arrondo, 2010).

The survey was conducted between 10:00 a.m. and 1:00 p.m. and between 5:00 p.m. and 8:00 p.m. on working days in October 2010. The overall interview usually required only about 5 minutes. The sampling design followed the principles of Manzano (1998).

We used a mixed sampling with two main characteristics: (1) non-probability sample structure: quota sampling, applied to sex and estimated age; and (2) random selection: systematic sampling with random starting and constant period of eleven units. Period was calculated after observations about crowd flow at the sampling zone (see Kish, 1965).

12.2.4. Data analysis.

The data collected were analysed using Sigma Plot for Windows v. 11.0. Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene test ($P > 0.05$). The level of significance applied to all tests was $P < 0.05$. Differences between sex, age and educational level were compared by three-way Anova. Tukey’s test was used as post-hoc analysis.

12.3. RESULTS

Salt marshes were considered a beneficial ecosystem by 75% of the responders (Question 1, Table 12.1), and 63% of respondents admitted to visiting Odiel Marshes at least once a year or more (Question 2, Table 12.1). The main use of Odiel Marshes by the population of Huelva City was hiking (81%), and running a less popular activity (4%). Odiel Marshes were used also by bird-watchers (14%), photographers (14%), fishers (13%) and cyclists (12%). People also used Odiel Marshes for relaxation, reading, inspiration and painting (Question 3, Table 12.1). On the other hand, 82% of respondents reported that salt marshes were a habitat for mosquitoes, but only 23% thought that marshes were a source of disease. Most of the citizens interviewed thought that salt marshes were a habitat for red list species (74%) and offered beautiful landscapes (80%). However, only about half of the respondents, or even less, recognized the ecological services provided by salt marshes, such as water purification and flood control, fighting climate change and being a habitat for breeding fish and shellfish species (Question 4, Table 12.1).

83% of respondents knew of the salt marsh restoration project in Huelva City, of which 60% had visited it, and more than half of the visitors had been there three or more times a year (Questions 5, 6 and 7, Table 12.1). 50% of respondents had visited the restored marshes since their restoration, increasing visits to the area by 27% after the restoration project was implemented (Question 8, Table 12.1). The most popular public uses given to the walkway over the restored marshes were hiking (77%) and cycling (15%). It was also used by Chemical Pole workers during lunch and rest breaks (10%). Other uses (11%) were reading, skating, barbecuing and relaxing (Question 9, Table 12.1).

Chenopodiaceae salt marsh was the favourite landscape for 80% of respondents versus 16% who chose the invasive *S. densiflora* prairie and only 4% who chose the native *S. maritima* prairie (Question 10, Table 12.1). *Spartina maritima* prairie was the least-liked landscape (54%) (Table 12.1).

The three-way Anova showed that age had a significant effect on the answers to Question 8 (visiting before restoration) and Question 10 (landscape preferences). Thus, people over 60 years visited the restored area before the implementation of the restoration project more frequently (78%) and they had a greater preference for the invasive *S. densiflora* prairie (30%) than other age groups (Table 12.2).

The educational level influenced the perception of salt marshes as beneficial ecosystems (Question 1): the high educational level group more frequently answered that salt marshes were beneficial (86%) and less frequently (25%) that they “smell bad” (Anova, $F = 7.770$, $P < 0.001$, df = 2; Tukey’s test, $P < 0.05$) and “attract trash” (Anova, $F = 7.669$, $P < 0.001$, df = 2; Tukey’s test, $P < 0.05$) (Question 4) (Tukey’s test, $P < 0.05$) (Table 12.2). The interaction between age, sex and educational level showed that men younger than 40 years with medium educational level preferred *S. densiflora* landscape more than other groups (88%) (Question 10) and that respondents older than 60 years with low educational level were more unaware of the marsh restoration project (37%) (Question 5) (Table 12.2).

The sex of the respondents influenced only the public use of salt marshes (Question 3), with men fishing more frequently (20%) than women (8%) (Anova, $F = 5.400$, $P < 0.05$, df = 1) (Table 12.2).

Summary of the answers to the										
Q 1										
Ans.	Benef.	Harm.	Indif.							
N (%)	296(75)	30(8)	68(17)							
Q 2										
Ans.	Never	Rarely	Often	VO						
N (%)	146(37)	128(32)	66(17)	54(14)						
Q 3										
Ans.	Hiking	Fishing	Cycling	R	W	BW	P	S	O	
N (%)	201(81)	32(13)	30(12)	10(4)	26(10)	35(14)	35(14)	17(7)	24(10)	
Q 4										
Ans.	BM	BF	PW	AT	FC	RLS	SB	FCC	B	SD
N (%)	322(82)	205(52)	151(38)	156(40)	163(41)	291(74)	155(39)	210(53)	315(80)	89(23)
Q 5										
Ans.	Yes	No								
N (%)	327(83)	67(17)								
Q 6										
Ans.	Yes	No								
N (%)	196(60)	131(40)								
Q 7										
Ans.	Rarely	Often	VO							
N (%)	84(43)	51(26)	61(31)							
Q 8										
Ans.	Yes	No								
N (%)	190(48)	204(52)								
Q 9										
Ans.	Hiking	Fishing	Cycling	R	W	BW	P	S	Others	
N (%)	151(77)	7(4)	29(15)	8(4)	20(10)	4(2)	12(6)	1(1)	21(11)	
Q 10										
Ans.	abc	acb	bac	bca	cab	cba				
N (%)	7(2)	9(2)	23(6)	39(10)	144(36)	172(44)				

Table 12.1. Summary of the answers to the questionnaire. Abbreviations: Q = Question, Ans. = Answer, N = number of people who responded to a particular response; % = percentage of people who responded to a particular response; Question 1: Benef. = Beneficial; Harm. = Harmful; Indif. = Indifferent. Question 2 and 7: VO = Very often. Question 3 and 9: R = Running; W = Working; BW = Bird-watching; P = Photography; S = Sailing. Question 4: BM = Breeding mosquitoes; BF = Breeding fish; PW = Purify water; AT = Attract trash; FC = Flood control; RLS = Red list species; SB = Smell bad; B = Beautiful; SD = Source of diseases; FCC = fighting climate change. In the multiple-choice questions (3, 4 and 9), sum of % is different than 100 due to people being able to answer with more than one option. Question 10 shows the order of photographs choice, the first letter corresponding to the photo that was most liked (a = *S. maritima* prairie; b = *S. densiflora* prairie and c = Chenopodiaceae salt marsh).

Factor		Sex	Age	E. level	Sex x Age	Age x E.level	Sex x Age x E. level	Total
df		1	2	2	2	4	4	393
Question 1	SS	0.668	0.999	0.342	0.0686	5.823	0.355	146.416
	MS	0.668	0.499	0.171	0.0343	1.456	0.0887	0.373
	F	1.891	1.412	0.484	0.097	4.118	0.251	
	P	0.17	0.245	0.617	0.908	**0.003**	0.909	
Question 5	SS	0.0196	1.79	1.196	0.195	5.625	1.108	219.777
	MS	0.0196	0.895	0.598	0.0973	1.406	0.277	0.559
	F	0.0351	1.603	1.071	0.174	2.519	0.496	
	P	0.851	0.203	0.344	0.84	**0.041**	0.739	
Question 8	SS	0.742	37.393	0.303	5.042	4.745	0.815	393.503
	MS	0.742	18.697	0.151	2.521	1.186	0.204	1.001
	F	0.846	21.337	0.173	2.877	1.354	0.233	
	P	0.358	**<0.001**	0.841	0.058	0.249	0.92	
Question 10	SS	3.426	1.536	1.113	1.513	8.353	4.985	114.596
	MS	3.426	0.768	0.557	0.756	2.088	1.246	0.295
	F	14.979	3.358	2.434	3.308	9.132	5.45	
	P	**<0.001**	**0.036**	0.089	**0.038**	**<0.001**	**<0.001**	

Table 12.2. Results of three-way ANOVAs that presented significant differences in the questions with simple responses (Question: 1, 2, 5, 6, 7, 8 and 10). E. Level = Educational level.

12.4. DISCUSSION

This work provided very positives responses by local residents of Huelva City to salt marsh restoration using *S. maritima* plantations. Most of Huelva's citizens recognized the benefits of coastal marshes (75%), a perception which increased with increasing educational level. Previous studies have pointed out that local people generally agree with river restoration, recognizing the benefits to restored ecosystems (Junker & Buchecker, 2008; Buijs *et al*., 2009).

The majority of the respondents showed a low level of knowledge about the ecological services and functions of salt marshes, a result in agreement with the low level of ecological knowledge typical of urban areas (Kellert, 1984). Previous works have illustrated that restoration projects increase the use of the ecosystem for wildlife, and that they are an educational and recreational resource for local people (e.g. Tunstall *et al.*, 1999; Burger, 2003; Natuhara *et al.*, 2005; Armitage *et al.*, 2007). In view of our results, environmental education campaigns should be carried out in Huelva City to increase its citizens' knowledge of the values and services provided by salt marshes, a threatened ecosystem that virtually surrounds the city. With this aim, environmental educational display boards, giving information about different elements and processes of the ecosystem, have been erected along the trail in the restored marshes: these are a very good location to carry out educational campaigns, since they are well-equipped and easily-accessible on foot from the city. Particular effort should be directed at school-age users (Anderson & Moss, 1993), which could be very effective when incorporated into local school curricula (Tanner *et al.*, 1992).

Respondents over 60 years with low educational level were those less aware about the studied restoration project. Anderson & Moss (1993) concluded that conditioning from literature, television and oral communication leads to negative adult perceptions about wetlands. Historically, people have considered wetlands to be unhealthy places and a source of disease (Borca, 2000), but this social perception seemed to be changing, since 77% of respondents thought that marshes were not a source of infection, 74% said that marshes had protected species, and 80% said that they are a beautiful ecosystem. According to Casagrande (1997), some interviewed fishermen were very knowledgeable about the restored area's wildlife and history, since they had frequently worked in those marshes in the past.

Regarding landscape preferences, most of the respondents preferred the native Chenopodiaceae salt marsh; the native *S. maritima* landscape was chosen less. The public's aesthetic preferences based on photographs were primarily influenced by perceived naturalness (Junker & Buchecker, 2008), plant colors, that is, preferring green to yellow or brown (Kaufman & Lohr, 2002), and the amount and diversity of colors (Hands & Brown, 2001). Then, the differences showed in our work could be related to the colors of our photographs, since the Chenopodiaceae salt marsh presented a darker green color and a higher diversity of tones of color than the two cordgrass prairies, which were more yellowish and homogeneous. The studied restoration project was based mainly in *S. maritima* plantations, which was the less favored landscape, but this native cordgrass facilitates the development of ecological succession, resulting in Chenopodiaceae salt marsh, the preferred landscape, at higher elevations (Castellanos *et al*., 1994; Figueroa *et al*., 2003).

The studied restoration project increased its visitor numbers by 27%. According to respondents' comments, this increase of visitors was related to improved access to the marshes by installation of a walkway, as well as to enhancing the landscape. In contrast, Tunstall *et al*. (1999) found significantly fewer visitors after the restoration of river banks in England. Respondents over 60 years visited the restored area more frequently than other age groups, which may be related to a greater amount of free time after retirement, and to their visits to the area before its degradation coinciding with the building of the Chemical Pole in the 1960s. In agreement with our results, Casagrande (1996) and Burger (2003) described that respondents highly valued passive activities, including walking, relaxing and enjoying views in urban wetlands. They tended to place lower values on active uses, including fishing (more popular for men than women) and boating.

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Appendix 1: Questionnaire to Huelva citizens about the perception and use of Odiel Marshes.

Sex … Age …

Education level (1 = low (middle school, elementary school or none), 2 = medium (secondary school diploma), 3 = high (university degree)).

1. Salt marshes are for you:

a. Beneficial.
b. Harmful.
c. Indifferent.

2. You visit Odiel Marshes:

a. Never.
b. Rarely (1-2 times per year).
c. Often (3-6 times per year).
d. Very often (> 6 times per year).

3. When you visit the salt marshes you go:

a. Hiking.
b. Fishing.
c. Cycling.
d. Running.
e. Working (catch bait, fishing ....)
f. Bird-watching.
g. Photography.
h. Sailing.
i. Others.

4. According to you, salt marshes:

a. Raise mosquitoes.
b. Raise fish and shellfish.
c. Purify water.
d. Attract trash.
e. Flood control.
f. Are habitat for red list species.
g. Smell bad.
h. Fight against climate change.
i. Are beautiful.
j. Are a source of diseases.

5. Do you know the marsh restoration project located between the “Nuevo Colombino” stadium and “Punta del Sebo”?

a. Yes
b. No

6. Have you ever visited the restored marshes since the restoration project was carried out?

a. Yes
b. No

7. If the above answer is “yes”, how often do you visit the restored marshes?

a. Rarely (1-2 times per year).
b. Often (3-6 times per year).
c. Very often (> 6 times per year).

8. Did you visit the restored marshes before the restoration project was carried out?

a. Yes
b. No

9. When you visited the restored marshes, you were...

a. Hiking.
b. Fishing.
c. Cycling.
d. Running.
e. Working (catch bait, fishing ....)
f. Bird-watching.
g. Photography.
h. Sailing.
i. Others.

10. Please order these images, with the first the most beautiful and the last the least attractive.

A

B

C

CHAPTER 13

General discussion

CHAPTER 13.- General discussion.

This general discussion present an overview of the contributions this PhD thesis makes to knowledge about salt marsh restoration, together with the applications to salt marsh management, and different aspects that could be the subject of future research.

Constructed wetlands have been proposed as an alternative method for mitigation of marsh habitat loss (Velásquez, 1992; Masero *et al*., 1999; Mitsch, 2010). This PhD thesis shows the positive effects of European salt marsh restoration using Small Cordgrass, *Spartina maritima* (Curtis) Fernald, Perennial Glasswort, *Sarcocornia perennis* (Mill.) A. J. Scott ssp. *perennis*, and Dwarf Eelgrass, *Zostera noltii* Hornem. At the same time, it also reports some negative effects of the invasion of Dense-flowered Cordgrass, *Spartina densiflora* Brongn. Our results show that Small Cordgrass plantations in the Gulf of Cádiz combat salt marsh losses and degradation, enhancing ecosystem services, plant and animal biodiversity and environmental quality of salt marshes, a threatened ecosystem. In addition, restoration of *S. maritima* marshes have clear social benefits, as has been shown in the public perception of Huelva citizens and in the increased number of visits to the restored salt marshes on which our studies have been focused.

Many works have described the negative effects of invasive species in wetlands (e.g. Benoit & Askins, 1999; Levin *et al*., 2006; Castillo *et al*., 2008, 2010; Gan *et al*., 2009; Zhou *et al*., 2009) and the benefits related to salt marsh restoration (e.g. Broome *et al*., 1988; Warren *et al*., 2002; Armitage *et al*., 2007; Utomo *et al*., 2010). Nevertheless, our results are the first based on a detailed monitoring of different aspects of a restoration project carried out in European *S. maritima* low salt marshes. A total of 70 species (10 plants, 34 birds and 26 macroinvertebrates) were recorded in the restored salt marshes, highlighting both their local and their regional value. These *S. maritima* marshes are the habitat of eight protected bird species, such as the Kentish Plover (*Charadrius alexandrinus*), the Osprey (*Pandion haliaetus*) and the Little Tern (*Sterna albifrons*). The maintenance of a healthy bird community in salt marshes would imply an improvement in ecological relations between different locations, since native populations of invertebrates and plants seem to be partly sustained through bird dispersion that maintains the connectivity between populations (Amezaga *et al*., 2002).

We have shown that *S. maritima* is a key factor in nutrient cycling in intertidal plains, showing rapid tissue decomposition (especially of leaves) and contributing to increasing the sediment organic matter. This increase in sediment organic matter favors biodiversity and abundance of different species of macroinvertebrates (Sacco *et al*., 1994), in turn favoring the bird community (Sánchez, 2005). At the same time, *S. maritima* structures the ecosystem, facilitating the development of ecological succession through *S. perennis* colonization (Castellanos *et al*. 1994; Figueroa *et al*. 2003; Bouma *et al*. 2009). Thus, plant and animal biodiversity is increased by the environmental heterogeneity that offers *S. maritima*, *S. perennis* and *Z. noltii* plantations *versus* extensive and homogeneous bare mudflats with low cover of biennial populations of *S. densiflora*. The beneficial effects of environmental heterogeneity on wetland biodiversity have previously been reported for the invertebrate community by de Szalay & Resh (2000) and for shorebirds by Armitage *et al*. (2007). Sánchez *et al*. (2006) reported that ecological diversity and abundance of avian and macroinvertebrate communities were related in artificial saltworks in the Odiel Marshes and, in turn, macroinvertebrate diversity and abundance seem to be related to vegetation complexity (Olson *et al*., 1995; Braga *et al*., 2009). However, the studied *S. maritima* restored marshes are in proximity to human infrastructures that have negative effects on the avian community, such as a busy road and the Huelva Chemical Pole (Rosa *et al*., 2003; Armitage *et al*., 2007). Given that they occupy less than 15 ha, they seem nonetheless to be of great importance for many shorebirds, such as the Ruddy Turnstone (*Arenaria interpres*), large numbers of which choose to use them as a feeding habitat. Regionally, *S. maritima* prairies are very scarce since they have been degraded or totally destroyed in recent decades, mainly due to anthropogenic erosion and habitat destruction by coastal infrastructures.

Moreover, our results showed that *S. maritima* prairies stabilize metals, concentrating them in the rhizosediments (especially iron and aluminum). At the same time, they promote sedimentation and act as a carbon and nitrogen sink, reducing eutrophication of estuary waters (Sousa *et al*., 2008). Thus, restoration efforts using *S. maritima* enhance not only restored areas, but increase the environmental quality of the whole estuary. The role of *S. maritima* in promoting sedimentation is especially important in the actual context of sea level rise, given many salt marsh restoration projects are being used to increase emerged areas (Vandenbruwaene *et al*., 2011). Furthermore, we have shown that *S. maritima* plantations sequestrate atmospheric carbon (Sousa *et al*., 2010), contributing to the mitigation of global climate change. All these attributes, together with a fast rate of growth, make *S. maritima* an excellent biotool for restoration of European coastal wetlands.

On the other hand, our results regarding invasion of *S. densiflora* show that this invasive cordgrass is able to colonize acidic ($4 < pH < 8$) and highly metal-polluted sediments in Tinto river. Its invasion is prevented by very acidic sediments ($pH < 4$) due to problems not in germination but in seedling establishment. Exotic species' invasions are, after habitat loss, the main problem affecting global diversity (Wilcove *et al*., 1998; Mooney & Cleland, 2001). In this context, *S. densiflora* has been described as a species with a high invasive capacity, since it produces many viable seeds (Nieva, 1996; Kittelson & Boyd, 1997), shows a high phenotypic plasticity (Nieva *et al*., 2001, 2005; Bortolus *et al*., 2004), grows fast and accumulates large quantities of aerial and subterranean biomass (Nieva *et al*., 2001; Castillo *et al*., 2008), and tolerates a wide range of environmental conditions (Nieva *et al*., 2001; Curado *et al*., 2010). This PhD thesis shows the limitation of the invasion of *S. densiflora* landward along the banks of the Tinto River, this being important knowledge for the future management of this peculiar and protected river.

13.1. Applications to Management and Conservation.

Restored and constructed salt marshes with a proper management regime can carry out the same or similar ecological services as natural marshes. Based on the results of our work in European marshes invaded by *S. densiflora* and in restored low marshes using *S. maritima*, we propose a series of management measures to improve the quality and services offered by European salt marshes.

The maintenance of invasive populations of *S. densiflora* in Tinto river could be beneficial, since they would prevent erosion of bare mudflats which they covered. However, the invasive nature of *S. densiflora* is a serious problem. Tinto river populations, and persistent individuals of *S. densiflora* in restored marshes, serve as sources of propagules (seeds and rhizomes) to other marshes. Then, *S. densiflora* should be removed totally from restored marshes in the Gulf of Cádiz and along the banks of the Tinto river. In Tinto river, *S. densiflora* should be replaced by native macrophytes with a high tolerance to acidity and metal pollution, such as *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha dominguensis* Pers.. In restored low marshes, it should be replaced with *S. maritima* and *S. perennis*, since *S. maritima* prairies seem to prevent *S. densiflora* invasion once they are well-established (Castillo *et al*., 2008). However, while *S. maritima* prairies are still not established vigorously and adjacent clumps have not yet coalesced, annual removal campaigns of new *S. densiflora* seedlings would be necessary to facilitate native vegetation development and avoid *S. densiflora* seed production *in situ*. Since *Spartina* shoots are semelparous (they die shortly after their first sexual reproduction event) and their mean shoot life span is about 2 yr (Vicari *et al*., 2002; Nieva *et al*. 2005), *S. densiflora* seedlings should be removed during the first two years after establishment to avoid seed production that would accelerate the invasion.

On the other hand, macroinvertebrate communities can be negatively affected by invasive cordgrasses, as described for invasive populations of *S. alterniflora* (Levin *et al*., 2006; Zhou *et al*., 2009). Thus, the fight against *S. densiflora*, together with native species plantations in the Gulf of Cádiz, would favor the macroinvertebrate community of salt marshes. Native vegetation provides organic matter, one of the abiotic variables that best explain macroinvertebrate distribution (Salgado *et al*., 2007). In this sense, *S. maritima* loses its leaves through the year (Castro & Freitas, 2000) contributing to the sedimentary organic matter, while *S. densiflora* keeps its dead leaves and tillers forming very dense tussocks (Nieva *et al*., 2005).

During low salt marsh restoration projects, permanent flooded intertidal ponds could be established within *S. maritima* prairies to establish *Z. noltii* populations, diversifying habitat types. These ponds would have anoxic sediments that would limit the establishment of most marsh plant species, being adequate for *Zostera* (Hemminga & Duarte, 2000). Habitat diversification with, for example, the creation of intertidal ponds within *S. maritima* prairies, the maintenance or creation of intertidal sand dunes as those present in the studied restored areas, or oyster cultch addition, would offer refuges to macroinvertebrates such as crabs, therefore attracting bird such as the Ruddy Turnstone (Ribeiro *et al*., 2004). In this context, removal of *S. densiflora* increases habitat diversity, which may favor bird communities, as has been shown by Patten & O'Casey (2007) in Willapa Bay with extensive invasive populations of *Spartina alterniflora* Loisel. Additionally, the installation of artificial perches for hunting, resting and feeding, such as those installed in the studied restored area, seems to make marshes more attractive for different bird species such as Osprey (*Pandion haliaetus*). At the same time, artificial perches also make it easier for marsh visitors to observe birds.

Extensive salt marsh plantations using *S. maritima* behave in a similar way to natural preserved marshes after approximately three years, therefore being a useful biotool to stabilize eroding areas since they reduce erosion and increase accretion. However, the reduction of erosion due to plant colonization is only local, operating within a static vegetation patch; friction exerted by the vegetation seems to increase erosion in bare areas such as drainage channels (Temmerman *et al*., 2007). Therefore, the location that *S. maritima* clumps will occupy must be well-panned before restoration, and plantations in natural drainage channels must be avoided since the sediment dynamics will change after planting. In very erosive salt marshes with erosive banks, other action may be necessary to fight erosion, such as installation of breakwaters or oyster cultch addition (Meyer *et al*., 1997).

The studied restored salt marshes are very close to Huelva city, offering a great opportunity for environmental education. With this aim, environmental interpretational panels about different aspects of salt marshes have been placed along a path by the Port Authority of Huelva. Now that the restored marshes are developing correctly, it is viable to organize guided visits for Huelva citizens, including school and high school students.

13.2 Future research.

This PhD thesis adds new knowledge to our understanding of salt marsh restoration, especially for European *S. maritima* marshes, but also opens new questions to be tackled in future studies. For example, future studies are still necessary to determine if the landward areas of Tinto river can be colonized by *S. densiflora* rhizome fragments.

Constructed wetlands are used for water purification (e.g. Samecka-Cymerman *et al*., 2004; Wu *et al*., 2010; Adhikari *et al*., 2011). However, little it is known about water purification in European constructed salt marshes. The combined effects of the capacities of *S. maritima* and *S. perennis* for reducing eutrophication, together with their metal phytoremediation potential reported in this thesis, should be studied in detail, focusing on the construction of salt marshes to provide treatment of domestic sewage and industrial effluents, as well as of running water coming from crop fields carrying high nitrate concentrations (Greenway, 2005). *Spartina maritima* and *S. perennis* constructed wetlands could be used as green filters, especially for small coastal towns where they would be a cheaper option than other depuration methods (Sundaravadivel & Vigneswaran, 2001).

Research needs to focus on optimizations which enhance applicability, and to address the economic feasibility of phytoremediation (Bert *et al*., 2009), since this is still an emerging technology that has to prove its sustainability on the field scale. In this sense, it would be interesting to study the metal phytoextraction capacity of *S. perennis* and *S. maritima* in metal polluted sediments without continuous inputs. This would allow an assessment of their metal sequestration efficiency and the time required to decontaminate metal polluted sediments, as has been studied for other species in other ecosystems (e.g. Inoue & Saeki, 2004; Meers *et al*., 2007).

To improve the methodology for the restoration of *S. maritima*, it would be interesting to develop an effective way to create intertidal ponds planted with the threatened species, such as *Z. noltii*, within *Spartina* prairies. This habitat diversification would favor *Z. noltii* conservation and could be very beneficial for bird and macroinvertebrate communities (Spruzen *et al*., 2008; Bouma *et al*., 2009). Intertidal ponds may act as *Z. noltii* seed sources to nearby low elevation areas below the *S. maritima* lower distribution limit in the tidal gradient.

On the other hand, it is important to carry out long-term continuous monitoring of newly *S. maritima* restored marshes to improve restoration methodology, avoiding mistakes in planning and implementing of future restoration projects.

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CONCLUSIONS

Conclusions.

1. *Spartina densiflora* can invade salt marshes with sediment pH higher than 4 along the **Tinto River**, where it finds a very low level of interspecific competition due to the absence of most of the native halophytes. However, it is not able to colonize by seeds riverbanks in more landward areas out the estuary where the establishment of seedlings is prevented.

2. *Zostera noltii*, *Spartina maritima* and *Sarcocornia perennis* ssp. *perennis* **plantations** develop correctly in restored salt marshes in Odiel Marshes, creating the typical zonation pattern of the Gulf of Cadiz.

3. *Spartina maritima* restored wetlands sequestrated 2480g C m^{-2} in *Spartina* areas and 2821g C m^{-2} in *Sarcocornia perennis* areas in the first 20 cm of sediment and 775 ± 117 g C m^{-2} in *Spartina maritima* biomass and 698 ± 77 g C m^{-2} in *Sarcocornia* biomass. This corresponded with a total carbon budget of 30.2 t C ha^{-1}. The **carbon-stock** in *Spartina maritima* restored marshes is still low 28 months after plantations, showing a potential capacity to sequestrate more carbon in above-ground biomass by expanding its cover, and especially in sediments and below-ground biomass.

4. Restored marshes using *Spartina maritima* reduce **eutrophication** of estuaries waters, retaining 329.0 g N m^{-2} in *Spartina* areas and 373.1 g N m^{-2} in *Sarcocornia perennis* areas in the first 20 cm of sediment and 47 ± 7 g N m^{-2} in *Spartina* biomass and 59 ± 6 g N m^{-2} in *Sarcocornia* biomass.

5. *Spartina maritima* leaves show a high **decomposition rates** (between 0.0043 k day^{-1} in winter and 0.0060 k day^{-1} in summer) in Odiel Marshes, facilitating a rapid cycling of nutrients.

6. *Sarcocornia perennis* and *Spartina maritima* are useful biotools for **phytoremediation** (phytostabilization and phytoextraction of metals) projects in European polluted salt marshes at low elevations in the tidal gradient. *Sarcocornia perennis* accumulates ca. 15 times more metals per square meter than *Spartina maritima*.

7. Extensive salt marsh restoration projects using *Spartina maritima* fight **erosion** and favor accretion in a similar way than natural populations ca. 2 yr after plantations, showing mean accretion rate between +10 and +27 mm yr^{-1}.

8. Salt marsh restoration using *Spartina maritima* increases the complexity and abundance of the **benthic macroinvertebrate community** ca. 3 yr after plantations in Odiel Marshes. Restored marshes achieve similar ecological diversity values and higher specific richness than preserved marshes (although with differences in species composition) and higher values for both parameters than non-restored marshes invaded by *Spartina densiflora.* In addition, restored and preserved marshes do not differ in total macroinvertebrate abundance neither in total biomass, being both higher than in non-restored marshes

9. Restored salt marshes using *Spartina maritima* and *Zostera noltii* showed higher **bird ecological diversity** than non-restored marshes invaded with *Spartina densiflora* ca. 2 yr after plantations in Odiel Marshes.

10. **Huelva’s citizens** show a low level of knowledge about ecological services and functions of salt marshes. Nevertheless, the restoration of urban salt marshes using *Spartina maritima* increased their visitor number in 27%.

ACKNOWLEDGEMENTS

Acknowledgements.

This thesis would not be complete without thanking all those people, institutions and services that have made it possible.

I am especially grateful to Dr. Jesús M. Castillo Segura for his guidance, encouragement, patience and excellent supervision during my study toward this degree. I am also grateful for his interest in my work, the time spent on my manuscripts and for the effort trying to teach me scientific thinking. I would also like to thank Dr. Enrique Figueroa Clemente and Dr. Alfredo E. Rubio Casal, who served on my advisory committee, for their critical reading and constructive suggestions on my thesis.

I am grateful to Ahmed M. Abbass and Jorge Carrión-Tacuri for their invaluable support during samplings, and Raúl García Varela, Antonio Luis Florencio Nieto, Jesús Villadeamigo Arrayás, Quique Muñoz and “my” students Laura, Juan Carlos and the two “Juanmas”, for making field trips unforgettable days. Thanks are owed to them for the big effort they made and for working at unusual times. All of them contributed a greatly to what is presented in this thesis.

I sincerely thank Dr. Vicente Manzano for planning the survey and sampling methods with me in order to understand the public perception of Huelva’s citizens about marshes; and also Anita Dante (for everything that she gives me), Marta Prieto, Cinta Barahona, and all the students and friends who helped me during the population survey in Huelva city.

Special thanks go to Dr. Emilio Sánchez Moyano for teaching me about the wonderful world of invertebrates. I cannot forget the invaluable help of Jose, Aida and Bibi in the study of bird communities. Thank you also to Dionisio Leva Soldado for his formidable cover illustration.

I thank the Port Authority of Huelva for their sponsorship and support of the monitoring ecological engineering project in the Odiel Marshes, and also the Directorate of the Odiel Salt Marshes Natural Park for collaboration. We also thank Proof-Reading-Service.com for the language editing; and Seville University Greenhouse Service and the microanalysis service of CITIUS for collaboration.

I also want to thank my brothers (The Kurattis) for their support; it has been a long road to get to this point, but, finally, I am now here.

Finally, I would like to thank my parents, Paquita Ferrera and Rafael Curado, who have always encouraged me, over the years, to pursue my dreams and ambitions in both science and in all other aspects of my life.