Native plant restoration combats environmental change: development of carbon and nitrogen sequestration capacity using small cordgrass in European salt marshes

**Guillermo Curado · Alfredo E. Rubio-Casal ·
Enrique Figueroa · Brenda J. Grewell ·
Jesús M. Castillo**

Abstract Restoration of salt marshes is critical in the context of climate change and eutrophication of coastal waters because their vegetation and sediments may act as carbon and nitrogen sinks. Our primary objectives were to quantify carbon (C) and nitrogen (N) stocks and sequestration rates in restored marshes dominated by *Spartina maritima* to provide support for restoration and management strategies that may offset negative aspects of eutrophication and climate change in estuarine ecosystems. Sediment C content was between ca. 13 mg C g^{-1} and sediment N content was ca. 1.8 mg N g^{-1}. The highest C content for *S. maritima* was recorded in leaves and stems (ca. 420 mg C g^{-1}) and the lowest in roots (361 ± 4 mg C g^{-1}). *S. maritima* also concentrated more N in its leaves (31 ± 1 mg N g^{-1}) than in other organs. C stock in the restored marshes was 29.6 t C ha^{-1}; ca. 16 % was stored in *S. maritima* tissues. N stock was 3.6 t N ha^{-1}, with 8.3 % stored in *S. maritima*. Our results showed that the *S. maritima* restored marshes, 2.5 years after planting, were sequestering atmospheric C and, therefore, provide some mitigation for global warming. Stands are also capturing nitrogen and reducing eutrophication. The concentrations of C and N contents in sediments, and cordgrass relative cover of 62 %, and low below-ground biomass (BGB) suggest restored marshes can sequester more C and N. *S. maritima* plantations in low marshes replace bare sediments and invasive populations of exotic *Spartina densiflora* and increase the C and N sequestration capacity of the marsh by increasing biomass production and accumulation.

Keywords Carbon sink · Climate change · Eutrophication · Odiel Marshes · Salt marsh restoration · *Spartina maritima*

G. Curado · A. E. Rubio-Casal · E. Figueroa ·
J. M. Castillo (✉)
Departamento de Biología Vegetal y Ecología,
Universidad de Sevilla, Ap. 1095,
41080 Sevilla, Spain
e-mail: manucas@us.es

B. J. Grewell
Department of Plant Sciences, USDA-ARS Exotic and
Invasive Weeds Research Unit University of California,
Davis, MS-4 One Shields Avenue,
Davis, CA 95616, USA

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). At the same time, eutrophication is an important conservation problem in coastal ecosystems worldwide, altering habitat, energy fluxes, trophic networks, and community composition (deJonge et al. 2002; Bertness et al. 2007; Caffrey et al. 2007). Coastal marshes are among those ecosystems that are greatly affected by global climate change and eutrophication. For example, sea-level rise is causing extensive loss of salt marshes (Hartig et al. 2002; Morris et al. 2002; Nicholls 2004)

and eutrophication may provoke dense algal growth that has been related to decline of salt marsh vegetation (Adam 1990).

Conservation and restoration of salt marshes is critical in the context of climate change because vegetation and sediments of salt marshes may act as carbon (C) sinks, reducing atmospheric carbon dioxide concentration (Caçador et al. 2004; Sousa et al. 2010a,b). Under global warming conditions, coastal wetlands are more valuable C sinks than any other wetland ecosystem because of their relatively high C sequestration rates and low methane emissions (Choi and Wang 2004). Moreover, salt marshes have been identified as strategically located nutrient sinks, mainly nitrogen (N), at the mouth of rivers that may reduce eutrophication impacts in estuaries and coastal waters (Romero et al. 1999; Wigand et al. 2007; Castro et al. 2009; Caetano et al. 2012).

Spartina species (cordgrasses) are among the most widespread halophytes in salt marshes around the world. *Spartina alterniflora* Loisel. (smooth cordgrass) has been widely studied showing that its prairies accumulate higher quantities of C than most grasses (Liao et al. 2007, Elsey-Quirk et al. 2011). *S. alterniflora* is also very efficient at N uptake, reducing eutrophication of estuaries in the Eastern North American portion of its native range (Patrick and DeLaune 1976; White and Howes 1994; McFarlin et al. 2008) and in China, where it is an invasive species (Wan et al. 2009; Zhang et al. 2009). In European salt marshes, native stands of perennial *Spartina maritima* (Curtis) Fernald (small cordgrass) also act as C sinks (Cartaxana and Catarino, 1997; Caçador et al. 2004; Lillebo et al. 2006). Populations of cordgrass can also reduce eutrophication through N accumulation (Caçador et al. 2007; Sousa et al. 2008; Castro et al. 2009; Sousa et al. 2010b) as the invasive *Spartina densiflora* Brongn. (dense-flowered cordgrass), an invasive species from South America, does in the Southwest Iberian Peninsula (Neves et al. 2010). However, the capacity of *S. maritima* to accumulate N in restoration plantings and expanding marshes is unknown. Furthermore, despite the fact that salt marshes are highly productive systems, there is an important lack of data about the potential of salt marshes for C storage (Chmura et al. 2003).

In this context, a specific goal of salt marsh creation and restoration projects may be to implement actions that will enhance C and N stocks to mitigate and offset negative effects of global climate change and eutrophication

(Crooks et al. 2011). An innovative restoration project was carried out in the Odiel Marshes (Southwest Iberian Peninsula). Prior to our study, *S. densiflora* dominated this site and had been eradicated from most places. The overall objectives of the restoration project were to restore native vegetation and to phytostabilize sediments following removal of *S. densiflora* (Castillo and Figueroa 2009a). *S. maritima* was planted throughout the site to establish a native cordgrass-dominated community because *S. maritima* contributes effectively to sediment stabilization, while density and biomass of stands increase (Castillo et al. 2008a,b; Sousa et al. 2008; Caçador et al. 2009) at the same time that they facilitate development of ecological succession (Figueroa et al. 2003).

The primary objectives of this study were to quantify C and N stocks and sequestration rates in sediment and in vegetation dominated by restored stands of *S. maritima*. Along with these aims, our goal was to quantify biomass accumulation, sedimentation rate, and C and N content for sediments and plant tissue of *S. maritima*. Overall, our intent was to provide support for restoration and management strategies that may offset negative aspects of eutrophication and climate change in estuarine ecosystems.

Material and methods

Study site

The study was carried out in a restored salt marsh area, known locally as "Punta del Sebo", which borders the main channel of the Huelva Estuary at the confluence of the Odiel and Tinto rivers in the European Atlantic basin (Southwest Iberian Peninsula; 37°08′–37°20′N, 6°45′–7°02′W) (Fig. 1). The Odiel Marshes are a significant wetland ecosystem recognized for their global significance with designations as a United Nations Educational, Scientific and Cultural Organization (UNESCO) Biosphere Reserve and the Ramsar Convention on Wetlands. The estuary has great historical significance for its shipping ports used by Phoenicians and ancient Greeks and as the origin of the 1492 and later expeditions led by Columbus to and from the Americas. These and later voyages are the likely source of accidental introductions of invasive South American *S. densiflora* that formed monospecific stands, displaced indigenous species, and transformed Odiel salt marshes (Nieva et al. 2001, Davy et al. 2009).The Odiel Marshes
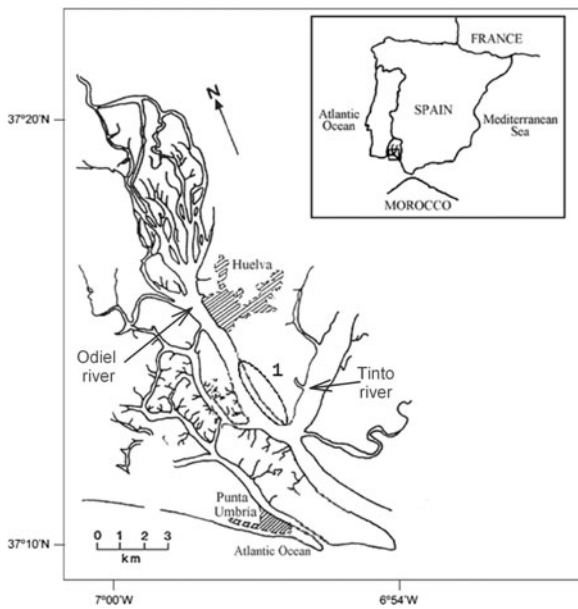
Fig. 1 Location of Odiel Marshes on the Atlantic coast of Southwest Iberian Peninsula (37°08′–37°20′N, 6°45′–7°02′W) and the restored area where our work was carried out (*1*)

are now highly eutrophic due to nutrient loading from urban and agricultural runoff, and industrial processes (Elbaz-Poulichet and Dupuy 1999, Davy et al.). The plant community in our study site was restored from November 2006 to January 2007 with plantations of *S. maritima* and *S. perennis*. *S. maritima* clumps coming from natural populations were planted manually at a density of one clump per square meter (ca. 20 shoots per clump) after the invasive *S. densiflora* was eliminated manually from 2.00 ha around the site (Castillo and Figueroa 2009a).

Every *S. maritima* population at channel edges in the Odiel Marshes have disappeared or have been degraded due to a combination of different anthropogenic impacts, erosion, and a limitation in the dispersion of *S. maritima* that very rarely establishes new tussocks by seeds (Castillo, Ayres et al. 2010). Thus, we decided to compare C and N stocks and sequestration for restored marshes with data from previous studies on *Spartina* marshes around the world.

Sediment and vegetation sampling

Sediment elevation relative to the Spanish Hydrographic Zero (SHZ) datum, redox potential, pH, electrical conductivity of interstitial water of sediments, organic matter content, and bulk dry density was recorded in *S. maritima* areas in May–June 2009 ($n=20$). Every sediment characteristic was recorded between 0 and 10 cm deep, except for the redox potential that was sampled in surface (0–2 cm) and depth (2–10 cm). Elevation was surveyed to a resolution of 2 cm with a Leica NA 820 Theodolite (Singapore), and 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). We recorded pH and electrical conductivity in the laboratory (Crison meter fitted with M-506 and 522 probes) after adding distilled water to the sediment (pH 1:1, v/v; conductivity 1:2, v/v). Sediment organic content was analyzed from triplicate subsamples by loss-on-ignition method after 4 h at 450 °C. Sediment bulk density was recorded by weighting (dry weight, DW) volume of sediments in 5×5-cm cylindrical cores. Sedimentation was determined by markers consisting in 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 *S. maritima* areas. The distance from the middle of crossbar to the sediment surface was measured quarterly from March 2009 to March 2010 ($n=9$). This prevents measurement errors due to the erosive vortex generated in the base of the markers (Curado et al. 2012). Sedimentation rate between consecutive measurements was calculated as the difference between the initial and final distance (in centimeters) divided by the number of years.

S. maritima plant material and sediment samples (between 0–2 cm and 2–20 cm deep, where roots were concentrated) for C and N content analysis were collected in July 2008 in *S. maritima* areas in two 10-m-long rows established parallel to the tidal line between +2.16 and +2.67 m over SHZ ($n=10$; five equidistant sampling points per row). Superficial sediments (0–2 cm deep) were collected using nylon horizons set up 6 months earlier to ensure they were recently deposited. Previous studies have reported that *S. maritima* C concentration does not change seasonally (Cartaxana and Catarino 1997; Caçador et al. 2004).

S. maritima cover was recorded by contact every 2 m along nine 60- to 80-m-long stratified random 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 *Spartina* band ($n=9$). Total area occupied by *S. maritima* was determined by the software ArcGIS 9 (ESRI 2008) after recording the distribution of

small cordgrass in the field using a global positioning system (GPS) (model eTrex Vista Garmin). Above- and belowground biomass (AGB and BGB, respectively) were recorded in October, coinciding with the period of maximum biomass accumulation (Castellanos 1992), in 10-cm quadrant plots with monospecific cover of 100 % ($n=10$). *S. maritima* biomass was washed carefully, separated into leaves, stems, roots, and rhizomes and dried for 48 h at 80 °C to achieve a constant DW. Then, plant material was weighed (Pinnacle P-403 balance, Denver Instrument, Denver, CO, USA) to quantify biomass. Net annual standing above- and belowground productivity (NAPP and NBPP) for *S. maritima* were calculated as the total AGB or BGB, respectively, divided by years since transplantation. Sampling plots for biomass were located in areas with bare sediments adjacent to clumps just after transplantation to ensure that all the standing biomass was effectively produced in situ after restoration plantings (Castillo, Leira-Doce et al. 2008).

Carbon and nitrogen content analysis

Plant samples were separated into photosynthetic organs, nonphotosynthetic stems, roots, and rhizomes. Sediment and plant samples were dried for 48 h at 80 °C and ground (Cyclotec, Foss Tecator AB, Höganäs, Sweden) to pass through an 80-μm sieve. Total C content (milligrams per gram DW) and total N content (milligrams per gram DW) were determined for undigested samples using an elemental analyzer (Leco CHNS-932, Spain). The value for each sample corresponded to the mean of three replicated measurements.

Carbon and nitrogen accumulation rates and stocks

C and N pools were calculated for every plant organ (biomass×N or C content) and for the sediment between 0–2 cm and 2–20 cm deep (sediment mass×N or C content). Sediment C and N stocks were calculated for each of two depths (0–2 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×sediment depth)) and the sediment C or N content. Annual C and N accumulation in the sediments was calculated as the product of the mean annual sedimentation rate and the sediment C and N content, respectively, in the upper 2 cm deposited on nylon horizons. Soil C and N density was calculated as the product of sediment C and N content and sediment bulk density.

S. maritima C and N stocks were calculated for each organ as the product of their mean biomass density (biomass×occupied area×relative cover) and C and N content, respectively. Annual C and N accumulation in *S. maritima* biomass was calculated taking into consideration both NAPP or NBPP and its C and N content, respectively.

Statistical analysis

Analyses were carried out using SPSS release 12.0 (SPSS Inc., Chicago, IL, USA). 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 $\sqrt{x}$. Student's t test for independent samples was applied to compare two means. If homogeneity of variance or normality was not achieved by data transformation, then means were compared using Mann–Whitney U test. One-way analysis of variance (ANOVA) was applied to compare more than two means. Tukey's honestly significant difference (HSD) test between means was calculated only if 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 nonparametric ANOVA.

Results

Carbon and nitrogen contents

Abiotic environment data for the restored *S. maritima* marshes are shown in Table 1. Sediment redox potential was similar at surface and at depth (t test, $P>0.05$). Sediment C content was lower at surface (0–2 cm: 11 ± 1 mg C g^{-1}) than at depth (2–20 cm: 16 ± 1 mg C g^{-1}) (t test$=-2.266$, d.f.$=18$, $P<0.05$). 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. In contrast, sediment N contents were similar at surface (1.6 ± 0.3 mg N g^{-1}) and at depth (2.1 ± 0.2 mg Ng^{-1}) (Mann–Whitney U test, $P>0.05$), varying between 0.8 and 3.1 mg N g^{-1}.

Restoration plantings of *S. maritima* showed significant differences in C content between organs (ANOVA or Kruskal–Wallis, $P<0.001$). The highest values were

Table 1 Elevation above Spanish Hydrographic Zero (m), sediment redox potential (mV), electrical conductivity (mS cm^{-1}), pH, organic content (%), bulk density (g cm^{-3}), and sedimentation rate (cm year^{-1}) in the *Spartina maritima* areas of restored salt marshes in the Odiel Marshes (Southwest Iberian Peninsula) ($n=9$–20)

	Spartina maritima areas	
Abiotic variables	Mean±SEM	Max–min
Elevation relative to SHZ	2.28±0.06	2.72/1.78
Redox potential (0–2 cm)	−5±18	164/−117
Redox potential (2–20 cm)	−44±20	172/−171
Conductivity	15.2±1.5	33.7/8.3
Organic matter content	4.6±0.7	9.18/0.31
pH	7.1±0.1	7.7/6.6
Bulk density	0.80±0.06	1.43/0.43
Sedimentation rate	2.2±0.3	0.3/0.1

recorded in leaves and stems (ca. 420 mg C g^{-1}) and the lowest in roots (361±4 mg C g^{-1}). *S. maritima* also concentrated more N in its leaves (31±1 mg N g^{-1}) than in the other organs (ANOVA, $F=19.415$, $P<0.001$, d.f.=39) (Table 2).

Carbon and nitrogen accumulation and stocks

The total volume of sediment colonized by *S. maritima* was 1,674 m^3 between 0 and 2 cm and 15,066 m^3 between 2 and 20 cm, accumulating 14.7 and 192.8 t C, respectively, corresponding to 2,480 g C m^{-2} in *S. maritima* areas in the first 20 cm of sediment. Sediment C density was 0.009 g C cm^{-3} at the surface and 0.013 g C cm^{-3} at depth of 2–20 cm. Sediment N pool of the *Spartina* areas was 2.14 t N in superficial sediments and 25.31 t N at depth. Sediments (2,176 m^3) were accumulated annually in areas colonized by *S. maritima* (8.37 ha) representing an annual accumulation of 19.1 t C and 2.8 t N.

No evidence of herbivory (that would reduce biomass and change allocation patterns) was observed during the study. 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, $\chi^2=24.905$, $P<0.05$, d.f.=3). Relating these biomass data with the respective C and N contents, *S. maritima* stems accumulated more C and N than the other organs (C: Kruskal–Wallis, $\chi^2=25.835$, $P<0.01$, d.f.=3; N: Kruskal–Wallis test, $\chi^2=28.143$, $P<0.001$, d.f.=3).

Twenty-eight months after transplanting, *S. maritima* colonized 8.37 ha with a relative cover of 62±6 %, accumulating ca. 40.2 t C and ca. 2.5 t N; ca. 50 % of these C and N stocks were stored in stems. Small cordgrass prairies showed a NBPP of 264±42 g DW m^{-2} year^{-1} and a NAPP of 553±83 g DW m^{-2} year^{-1}, which corresponded to 104 g C m^{-2} year^{-1} in BGB and 228 g C m^{-2} year^{-1} in AGB and to a total N accumulation of ca. 20 g N m^{-2} year^{-1} (Table 2).

Total C stock was 247.7 t C (29.6 t C ha^{-1}); ca. 16 % was stored in *S. maritima* tissues. The C stock was increasing annually at 19.1 t C from

Table 2 Carbon and nitrogen contents, biomass (dry weight), and carbon and nitrogen stock density for leaves, stems, roots, and rhizomes in restored salt marshes using *Spartina maritima* (8.37 ha with a relative cover of 62 %) 28 months after transplanting. Different coefficients indicate significant differences between organs (ANOVA, $P<0.05$)

Organ	C content (mg C g^{-1})	N content (mg N g^{-1})	Biomass (g m^{-2})	C stock (g C m^{-2})	N stock (g N m^{-2})
Leaves	419±1[a]	31±1[a]	356±53[a]	145±23[a]	11±2[a]
Stems	415±1[a]	24±1[b]	935±145[b]	388±60[b]	23±4[b]
Rhizomes	408±1[b]	19±1[c]	424±60[a]	173±25[a]	9±1[a]
Roots	361±4[c]	22±4[bc]	192±44[c]	69±16[c]	4±1[c]
Total	–	–	1,907±283	775±117	47±7

sedimentation and 17.2 t C due to *S. maritima* expansion. Total N stock in 8.37 ha of the restored salt marshes colonized by *S. maritima*, including their sediments in the first 20 cm depth, was 30.25 t N (3.6 t N ha^{-1}), with 8.3 % stored in the small cordgrass. N stock of 2.8 t N was being added annually by deposition of sediments and ca. 1.0 t N was sequestrated by *Spartina* colonization (Table 2).

Discussion

This study shows the capacity of European restored salt marshes planted with small cordgrass to sequestrate C and N in their vegetation and sediments.

Carbon and nitrogen in sediments

Sediment C content in the studied marshes was still low 28 months after transplanting in comparison with mature marshes of *S. maritima* and similar to the values recorded in young marshes of *Spartina anglica* C. E. Hubbard (common cordgrass) (Table 3). Sediment C budget in *Spartina* areas (2.5 kg C m^{-2} in the top 20 cm) was lower than those reported for *S. maritima*, *S. alterniflora*, *Spartina patens* (Aiton) Muhl. (salt meadow cordgrass) natural marshes (Table 3). Sediment C density (between 0.009 and 0.013 g cm^3) was also lower than the average soil C density of salt marshes (0.039 ± 0.003 g cm^{-3}) (Chmura et al. 2003).

Sediment N content was within the range reported for bare sediments in *S. maritima* marshes (1.8 mg N g^{-1} following Castro et al. (2009)) and higher than in *S. maritima* Portuguese marshes (Table 3). In this sense, sediment organic content was ca. 5 %, while values ca. 11 % have been recorded for other *S. maritima* marshes (Lillebo et al. 2006; Castillo, Leira-Doce et al. 2008). However, our sediment N contents were comparable to those reported for *S. alterniflora* marshes in the USA (Table 3). Sediment N stock in *S. maritima* restored marshes was similar to that recorded for *S. patens* and *S. alterniflora* marshes in the USA (Table 3).

Carbon and nitrogen in *S. maritima* aboveground biomass

C content for *S. maritima* AGB was similar to that reported for *S. maritima*, *S. alterniflora*, and *S. patens* natural populations (Table 3). High C contents are related to fairly rigid walls that limit the amount of water influx during hypoosmotic stress, which would be beneficial in relatively stable saline environments (Touchette 2007), such as low elevations in the tidal gradient colonized by *S. maritima* (Castillo and Figueroa 2009b).

N content in *S. maritima* leaves and shoots was higher than those recorded in natural populations in the same season (Caçador et al. 2007; Sousa et al. 2008; Castro et al. 2009) and than those recorded for *S. alterniflora* in USA marshes (Patrick and DeLaune (1976) and McFarline et al. (2008)) and in Chinese marshes (Liao et al. (2007)). Based on our results, it is not possible to identify the environmental factors determining the exposed interpopulation differences in N content. On one hand, N bioavailability in the sediments is a key factor determining N acquisition by halophytes (Darby and Turner 2008). On the other hand, N content depends on the age of the population, with young populations of *S. maritima* showing higher N contents in their AGB than mature populations (Caçador et al. 2007).

S. maritima AGB values in fully colonized patches 28 months after transplantation ($1,290\pm194$ g DW m^{-2}) were similar to those recorded for natural populations (Castillo, Rubio-Casal and Figueroa 2010). Created populations of *S. maritima* develop similar AGB to natural populations within ca. 2 years after transplantation (Castillo, Leira-Doce et al. 2008). AGB C stock in *S. maritima* biomass was also similar to those recorded in *S. patens* marshes and higher than in *S. alterniflora* prairies, while AGB N stock for restored *S. maritima* prairies was higher compared with other *Spartina* species (Table 3).

S. maritima NAPP (553 ± 83 g DW m^{-2} year^{-1}) was within the wide range recorded for natural populations (120–2,800 g DW m^{-2} year^{-1} following Sousa et al. (2010a,b)) and for created marshes (131–590 g DW m^{-2} year^{-1} following Castillo et al. (2008a)). C accumulation rate of *S. maritima* AGB in the restored marshes (228 g C m^{-2} year^{-1}) was also within the range reported for natural marshes (ca. 50–1,800 g C m^{-2} year^{-1}) (Sousa et al. 2010a,b) and for the seaside alkali grass (*Puccinellia maritima* (Huds.) Parl.) in European marshes (Bouchard and Lefeuvre 2000). The recorded NAPP of *S. maritima* corresponded to a N accumulation of ca. 15 g N m^{-2} year^{-1}, with relatively low sediment N

Table 3 C and N contents and stocks in sediment, above-, and belowground biomass of *S. maritima*-restored marshes in the Odiel Marshes (Southwest Iberian Peninsula) in comparison with natural marshes of other *Spartina* species

	Sediment C content	Sediment C stock	Sediment N content	Sediment N stock	AGB C content	AGB C stock	AGB N stock	BGB C content	BGB C stock	BGB N content	BGB N stock
S. maritima-restored marshes	11 mg C g⁻¹ at surface 16 mg C g⁻¹ at depth	2.5 kg C m⁻²	1.6±0.3 mg N g⁻¹ at surface 2.1±0.2 mg N g⁻¹ at depth	329.0 g N m⁻²	420 mg C g⁻¹	533 g C m⁻²	24 g N m⁻²	380 mg C g⁻¹	242 g C m⁻²	19–22 mg N g⁻¹	13 g N m⁻²
S. maritima marshes	22–75 mg C g⁻¹ (Cartaxana and Catarino 1997; Caçador et al. 2004; Lillebo et al. 2006)	9–22 kg C m⁻² (Caçador et al. 2004)	3.0–7.0 mg N g⁻¹ (Caçador et al. 2007; Castro et al. 2009)		400–450 mg C g⁻¹ (Cartaxana and Catarino 1997; Castro et al. 2009)					12–16 mg N g⁻¹ (Caçador et al. 2007)	
S. alterniflora marshes		7.5–21.0 kg C m⁻² (Liao et al. 2007; Elsey-Quirk et al. 2011)	0.2–1.9 mg N g⁻¹ (Tyler et al. 2003)	337–379 g N m⁻² (Elsey-Quirk et al. 2011)	400–430 mg C g⁻¹ (Hou et al. 2010)	200 g C m⁻² (Elsey-Quirk et al. 2011)	5–9 g N m⁻² (Darby and Turner 2008; Elsey-Quirk et al. 2011)	390–400 mg C g⁻¹ (Hou et al. 2010)	450 g C m⁻² (Elsey-Quirk et al. 2011)	6 mg N g⁻¹ (Liao et al. 2007)	10–68 g N m⁻² (Darby and Turner 2008; Elsey-Quirk et al. 2011)
S. patens marshes		7.5–8.5 kg C m⁻² (Liao et al. 2007; Elsey-Quirk et al. 2011)		337–379 g N m⁻² (Elsey-Quirk et al. 2011)		500 g C m⁻² (Elsey-Quirk et al. 2011)	9–11 g N m⁻² (Windham and Ehrenfeld 2003; Elsey-Quirk et al. 2011)		366 g C m⁻² (Elsey-Quirk et al. 2011)		8–10 g N m⁻² (Windham and Ehrenfeld 2003; Elsey-Quirk et al. 2011)
S. foliosa marshes										8–11 mg N g⁻¹ (Boyer et al. 2000)	
S. anglica young marshes	3–14 mg C g⁻¹ (Hemminga et al. 1998)		0.2–1.0 mg N g⁻¹ (Hemminga et al. 1998)								

content (ca. 2 mg N g^{-1}). Sousa et al. (2008, 2010b) described N sequestration by *S. maritima* in the AGB that varied between ca. 2 g N m^{-2} year^{-1} for matured marshes with ca. 6 mg N g^{-1} in sediment and ca. 48 g N m^{-2} year^{-1} with a sediment N content of ca. 3 mg N g^{-1}. Elsey-Quirk et al. (2011) also described similar AGB N accumulation for *S. patens* and *S. alterniflora* marshes (ca. 11 g N m^{-2}).

Carbon and nitrogen in *S. maritima* belowground biomass

Our C content data for *S. maritima* BGB coincided with that reported for *S. alterniflora* (Table 3). The root system of *S. maritima* was the organ that showed the lowest C content, coinciding with Cartaxana and Catarino (1997); however, their value was much lower than ours (179 vs. 361 mg C g^{-1}). N content of *S. maritima* BGB was slightly higher than that reported for Portuguese *S. maritima* marshes and much higher than for *Spartina foliosa* Trin. (California cordgrass) and *S. alterniflora* (Table 3).

S. maritima BGB values (616±98 g DW m^{-2}) were much lower than those recorded in matured populations (ca. 2,500–4,800 g DW m^{-2}) (Castillo, Rubio-Casal and Figueroa 2010). Therefore, the analyzed restored marshes had still not raised its maximum BGB even in totally colonized areas, in agreement with Castillo et al. (2008a), who reported that *S. maritima* transplants need between 2.5 and 4.0 years, depending on the sedimentation dynamic, to develop a similar BGB to natural marshes. The BGB C stock for *S. maritima* was lower than for *S. patens* and *S. alterniflora* marshes (Table 3). On the other hand, BGB N stock in the restored marshes was similar, and even higher, than in *S. alterniflora* and *S. patens* marshes in the USA (Table 3).

The NBPP of *S. maritima* (264± 42 g DW m^{-2} year^{-1}) was close to the minimum values reported for created populations in the Odiel Marshes (366–3,598 g DW m^{-2} year^{-1}) (Castillo, Leira-Doce et al. 2008) and lower than those recorded in natural populations (700–3,500 g DW m^{-2} year^{-1}) with similar C contents, resulting in lower C accumulation rates in BGB (104 g C m^{-2} year^{-1} vs. 239–1,008 g C m^{-2} year^{-1}) (Sousa et al. 2010a,b). The relatively low NBPP recorded in the restored marshes was consistent with an accretion rate of 2.2±0.3 cm year^{-1} (Castillo, Leira-Doce et al. 2008). The NBPP of *S. maritima* corresponded to a

N accumulation of ca. 6 g N m^{-2} year^{-1}. Belowground N sequestration in Portuguese estuaries varied between 6 g N m^{-2} year^{-1} in young marshes and 45 g N m^{-2} year^{-1} in mature marshes (Sousa et al. 2008). Elsey-Quirk et al. (2011) described BGB N accumulation between 17.5 and 22.5 g N m^{-2} for *S. patens* and *S. alterniflora* marshes.

Total carbon and nitrogen stocks in *S. maritima* restored marshes

C stock in *S. maritima* biomass 28 months after planting (ca. 0.8 kg C m^{-2}) was lower than that reported for *S. alterniflora* Chinese marshes (ca. 3.0 kg C m^{-2}) due to higher biomass accumulation with similar C content (Liao et al. 2007). *S. patens* (ca. 0.9 kg C m^{-2}) and the short-form of *S. alterniflora* (ca. 0.7 kg C m^{-2}) showed similar values to *S. maritima* restored marshes (Elsey-Quirk et al. 2011). Thus, total C stock in *S. maritima* restored marshes was still low 28 months after planting (29.6 t C ha^{-1}), when compared with natural marshes (e.g., 156–166 t C ha^{-1} for *S. patens* and *S. alterniflora* prairies 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)). On the other hand, Liao et al. (2007) reported total N stock for *S. alterniflora* stands of ca. 46 g N m^{-2}, very similar to our results (47± 7 g N m^{-2}). Sediment N stock (30.25 t N in the top 20 cm of 8.37 ha) was more than 10 times that stored in plant biomass (ca. 2.5 t N). Although the sediment retains N, the effect of cleaning estuarine waters is performed mostly by the biota through bacterial denitrification and N uptake by vegetation (White and Howes 1994; Dollhopf et al. 2005).

A proportion of the C and N contained in the biomass of the expanding *S. maritima* plantations will be accumulated gradually as dead matter in the sediments, where it is captured for the long-term, and a further proportion will be exported in the form of dead leaves and shoots, mainly following the second year after plantations (Castillo, Leira-Doce et al. 2008), since its shoots are semelparous and their mean shoot life span is about 2 years (Castellanos et al. 1998). Our results showed that the restored marshes, 2.5 years after planting, were sequestering atmospheric C, mitigating impacts of climate change, and capturing N-reducing eutrophication. Moreover, the relatively low C and N contents recorded in sediments colonized by

S. maritima, at cordgrass relative cover of 62 % and low BGB, showed that these marshes can still sequester much more C and N. Craft et al. (1988) reported that sediment C content in restored marshes changed more slowly than the development of *Spartina*. Cornell et al. (2007) suggested that, with proper construction, most major C fluxes can be established in created salt marshes with *S. alterniflora* in less than 5 years.

As sea level rises, if suspended sediments and primary production are sufficient, tidal marshes will also rise or move upslope. Thus, BGB influences soil elevation rise by subsurface expansion and sediment deposit stabilization (Ford et al. 1999) and plants' belowground accumulation of organic, rather than inorganic matter, governs the maintenance of mature salt marsh ecosystems in the vertical plane (Turner et al. 2004). In North America, estuarine salt marshes have been shown to sequester C at a rate more than 10 times higher per unit area that any other wetland ecosystem due to high soil C content and constant burial due to sea-level rise (Brigham et al. 2006). Since *S. maritima* is effective in uptake of atmospheric CO_2 and because it also counteracts salt marsh inundation caused by sea-level rise, restoration of European salt marshes using small cordgrass should be actively pursued as a method to sequester C, and environmental managers should provide conservation corridors for dispersal and migration of *S. maritima* with habitat shifts due to sea-level rise.

S. maritima was planted in the restored marshes after eliminating invasive *S. densiflora* clumps (Castillo and Figueroa 2009a). In low marsh areas as those restored, *S. densiflora* is not able to grow at the lowest elevations (Castillo et al. 2000) and it establishes biannual populations composed by sparse and small tussocks at higher elevations (Castillo and Figueroa 2009b). *S. maritima* prairies established through plantations in low-elevation marshes replace bare sediments and biannual populations of *S. densiflora*, raising the C and N sequestration capacity of the marshes by increasing biomass production and accumulation. Following eradication of invasive *S. densiflora* stands to restore native plant community diversity in an Iberian salt marsh, restoration plantings of *S. maritima* stabilize sediments and help retain the positive contribution of past cordgrass stands, the deep long-term storage of C in deep sediments, while the newly established *S. maritima* community replaces an exotic invader in the important C storage cycle.

References

Adam, P. (1990). *Salt marsh ecology*. New York: Cambridge University Press.

Bertness, M. D., Crain, C., Holdredge, C., & Sala, N. (2007). Eutrophication and consumer control of New England salt marsh primary productivity. *Conservation Biology, 22*, 131–139.

Bouchard, V., & Lefeuvre, J. C. (2000). Primary production and macro-detritus dynamics in a European salt marsh: Carbon and nitrogen budgets. *Aquatic Botany, 67*, 23–42.

Boyer, K. E., Callaway, J. C., & Zedler, J. B. (2000). Evaluating the progress of restored cordgrass (*Spartina foliosa*) marshes: Belowground biomass and tissue nitrogen. *Estuaries, 23*, 711–721.

Brigham, S. D., Megonigal, J. P., Keller, J. K., Bliss, N. P., & Trettin, C. (2006). The carbon balance of North American wetlands. *Wetlands, 26*, 889–916.

Caçador, I., Costa, A. I., & Vale, C. (2004). Carbon storage in Tagus salt marsh sediments. *Water, Air, and Soil Pollution, 4*, 701–714.

Caçador, I., Costa, A. I., & Vale, C. (2007). Nitrogen sequestration capacity of two salt marshes from the Tagus estuary. *Hydrobiologia, 587*, 137–145.

Caçador, I., Caetano, M., Duarte, B., & Vale, C. (2009). Stock and losses of trace metals from salt marsh plants. *Marine Environmental Research, 67*, 75–82.

Caetano, M., Bernárdez, P., Santos-Echeandia, J., Prego, R., & Vale, C. (2012). Tidally driven N, P, Fe and Mn exchanges in salt marsh sediments of Tagus estuary (SW Europe). *Environmental Monitoring and Assessment, 184*, 6541–6552.

Caffrey, J. M., Murrell, M. C., Wigand, C., & McKinney, R. (2007). Effect of nutrient loading on biogeochemical and microbial processes in a New England salt marsh. *Biogeochemistry, 82*, 251–264.

Cartaxana, P., & Catarino, E. (1997). Allocation of nitrogen and carbon in an estuarine salt marsh in Portugal. *Journal of Coastal Conservation, 3*, 27–34.

Castellanos, E.M. (1992). Colonización, dinámica poblacional y papel en la sucesión de *Spartina maritima* (Curtis) Fernald en las Marismas del Odiel. Ph.D. Thesis, Universidad de Sevilla.

Castellanos, E. M., Heredia, C., Figueroa, M. E., & Davy, A. J. (1998). Tiller dynamics of *Spartina maritima* in successional and non-successional Mediterranean salt marsh. *Plant Ecology, 137*, 213–225.

Castillo, J. M., & Figueroa, E. (2009a). Restoring salt marshes using small cordgrass, *Spartina maritima*. *Restoration Ecology, 17*, 324–326.

Castillo, J. M., & Figueroa, E. (2009b). Effects of abiotic factors on the life span of the invasive cordgrass *Spartina*

densiflora and the native *Spartina maritima* at low marshes. *Aquatic Ecology, 43,* 51–60.

Castillo, J. M., Fernández-Baco, L., Castellanos, E. M., Luque, C. J., Figueroa, M. E., & Davy, A. J. (2000). Lower limits of *Spartina densiflora* and *S. maritima* in a Mediterranean salt marsh determined by different ecophysiological tolerances. *Journal of Ecology, 88,* 801–812.

Castillo, J. M., Leira-Doce, P., Rubio-Casal, A. E., & Figueroa, M. E. (2008). Spatial and temporal variations in aboveground and belowground biomass of *Spartina maritima* (small cordgrass) in created and natural marshes. *Estuarine, Coastal and Shelf Science, 78,* 819–826.

Castillo, J. M., Mateos-Naranjo, E., Nieva, F. J., & Figueroa, M. E. (2008). Plant zonation at salt marshes of the endangered cordgrass *Spartina maritima* invaded by *Spartina densiflora. Hydrobiologia, 614,* 363–371.

Castillo, J. M., Ayres, D. R., Leira-Doce, P., Bailey, J., Blum, M., Strong, D. R., et al. (2010). The production of hybrids with high ecological amplitude between exotic *Spartina densiflora* and native *S. maritima* in the Iberian Peninsula. *Diversity and Distributions, 16,* 547–558.

Castillo, J. M., Rubio-Casal, A. E., & Figueroa, E. (2010). Cordgrass biomass in coastal marshes. In M. Momba & F. Bux (Eds.), *Biomass (pp 1–26).* Rijeka: Sciyo.

Castro, P., Valiela, I., & Freitas, H. (2009). Sediment pool and plant content as indicators of nitrogen regimes in Portuguese estuaries. *Journal of Experimental Marine Biology and Ecology, 380,* 1–10.

Chmura, G.L., Anisfeld, S.C., Cahoon, D.R. & Lynch, J.C. (2003). Global carbon sequestration in tidal, saline wetland soils. Global Biogeochemical Cycles, 17, article 11.

Choi, Y., & Wang, Y. (2004). Dynamics of carbon sequestration in a coastal wetland using radiocarbon measurements. *Global Biogeochemical Cycles, 18,* GB4016.

Cornell, J. A., Craft, C. B., & Megonigal, J. P. (2007). Ecosystem gas exchange across a created salt marsh chronosequence. *Wetlands, 27,* 240–250.

Craft, C. B., Broome, S. W., & Senica, E. D. (1988). Nitrogen, phosphorus, and organic carbon pools in natural and transplanted marsh soils. *Estuaries, 11,* 272–280.

Crooks, S., Herr, D., Tamelander, J., Laffoley, D., & Vandever, J. (2011). *Mitigating climate change through restoration and management of coastal wetlands and near-shore marine ecosystems: Challenges and opportunities* (Environment Department Paper 121 (69 pp.)). Washington, DC: World Bank.

Curado, G., Figueroa, M. E., & Castillo, J. M. (2012). Sediment dynamics in *Spartina maritima* restored, non-restored and preserved salt marshes. *Ecological Engineering, 47,* 30–35.

Darby, F. A., & Turner, R. E. (2008). Below- and aboveground biomass of *Spartina alterniflora*: Response to nutrient addition in a Louisiana salt marsh. *Estuaries and Coasts, 31,* 326–334.

Davy, A. J., Bakker, P., & Figueroa, M. E. (2009). Human modification of European salt marshes. In B. R. Silliman, E. D. Grosholz, & M. D. Bertness (Eds.), *Human impacts on salt marshes: A global perspective* (pp. 311–335). Berkeley: University of California Press.

deJonge, V. N., Elliott, M., & Orive, E. (2002). Causes, historical development, effects and future challenges of a common environmental problem: Eutrophication. *Hydrobiologia, 475,* 1–19.

Dollhopf, S. L., Hyun, J. H., Smith, A. C., Adams, H. J., O'Brien, S., & Kostka, J. E. (2005). Quantification of ammonia-oxidizing bacteria and controls of nitrification in saltmarsh sediments. *Applied and Environmental Microbiology, 71,* 240–246.

Elbaz-Poulichet, F., & Dupuy, C. (1999). Behaviour of rare earth elements at the freshwater-seawater interface of two acid mine rivers: The Tinto and Odiel (Andalucia, Spain). *Applied Geochemistry, 14,* 1063–1072.

Elsey-Quirk, T., Seliskar, D. M., Sommerfield, C. K., & Gallagher, J. L. (2011). Salt marsh carbon pool distribution in a mid-Atlantic Lagoon, USA: Sea level rise implications. *Wetlands, 31,* 87–99.

Figueroa, M. E., Castillo, J. M., Redondo, S., Luque, T., Castellanos, E. M., Nieva, F. J., et al. (2003). Facilitated invasion by hybridization of *Sarcocornia* species in a salt-marsh succession. *Journal of Ecology, 91,* 616–626.

Ford, M. A., Cahoon, D. R., & Lynch, J. C. (1999). Restoring marsh elevation in a rapidly subsiding salt marsh by thin-layer deposition of dredged material. *Ecological Engineering, 12,* 189–205.

Hartig, E. K., Gornitz, V., Kolker, A., Mushacke, F., & Fallon, D. (2002). Anthropogenic and climate-change impacts on salt marshes of Jamaica Bay, New York City. *Wetlands, 22,* 71–89.

Hemminga, M. A., van Soelen, J., & Maas, Y. E. M. (1998). Biomass production in pioneer *Spartina anglica* patches: Evidence for the importance of seston particle deposition. *Estuarine, Coastal and Shelf Science, 47,* 797–805.

Hou, L., Liu, M., Yang, Y., Ou, D., Lin, X., & Chen, H. (2010). Biogenic silica in intertidal marsh plants and associated sediments of the Yangtze Estuary. *Journal of Environmental Sciences, 22,* 374–380.

IPCC. (2007). *Climate change 2007: Synthesis report. Contribution of working groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Core Writing Team.* Switzerland: Geneva.

Liao, C., Luo, Y., Jiang, L., Zhou, X., Wu, X., Fang, C., et al. (2007). Invasion of *Spartina alterniflora* enhanced ecosystem carbon and nitrogen stocks in the Yangtze Estuary, China. *Ecosystems, 10,* 1351–1361.

Lillebo, A. I., Flindt, M. R., Pardal, M. A., & Marques, J. C. (2006). The effect of *Zostera noltii*, *Spartina maritima* and *Scirpus maritimus* on sediment pore-water profiles in a temperate intertidal estuary. *Hydrobiologia, 555,* 175–183.

McFarlin, C. R., Brewer, J. S., Buck, T. L., & Pennings, S. C. (2008). Impact of fertilization on a salt marsh food web in Georgia. *Estuaries and Coasts, 31,* 313–325.

Morris, J. T., Sundareshwar, P. V., Nietch, C. T., Kjerfve, B., & Cahoon, D. R. (2002). Responses of coastal wetlands to rising sea level. *Ecology, 83,* 2869–2877.

Neves, J. P., Simoes, M. P., Ferreira, L. F., Madeira, M., & Gazarini, L. C. (2010). Comparison of biomass and nutrient dynamics between an invasive and a native species in a Mediterranean saltmarsh. *Wetlands, 30,* 817–826.

Nicholls, R. J. (2004). Coastal flooding and wetland loss in the 21st century: Changes under the SRES climate and socio-economic scenarios. *Global Environmental Change, 14,* 69–86.

Nieva, F. J., Díaz-Espejo, A., Castellanos, E. M., & Figueroa, M. E. (2001). Field variability of invading populations of *Spartina densiflora* Brong. in different habitats of the Odiel Marshes (SW Spain). *Estuarine, Coastal and Shelf Science, 52*, 515–527.

Patrick, W. H., Jr., & DeLaune, R. D. (1976). Nitrogen and phosphorus utilization by *Spartina alterniflora* in a salt marsh in Barataria Bay, Louisiana. *Estuarine and Coastal Marine Science, 4*, 58–64.

Ranwell, D. S., Bird, E. C. F., Hubbard, J. C. R., & Stebbings, R. E. (1964). *Spartina* salt marshes in Southern England. V. Tidal submergence and chlorinity in Poole Harbour. *Journal of Ecology, 52*, 627–641.

Romero, J. A., Comin, F. A., & Garcia, C. (1999). Restored wetlands as filters to remove nitrogen. *Chemosphere, 39*, 323–332.

Sousa, A. I., Lillebø, A. I., Caçador, I., & Pardal, M. A. (2008). Contribution of *Spartina maritima* to the reduction of eutrophication in estuarine systems. *Environmental Pollution, 156*, 628–635.

Sousa, A. I., Lillebø, A. I., Pardal, M. A., & Caçador, I. (2010a). The influence of *Spartina maritima* on carbon retention capacity in salt marshes from warm–temperate estuaries. *Marine Pollution Bulletin, 61*, 215–223.

Sousa, A. I., Lillebø, A. I., Pardal, M. A., & Caçador, I. (2010b). Productivity and nutrient cycling in salt marshes: Contribution to ecosystem health. *Estuarine, Coastal and Shelf Science, 87*, 640–646.

Touchette, B. W. (2007). Seagrass–salinity interactions: Physiological mechanisms used by submersed marine angiosperms for a life at sea. *Journal of Experimental Marine Biology and Ecology, 350*, 194–215.

Turner, R. E., Swenson, E. M., Milan, C. S., Lee, J. M., & Oswald, T. A. (2004). Below-ground biomass in healthy and impaired salt marshes. *Ecological Research, 19*, 29–35.

Tyler, A. C., Mastronicola, T. A., & McGlathery, K. J. (2003). Nitrogen fixation and nitrogen limitation of primary production along a natural marsh chronosequence. *Oecologia, 136*, 431–438.

Wan, S., Qin, P., Liu, J., & Zhou, H. (2009). The positive and negative effects of exotic *Spartina alterniflora* in China. *Ecological Engineering, 35*, 444–452.

White, D. S., & Howes, B. L. (1994). Long-term ^{15}N-nitrogen retention in the vegetated sediments of a New England salt marsh. *Limnology and Oceanography, 39*, 1878–1892.

Wigand, C., McKinney, R. A., Cole, M. L., Thursby, G. B., & Cummings, J. (2007). Varying stable nitrogen isotope ratios of different coastal marsh plants and their relationships with wastewater nitrogen and land use in New England, USA. *Environmental Monitoring and Assessment, 131*, 71–81.

Windham, L., & Ehrenfeld, J. G. (2003). Net impact of a plant invasion on nitrogen-cycling processes within a brackish tidal marsh. *Ecological Applications, 13*, 883–897.

Zhang, W., Zeng, C., Zhang, L., Wang, W., Lin, Y., & Ai, J. (2009). Seasonal dynamics of nitrogen and phosphorus absorption efficiency of wetland plants in Minjiang River estuary. *Ying Yong Sheng Tai XueBao, 20*, 1317–1322 (In Chinese).