Thiago Drumond Teixeira Correia Couto

Carbon budget in a temperate estuary salt marsh. Influence of temperature increase in carbon sequestration

Doctoral dissertation in the scientific area of Bioscience (specialty Marine Ecology), supervised by Professor João Carlos Marques and co-supervised by Professor Isabel Caçador, presented to the Department of Life Sciences of the Faculty of Sciences and Technology of the University of Coimbra.

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Dissertação apresentada á Universidade de Coimbra para a obtenção do grau de Doutor em Biociências (especialidade Ecologia Marinha)

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This thesis was developed with the support of:

"I know that I know nothing"

(Socrates)

Contents

This thesis is based on the following manuscripts, either published or submitted for publication in international scientific journals. Additionally, when developing the work to achieve the results regarding each chapter, other subsidiary publications were also prepared. The whole set of publications is listed bellow.

Chapter 1.   Submitted to Aquatic botany as: Couto, T., Duarte, B., Caçador, I., Marques, J.C., 2013. Use of PSII photochemistry to evaluate carbon storage in salt marsh plants in a temperate Atlantic estuary.

Additionally, the following papers were published using also data from the work developed.

Duarte, B., Couto, T., Marques, J.C., Caçador, I. 2012. Scirpus maritimus leaf pigment profile and photochemistry during senescence: Implications on carbon sequestration. Plant Physiology and Biochemistry 57, 238-244.

Duarte, B., Couto, T., Valentim, J., Silva, H., Marques, J.C., Dias, J.M., Caçador, I, 2013. Abiotic modulation of *Spartina marítima* photobiology in different latitudinal populations. Estuarine, Coastal and Shelf Science 130, 127- 137.

Chapter 2.   Published as: Couto, T., Duarte, B., Caçador, I., Baeta, A., Marques, J.C., 2013. Salt marsh plants carbon storage in a temperate Atlantic estuary illustrated by a stable isotopic analysis based approach. Ecological Indicators 32, 305-311.

Additionally, the following papers were published using also data from the work developed.

Couto, T., Duarte, B., Barroso, D., Caçador, I., Marques, J.C., 2013. Halophytes as sources of metals in estuarine systems with low level of contamination. Functional Plant Biology 40, 931-939.

Duarte, B., Freitas, J., Couto, T., Valentim, J., Dias, J.M., Silva, H., Marques, J.C., Caçador, I., 2013. New multi-metric Salt Marsh Sediment Microbial Index (SSMI) application to salt marsh sediments ecological status assessment. Ecological Indicators 29, 390-397.

Chapter 3.   Submitted to Ecological Modelling as: Couto, T., Martins, I., Duarte, B., Caçador, I., Marques, J.C., 2013. Modelling the effects of global temperature increase on the growth of salt marsh plants

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Resumo

Devido ao aumento do uso de combustíveis fósseis pelo homem desde que começou a atividade industrial, o mundo tem sofrido com o aumento do CO_2 atmosférico e com o aumento da temperatura. Zonas húmidas costeiras, como os sapais são excelentes acumuladores de carbono, pois retiram CO_2 da atmosfera, que se acumula nos tecidos das plantas. Modelos ecológicos têm sido utilizados para tentar entender quais os resultados de impactos antropogénicos numa escala global. O aquecimento global pode ameaçar áreas de sapal nomeadamente, por exemplo, através do aumento do nível do mar.

O presente trabalho, realizado no sapal do estuário do rio Mondego, localizado na costa Atlântica de Portugal, teve os seguintes objetivos principais:

1. Comparar mudanças sazonais e funções associadas aos diferentes mecanismos fotossintéticos de três espécies de plantas de sapal (*Scirpus maritimus, Spartina maritima and Zostera noltii*) no estuário do Mondego e avaliar as implicações na sua capacidade de acumular carbono.

2. Com recurso à análise dos isótopos estáveis de carbono, avaliar as concentrações de carbono no sedimento, assim como em folhas, caules e raízes de três espécies de plantas de sapal do estuário do Mondego, considerando suas diferenças metabólicas, e

olhando para tecidos e sedimento como diferentes compartimentos, com distintas capacidades para armazenar carbono.

3. Avaliar, através de um modelo, como o aumento da temperatura influencia o crescimento vegetal e quais os feitos com respeito ao armazenamento de carbono nos tecidos foliares das três espécies de plantas selecionadas. Simultaneamente, avaliar se a taxa de sedimentação nas áreas ocupadas por cada espécie conseguirá acompanhar a subida do nível do mar.

Em função destes objetivos, a tese foi estruturada em três capítulos, sendo os principais resultados referidos a seguir de forma sucinta.

No tocante ao capítulo 1 - Use of PSII photochemistry to evaluate salt marsh plants carbon storage in a temperate Atlantic estuary - o trabalho desenvolvido permitiu verificar que os valores de biomassa radicular foram maiores do que os valores foliares e do caule juntos, ocorrendo geralmente os valores mais altos nas estações mais quentes, com os valores de carbono e pigmentos a seguirem a mesma tendência. *Spartina martima* mostrou altos valores de atividade do fotossistema II (PSII) máximo e operacional e também altos valores de rETR (taxa relativa de transporte de eletrões) durante a maior parte do ano. *Scirpus maritimus* mostrou altos valores de sequestro de carbono apenas numa janela temporal reduzida, enquanto as características fotoquímicas de *Spartina maritima* e *Zostera*

noltii permitem que estas espécies absorvam carbono ao longo de todo o ano.

No tocante ao capítulo 2 - Salt marsh plants carbon storage in a temperate Atlantic estuary illustrated by a stable isotopic analysis based approach - os resultados obtidos permitiram verificar que as proporções de carbono variaram de 27 a 39%, em *S. maritimus* e *S. Maritima*, e entre *30 e 39%* em *Z. noltii*. *S. maritimus* teve a maior produção de carbono nas folhas e caule, sendo os valores similares aos obtidos para *S. maritima* no que diz respeita à produção radicular. As três espécies juntas ocupam aproximadamente 50% da área de sapal, o que corresponde a 29.77 ha, e acumularam 24000 Kg de carbono em seus tecidos em 21 meses de estudo. A área ocupada por *Z. noltii* apresentou a maior concentração de carbono no sedimento e *S. maritimus* a menor. A análise dos isótopos de carbono mostraram que aparentemente, a matéria orgânica presente no sedimento é composta por uma mistura de fontes terrestres, macro e microalgas. Apesar dos altos valores de exportação de carbono, *S. maritima* e *Z. noltii* acumulam carbono de forma constante ao longo do tempo. As espécies estudadas comportaram-se simultaneamente como acumuladores e fontes de carbono.

Finalmente, no capítulo 3 - Modelling the effects of global temperature increase on the growth of salt marsh plants - apresentam-se os resultados relativos à utilização de um modelo para simular os processos de crescimento em espécies de plantas C_3 e C_4. O modelo foi calibrado com dados fornecidos pelo trabalho de terreno, tendo as simulações efetuadas no que respeita à variação de biomassa sido consistentes com as

observações no terreno. Num cenário de aumento de temperatura, deverá esperar-se um aumento da biomassa do caule e foliar em relação à observada com a temperatura atual, nomeadamente em *S. maritimus*. A taxa de sedimentação das áreas ocupadas por *S. maritima* e *Z. noltii* será, previsivelmente, capaz de acompanhar a subida do nível do mar, não sendo expectável que o mesmo se verifique em relação à área coberta por *S. maritimus*. Um eventual desaparecimento de S. *maritimus* em função da subida do nível do mar diminuiria a capacidade de sequestro de carbono do ecossistema.

Abstract

Because of the increase of the fossil fuel usage by the mankind since the industrial activity, the world has been suffered with the atmospheric CO_2 and temperature increase. Coastal wetlands, as salt marshes are excellent carbon sinks as they withdraw CO_2 from the atmosphere and store it in living plant tissue. Ecological models have been used to try understanding what the results of the anthropogenic impacts in a global scale. Global warming could threat the salt marsh areas through the sea level increase for example. The study site is the salt marsh of the Mondego estuary, located in the central Atlantic coast of Portugal, which is considered as a rich estuarine habitat in terms of productivity and biodiversity.

The main objectives of this work were the following:

1.   Compare seasonal changes and functions associated with the different photochemical mechanisms of three salt marsh plant species (*Scirpus maritimus, Spartina maritima* and *Zostera noltii*) in the Mondego estuary and to assess the implications for their carbon storage capability.

2.   Using stable isotopic analysis, study the differences in carbon concentration in the sediments, aboveground and belowground organs of three plant species in the Mondego estuary, considering their metabolic differences, and look to these three species

(tissues and sediment in the areas covered by them) as different carbon compartments with different carbon storage capabilities.

3. Simulate, through the use of a model, how the temperature increase influences plants growth and what could happen regarding carbon storage in capacity of the three plant species leaves tissues, analyzing simultaneously if sedimentation rates in the areas occupied by these three species can keep pace of the sea level increase.

As a function of these objectives, the thesis was structured in 3 chapters, results being briefly described below.

In chapter 1 - Use of PSII photochemistry to evaluate salt marsh plants carbon storage in a temperate Atlantic estuary - we found that biomass values were higher in the belowground than in the aboveground organs, and usually the higher values occurred in the warmer seasons, with carbon and pigments contents following the same trend. *Spartina maritima* showed high values of PSII activity both maximal and operational, leading to also high values of rETR during almost all the year. *Scirpus maritimus* showed a reduced temporal window with high carbon sequestration ability, while *Spartina maritima* and *Zostera noltii* PSII photochemical characteristics allow them to constantly absorb carbon during all the year.

In chapter 2 - Salt marsh plants carbon storage in a temperate Atlantic estuary illustrated by a stable isotopic analysis based approach – we found that carbon contents, ranged from 27–39% in *S. maritimus* and *S.*

maritima to 30–39% for *Z. noltii*. *S. maritimus* had the highest carbon production in the aboveground organs and had similar results with *S. maritima* in the belowground carbon production. These three species together occupied about 50% of the salt marsh area, corresponding to 29.77 ha and they stored in 21 months of study 24000 Kg of carbon in their aboveground and belowground organs. *Z. noltii* presented highest carbon concentration in the sediment and *S. maritimus* the lowest. Stable carbon isotopic analysis showed that apparently, the sedimentary organic matter is composed by a mix of terrestrial sources, macro and microalgae. Despite the high carbon exportation, *S. maritima* and *Z. noltii* are constantly accumulating carbon. The studied species have both a sink and source behaviour simultaneously.

In chapter 3 - Modelling the effects of global temperature increase on the growth of salt marsh plants - we used a growth model based on C_3 and C_4 plant species. The model was calibrated using data provided by field work, and results regarding simulations of biomass variations were consistent with field measurements. With an increase in temperature one should expect that the aboveground biomass increase in relation to the observed with the present temperature, especially in *S. maritimus*. Sedimentation rates in areas covered by *S. maritima* and *Z. noltii* are expected to compensate sea level increase, but that is apparently not the case in those covered by *S. maritimus*. An eventual disappearance of *S. maritimus* from the Mondego estuary will of course decrease the carbon sequestration capability of the ecosystem as a whole.

General Introduction

General Introduction

1. Estuarine ecosystems

Estuarine systems represent important ecosystems in the coastal landscape, providing important ecosystems services such as water quality improvement, fisheries resources, habitat and food for migratory and resident animals, and recreational areas for human populations. In the past 40 years, estuarine conservation was recognized as a priority at the national and international levels through several acts as the Ramsar Convention, 1977 (Munari & Mistri, 2008), and Water Framework Directive, 2000. Regard the tentative of conservation; on a worldwide-scale coastal, estuarine and transitional waters have been affected by man's activities. Historically, developing human civilizations has often been concentrated in coastal areas where access to water promoted trade and commerce. As a consequence, human alteration of natural ecosystems is profound in coastal areas and a central theme of environmental management is to develop policy to balance socio-economic growth and environmental protection (Borja & Dauer, 2008).

In the last 250 years, industrial activity has increased with a concomitant increase of the fossil fuel usage (Houghton, 1999) and consequent atmospheric CO_2 increase. This has recognized consequences on climate change, namely increasing the global surface temperature (Bluemle *et al.,* 1999; IPCC, 2007), which can put in risk some parts of the estuarine systems, as salt marshes, with the sea level increase.

2. Mondego estuary

The Mondego estuary (Figure 1) is located in the Portuguese Atlantic coast (40°08N, 8°50W) (Marques and Nogueira, 1991) ending in the city of Figueira da Foz. The estuary has approximately 8.6 km^2 and its upstream limit, defined as a function of the tidal influence, was settled 21 km upstream from the mouth (Teixeira *et al.*, 2008). The final part of the estuary, that has 7 Km is divided in two arms (north and south) by the Murraceira Island (Marques *et al.*, 2003). The north arm is deeper than the south arm and is the main navigation channel.

Figure 1: Final part of the Mondego estuary. Image: Google Earth.

From the 1980's until the end of 1990's, the Mondego estuary suffered with intense anthropogenic disturbance mainly in the lasts years of the 1990's, until 1997, where the upstream connection between the north and south arm was closed (Neto *et al.,* 2013) and the effect were showed with the eutrophication of the south arm (Marques *et al.,* 2003). In 1997 and 1998, mitigation measures were made. The upstream communication between the two arms was re-established, and the water discharged through the Pranto River (a small tributary draining 2300 ha of agricultural fields and flowing directly into the south arm through a sluice) was partially diverged from the south arm to an alternative sluice placed upstream in the north arm (Neto *et al.*, 2013). The mitigation measures showed good results, improving the conditions in the south arm. In a recent heavy metals study, Couto *et al.*, 2012, showed the cycling of six heavy metals (zinc, copper, lead, cobalt, cadmium and chrome) in the Mondego estuary and concluded that the system have a low level of these contaminants and can be used as reference for non heavy metal contaminated salt marshes. The clime in Mondego estuary and in the city of Figueira da Foz are usually dry in the summer and humid in winter. In the twenty-one months of this study, the clime followed the same pattern (Figure 2), with an average temperature above 20 ^{o}C in summer and around 10 ^{o}C in winter.
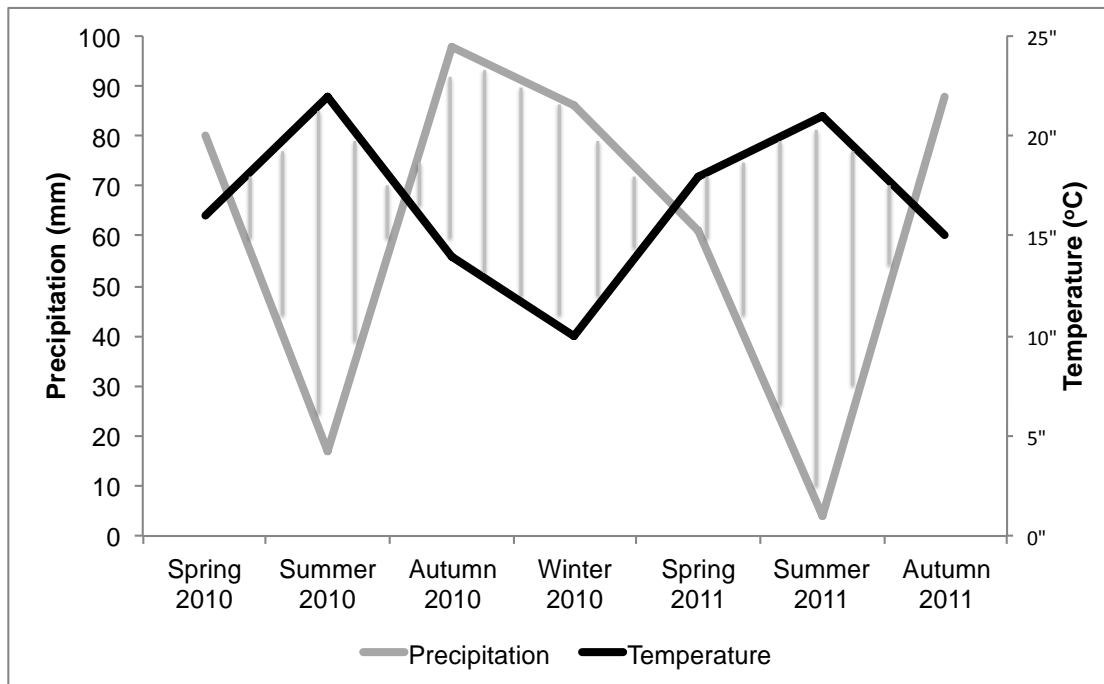
Figure 2: Average seasonal precipitation and temperature in the Mondego estuary during the study period.

The species *Scirpus maritimus* (Figure 3)*, Spartina maritima* (Figure 4) and *Zostera noltii* (Figure 5) together occupy approximately 50% of salt marsh vegetated area in the Mondego salt marsh, corresponding to 29.77 ha (Neto, unpublished data). The genus *Scirpus* is one between others that is common to all temperate wetlands (Scholz, 2011). *Scirpus maritimus* is usually found in monospecific stands in wetlands (Lieffers and Shay, 1980) and cannot support high salinity (Lillebo *et al.,* 2003). *Scirpus maritimus* occurs upstream areas in the south arm of Mondego estuary, since in that area the salinity is lower than in the downstream and although it is highly productive, this specie also undergoes a senescence process during the autumn, where its productivity dramatically declines (Duarte *et al.,* 2012) because the lost of the aboveground organs. The genus *Spartina* is the most frequent primary colonizers of coastal mudflats, leading to development of salt marsh communities (Long and Mason, 1983). This genus have good

tolerance to flooded conditions, which allows it to colonize low marsh areas (Maricle and Lee, 2007). *Spartina maritima* is a rhizomatous grass with a continuous but very slow growth, it mainly occurs in the Atlantic coast of Europe and Africa (Santín *et al.*, 2009), at the Mondego estuary it form extensive monotypic stands, occupies downstream intertidal mudflats (Sousa *et al.* 2008). The genus *Zostera* live in intertidal and subtidal inshore waters, forming a critical habitat and a basis of the food web (Larkum *et al*, 2006). *Zostera noltii* occurs along the coasts of Europe and northern Africa, growing in the intertidal region (Green and Short, 2003). In the Mondego estuary, *Z. noltii* occurs downstream. These three species have different pathways, *Spartina maritima* and *Zostera noltii* are C_4 plants; *S. maritimus* have a C_3 pathway. The main differences between these two pathways are that the photosynthesis in C_3 plants occurs in the mesophyll cells, while in C_4 plants occurs in the mesophyll and bundle sheath cells (Taiz and Zeiger, 2009), which allow C_4 plants live in more stressful systems. Couto *et al.,* 2013 showed that this species together can accumulate about 38 Kg of carbon per day in their tissues, and occupying only 50% of the salt marsh area, the carbon sequestration ability of the system can be higher.

3. Salt marshes

Salt marshes occupy the transition zone between terrestrial and marine ecosystems and are characterized by a high productivity, which is considered essential in maintaining the detritus-based food chain supporting estuarine and coastal ecosystems (Marinucci 1982). Salt marshes are key

areas for the estuarine system, namely for primary production and nutrient regeneration (Caçador *et al.*, 2009), becoming this way one of the most productive ecosystems in the planet (Lefeuvre *et al.*, 2003). Estuarine wetlands, as salt marshes, constitute good carbon sinks having simultaneously reduced rates of greenhouse gases emissions (Magenheimer *et al.*, 1996), with a carbon sequestration capacity per unit area of about one order of magnitude higher than other wetland systems (Bridgham *et al.*, 2006). Salt marshes are usually located in estuarine systems and their primary production allows for a greater reduction of CO_2 in the atmosphere and incorporation on organic tissues through photosynthesis (Sousa *et al.* 2010). Wetlands represent the largest carbon pool with a capacity of 770 Gt of carbon, overweighing the total carbon storage of farms and rain forests (Han *et al.,* 2005).

Figure 3: *Scirpus maritimus.* Photo: Thiago Couto

Figure 4: *Spartina maritima.* Photo: Thiago Couto

Figure 5: *Zostera noltii.* Photo: Thiago Couto

Chapter 1: Use of PSII photochemistry to evaluate carbon storage in salt marsh plants in a temperate Atlantic estuary

Chapter 1

Use of PSII photochemistry to evaluate carbon storage in salt marsh plants in a temperate Atlantic estuary

Abstract

Salt marshes become key areas for the estuarine system, namely for primary production and nutrient regeneration, becoming this way one of the most productive ecosystems in the planet. These systems constitute good carbon sinks, having simultaneously reduced rates of greenhouse gases emissions. The PSII photochemistry of three salt marsh species was examined to assess their carbon storage capacity during the year in the Mondego estuary (Portugal). This system is located on the central Atlantic coast of Portugal and is considered to be a rich estuarine habitat in terms of productivity and biodiversity. All of the analysed species presented greater biomass in their belowground organs in all seasons of the year, particularly during the warmer seasons. The pigment data obtained for *Scirpus maritimus* followed the same pattern as the biomass results, and *Spartina maritima* and *Zostera noltii* showed higher pigment levels in spring and summer. Additionally, *Spartina maritima* displayed high values for both maximal and operational PSII activity, leading to high rETR values throughout almost the entire year. *Scirpus maritimus* exhibited a reduced temporal window associated with a high carbon sequestration ability, while the PSII photochemical characteristics of *Spartina maritima* and *Zostera*

noltii allowed them to continuously absorb carbon throughout the entire year.

1. Introduction

Salt marshes are key areas for estuarine systems, particularly for primary production and nutrient regeneration (Caçador *et al*., 2009), thus representing one of the most productive ecosystems on the planet (Lefeuvre *et al*., 2003). These systems constitute good carbon sinks, simultaneously showing reduced rates of greenhouse gas emissions (Magenheimer *et al*., 1996). This ability mainly depends on the photosynthetic mechanisms of salt marsh colonising plants, which allow them to absorb large amounts of CO_2, even under the adverse conditions inherent to these ecosystems. Despite the essential role of light in this process, during the warmer seasons, the light intensity can lead to photoinhibition and consequently damage to photosystems (Baker and Bowyer 1994, Ralph *et al*. 2002). To avoid this photo-oxidative damage and to protect plant photosystems, carotenoids such as violaxanthin, antheraxantin and zeaxanthin act as photo-protective pigments against light damage, dissipating excess radiation energy (Horton *et al*., 1996). Other pigments, such as luthein and neoxanthin, act in the light harvesting process (Thayer and Bjorkman, 1990). The most abundant species colonising Mondego estuary include *Scirpus maritimus*, which

exhibits a C_3 photosynthetic mechanism (Boschker *et al.*, 1999), *Spartina maritima*, which is a C_4–type plant (Adam, 1990), as is *Zostera noltii* (Jiménez *et al.*, 1987; Larkum *et al.*, 2006). Compared to C_3 plants, the C_4–type photosynthetic pathway has been shown to be to advantageous in areas with high irradiance, high temperatures and intermittent water stress (Ehleringer and Monson, 1993) and is associated with adaptations to avoid stress, such as that induced in high salinity salt marsh systems (Chmura and Aharon, 1995). The three selected species present certain unique ecological characteristics. *S. maritima* and *Z. noltii,* that is a seagrass, have the pigments compositions similar to that of most angiosperms, including chlorophylls a and b, which function directly in photosynthesis, and carotenoids, which assist in the absorption of ultraviolet light and excess oxygen and in other protective functions (Beer *et al.* 1998). *S. maritimus* presents marked senescence beginning in autumn, leading to a dramatic reduction of its photosynthetic ability during a large portion of the year (Duarte *et al.* 2012), accompanied by declines in photosynthetic and photo-protective pigments (Biswal *et al.* 1994) until the complete lost of the aboveground organ. These characteristics imply that photosynthetic activity ceases during part of the year.

Considering the differences in the target species, the main goal of this work was to compare seasonal changes and functions associated with the different photochemical mechanisms of these three salt marsh plant species in the Mondego estuary and to assess the implications for their carbon storage capability.

2. Methods

2.2. Study area and sample collection

The Mondego Estuary (Figure 1) is located on the central Atlantic coast of Portugal (40º08`N, 8º50`W) (Marques and Nogueira, 1991).
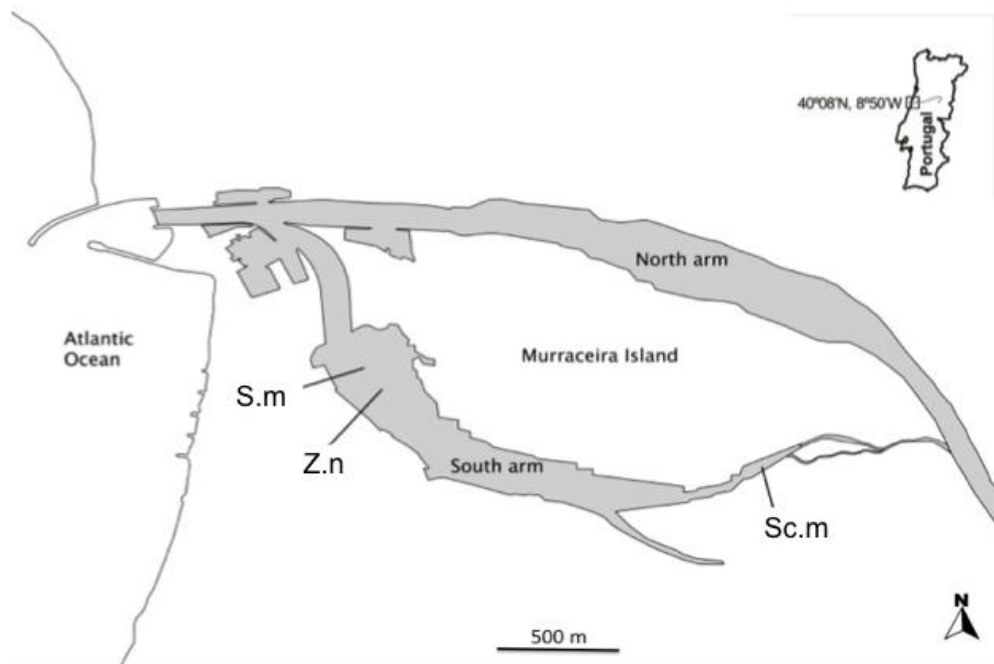

Figure 1: Mondego estuary and sample locations of the studied species. S.m: *Spartina maritima*; Z.n: *Zostera noltii* and Sc.m: *Scirpus maritimus.*

Its terminal portion consists of two arms (North and South), separated by Murraceira Island (Marques *et al.*, 2003). The South arm is shallower than the North arm (2–4 m during high tide) (Neto *et al.*, 2008) and is considered to be a rich estuarine habitat in terms of productivity and biodiversity (Marques *et al.*, 1993).

During each sampling event, leaves were harvested from pure stands of *S. maritimus*, *Z. noltii* and *S. maritima* in each season, from spring of 2010 until winter of 2011. All of the collected leaves were flash-frozen in the field in

liquid nitrogen and then maintained at – 80 ℃ until analysis. For the quantification of aboveground biomass, three squares (0.3 x 0.3 m) randomly placed and located at a minimum of 10 m distance from each other were subjected to sampling via clipping in each area. The aboveground inside the squares were clipped at the sediment level. To assess belowground biomass, within each clipped square, a core with an 8 cm diameter and 30 cm length was collected (Caçador *et al.,* 2004). In the laboratory, the aboveground samples were washed and passed by ultrapure water (18.2 MΩ cm) to remove dust. The belowground samples were cleaned from the sediments by water flux inside a sieve with a mesh size of 212 µm and subsequently passed by ultrapure water. Above- and belowground samples were dried in an oven at a 60 ℃ until a constant weight. Above- and belowground biomass were after expressed in a square metre basis (g m^{-2}) All field samples and analyses were performed first for *Z. noltii*, as Leuschner *at al.*, 1998, demonstrated that *Z. noltii* photosynthesis is limited by desiccation during low tide. Thus, the sampling and analyses of this species were conducted immediately after the tide had receded.

2.3. Leaf Photochemistry

Modulated chlorophyll fluorescence measurements were performed in attached leaves in the field with a FluoroPen FP100 PAM (Photo System Instruments, Czech Republic). All measurements in the dark-adapted state were conducted after the leaves had been subjected to darkness for at least 30 min. The minimal fluorescence (F_0) in the dark-adapted state was

quantified by measuring modulated light, which was sufficiently low (< 0.1 $\mu mol\ m^{-2}\ s^{-1}$) to avoid inducing any significant variation in fluorescence. The maximal fluorescence level (F_M) in the dark-adapted state was measured using a 0.8 seconds saturating pulse at 8000 $\mu mol\ m^{-2}\ s^{-1}$. The maximum photochemical efficiency ($\Phi PSII$) was assessed as (F_M-F_0)/F_M. These parameters were also measured in light-adapted leaves, where F'_0 represented the minimum fluorescence and F'_M the maximum fluorescence and $\Phi'PSII$ the operational photochemical efficiency. Rapid light curve (RLC) measurements were conducted in dark-adapted leaves using the pre-programmed LC1 protocol of the FluoroPen, consisting of a sequence of pulses from 0 to 500 $\mu mol\ m^{-2}\ s^{-1}$. During this protocol, F_0 and F_M as well as the maximum photochemical efficiency were measured. Each $\Phi PSII$ value was employed to calculate the electron transport rate (ETR) through photosystem II using the following equation: ETR = $\Phi PSII$ × PAR × 0.5, where PAR is the actinic photosynthetically active radiation generated by the FluoroPen, and the value of 0.5 assumes that the absorbed photons are equally partitioned between PSII and PSI (Genty *et al.*, 1989). Rapid light curves (RLC) were generated from the calculated ETRs and the irradiances applied during the rapid light curve steps. Each RLC was fitted to a double exponential decay function to quantify the characteristic parameters, alpha and ETR_{max} (Platt *et al.*, 1980). The initial slope of the RLC (alpha) is a measure of the light harvesting efficiency of photosynthesis, while the asymptote of the curve, i.e., the maximum electron transportation rate (ETR_{max}), is a measure of the capacity of the photosystems to utilise the absorbed light energy (Marshall *et al.*, 2000).

2.4. Pigment Profile Analysis

Leaf samples for pigment analysis were freeze-dried in the dark for 48 h, after which they were ground in pure acetone with a glass rod. To ensure complete disaggregation of the leaf material, samples with acetone were subjected to a cold ultrasound bath for 2 min. Extraction was performed at – 20 °C over 24 h in the dark to prevent pigment degradation. Following extraction, the samples were centrifuged at 4000 rpm for 15 min at 4 °C. For pigment analysis, the Gauss-Peak Spectra (GPS) method was employed (Küpper *et al.,* 2007). Samples were scanned in a dual beam spectrophotometer from 350 nm to 750 nm in 0.5 nm steps. The absorbance spectrum was introduced in the GPS fitting library using SigmaPlot Software. The application of this library allowed us to identify and quantify Chlorophyll a (Chl a), Chlorophyll b (Chl b), Pheophytin a (Pheo a), Antheraxanthin, β-carotene, Lutein, Violoxanthin and Zeaxanthin contents. The chlorophyll degradation index (CDI) and de-epoxidation state (DES) were calculated using the follow equations:

$$CDI = \frac{[Chl\ a]}{[Chl\ a + [Pheo\ a]}$$

$$DES = \frac{[Anthera] + [Zea]}{[Viola] + [Anthera] + [Zea]}$$

2.5. Statistical analysis

To perform comparisons between different groups of plant species and seasons of the year, one-way ANOSIM tests were used (Clarke, 1993). Non-metric multidimensional scaling (nMDS) was employed together with ANOSIM to obtain a better understanding of the results. The statistical analyses were performed using PRIMER version 6 (Clarke and Warwick, 2001).

3. Results

3.1. Biomass

Figure 2 shows the biomass results for all of the studied species in all seasons. All three species presented a higher biomass in their belowground than in their aboveground organs in all seasons of the year ($R > 0.7$; $p < 0.01$). *Spartina maritima* displayed the highest aboveground biomass values in all seasons, except in spring, when *S. maritimus* presented the highest values. The three species exhibited significant differences in their aboveground biomass values ($R > 0.7$; $p < 0.01$), and *S. maritimus* and *Z. noltii* showed a seasonal pattern ($R > 0.7$; $p < 0.01$), which was not as clear in *S. maritima* ($R < 0.2$; $p < 0.05$). Regarding belowground biomass, *S. maritimus* always presented the highest values in all seasons of the year, followed by *S. maritima* and *Z. noltii*. Similarly, the belowground results showed significant differences ($R > 0.7$; $p < 0.01$) between species. *Scirpus*

maritimus and *S. maritima* displayed small differences in their seasonal belowground biomass patterns (R < 0.2; p < 0.05), while *Z. noltii* exhibited a similar pattern in its above- and belowground organs (R > 0.7; p < 0.01). The nMDS (Figure 3) revealed that *S. maritimus* and *Z. noltii* exhibited the greatest differences in their aboveground and belowground biomass, as Figure 3 shows that their symbols are distant from one another. Total biomass showed the same pattern observed for belowground biomass.
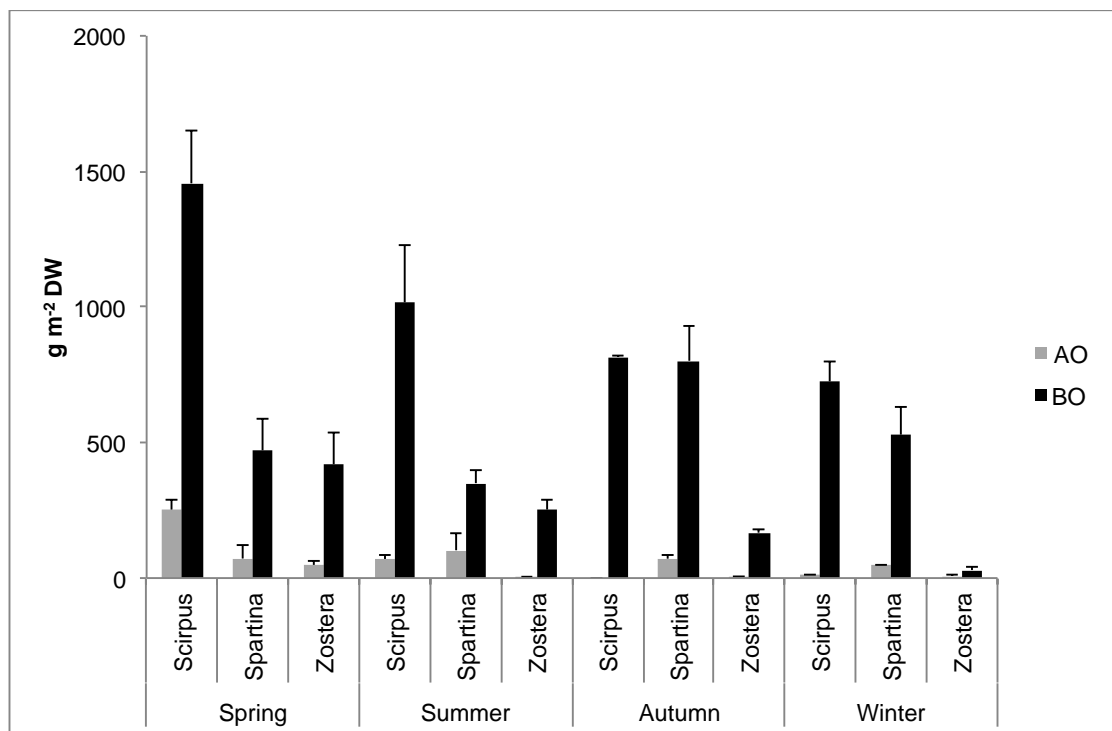

Figure 2: Biomass for all studied species in all seasons of the year in the Mondego salt marsh and standard deviation. AO: Aboveground organs and BO: Belowground organs.
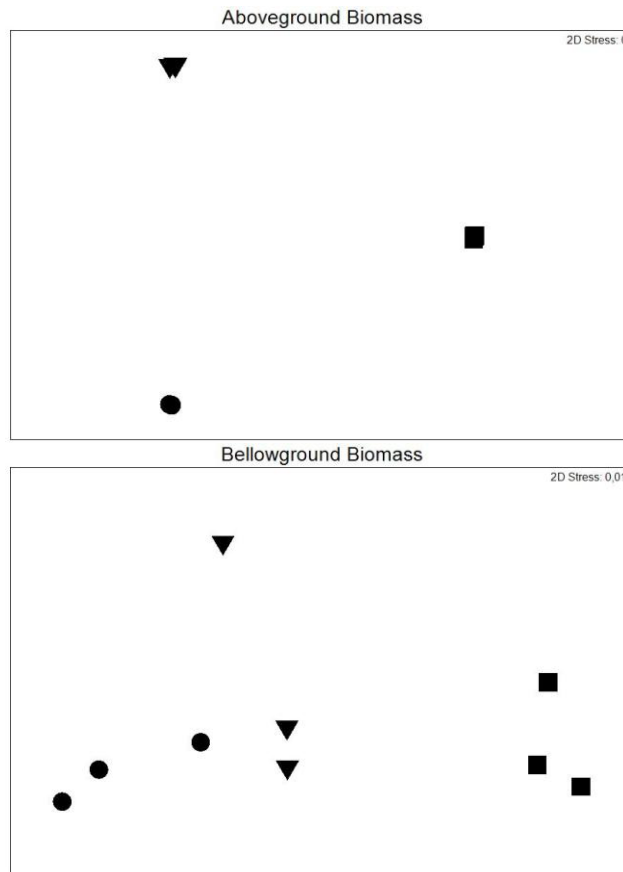
Figure 3: Non-metric multidimensional scaling (nMDS) plot based on above and belowground biomass of all study period in the Mondego salt marsh. Circle: *S. maritimus*, triangle: *S. maritima* and square: *Z. noltii*.

3.2. Pigments analysis

Table I shows pigment results obtained in each season over one year. With respect to Chl a, Chl b and total Chl, *Spartina maritima* presented the highest values in all seasons, displaying maximum levels in spring and summer and minimum levels in autumn and winter. The annual mean Chl a, Chl b and total Chl contents showed moderate differences (0.7 > R > 0.3; p<0.01) when compared between species, and from a seasonal perspective, similar differences were found for *S. maritima* and *Z. noltii*; these differences between seasons were especially evident in *S. maritimus*

(R > 0.8; p < 0.01). Chl a and b exhibited similar behaviour, with higher values being recorded in warmer seasons. *S. maritimus* and *S. maritima* presented high and similar (R<0.3; p<0.01) values for Pheo a and violaxanthin in spring and summer, while *Zostera noltii* showed low contents of the violaxanthin pigment, which exhibited seasonal differences (R=0.424; p<0.05). These two pigments presented a similar pattern, with higher levels occurring in the spring and summer. *Zostera noltii* displayed high levels of antheraxanthin in summer. The other two species showed low levels of this pigment, with very little difference being detected between them (R<0.15; p<0.01). The β-carotene, lutein and zeaxanthin pigments showed high levels in *S. maritimus* in spring and high levels in *S. maritima* in spring and summer. The levels of the β-carotene and zeaxanthin pigments were also similar in *S. maritimus* and *S. maritima* in spring. In contrast, only *Z. noltii* did not exhibit seasonal variation in the zeaxanthin pigment (R=0.13; p<0.05). *Zostera noltii* presented the highest CDI values in spring, summer and autumn, but ANOSIM indicated almost no difference between species (R<0.2; p<0.01). *Spartina maritima* displayed the smallest seasonal differences in CDI values (R=0.198; p<0.05). For DES, *Z. noltii* showed higher values in all seasons compared to the other two species, though the same behaviour was observed among all species for this parameter, increasing in summer, decreasing in autumn and increasing again in winter. Significant seasonal variation was detected for *S. maritimus* (R=0.938; p<0.01).

3.3. Chlorophyll fluorescence

The three species exhibited different behaviour in terms of the maximum quantum yield results (Figure 4). In *S. maritimus*, the values for this parameter decreased until reaching zero in autumn (because of the loss of its aboveground components in this season). *Spartina maritima* and *Z. noltii* exhibited stable results during spring, summer and autumn, after which the values for *S. maritima* decreased, while those for *Z. noltii* increased. Only small differences were detected between the three species shown (R<0.2; p<0.01). Considering the seasonal pattern underlying the maximum quantum yield, all three species presented significant differences between the seasons of the year, with *S. maritimus* showing the largest difference (R=0.712; p<0.01). Concerning the operational quantum yield (Figure 4), *S. maritimus* and *Z. noltii* displayed the same behaviour, exhibiting decreasing values until autumn, followed by increases in winter.

The statistical analysis between species did not reveal statistically significant differences (R<0.1; p<0.01), though such differences were found in the seasonal analysis (R>0.7; p<0.01). The alpha values (Figure 5) exhibited similar behaviour to the operational quantum yield values, with the exception of those obtained for *Z. noltii* in autumn and *S. maritima* in winter, when the values reached zero. The alpha results did not display large annual mean differences between species (R<0.2; p<0.01), but they did present seasonal differences, although the difference was small in *S. maritima* (R=0.383; p<0.05).

Table I: Pigment values for each studied species for each season of the year (µg g^{-1}) in the Mondego salt marsh and standard deviation.

	Scirpus maritimus				*Spartina maritima*				*Zostera noltii*			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Chl a	168 ± 41	108 ± 24		9 ± 2	264 ± 28	316 ± 74	128 ± 33	163 ± 36	25 ± 7	37 ± 4	12 ± 3	15 ± 2
Chl b	85 ± 24	50 ± 7		0.5 ± 0.1	113 ± 13	94 ± 16	54 ± 13	59 ± 9	7 ± 2	7 ± 2	9 ± 1	3 ± 0.07
Total Chl	253 ± 53	158 ± 32		9 ± 3	377 ± 40	410 ± 90	183 ± 40	215 ± 63	30 ± 8	44 ± 6	21 ± 3	50 ± 15
β-Carotene	41 ± 5	36 ± 7		0.9 ± 0.4	38 ± 4	40 ± 7	9 ± 0.92	13 ± 0.8	2 ± 0.1	3 ± 0.7	1 ± 0.3	$4^{-8} \pm 0.9^{-8}$
Antheraxanthin	$7^{-13} \pm 1^{-13}$	$3.5^{-6} \pm 0.8^{-6}$		1 ± 0.5	$2.5^{-13} \pm 0.08^{-13}$	$1^{-6} \pm 0.07^{-6}$	$9^{-8} \pm 1^{-8}$	$3^{-12} \pm 0.4^{-12}$	$9^{-18} \pm 2^{-18}$	4 ± 1	0.6 ± 0.09	$6^{-17} \pm 1^{-17}$
Lutein	38 ± 4	38 ± 7		0.3 ± 0.07	21 ± 2	43 ± 7	8 ± 1	11 ± 2	2 ± 0.2	0.4 ± 0.1	0.3 ± 0.06	$5^{-17} \pm 0.7^{-17}$
Neoxanthin	$6^{-13} \pm 1^{-13}$	$2^{-6} \pm 0.3^{-6}$		$2^{-13} \pm 1^{-13}$	$1^{-5} \pm 0.2^{-5}$	16 ± 4	0.9 ± 0.2	4 ± 0.8	$4^{-18} \pm 0.9^{-18}$	$0.6^{-2} \pm 0.1^{-2}$	$2^{-14} \pm 0.1^{-14}$	$7^{-17} \pm 2^{-17}$
Violaxanthin	40 ± 1	27 ± 5		0.1 ± 0.02	37 ± 1	18 ± 4	11 ± 3	9 ± 2	2 ± 0.4	2 ± 0.7	0.6 ± 0.08	$6^{-17} \pm 0.4^{-17}$
Zeaxanthin	44 ± 6	38 ± 7		4 ± 1	40 ± 4	43 ± 7	10 ± 0.9	14 ± 0.9	6 ± 1	15 ± 4	2 ± 0.4	4 ± 0.2
Pheo a	110 ± 29	95 ± 17		2 ± 0.7	88 ± 5	67 ± 0.7	50 ± 7	57 ± 8	$5^{-18} \pm 1^{-18}$	10 ± 3	2 ± 0.6	21 ± 4
CDI	0.6 ± 0.1	0.54 ± 0.03		0.8 ± 0.09	0.7 ± 0.02	0.8 ± 0.03	0.7 ± 0.04	0.7 ± 0.06	1 ± 0	0.8 ± 0.07	0.8 ± 0.1	0.4 ± 0.06
DES	0.5 ± 0.02	0.59 ± 0.03		0.9 ± 0.03	0.5 ± 0.02	0.7 ± 0.01	0.5 ± 0.07	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.04	0.8 ± 0.2	1 ± 1^{-9}

The rETR results (Figure 5) showed that the three studied species generally displayed signs of saturation when the PAR values reached 100 to 200 µmol photons m^{-2} s^{-1}. *Spartina maritima* presented higher rETR values in autumn, but lower values in winter than the other two species. *Spartina maritima* and *S. maritimus* showed similar behaviour for rETR in spring and summer, which was also similar to *Z. noltii* in autumn and winter.
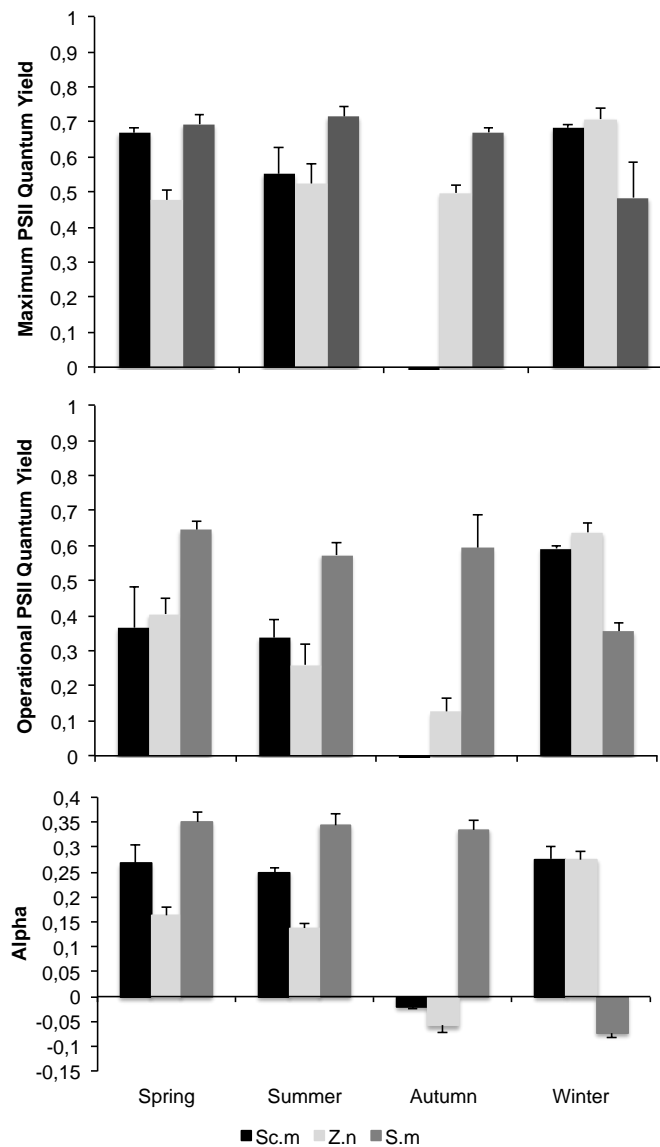

Figure 4: Maximum PSII Quantum Yield, Operational PSII Quantum Yield and Alpha results through one year in the Mondego salt marsh, with standard deviation. Black: *S. maritimus*, Light grey: *Z. noltii* and *S. maritima*: Dark grey.
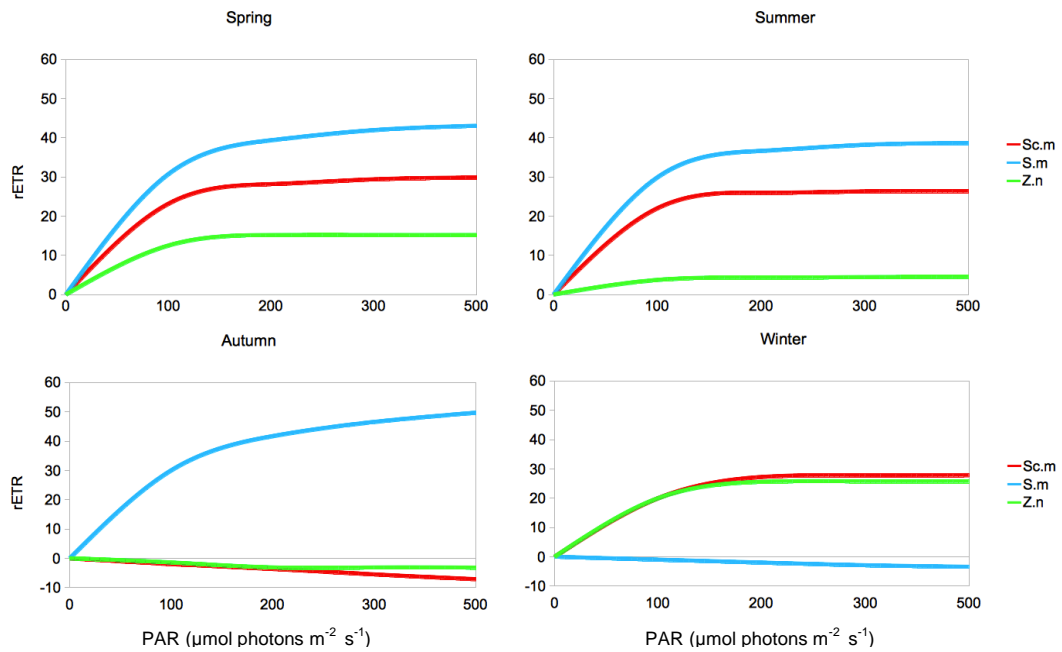
Figure 5: Rapid light curves for all studied species in the Mondego salt marsh through one year. Sc.m: *S. maritimus*, S.m: *S. maritima* and Z.n: *Z. noltii*.

4. Discussion

Light and temperature influence photosynthetic pigments and consequently affect plant photosynthetic behaviour (Wang *et al.* 2009). The results of the present study appear to demonstrate this phenomenon well, where high levels of pigments were observed in warmer seasons. Consistent with the findings of Duarte *et al.*, 2012, the pigment contents of *S. maritimus* recorded in the present study displayed similar behaviour to the biomass values, whereas those of *S. maritima* and *Z noltii* did not.

The maximum quantum yield, operational quantum yield and alpha values obtained for *S. maritimus* and *Z. noltii* followed a similar pattern, showing low values in autumn, but *S. maritima* did not follow this pattern. This finding in *S. maritimus* can be explained by the fact that this species lacks its aboveground components during autumn and part of the winter.

The senescence of its aerial parts, that starts in the autumn, could explain the similar behaviour of the photosynthetic efficiencies and pigments values, which decreased until the complete loss of the aerial components of this species. Seagrasses as *Z. noltii* also have a period of senescence, that starts in the late summer and continuous until the autumn (Larkum, *et al.,* 2006), what also explain the low photosynthetic efficiencies and pigments values in autumn, but this species do not lose the aboveground organs completaly. This annual mechanism has evident consequences for the ecosystem, leading to a decreased carbon harvesting ability and, thus, lower biomass production, resulting in the provision of reduced ecosystem services during a long period each year (Duarte *et al.*, 2012). Because *S. maritima* and *Z. noltii* do not lose all of their aboveground parts and maintain a portion of their aboveground components throughout the year, they did not show the type of behaviour of *S. maritimus.*

The rETR results followed a similar pattern for all species, displaying apparent saturation at approximately 200 µmol photons m^{-2} s^{-1}, which also occurred in other studies, such as that of Edwards and Kim, 2010. The results showed that *S. maritima* exhibited the highest rETR value in autumn and similar rETR values in spring and summer. *Zostera noltii* presented low values for this parameter in all seasons of the year, except in winter. However, the measurements for *Z. noltii* were performed during low tides, meaning that this species was out of water, which could be the reason for the low rETR values obtained, even though the measurements were conducted immediately after the tide had receded. *Zostera noltii* exhibits growth and physiological activity concentrated during periods of submersion

(Beer and Rehnberg, 1997). This species presented the highest chlorophyll degradation index, which could contribute to explaining the lower alpha, rETR and pigment values recorded. Duarte *et al.* 2012 noted that *S. maritimus* shows behaviour that is typical of shaded plants, exhibiting signs of photoinhibition under high irradiance, and indicated that this outcome could be due to its vertical orientation, which allows only the tops of the leaves to be exposed to high irradiance. Furthermore, Huner *et al.* 1998 reported that at low temperatures, photosynthesis rates can decrease, affecting the PSII ETR, which could explain the low rETR values recorded for *S. maritima* in winter. In fact, the pigment values obtained for the three species were usually highest in spring and summer, the warmer seasons, when the levels of chlorophylls, which are involved in photosynthesis, together with those of photo-protective pigments, were high.

Baerlocher *et al.* 2004 found that *Spartina alterniflora* could assimilate 0.22 mol CO_2 per mole of electrons transported through PSII. This finding indicates that higher amounts of carbon are absorbed in association with a higher ETR. Assuming that species that exhibit high ETRs also show high carbon assimilation, in the present study, *S. maritima* was expected to be the species that stores the highest amounts of carbon in its aboveground tissues. *Spartina maritima* displayed the highest ETR throughout most of the year; Couto *et al.,* 2013, reported aboveground carbon pool data for the same three species over almost two years (from spring of 2010 to autumn of 2011), and with the exception of the winter of 2010, *S. maritima* exhibited the highest aboveground carbon pool values in all seasons. In both Couto *et al.*'s study and the present study, *S. maritima*

showed the highest aboveground biomass values in almost all seasons of the year, except in the winter of 2010. Additionally, in the present work, this species usually displayed the highest ETR values, corroborating the notion that ETR values are related to aboveground biomass and, consequently, to carbon assimilation.

5. Conclusions

The biomass results were highest in the warmer seasons, with significant differences being detected during the year. The levels of the majority of pigments examined here presented significant differences between species and seasons. The pigment values recorded for *S. maritima* and *Z. noltii* did not show a trend of variation similar to the biomass values, while in *S. maritimus*, the two parameters varied concomitantly, which was most likely related to the annual loss of the aboveground biomass in the cold season. *Spartina maritima* was the species that exhibited the highest photosynthetic efficiency, except in winter. Furthermore, with the exception of *S. maritimus* (which could absorb carbon only in the warmer seasons), the other species were able to store carbon throughout the whole year, therefore acting as an efficient sink.

Chapter 2: Salt marsh plants carbon storage in a temperate Atlantic estuary illustrated by a stable isotopic analysis based approach

Chapter 2

Salt marsh plants carbon storage in a temperate Atlantic estuary illustrated by a stable isotopic analysis based approach

Abstract

The biomasses, carbon standing stocks, and exportations of three salt marsh species – *Scirpus maritimus*, *Spartina maritima* and *Zostera noltii* – were determined and their isotopic composition analysed to illustrate their role in carbon storage in a temperate Atlantic estuary (Mondego, Portugal). Biomass values were higher in the warmer seasons than in the cold seasons, with carbon contents following the same trend. Carbon content ranged from 27–39% in *S. maritimus* and *S. maritima* to 30–39% for *Z. noltii*. *Scirpus maritimus* had the highest carbon production in the aboveground organs and had similar results with *S. maritima* in the belowground carbon production. These three species together occupied about 50% of the salt marsh area and they stored in 21 months of study 24000 Kg of carbon in their aboveground and belowground organs. *Zostera noltii* presented highest carbon concentration in the sediment and *S. maritimus* the lowest. Stable carbon isotopic analysis showed that apparently, the sedimentary organic matter is composed by a mix of terrestrial sources, macro and microalgae. Despite the high carbon exportation, *S. maritima* and *Z. noltii* are constantly accumulating carbon. The studied species have both a sink and source behaviour simultaneously.

1. Introduction

In the last 250 years, industrial activity has increased with a concomitant increase of the fossil fuel usage (Houghton, 1999) and consequent atmospheric CO_2 increase. This has recognized consequences on climate change, namely increasing the global surface temperature (Bluemle *et al.,* 1999; IPCC, 2007). As a way to mitigate the high concentration of CO_2 in the atmosphere it is important to look to plant ecosystems (for e.g. salt marshes) that remain in a good ecological state and try to preserve or in many cases restore them. Coastal wetlands, such as salt marshes have high productivity, being one of the most productive ecosystems in the world (Mitsch and Gosselink, 2000), thus being excellent carbon sinks as they withdraw CO_2 from the atmosphere and store it in living plant tissue (Williams, 1999). Salt marshes are usually located in estuarine systems and their primary production allows for a greater reduction of CO_2 in the atmosphere and incorporation on organic tissues through photosynthesis (Sousa *et al.* 2010). Wetlands represent the largest carbon pool with a capacity of 770 Gt of carbon, overweighing the total carbon storage of farms and rain forests (Han *et al.,* 2005). Plants can fix carbon through photosynthesis, displaying different mechanisms. The photosynthesis in C_3 plants occurs in the mesophyll cells, while in C_4 plants occurs in the mesophyll and bundle sheath cells (Taiz and Zeiger, 2009), allowing a high

efficiency under stressful conditions. The carbon fixation occurs through Calvin cycle, where CO_2 and water are combined with ribulose-1,5-biphosphate into two molecules of 3-phosphoglycerate through ribulose-1,5-biphosphate carboxylase (rubisco), that is converted in carbohydrates. Rubisco can act as a oxygenase, producing 2-phosphoglycolate and 3-phosphoglycerate instead of two molecules of 3-phosphoglycerate, decreasing the photosynthetic efficiency; but some plants have mechanisms to exceed this decrease, like the C_4 photosynthetic pathway (Taiz and Zeiger, 2009).

In the present work the authors utilized a stable isotopic approach to study differences in the carbon concentration in the sediments, aboveground and belowground organs of three plant species in a temperate estuary salt marsh - Mondego estuary (Portugal) - considering their metabolic differences, and look to these three species (tissues and sediment) as different carbon compartments with different carbon storage abilities.

Among the most abundant salt marsh plant species in the Mondego estuary are *Scirpus maritimus* with a C_3 photosythetic mechanism (Boschker *et al.*, 1999), *Spartina maritima* with a C_4 type (Adam, 1990) as well as the seagrass *Zostera noltii* (Jiménez *et al.*, 1987; Larkum *et al.*, 2006). When compared with C_3 plants, the C_4 type have a photosynthetic pathway that has been shown to be to advantageous in areas with high irradiance, high temperatures and intermittent water stress (Ehleringer and Monson, 1993) and is associated with adaptations to avoid the stress, and an advantage in elevated-salinity salt marsh systems (Chmura and Aharon, 1995). The fixed carbon is used to plants needs, but the majority of biomass produced by

plants is degraded or exported, only a small part is retained in the sediment (Howarth, 1993).

2. Methods

2.1. Study site

Mondego estuary (Figure 1) is located in the Portuguese Atlantic coast (40°08N, 8°50W) (Marques and Nogueira, 1991) ending in the city of Figueira da Foz. The estuary has approximately 8.6 km^2 and its upstream limit, defined as a function of the tidal influence, was settled 21 km upstream from the mouth (Teixeira *et al.*, 2008). The final part of the estuary, that has 7 Km is divided in two arms (north and south) by the Murraceira Island (Marques *et al.*,2003). The north arm is deeper than the south arm and is the main navigation channel. The areas where the samples of *Spartina maritima* and *Zostera noltii* were collected are relatively close, being the sample station hereafter denominated as Gala. The *Scirpus maritimus* colonized area is more upstream the estuary in an area hereafter denominated as Montante.

2.2. Sampling and laboratory procedures

For each species were sampled three pure stands cores with 50 cm depth and 9 cm diameter, located at a minimum of 10 m distance from each other during almost two years (spring of 2010 to autumn of 2011). For the

aboveground biomass three 0.3 x 0.3 m squares of each species were randomly selected in each area and clipped out (the aboveground biomass of *S. maritimus* in autumn of 2011 was calculated with dead aboveground organs).
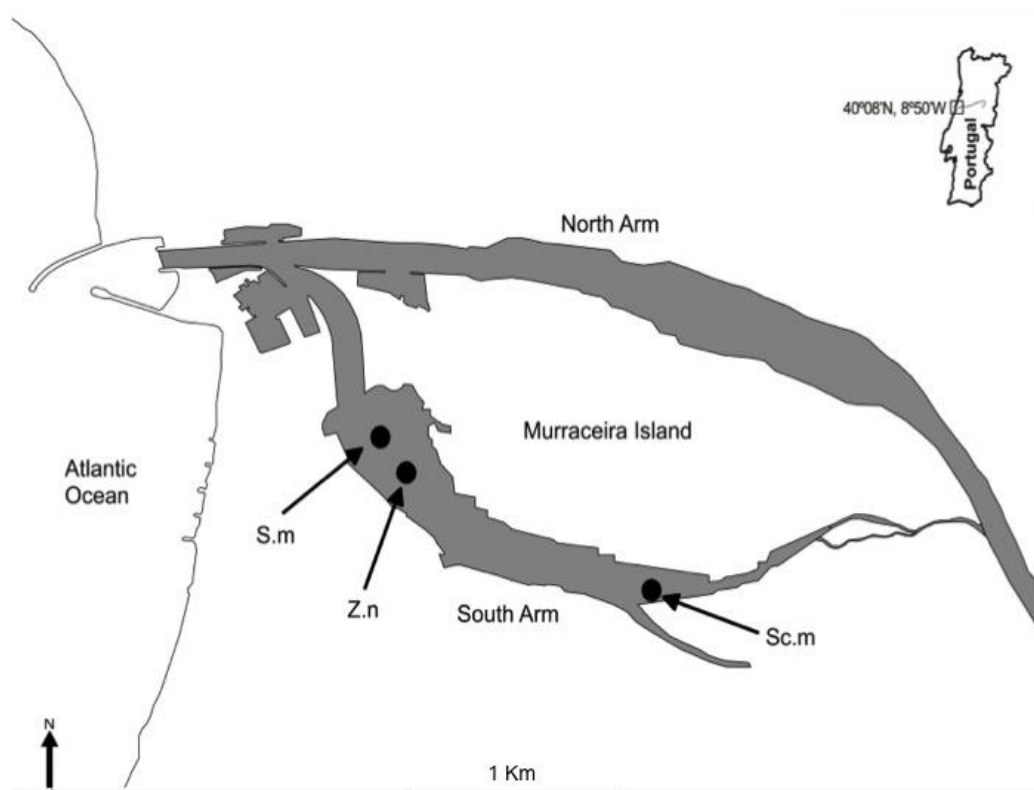

Figure 1: Terminal part of the Mondego estuary and *Zostera noltii* (Z.n), *Spartina maritima* (S.m) and *Scirpus maritimus* (Sc.m) sampling location.

To assess belowground biomass, inside each clipped square a core was taken, with 8 cm diameter and 30 cm long (Caçador *et al.,* 2004). In the laboratory, the aboveground samples were washed and passed by ultrapure water (18.2 MΩ cm). The belowground organs were cleaned from the sediments by water flux inside a sieve with a mesh size of 212 μm and subsequently passed by ultrapure water. Both above and belowground tissues were dried at 60 °C until constant weight pulverized with the help of a grinding ball mill (Glen CrestomMM2000) (Gross *et al.*, 1991). Sediment

samples were oven dried at 60 ⁰C until constant weight. After, the sediment was cleaned of roots, passed through a 0.25 mm mesh, homogenised and ground with an agate mortar. Pore water salinity was measured using a refractometer (Atago, S/Mill-E). The sediment organic matter content was determined in dried samples by loss of ignition (LOI) at 600 ⁰C for 2 h (Caçador *et al.,* 2000). Sediment grain size was determined by mechanical sequential sediment sieving, using analytical sieves housed in a shaker, to evaluate the relative abundance (Folk, 1954). Sedimentation rates were measured using lengths of wood with millimetre marks, which were buried up to the zero mark level in the area occupied by each species. This procedure was performed in February 2011 in the sampling areas corresponding to the three salt marsh plants. One year later (February 2012), the wooden markers were checked to measure the level corresponding to sediment accumulation in each of the three sampling areas. Three woodpiles were buried in each species zone.

2.3. Carbon analysis

Total carbon content was determined for both aboveground and belowground species organs a CHNS/O analyzer (Fisons Instruments Model EA 1108). The Net Primary Production (NPP, g) was determined using the equation 1, where the minimum biomass found in the study period is subtracted from the maximum biomass in the same period.

$$NPP = Maximum\ biomass - Minimum\ biomass \qquad (1)$$

The root decomposition was calculated using equation 2, and the aboveground biomass losses (grams) were assessed for the biomass lost during senescence.

$$Root\ decomposition = \left(1 - \frac{Minimum\ root\ biomass}{Maximum\ root\ biomass}\right) \times Root\ NPP \qquad (2)$$

The carbon pool (grams) for each species and for each season analyzed was calculated multiplying the results in percentage (%) from the CHNS/O analyzer by the biomass (equation 3).

$$Carbon\ pool = [Carbon]_t \times Biomass_t \qquad (3)$$

Carbon primary production was determined applying equation 4, using the same procedure as for the biomass NPP, but using the carbon pool values.

$$CNPP = Maximum\ carbon\ pool - Minimum\ carbon\ pool \qquad (4)$$

For the carbon exports (g) calculations were applied the equations 5 and 6, where values were calculated as a percentage of CNPP (Eq. (4)) as described above, taking into account the percentage of mass losses due to decomposition of the belowground (carbon export$_{dec}$) or senescence of the aboveground organs (carbon export$_{sen}$).

$$Carbon\ export_{dec} = Root\ decomposition \times Root\ CNPP \qquad (5)$$

$$Carbon\ export_{sen} = Aboveground\ senescence \times Aboveground\ CNPP \qquad (6)$$

The turnover rate was calculating using the CNPP divided by the carbon pool (equation 7).

$$Turnover\ rate = \frac{CNPP}{Carbon\ pool} \qquad (7)$$

The carbon in the sediment of each species was calculated with basis on the sedimentation rate of each species area and the carbon content in the 10–30 cm depth; with the sediment carbon results and the belowground production of each specie, were estimated the imported and exported carbon from the sediment (belowground production minus the carbon in the sediment) (Sousa *et al.*, 2010).

2.4. Isotopic analysis

The carbon isotopic composition of the pulverized plants and sedimentary organic matter samples was determined using a Flash EA 1112 Series elemental analyser coupled on line via Finningan conflo III interface to a Thermo delta V S mass spectrometer. The carbon isotope ratio are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PDB limestone for $\delta^{13}C$); $\delta^{13}C$ = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where $R = {}^{13}C/{}^{12}C$. Precision in the overall preparation and analysis was better than 0.2‰. The isotopic results were

used to make a link between the plants species and the sedimentary organic matter values.

2.5. Statistical analysis

To check for differences between the biomasses and the carbon contents, one-way ANOSIM tests (Clarke, 1993) were employed. Pairwise and non-metric multidimensional scaling (nMDS) were analysed together with the ANOSIM for a better understanding of the results. The statistical analyses were performed using the PRIMER version 6 (Clarke and Warwick, 2001).

3. Results

3.1. Sediment characteristics

The salinity in the pore water was higher for *S. maritima* (28 ± 4) and *Z. noltii* (27 ± 3) than for *S. maritimus,* that presented the lowest value (21 ± 8). On the other hand *S. maritimus* showed the highest LOI results (13.6% ± 0.01) followed by *S. maritima* (9.3% ± 0.001) and *Z. noltii* (7.8% ± 0.009). In the area colonized by *S. maritima* and *S. maritimus*, the sediment had approximately 50% of sand between 100-50 µm (fine sand) while the sediment colonized by Z. noltii present about 70%. More than 20% of silt and clay (< 63 µm) was found in the *S. maritima* and *Z. noltii* sediment and 13% in the *S. maritimus* area. The sedimentation rate for one year was highest in

Z. noltii area (2.7 cm y^{-1} ± 0.2), followed by *S. maritima* stands with 1 cm y^{-1} ± 0.1 and *S. maritimus* with the lowest sedimentation rate (0.2 cm y^{-1} ± 0.04).

3.2. Aboveground and belowground biomass

The *Scirpus maritimus* aboveground biomass (Figure 2A) showed high values during the warmer seasons, decreasing towards autumn, where it is absent. With a similar pattern, *S. maritima* had also high values in the warmer seasons (Figure 2A). With the lowest aboveground biomass, *Z. noltii* had in spring its highest biomass (Figure 2A). *S maritimus* and *Z. noltii* showed significant differences (R>0.8; p<0.01) seasonally, but *S. maritima* almost did not have difference (R=0.127; p<0.05). The species *Z. noltii*, when compared the aboveground biomass with the others two species had high statistical differences (R>0.7; p<0.01) with both in all seasons analysed. Between *S. maritimus* and *S. marítima*, the differences (R> 0.7; p<0.01) occurred in the majority of the seasons, with exception of summer of 2010, spring and autumn of 2011, where the differences were not so high (R<0.7; p<0.05). Following the same pattern that in the aboveground biomass, the belowground biomass in *S. maritimus* decreased in the cold seasons and increased in the warmer seasons (Figure 2B) and *S. maritima* had the high values of belowground biomass in the spring and autumn (Figure 2B), which is not similar with the aboveground biomass, and *Z. noltii* showed similar behaviour (Figure 2B) that showed in the aboveground biomass. Only *Z. noltii* showed high seasonally differences (R=0.769; p<0.01). When

compared the species, all three showed differences between each other, but *S. maritima* and *S. maritimus* comparisons had the lowest differences.

Scirpus maritimus and Spartina maritima had high differences (R>0.8; p<0.01) seasonally when compared the aboveground and belowground biomass, but the difference for *Z. noltii* was lower (R=0.514; p<0.01).

Aboveground Biomass (A)
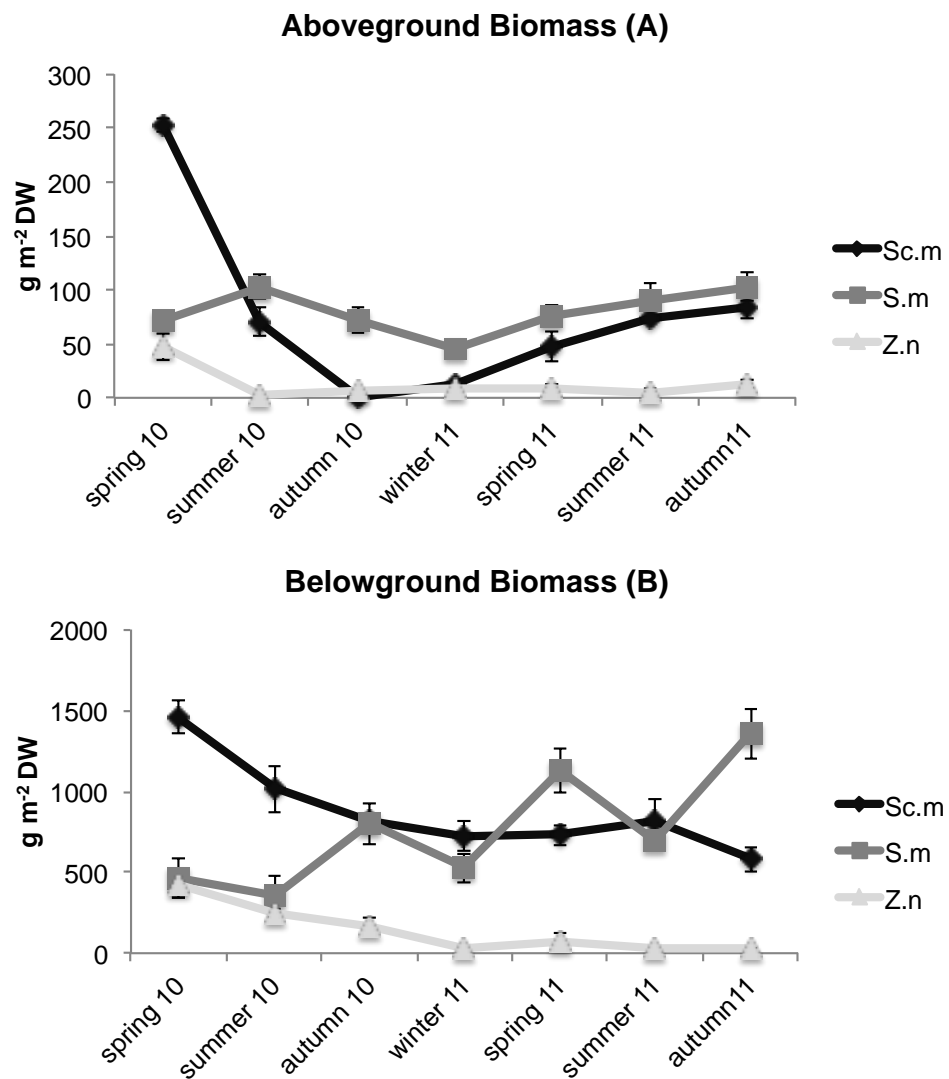

Belowground Biomass (B)

Figure 2: Aboveground and belowground biomass and standard deviation for each species studied in the Mondego salt marsh for each season analysed (attention to the different scales in the graphs). Sc.m (*S. maritimus*), S.m (*S. maritima*) and Z.n (*Z. noltii*).

3.3. Carbon in plants and sediment

Table I contain the average carbon percentage of the three species, by season for the sediment, aboveground and belowground organs, being the range approximately between 27% and 39% for the plant organs and 2.2% to 4.4% for the sediment. Since the carbon results for the above and belowground organs are dependent of the biomass, it followed the same pattern. With exception in spring of 2010, *S. maritima* had always the highest carbon pool for the aboveground organs and *Z. noltii* the lowest (Figure 3A), with the lower values occurring in the cold seasons in the three species. Seasonally, *S. maritima* showed the lowest differences ($R=0.241$; $p<0.05$), and when compared the results for the aboveground carbon pool between species, all results were significant different ($R>0.6$; $p<0.05$) in all seasons, showing that each species had different values for carbon pool in the aboveground organs; which figure 4A show clearly. The carbon pool values were highest in the belowground organs for all species (Figure 3B). Only *Z. noltii* showed statistical difference ($R=0.635$; $p>0.01$) between seasons. *Zostera noltii* had different carbon values ($R>0.7$; $p<0.01$) for the belowground organs when compared with the other two species; in the figure 4B is possible to see that *Z. noltii* belowground carbon pool results were placed distant from the results of the other two species.

Table I: Average percentage (%) and standard deviation of the carbon found in the aboveground and belowground organs for each species analysed in the Mondego salt marsh, for each season and the average for all studied period (Total Av.). Sc.m (*S. maritimus*), S.m (*S. maritima*) and Z.n (*Z. noltii*).

	Spring 10	Summer 10	Autumn 10	Winter 11	Spring 11	Summer 11	Autumn 11	Total Av.
Aboveground Organs								
Sc.m	34.4 ± 0.009	36.9 ± 0.050	33.3 ± 0.113	35.4 ± 0.016	33.4 ± 0.006	27.4 ± 0.017	28.9 ± 0.052	32.8 ± 0.012
S.m	36.5 ± 0.022	38.2 ± 0.009	38.9 ± 0.024	37.3 ± 0.005	37.3 ± 0.015	38.5 ± 0.006	35.8 ± 0.020	32.1 ± 0.008
Z.n	35.1 ± 0.006	38.1 ± 0.009	38.7 ± 0.011	35.3 ± 0.016	34.7 ± 0.015	35.4 ± 0.010	39.9 ± 0.048	36.7 ± 0.032
Belowground Organs								
Sc.m	35.7 ± 0.022	38.8 ± 0.008	39.5 ± 0.011	39.4 ± 0.008	33.5 ± 0.033	39.2 ± 0.005	39.1 ±0.006	37.8 ± 0.021
S.m	27.2 ± 0.078	39.1 ± 0.015	32.2 ± 0.021	34.5 ± 0.014	27.4 ± 0.056	34.4 ± 0.018	29.4 ± 0.011	32.0 ± 0.004
Z.n	31.4 ± 0.003	31.7 ± 0.003	30.9 ± 0.025	31.3 ± 0.012	31.4 ± 0.003	31.7 ± 0.003	30.9 ± 0.025	31.3 ± 0.037
Sediment								
Sc.m	4.2 ± 0.32	3.6 ± 0.43	3.3 ± 0.27	3.2 ± 0.15	2.9 ± 0.29	3.1 ± 0.26	3.0 ± 0.29	3.3 ± 0.44
S.m	4.0 ± 0.39	2.9 ± 0.17	2.2 ± 0.26	2.6 ± 0.43	2.8 ± 0.22	3.2 ± 0.40	2.4 ± 0.22	2.8 ± 0.59
Z.n	4.4 ± 0.11	2.3 ± 0.38	2.2 ± 0.15	2.9 ± 0.49	2.3 ± 0.19	2.6 ± 0.20	2.4 ± 0.40	2.7 ± 0.77

Aboveground Carbon Pool (A)

Belowground Carbon Pool (B)
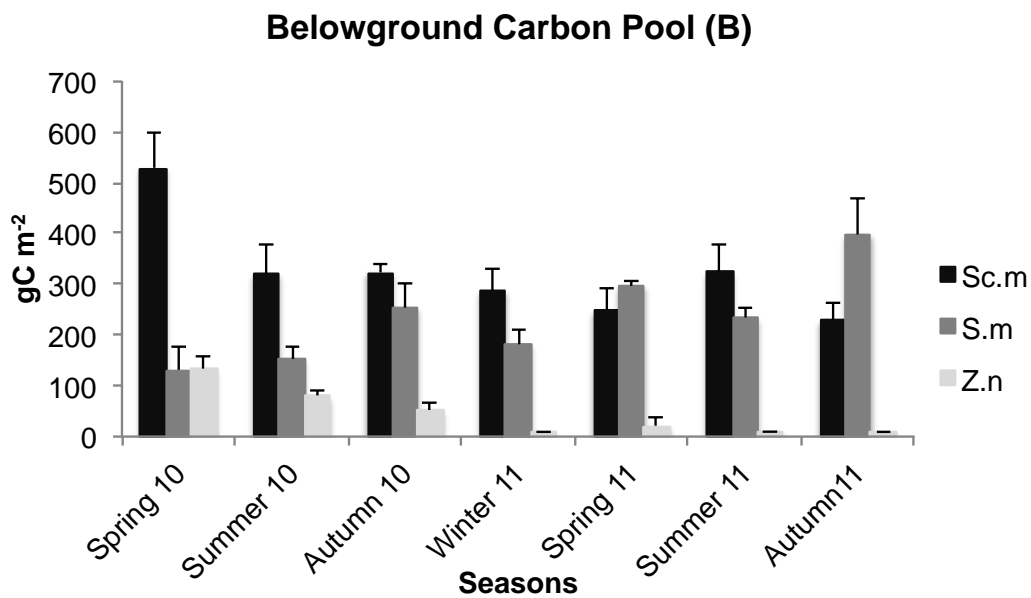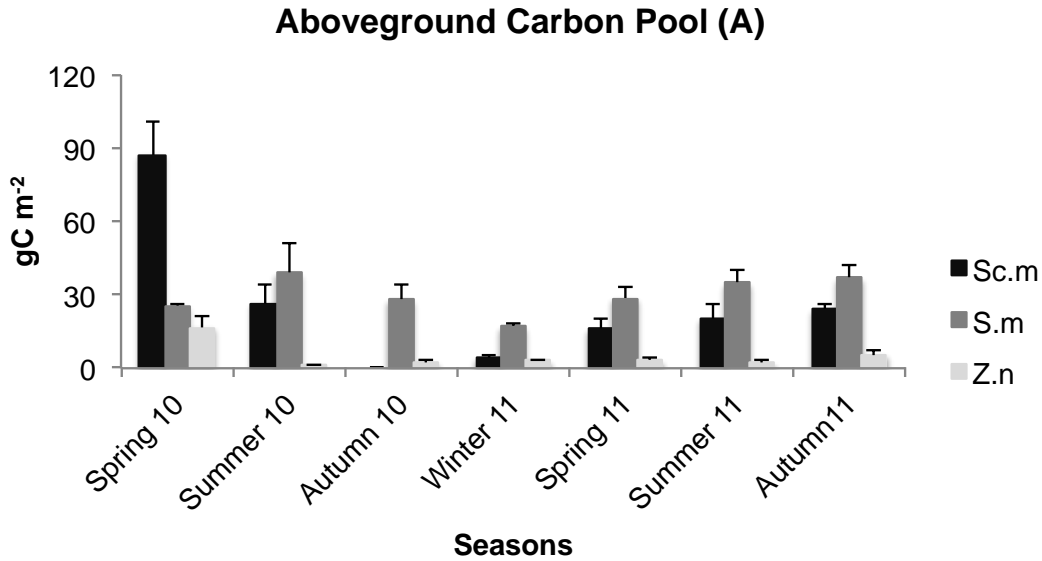
Figure 3: Aboveground and belowground carbon pool values and standard deviation for *S. maritimus* (Sc.m), *S. maritima* (S.m) and *Z. noltii* (Z.n) in each season analysed in the Mondego salt marsh.

Usually, all species showed significant differences ($R > 0.7$; $p < 0.01$) when compared the carbon pool values between the aboveground and belowground organs. The percentages of carbon found in the above and belowground organs were not so different when the whole studied period is seen, so its been expected that the carbon pool had similar behaviour as biomass.

A Aboveground Carbon Pool

2D Stress: 0

B Bellowground Carbon Pool

2D Stress: 0,01
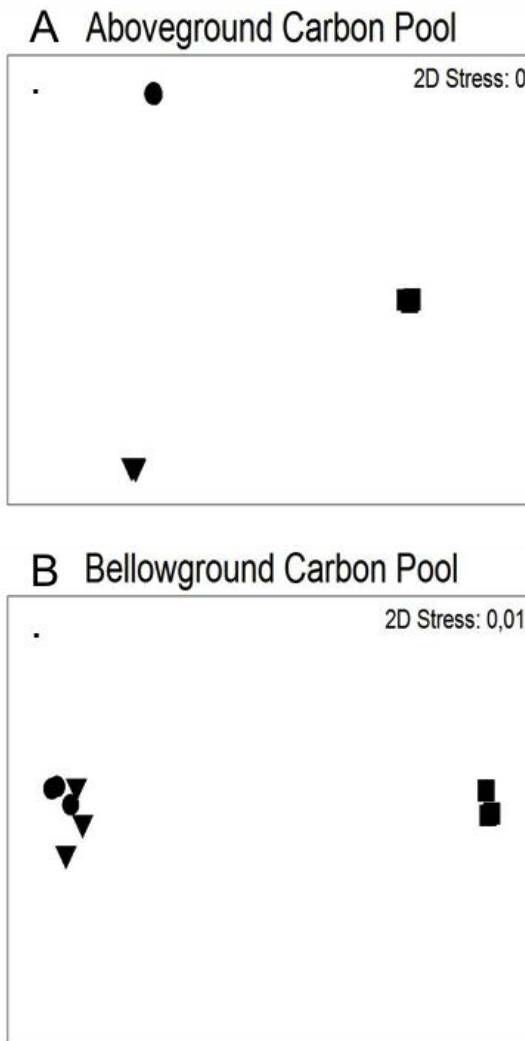
Figure 4: Non-metric multidimensional scaling (nMDS) plot based on above (A) and belowground (B) carbon pool of all studied species in the Mondego salt marsh for all study period. Circle: *S. maritimus*, triangle: *S. maritima* and square: *Z. noltii*.

Spartina maritima and *Zostera noltii* had CNPP values (table II) in the belowground organs about 90% and *S. maritimus* about 79%. When compared the CNPP of the above and belowground organs, the last one was higher. CNPP results were different between the above and belowground organs ($R > 0.7$; $p < 0.05$) and between species ($R > 0.6$; $p < 0.05$). Only *S. maritimus* showed relatively high difference ($R = 0.556$, $R < 0.05$) between organs for the turnover results (table II). Between species, the turnover

results had high differences in the aboveground organs (R>0.7; p<0.05) but in the belowground organs the species differences were lower (R<0.4; p<0.05).

The export values for all species and organs are showed in table II. For the aboveground organs, the species had differences (R>0.6; p<0.05), being *S. maritima* and *Z. noltii* the more similar. For the belowground organs all species showed low differences (R<0.3; p<0.05). The statistical analyses comparing the aboveground and belowground organs for the exports results showed significant differences (R>0.4; p<0.01). *Scirpus maritimus* had high turnover values in the aboveground organs (table II), in otherwise, *S. maritima* showed high values in belowground organs and *Z. noltii* same values for both organs.

Table II: Average total, aboveground and belowground CNPP, exports and turnover values for each species analysed in the Mondego salt marsh (gC.m^{-2}) and standard deviation for all study period.

Species	Organ	CNPP	Total CNPP	Export	Turnover
S. maritimus	Aboveground	87 ± 5	421 ± 75	87.2 ± 5	1 ± 0
	Belowground	334 ± 53		206 ± 25	0.57 ± 0.08
S. maritima	Aboveground	31 ± 3	380 ± 83	21.2 ± 3	0.65 ± 0.06
	Belowground	349 ± 88		292.9 ± 73	0.75 ± 0.08
Z. noltii	Aboveground	15 ± 0.6	139 ± 22	14.8 ± 0.4	0.94 ± 0.01
	Belowground	124 ± 15		116.9 ± 13	0.94 ± 0.01

Table III shows the CNPP, exports and the carbon accumulated for each species area. The table also shows the hectares that each species occupies in the Mondego estuary salt marsh; which is about 50% of the total salt marsh area (unpublished data). All species had losses of more than 60% of the CNPP, even more for *S. maritimus*, but this species accumulated more

carbon than the other two in the tissues (21104 Kg), because of the area that its occupied it is larger than the others two. *Spartina maritima* have the smaller area but accumulated more carbon (1951 Kg) than the *Z. noltii* (900 Kg).

Table III: Average of Carbon Primary Accumulation (CPA) and Exports (kg) for each species area (ha) and organ analysed for all study period in the Mondego salt marsh.

Species	CPA/Exports	Organ	Carbon
Scirpus maritimus (16.40 ha)	CPA	Aboveground	14304 ± 835
		Belowground	54903 ± 8734
	Exports	Aboveground	14304 ± 2200
		Belowground	33798 ± 4193
	Acumulated	Total	21104 ± 4541
Spartina maritima (2.89 ha)	CPA	Aboveground	918 ± 88
		Belowground	10114 ± 2570
	Exports	Aboveground	613 ± 85
		Belowground	8466 ± 1751
	Acumulated	Total	1951 ± 297
Zostera noltii (10.48 ha)	CPA	Aboveground	1655 ± 66
		Belowground	13065 ± 1654
	Exports	Aboveground	1561 ± 42
		Belowground	12258 ± 1460
	Acumulated	Total	900 ± 124

The carbon content in sediment (Figure 5) shows that *Z. noltii* had the highest carbon values in the sediment, followed by *S. maritima* and *S. maritimus*. All species presented differences between each other ($R > 0.5$; $p < 0.05$). Approximately 62% of the *S. maritimus* belowground CNPP is washed out of the sediment, 52% for *S. maritima*, but all the belowground CNPP of *Z. noltii* was retained in the sediment. The carbon content in the

sediment of the three species together correspond approximately 1679 Kg of C per hectare per year, corresponding a total of 50000 Kg of C in the sediment.
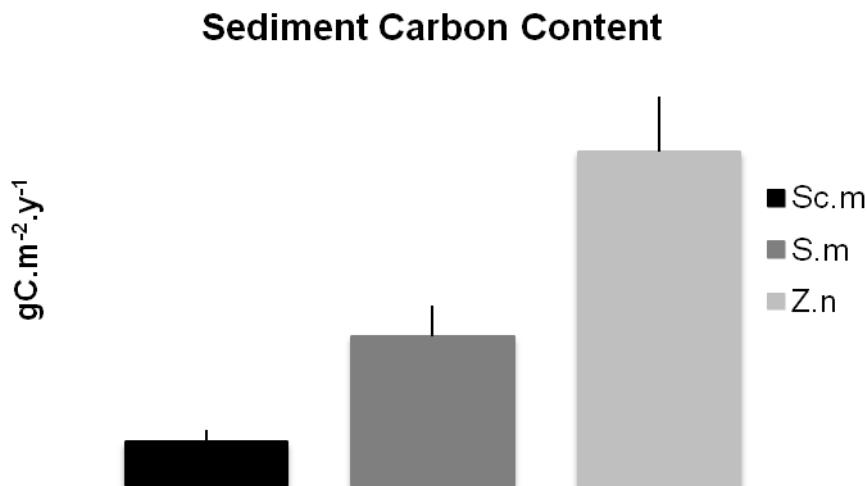
Figure 5: Sediment carbon content values and standard deviation for *S. maritimus* (Sc.m), *S. maritima* (S.m) and *Z. noltii* (Z.n) in the Mondego salt marsh.

3.4. Isotopic analysis

The average for the δ^{13}C results for each season is showed in table IV. For above and belowground organs, *S. maritima* and *Z. noltii* were more similar in the δ^{13}C values than with *S. maritimus*. The *S. maritima values* varied between -14.5‰ and -14.8‰, *Z. noltii* values range was between -13‰ and -16‰ and -25‰ and -26‰ to *S. maritimus*. In the sediment values, all three species had similar results; *S. maritimus* presented lower values and the three species together had a δ^{13}C range from -19.88‰ to -24.45‰.

Table IV: average $\delta^{13}C$ (‰) and standard deviation for each species and season analysed in the Mondego salt marsh. Sc.m (*S. maritimus*), S.m (*S. maritima*) and Z.n (*Z. noltii*).

	Winter 11	Spring 11	Summer 11	Autumn11
Average Aboveground $\delta^{13}C$				
Sc.m	-25.51 ± 0.29	-25.87 ± 0.11	-25.55 ± 0.21	-25.38 ± 027
S.m	-14.55 ± 0.12	-14.70 ± 0.10	-14.51 ± 0.16	-14.80 ± 0.20
Z.n	-16.00 ± 0.11	-14.43 ± 0.24	-13.57 ± 0.30	-15.52 ± 1.65
Average Bellowground $\delta^{13}C$				
Sc.m	-25.58 ±0.61	-25.82 ± 0.18	-26.05 ± 0.08	-25.68 ± 0.44
S.m	-14.46 ± 0.31	-14.93 ± 0.73	-14.58 - 0.45	-14.85 ± 0.74
Z.n	-14,80 ± 0.15	-15.33 ± 0.04	-13.58 ± 0.17	-13.85 ± 0.13
Average Sediment $\delta^{13}C$				
Sc.m	-22.81 ± 0.66	-23.06 ± 0.08	-24.45 ± 0.12	-22.86 ± 0.06
S.m	-21.80 ± 0.06	-21.82 ± 0.42	-21.73 ± 0.30	-22.03 ± 0.22
Z.n	-21.24 ± 0.17	-19.88 ± 0.53	-20.07 ± 0.39	-19.97 ± 0.54

4. Discussion

In agreement with others studies (Lillebø *et al.*, 2003 Duarte *et al.*, 2012) *S. maritimus* aboveground biomass showed high values during the warmer seasons. This species loses its aerial parts in cold seasons, so it has normally higher values in spring and summer than in autumn and winter. *Spartina maritima* had similar behaviour and Caçador *et al.* 2004 showed same pattern for *S. maritima* aboveground biomass in warmer seasons in Tagus estuary. Auby and Labourg, 1996 showed that *Z. noltii* had higher belowground biomass than the aboveground biomass, the same occurred in this study, but not only for *Z. noltii*, the same happened to the three species.

Caçador *et. al.* 2004 showed that in Tagus estuary the belowground biomass of *S. maritima* exceeded the aboveground biomass, contributing

more than 90% of the total biomass in some cases. A similar pattern occurred in Mondego, when observed the average biomass for the whole analysed period, not only *S. maritima* presented 90% of the total biomass in the belowground organs, but also the remaining other two analysed species showed a similar behaviour.

In a previous study in the Mondego salt marsh, Sousa *et al.* 2010 showed that the ratio between the aboveground and belowground carbon pool for *S. maritima* is high in autumn, which is corroborated by this study, where even when compared individually, this species had high carbon pool values for above and belowground organs in the same season.

In Tagus estuary, *S. maritima* have a CNPP in belowground biomass, in some areas, with more than 90% of the total value (Caçador *et al.*, 2004), which occurred in Mondego with the species *S. maritima* and *Z. noltii* being a little lower in *S. maritimus*. The aboveground carbon estimate for seagrasses can range from 0.1 to 18.7 g C m^{-2} day^{-1}, but the average is between 0.5–2.0 g C m^{-2} day^{-1} for most beds with aboveground biomass higher than 50 g DW m^{-2} (Dawes, 1998). This is not the case of the *Z. noltii* beds in this study, which had an average biomass of 13.1 g DW m^{-2} during the studied period; nevertheless, this species had an average production of 0.023 g C m^{-2} day^{-1}. *Z. noltii*, depending of the tide, stay more or less hours out of the water. Leuschner and Rees, 1993, showed that even while is emersed, this species could have high carbon assimilation if the desiccation were not to high. The senescence of *S. maritimus* aboveground organs reflects its turnover values, being very high. *Spartina maritima* presented the lower aboveground turnover values and the second lower belowground turnover values, and

comparing to the other two species *S. maritima* occupies less area, but can hold for more time the carbon in its tissues before export to sediment or water column.

Even with great losses of carbon by the three species, they can still be considered as carbon accumulators, acting as a sink. The three species together accumulated approximately 24000 Kg of carbon in the tissues in twenty-one months (what corresponds to an average of 38 Kg of carbon per day) and 50000 Kg of C in the sediment in one year, and since these three species occupy 50% of the estuarine salt marsh area, the carbon accumulation in the salt marsh of the Mondego estuary can even be higher.

The isotopic values confirm that *S. maritima* and *Z. noltii* share the same plant metabolism; in fact, Wang *et al.*, 2003 showed similar results for *Spartina alterniflora* and Baeta *et al.* 2009a had similar results for *Z. noltii*. Zhou *et al.* 2006 found close values for *S. triqueter, S. mariquete* and *S. tabernaemontani*, which correspond to the values showed for *S. maritimus* in this work. The isotopic results indicated that *S. maritimus* have a C_3 plant metabolism, that can vary between -23‰ and -34‰ (Ogrinc *et al.*, 2005) and *S. maritima* and *Z. noltii* a C_4 plant metabolism, which can range from -9‰ to -17‰ (Ogrinc *et al.*, 2005). Middelburg *et al.*, 1997 showed that in Georgia and Waarde marshes, the C_4 plants had a range of $\delta^{13}C$ between -12‰ and -17‰, corroborating the isotopic results of this work. Baeta *et al.*, 2009b showed that the sedimentary organic matter is not composed only by the material from the species observed in this study, but for a mix of terrestrial source, micro and macroalgae. This show that part of the carbon exported by these species remain in the sediment, reinforcing that the estuary act as a

carbon sink, although it can act as source of carbon too, since the major part of carbon is exported to the water column.

6. Conclusions

Usually the biomass values were higher in the warmer seasons than in the cold seasons. The carbon results are dependent of the biomass results, for this reason they followed a similar pattern. The plants carbon percentage had a range of 27-39% in *S. maritimus* and *S. maritima* and 30-39% for *Z. noltii*. *Scirpus maritimus* had the highest carbon production in the aboveground organs and had similar results with *S. maritima* in the belowground carbon production. The studied species had losses of carbon higher than 60% of the production. Despite the high exportation of carbon, when considering the whole area occupied by these species, were accumulated approximately 24000 Kg of carbon in twenty-one months in the species tissues, which have an average of 38 Kg of carbon per day and 50000 Kg in the sediment in one year. Although the stable isotope analysis together with the carbon calculations indicates that a part of the exported plant material remains in buried in the sediment; the studied species have both a sink and source behaviour simultaneously.

Chapter 3: Modelling the effects of global temperature increase on the growth of salt marsh plants

Chapter 3

Modelling the effects of global temperature increase on the growth of salt marsh plants

Abstract

Gradual increases in temperature and atmospheric CO_2 concentrations have resulted from the increased human use of fossil fuels since the beginning of industrial activity. In coastal wetland ecosystems, salt marshes constitute important habitats because they play important ecological roles, acting as carbon sinks by capturing atmospheric CO_2 and storing it in living plant tissue. Ecological models are important tools for understanding the results of anthropogenic impacts on a global scale. Global warming poses threats to salt marshes through different effects, e.g., increases in sea level. The objectives of this study were i) to assess how temperature increases will influence the growth of salt marsh plants, ii) to infer the carbon budget of salt marshes under temperature increase scenarios and iii) to predict how salt marsh plants will keep pace with increases in sea level. These goals were achieved by developing growth models of three different plants (*Spartina maritima*, *Scirpus maritimus* and *Zostera noltii*) found in the Mondego estuary. Models were developed for C_3 and C_4 plant species. The results suggest that a temperature increase enhances the aboveground biomass of salt marsh plants. According to the predictions of the models, the sedimentation rate of *S. maritima* and *Z. noltii* can keep pace with increases

in sea level, but this is apparently not the case for *S. maritimus*. If *S. maritimus* disappears from the Mondego estuary, the carbon sequestration ability of the system should decrease due to the loss of active plant tissue. This conclusion is based on the fact that *S. maritimus* accumulated more than 80% of the total carbon sequestered in the tissues by the three studied species.

Key words: growth model, sea level increase, sedimentation, salt marsh, estuaries

1. Introduction

Because of increasing human fossil fuel use since the beginning of intensive industrial activity (Houghton, 1999), the world has experienced an increase in atmospheric CO_2. It is widely believed that anthropogenic additions of CO_2 to the atmosphere are contributing to increase surface temperatures worldwide, a phenomenon known as the ''greenhouse effect'' (Bluemle *et al.,* 1999). IPCC (2007) reports show that the global temperature increased approximately 0.3 °C per decade from 1979 to 2005. Coastal wetlands such as salt marshes are excellent carbon sinks, as they withdraw CO_2 from the atmosphere and store it in living plant tissue (Williams, 1999). Salt marshes reduce the rates of greenhouse gas emissions (Magenheimer *et al.*, 1996) and have a carbon sequestration capacity per unit area approximately one order of magnitude higher than other wetland systems (Bridgham *et al.*, 2006), with the potential to sequester carbon continuously

over thousands of years (Brevik et al, 2004). In addition to their carbon storage capacity, salt marshes are key areas for estuarine systems because of their role in primary production and nutrient regeneration (Caçador *et al.*, 2009). They represent one of the most productive ecosystems on the planet (Lefeuvre *et al.*, 2003). However, global warming can threaten salt marsh areas through, e.g., increases in sea level. Salt marsh ecosystems play a vital role in the dissipation of wave energy, accretion of sediment and filtration of nutrients and as habitats supporting commercially important fisheries (Mudd et al. 2009). These ecosystems are vulnerable to changes in sea level (Cundy & Croudace, 1996, Rybczyk & Cahoon, 2002), and sea level increases have become a severe threat to coastal zones and estuaries at low elevations (Vandenbruwaene et al. 2011). It is recognised that many marshes are able to keep pace with increases in sea level (Friedrichs and Perry, 2001); the response of coastal marshes to relative increases in sea level depends upon their ability to maintain their relative elevation through sedimentation (Reed, 1990). IPCC (2007) reports show that the most pessimistic prediction for sea level change is an increase of approximately 0.97 cm per year, which will endanger certain areas around the world that are influenced by the tides, including, in particular, the Mondego salt marsh. Ecological models have been used to clarify the effects of anthropogenic impacts on a global scale by integrating processes related to the biota of the ecosystem in its conceptual structure (Fragoso Jr., *et al.* 2009). Accordingly, an ecological model focuses on the objects of interest for a considered and well-defined problem. There can be many different ecological models of the same ecosystem, as the model version is selected according to the goals of

modelling (Jørgensen & Fath, 2011). Because *Spartina maritima, Scirpus maritimus* and *Zostera noltii* together occupy approximately 50% of the salt marsh vegetation of the Mondego estuary, corresponding to 29.77 ha (Neto, unpublished data), these species were selected as the focus of the present study. Our goal was to examine the influence of temperature increase on the carbon accumulation of the salt marsh area of the Mondego estuary. Specifically, our objectives were to investigate how temperature increase influences the growth of salt marsh plants (*Spartina maritima*, *Scirpus maritimus* and *Zostera noltii*) and their carbon storage capacity. Furthermore, we attempted to estimate whether the sedimentation rate of the three salt marsh plants can keep pace with the increase in sea level.

2. Materials and Methods

2.1. Study site

The Mondego estuary (Figure 1) is located on the Portuguese Atlantic coast (40°08 N, 8°50 W) (Marques and Nogueira, 1991). The estuary is approximately 8.6 km^2 in area, and its upstream limit extends up to 21 km from the mouth of the estuary (Teixeira *et al.*, 2008). The final portion of the estuary (approximately 7 km) is divided into two arms (north and south) by Murraceira Island (Marques *et al.*, 2003). The sample sites for this study (Figure 1) were located in the south arm of the estuary. The south arm is shallower than the north arm (2–4 m during high tide) and is characterised by large areas of intertidal mudflats (almost 75% of the area) (Neto *et al.*, 2008).

The sediments of the south arm contain a high percentage of silt and clay, and the area is considered to be a rich estuarine habitat in terms of productivity and biodiversity (Marques *et al.*, 1993).
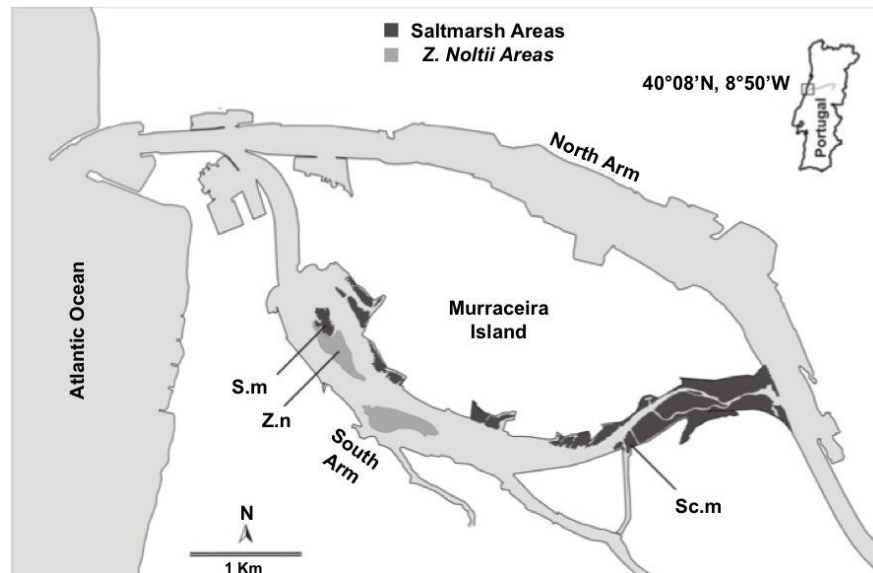

Figure 1: Locations where the observed data were gathered. S.m (*Spartina maritima*), Z.n (*Zostera noltii*) and Sc.m (*Scirpus maritimus*).

2.2. Field data sampling and laboratory procedures

Field data sampling was conducted seven times, once in each season, between 2010 (spring, summer and autumn) and 2011 (winter, spring, summer and autumn). For the aboveground biomass, three replicates (size= 0.3 x 0.3 m squares) of each salt marsh plant species were randomly selected in each area and clipped (Caçador *et al.,* 2004).

In the laboratory, all samples were washed with ultrapure water (18.2 MΩ cm) to remove dust. The aboveground tissues were then dried at 60 °C until a constant weight was achieved, and the dried tissues were pulverised with a grinding ball mill (Glen Creston MM2000) (Gross *et al.*, 1991). Total

carbon content was determined using a CHNS/O analyser (Fisons Instruments Model EA 1108). The carbon pool (grams) for each species and for each season analysed was calculated by multiplying the results from the CHNS/O analyser, expressed as a percentage (%), by the biomass according to equation 1.

$$Carbon\ pool = [Carbon]_t \times Biomass_t \qquad \text{(Eq. 1)}$$

Sedimentation rates were measured using lengths of wood with millimetre marks, which were buried up to the zero mark level in the area occupied by each species. This procedure was performed in February 2011 in the sampling areas corresponding to the three salt marsh plants. One year later (February 2012), the wooden markers were checked to measure the level corresponding to sediment accumulation in each of the three sampling areas. Three woodpiles were buried in each species zone.

2.3. Model

To simulate the growth of a primary producer, a model must include the principal physiological processes, such as photosynthesis, respiration and exudation (Duarte, 2011). The present study follows the work of Simas *et al.* (2001), where fluxes are expressed as gC dry weight m^{-2}; the key equation is

$$\frac{dB}{dt} = \mu - R - L_m - E \qquad \text{(Eq. 2)}$$

where B is plant biomass, μ is gross productivity, R is respiration, L_m is leaf mortality and E is exudation. Gross production (μ) is represented by equation 3:

$$\mu = P_{max} \cdot f(T) \cdot f(I) \cdot f(N)$$ (Eq. 3)

where P_{max} is the plant's maximum production, $f(T)$ is the air temperature function, $f(I)$ is the light function and $f(N)$ is the nutrient function.

P_{max} values for each studied plant species are shown in table I. The air temperature function $f(T)$ is described in equation 4 (Bach, 1993):

$$f(T) = \gamma_1^{(T - T_{opt})},$$ (Eq. 4)

where γ_1 is the temperature coefficient for growth, T is the daily air temperature and T_{opt} is the optimum temperature for growth.

The daily temperature variation (T) throughout the year was obtained from a modified sine equation (Anastácio *et al.*, 1995) (Equation 5):

$$T = \frac{T_{max} + T_{min}}{2} + \frac{T_{max} - T_{min}}{2} \times SIN\left(\left(\frac{time}{365} - 0.23\right) \times 2 \times \pi\right)$$ (Eq. 5)

where T_{max} is the maximum air temperature (39.2 °C) and T_{min} the minimum air temperature (-2.4 °C) during the study period. The maximum and minimum temperature values were obtained from the Portuguese Meteorology Institute (IPMA).

The light function was defined as a Michaelis-Menten equation:

$$f(I) = \frac{I}{(I_k + I)} \; ,$$

where I is the light intensity and I_k is the half-saturation constant. Daily values of light intensity at 40⁰ latitude were obtained from the Portuguese Environment Agency (APA), and half-saturation constants for the three plant species were obtained from the literature (Morris, 1982).

The regulation of growth by nitrogen ($f(N)$) is expressed in equation 7:

$$f(N) = \frac{N}{(N + \eta)} \; ,$$

(Eq. 7)

where N is the concentration (%) of nitrogen in the leaves and η is the half-saturation constant (%) for nitrogen.

Leaf mortality (L_m) is dependent on temperature (equation 8):

$$L_m = L_{max} \left(\gamma_2^{T - T_{opt}} \right) H$$

(Eq. 8)

where L_{max} is the maximum leaf mortality rate, γ_2 is the temperature coefficient for leaf mortality and H is the loss of aboveground biomass due to wave motion, calculated using equation 9:

$$H = 1 + W_1 \, e^{-W_2 h}$$

(Eq. 9)

where W_1 and W_2 are constants and h is the tidal height.

Exudation (E) (equation 10) is the release of dissolved organic carbon and can be calculated as a fraction of gross production (Baretta-Bekker *et al.* 1997):

$$E = \mu[\alpha + (1 - \alpha)(1 - f(N))]$$ (Eq. 10)

where α is the fraction of gross production that is excreted under nutrient stress conditions (Simas *et al.* 2001).

The respiration rate (R) is dependent on the temperature and on the assimilated biomass (Baretta-Bekker *et al.*, 1997) and can be calculated as in equation 11:

$$R = r^{bas} \, Q_{10}^{T/(T_{opt}-1)} + q^{res} (\mu - E)$$ (Eq. 11)

where r^{bas} is the basal respiration, Q_{10} is the Q_{10} value and q^{res} is the fraction of production lost by respiration.

2.4. Model calibration and scenario simulations

The model was built using STELLA software (version 9.0) (Isee systems). As in Simas *et al.* 2001, the model was calibrated independently for C$_3$ (*Scirpus maritimus*) and C$_4$ (*Spartina maritima*, *Zostera noltii*) species. The model was run for a two-year period corresponding to the sampling period, and the predictions were compared with the observations. The parameter values used in the model are shown in table I.

To test the influence of temperature on the biomass production of the three plant species in the model, the temperature was increased by 1.5 °C.

2.5. Sensitivity analyses

A sensitivity analysis was performed on selected key parameters to assess the variation in the biomass of the three salt marsh plants when the parameters were changed. The parameters were changed in ±10% (Jørgensen and Fath, 2011).

2.6. Sea level increase

To study the sedimentation rate associated with the area occupied by each studied species relative to sea level increase, the bathymetry of the area occupied by each plant was considered along with the average height of each plant species, namely, 1.20 m for *S. maritimus*, 0.7 m for *S. maritima* and 0.3 m for *Z. noltii*. A sea level increase of 0.97 cm per year was simulated and compared with the sedimentation rate of each species. Bathymetry data for the Mondego estuary were extracted from Kenov *et al*. (2012), and the average maximum and minimum tidal heights were obtained from the Portuguese Hydrographic Institute (IH). A constant sedimentation rate (2.7 cm y^{-1} for *Z. noltii*, 1 cm y^{-1} for *S. maritima* and 0.2 cm y^{-1} for *S. maritimus*) and constant rate of sea level increase (0.97 cm y^{-1}) were assumed.

Table I: Parameters values used in the model. A major part of the values were gathered from the works of Simas *et al.* 2001 and 2007.

Code	Description	Units	*Scirpus maritimus*	*Spartina maritima*	*Zostera noltii*	References
P_{max}	Maximum production rate	gC dw m^{-2} d^{-1}	1.23	2.4	0.27	Curtis *et al.*, 1989a; Curtis *et al.,* 1989b; Sousa *et al.,* 2010; Bach, 1993
γ_1	Temperature coefficient for growth	-	1.1	1.08	1.08	Bach 1993
T_{opt}	Optimum temperature for growth	°C	21	25	25	Giurgevich and Dunn 1981
I_k	Light half saturation constant	W m^{-2}	177	250	250	Morris 1982
η	Nitrogen half saturation constant	%	0.36	0.36	0.36	Morris 1982
L_{max}	Maximum leaf mortality rate	gC dw d^{-1}	0.812	0.5774	0.0641	*
γ_2	Temperature coefficient for leaf mortality	-	1.07	1.07	1.07	Bach 1993
W_1	Coefficient for H (Eq. 8)	-	30	30	30	Bach 1993
W_2	Coefficient for H (Eq. 8)	m^{-1}	1.5	1.5	1.5	Bach 1993
α	Exudation under nutrient stress	-	0.2	0.2	0.2	Bach 1993
r^{bas}	Basal respiration	d^{-1}	8.4 x 10^{-4}	5.52 x 10^{-4}	5.52 x 10^{-4}	Simas et al. 2001
Q_{10}	Q_{10} value	-	2	2	2	Baretta-Bekker et al. 1997
q^{res}	Production respired fraction	-	0.1	0.1	0.1	Baretta-Bekker et al. 1997

* Values estimated at calibration.

2.7. Statistical analysis

To compare the biomass results between the model simulation using the actual temperature and the simulation with the 1.5 °C increase in temperature, a one-way ANOSIM test using Primer software, version 6 (Clarke, 1993) was performed. The model predictions and observations were compared using a regression analysis.

3. Results and discussion

3.1. Model predictions vs. observed biomass

The model results followed the same pattern as the observed values (Figure 2), with plant aboveground biomass values usually higher in the warm seasons than in the cold seasons, as also shown by several other studies (Lillebø *et al.,* 2003; Caçador *et al.*, 2004; Duarte *et al.* 2012; Couto *et al.,* 2013). Because the observed values in the spring of 2010 for *S. maritimus* and *Z. noltii* were unusually high, they were excluded from the linear regression calculations. The regression (Figure 2) demonstrated that the observed and model results showed similar variation, although the data for *S. maritima* showed the best fit, followed by *S. maritimus* and *Z. noltii.* The sensitivity analysis (table II) showed that the parameters with the greatest influence on the biomass values of the three plant species were the maximum temperature (T_{max}) and the maximum leaf mortality rate (L_{max}). A change of ±10% in these parameters could increase or decrease the average

biomass by more than 50%.
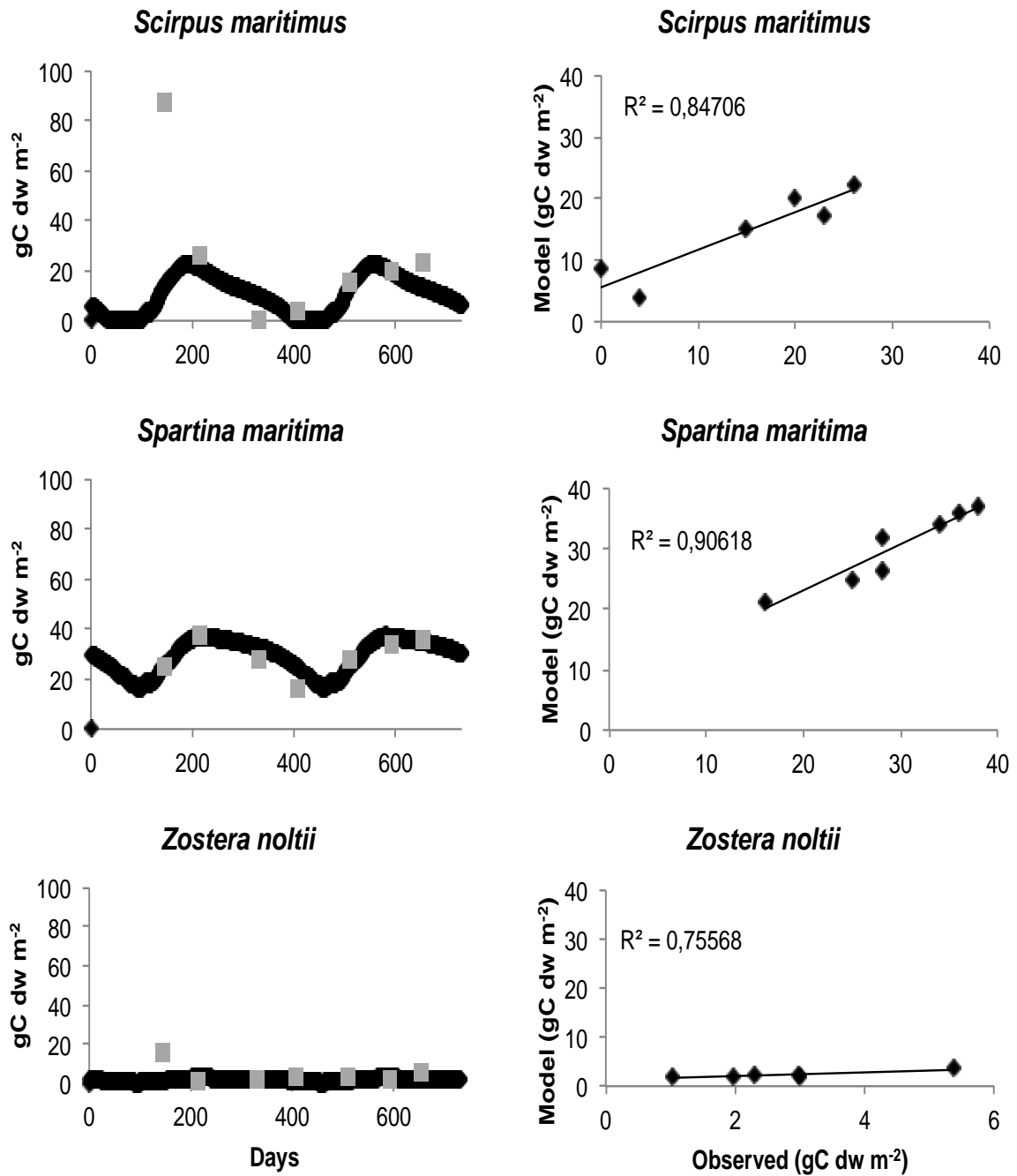
Figure 2: Observed (grey squares) and model (black line) values during 730 days and linear regression comparing both results for each species.

Table II: Sensitivity analyses of the average biomass of the total study period in relation to key parameters. The results are in percentage of the standard model.

Parameters	Sc.m		S.m		Z.n	
	10%	-10%	10%	-10%	10%	-10%
I_k	-40	52	-37	40	-49	76
T_{max}	322	-83	43	-30	79	-44
T_{min}	0.2	-0.1	-4	1	-1	0
N	41	-41	23	-30	44	-40
η	-38	46	-27	27	-37	49
r^{bas}	-0.3	-0.3	-4	-4	-3	2
Q_{10}	-0.4	-0.3	-4	-4	-3	2
q^{res}	-14	14	-10	6	-17	16
L_{max}	-72	187	-70	80	-76	145

3.2.   Temperature increase

IPCC reports (IPCC, 2007) state that the global temperature has increased approximately 0.3 °C every 10 years since 1979. Figure 3 shows the results of a comparison between the model results with the actual temperature and the model results assuming an increase of 1.5 °C. The results indicate that in such a scenario (a temperature increase of 1.5 °C), *S. maritimus* and *Z. noltii* would show an increase of approximately 30% in maximum biomass compared with the simulation using the actual temperature, whereas *S. maritima* would show an increase of 10% in maximum biomass.

The increase in temperature apparently has a positive effect in the model, as the increased plant biomass promoted by the increased temperature will absorb more carbon from the atmosphere. However, temperature increase will certainly affect the system in other ways. For example, it will cause sea level to increase due to oceanic thermal

expansion, and also due to the melting of Arctic and Antarctic ice (Titus *et al.*, 1991). This process could be detrimental to salt marshes because the sea level increase would place increased stress on certain marshes; these marshes might ultimately be submerged (Silliman *et al.*, 2009).
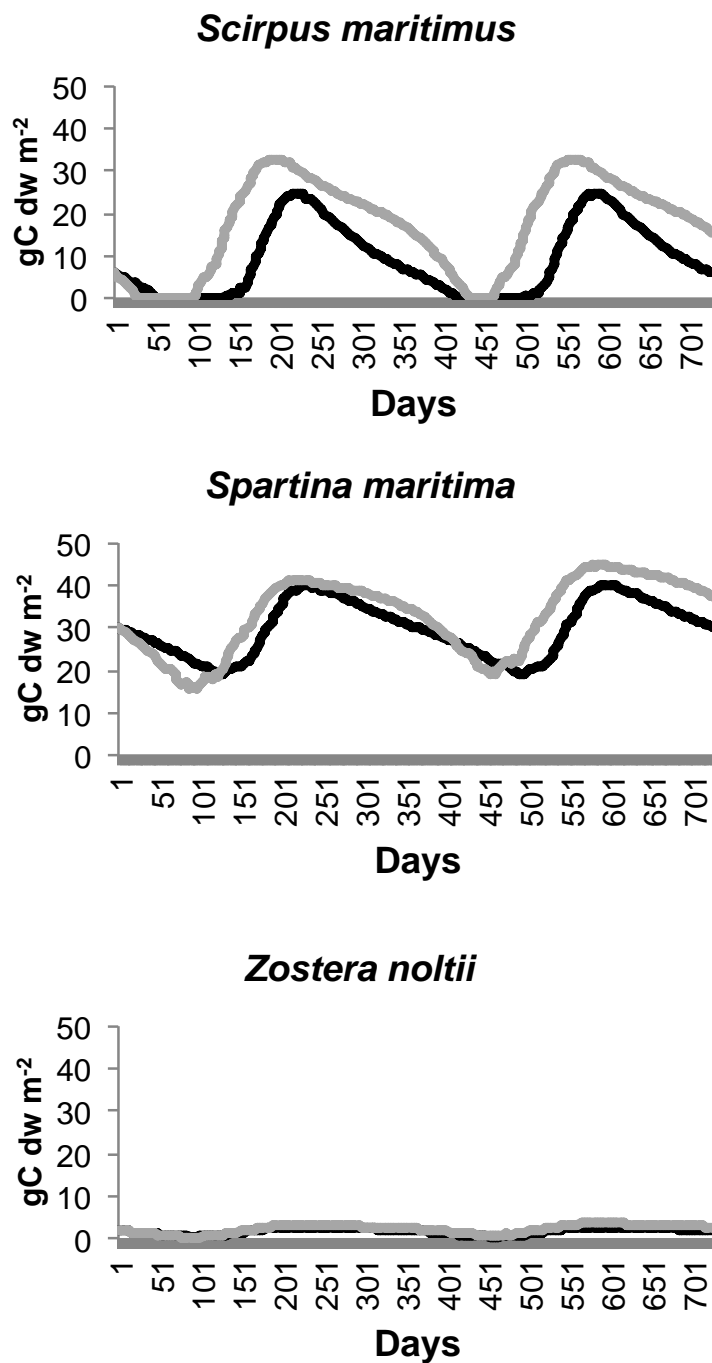
Scirpus maritimus

Spartina maritima

Zostera noltii

Figure 3: Model results for the actual temperature (black line) and for the 1.5 °C increase in the temperature (grey line) during 730 days.

3.3. Sea level increase and sedimentation

Kenov *et al.* (2012) have shown that the bathymetry data of the sand banks for the three studied species in the Mondego estuary correspond to an elevation of approximately 2 m above sea level. The average heights of the studied species are 1.20 m for *S. maritimus*, 0.7 m for *S. maritima* and 0.3 m for *Z. noltii*. The sum of the heights of the banks and the average height of each species is approximately 3.2 m for *S. maritimus*, 2.7 m for *S. maritima* and 2.3 m for *Z. noltii*. In 2011, the average low tide was 0.9 m, and the average high tide was 3 m. *Z. noltii* had the highest sedimentation rate for a one-year period (2.7 cm y^{-1} ± 0.2), followed by *S. maritima* with 1 cm y^{-1} ± 0.1 and *S. maritimus* with the lowest sedimentation rate (0.2 cm y^{-1} ± 0.04). Along western European coasts, the sea level has been increasing at least since the Roman Period, and this increase has accelerated during the Modern Period (Pye, 1995). Salt marshes can keep pace with the sea level increase, but this ability depends on the sedimentation rate (Reed *et al.,* 1990; Pont *et al.*, 2002). In this case, *S. maritimus* could be at risk in the Mondego estuary. Figure 4 shows that in approximately 37 years, if the sedimentation rates and rates of sea level increase remain the same, the *Z. noltii* area would not be immersed during a high tide. As a result, this species would, most likely, disappear from this area. The *S. maritima* sedimentation rate (1 cm y^{-1}) and sea level (0.9 cm y^{-1}) are increasing to a similar extent, and the *S. maritima* area should not be greatly influenced by the increasing sea level during the next 50 years. Unlike *Z. noltii*, *S. maritimus* could be completely covered during every high tide in approximately 40 years.
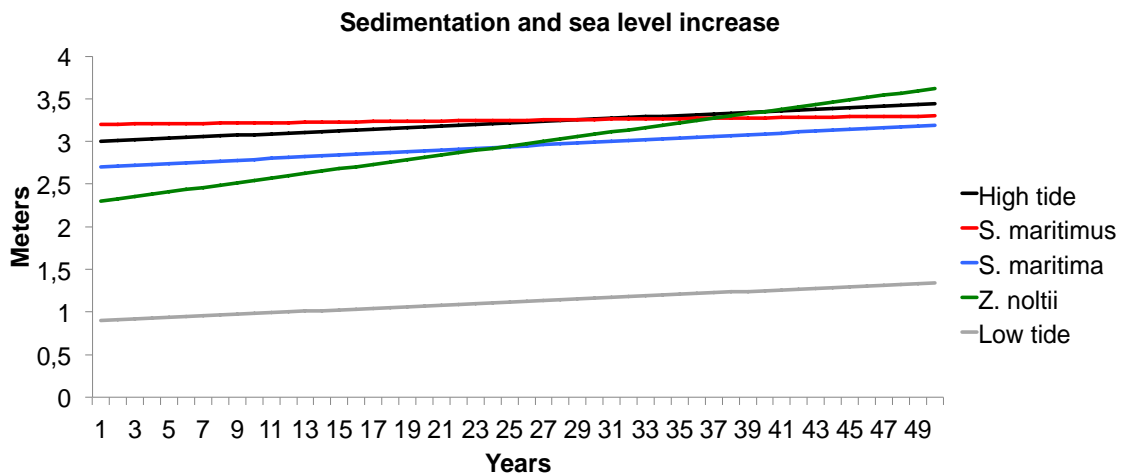
Sedimentation and sea level increase

Figure 4: Predicted sea level increase and sedimentation of the three studied species through 50 years.

Of the three study species, *S. maritimus* should show the greatest effects of the sea level increase because its sedimentation rate (0.2 cm y^{-1}) is lower than the expected rate of increase of sea level. Before the *S. maritimus* area becomes completely submerged, this species will, most likely, be influenced by the salinity increase that accompanies an increase in sea level. Lillebø *et al.* (2003), working with *S. maritimus* in the same area, showed that salinity affects the dynamics of *S. maritimus*. In fact, the findings of previous studies have indicated that lower biomass values are associated with high salinity, showing that the survival of this species could be threatened by high salinity. Couto *et al.* (2013) showed that *S. maritimus* accumulated approximately 21000 Kg of carbon in the tissues in 21 months, compared with 1900 Kg of carbon accumulated in the tissues by *S. maritima* and 900 Kg by *Z. noltii*. If the species that accumulates the highest amount of carbon of the three species disappears, the carbon sequestration ability of the Mondego estuary may decrease. Silliman *et al.*, 2009 suggest that as much

as 90% of tidal marshland worldwide could be in jeopardy by 2100 because of the increase in sea level.

4. Conclusions

The fit of the models for the C_3 and C_4 plants to the observed biomass values was satisfactory. The assumption of a temperature higher than the actual temperature yielded simulated plant biomass values higher than the actual values. This result indicates that the biomass of both C_4 and C_3 plants would increase and that as a result, carbon sequestration would also increase. The increase in sea level should be prejudicial to salt marsh species. The *S. maritima* sedimentation rate can apparently keep pace with the sea level increase, and *Z. noltii* shows a high sedimentation rate. In contrast, because the *S. maritimus* sedimentation rate is very low, this species could disappear from the Mondego estuary. This scenario would have important implications for the system's carbon sequestration ability because, of the three species examined, *S. maritimus* accumulates the highest quantity of carbon in the tissues.

General discussion and conclusions

General discussion and conclusions

In agreement with others studies (Lillebø *et al.*, 2003 Duarte *et al.*, 2012) *Scirpus maritimus* aboveground biomass showed high values during the warmer seasons. This species loses its aerial parts in cold seasons, so it has normally higher values in spring and summer than in autumn and winter. *Spartina maritima* had similar behaviour and Caçador *et al.* 2004 showed same pattern for *S. maritima* aboveground biomass in warmer seasons in Tagus estuary. As the biomass were higher in warmer seasons then in the cold ones, the photo-protective piments had similar behaviour, which is normal, since at high solar radiation the relative electron transportation rate stop growing. Auby and Labourg, 1996 showed that *Z. noltii* had higher belowground biomass than the aboveground biomass, the same occurred in this study, but not only for *Z. noltii*, the same happened to the three species.

Caçador *et. al.* 2004 showed that in Tagus estuary the belowground biomass of *S. maritima* exceeded the aboveground biomass, contributing more than 90% of the total biomass in some cases. A similar pattern occurred in Mondego, when observed the average biomass for the whole analysed period, not only *S. maritima* presented 90% of the total biomass in the belowground organs, but also the remaining other two analysed species showed a similar behaviour.

Working with *Spartina alterniflora*, Baerlocher *et al.* 2004 found that this species could assimilate 0.22 mol CO_2 per mole of electrons transported through PSII. Looking to our results, the species that should have a higher carbon accumulation is *S. maritima*, since it have higher ETR than the other

two, the aboveground carbon pool followed this pattern; *S. maritimus* had the higher carbon accumulation in the tissues per hectare, even with its seasonal total aboveground organs lost. The answer for that could be in the different metabolisms, enhanced levels of CO_2 in the atmosphere has little effect on C_4 plants, but most C_3 plants increase their productivity (Körner, 2006), but *S. maritima* turnover results showed that this species is able to hold carbon for more time. In the other hand, the ETR results were usually higher in the warmer seasons than in the cold seasons, and the carbon pool results showed same pattern. The isotopic values indicate that part of the carbon exported by the studied species remain in the sediment, reinforcing that the estuary act as a carbon sink, although it can act as source of carbon too, since the major part of carbon is exported to the water column or sediment. In fact, Howarth, 1993 says that the fixed carbon is used to plants needs, but the majority of biomass produced by plants is degraded or exported, only a small part is retained in the sediment. Even with great losses of carbon by the three species, they can still be considered as carbon accumulators, acting as a sink. The three species together accumulated approximately 24000 Kg of carbon in twenty-one months in the tissues, what corresponds to an average of 38 Kg of carbon per day, and since these three species occupy 50% of the estuarine salt marsh area, the carbon accumulation in the salt marsh of the Mondego estuary can even be higher.

Light and temperature have influence in the photosynthetic pigments, and consequently affect the plant photosynthetic behavior (Wang *et al.* 2009) and consequently the capability of carbon sequestration. Huner *et al.* 1998 said that at low temperatures the photosynthesis rate could decrease. It is

now widely believed that anthropogenic additions of CO_2 to the atmosphere are contributing to increased mean global surface temperatures (Bluemle et al., 1999), what help increase the sea-level, and despite these valuable ecosystem functions, there has been a significant loss in tidal marsh area on a global scale in the last decades (Lotze *et al.*, 2006) by direct or indirect anthropogenic influence. To test the response of the three species in relation with the global temperature increasing, a model for each species was run. The regressions show that models had consistent results when compared with the observed results. As the model was based in the work of Simas *et al,* 2001, the results showed similar and expected values, showing higher biomass in the warmer seasons. IPCC, 2007 reports said that every 10 years the world temperature have being increasing about 0.3 °C. The model results with an increased temperature were consistent with the observed and with the actual temperature modelled values. *S. maritimus* showed a higher biomass increasing then the *S. maritima* and *Z. noltii* with an increased temperature, with means that the carbon sequestration could be higher if we looking at only to the temperature parameter, without its consequences, as the sea level increase. Looking to the sedimentation rate of each species and to the increase of the sea level, is possible to see that in the future, the Mondego estuary carbon dynamics could be changed, mainly because *S. maritimus.* It is knower that more than 90% of the plants species in the world are C_3 plants, but where the system is more stressful, like in the salt marsh areas near to the ocean, with higher daily tide and salinity changes, the C_4 species appear to be more dominant than in the upstream of the Mondego estuary. The sedimentation rate in the *S. maritima* area can probably

continuing keeping peace with the sea level, and the sedimentation rate at *Z. noltii* area is actually higher then the sea level increase rate; but for *S. maritimus* area, if the sedimentation and sea level rates continues similar then the actual rate, can be fully flooded, which implies in less carbon sequestration. *Scirpus maritimus* does not occur in the north arm of the estuary and with the sea level increase, this species could be completely flooded in the Mondego estuary in about 350 years, but before that, its growing ability could be compromised because of the salinity increase that came together with the sea level increase, with is prejudicial to the system carbon sequestration ability. Not only the salt marsh of the Mondego estuary will lose carbon absorption capacity, in fact, Silliman *et al.*, 2009 suggests that as much as 90% of the tidal marshes worldwide could be in jeopardy by 2100 because of the increase of the sea level.

The Mondego estuary salt marsh has about 50% of its area colonized by the three species studied in this work. Usually the biomass values were higher in the warmer seasons than in the cold seasons, as the photo-protective pigments. The carbon results are dependent of the biomass results, for this reason they followed a similar pattern. *Spartina maritima* was the species with higher photosynthetic efficiency, except in the winter season, but *S. maritimus* was the one that accumulated more carbon. The studied species had losses of carbon higher than 60% of the production. Regarding the high exportation of carbon, when considering the whole area occupied by these species, were accumulated approximately 24000 Kg of carbon in twenty-one months, which have an average of 38 Kg of carbon per day. Although the stable isotope analysis together with the carbon

calculations indicates that a part of the exported plant material remains buried in the sediment; the studied species have both a sink and source behaviour simultaneously. The model results were consistent with the observed results. The model also showed that *S. maritimus* should have a higher carbon production than the other two with a temperature increase but it is the species that probably will more affected by the sea level increase, affecting the carbon sequestration dynamics in the salt marsh of the Mondego estuary.

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