

Abiotic modulation of *Spartina maritima* photobiology in different latitudinal populations



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ABSTRACT

Spartina maritima has a very wide distribution in the northern hemisphere salt marshes crossing a wide variety of climatic environments. Therefore, it is not strange that some differences arise when observing the photosynthetic mechanisms of different populations inhabiting different latitudes. During this study it could be observed that climate is the most important factor controlling the photosynthetic traits of different populations distributed along a climatic gradient, namely the air temperature, humidity and light environment. Also some sediment physicochemical parameters such as pH and pore water salinity showed important influences driving the photosynthetic mechanisms in *S. maritima*. Furthermore *S. maritima* is one of the most abundant halophytes colonizing the Portuguese salt marshes. These facts have greater importance if one considers the large abundance of this halophytic species and how climate change will affect their metabolism and thus the ecosystem services provided by this species to the estuarine system.

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Salt marsh vegetation has a wide geographic distribution due, not only to its long-distance dispersal, but also to the similarities between salt marsh environments around the globe (Adam, 2002). Although these similarities, the key abiotic factors (for e.g. salinity, radiation, air temperature and humidity) affecting these ecosystems have a latitudinal gradient, even at small scales (Pennings et al., 2002). In this context, halophytes with a wide latitudinal distribution are likely to show intra-specific differences between partially and totally isolated populations (Álvarez et al., 2010). Genetic differentiation among populations within a plant species is well documented, with certain populations being better adapted than others to specific environmental stressors (Keeley, 1979; Silander and Antonovics, 1979; Davies and Singh, 1983; Chung, 1989). This natural genetic variation provides the potential for selection of plant populations exhibiting improved stress tolerance. The *Spartina* genus is one of the more successful halophytes, being present in a wide range of latitudes across the globe. Some works have already described latitudinal population differentiation of *Spartina* species (Lessmann et al., 1997; Daehler et al., 1999; Otero et al., 2000; Seliskar et al., 2002; Proffitt et al., 2005; Álvarez

et al., 2010). The members of the *Spartina* genus have C4 photosynthesis using phosphoenolpyruvate carboxylase (PEPC) to concentrate CO₂ at higher amounts than it would be observed in a C3 organism (Hatch, 1992; Álvarez et al., 2010). Although this metabolic advantage PEPC is controlled by light-dependent phosphorylation (Echevarría et al., 1990; Vidal et al., 1996), making it very dependent on the surrounding climatic environment. Differences are likely to be found since leaves have high sensitivity to environmental pressures, such as climatic variations, contaminants and grazing (Heide, 2005; Stephenson et al. 2006). In addition, the activation of PEPC by its specific kinase is poor in low light (Bailey et al. 2007) and at low temperatures (Lara et al. 2001), both of which abiotic factors change with latitude.

Spartina maritima (Curtis) Fernald, has a very wide distribution in the northern hemisphere native to the coasts of western and northern Europe and western Africa, with also a disjunct population on the Atlantic coasts of Namibia and South Africa (Marchant and Goodman, 1969). Recently, it has been withdrawing from several salt marshes either due to dieback or to hybridization with other *Spartina* species, originating polyploid species (Ainouche et al., 2003).

The study described in this paper aimed to identify the differences in the seasonal photobiological traits (PSII photochemistry

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and pigment profiles) between three populations of *Spartina maritima* from three transitional systems across Portugal latitudinal and climatic gradient, as well as the abiotic factors underlying the differences observed.

1. Material and methods

1.1. Study area and sampling

Spartina maritima field sampling (Fig. 1) was carried out from June 2010 to May 2011 in Alcochete salt marsh (Tagus estuary), in a large *S. maritima* extension at Mondego estuary and in Barra salt marsh (Aveiro lagoon). All plant leaves were immediately stored in liquid nitrogen and brought back to the laboratory where they were kept at $-80\text{ }^{\circ}\text{C}$ until analysis. Simultaneously from April 2010 to February 2011 the aboveground biomass was assessed for each species by clipping out five squares of $0.3 \times 0.3\text{ m}$. Samples were stored in plastic bags and quickly transported to the laboratory. The aboveground plant biomass collected was washed with Milli-Q water to remove dust and sediment. Plant organs were oven dried at $60\text{ }^{\circ}\text{C}$ until constant weight. Aboveground biomass was after expressed in a square meter basis (kg m^{-2}). The ratio of belowground to aboveground biomass (R/S) was also determined in order to assess the compartmentalization of the total biomass. Sediment samples ($n = 3$) were collected in pure stands of the selected species, using PVC tubular cores (9 cm diameter, 50 cm long) and transported to the lab in refrigerated bags where they were sliced between 5 and 8 cm depths.

1.2. Sediment physical-chemistry

All the analyses were carried out in sediment samples from 5 to 8 cm depth, due to the high influence of the root system at this depth (Duarte et al., 2008, 2009). Sediment water content was determined by drying sediment samples at $60\text{ }^{\circ}\text{C}$ until constant weight. Pore water salinity was measured with a hand

refractometer, after pore water extraction by centrifugation at $14,000\text{ g}$ for 15 min at $4\text{ }^{\circ}\text{C}$. Organic matter was determined by the loss on ignition (LOI) method by burning 1 g of air-dried sediment at $600\text{ }^{\circ}\text{C}$ for 2 h. Total N was determined in air-dried and burned sediment samples, using a CHNS/O analyser (Fisons Instruments Model EA 1108).

Sediment pH was determined by resuspending sediment sub-samples in ultra pure water at a ratio of 1:2.5 (w/v).

1.3. Climatic data mining

Climatic data collection was withdrawn from the public database from the Portuguese National Institute of Water (www.snirh.pt). For a more robust dataset, the data regarding the last 10 years was used where available and seasonal averages were calculated, in order to characterize climatically the studied periods. Data relative to air temperature, relative humidity, precipitation and radiation were consulted directly in the database, while potential evapotranspiration was calculated using a modified Turc method (Turc, 1961; Douglas et al., 2009):

$$ET = 0.013 \times (23.88 \times R_S + 50) \times \left(\frac{T}{T + 15} \right)$$

where ET is potential evapotranspiration in mm/day, R_S is solar radiation in $\text{MJ m}^{-2} \text{d}^{-1}$, and T is average daily air temperature.

1.4. PAM fluorometry

Modulated chlorophyll fluorescence measurements were made in attached leaves in the field with a FluoroPen FP100 PAM (Photo System Instruments, Czech Republic). All the measurements in the dark-adapted state were made after darkening of the leaves for at least 30 min. The minimal fluorescence (F_0) in dark-adapted state was measured by the measuring modulated light, which was sufficiently low ($<0.1\text{ }\mu\text{mol m}^{-2} \text{s}^{-1}$) to induce any significant variation

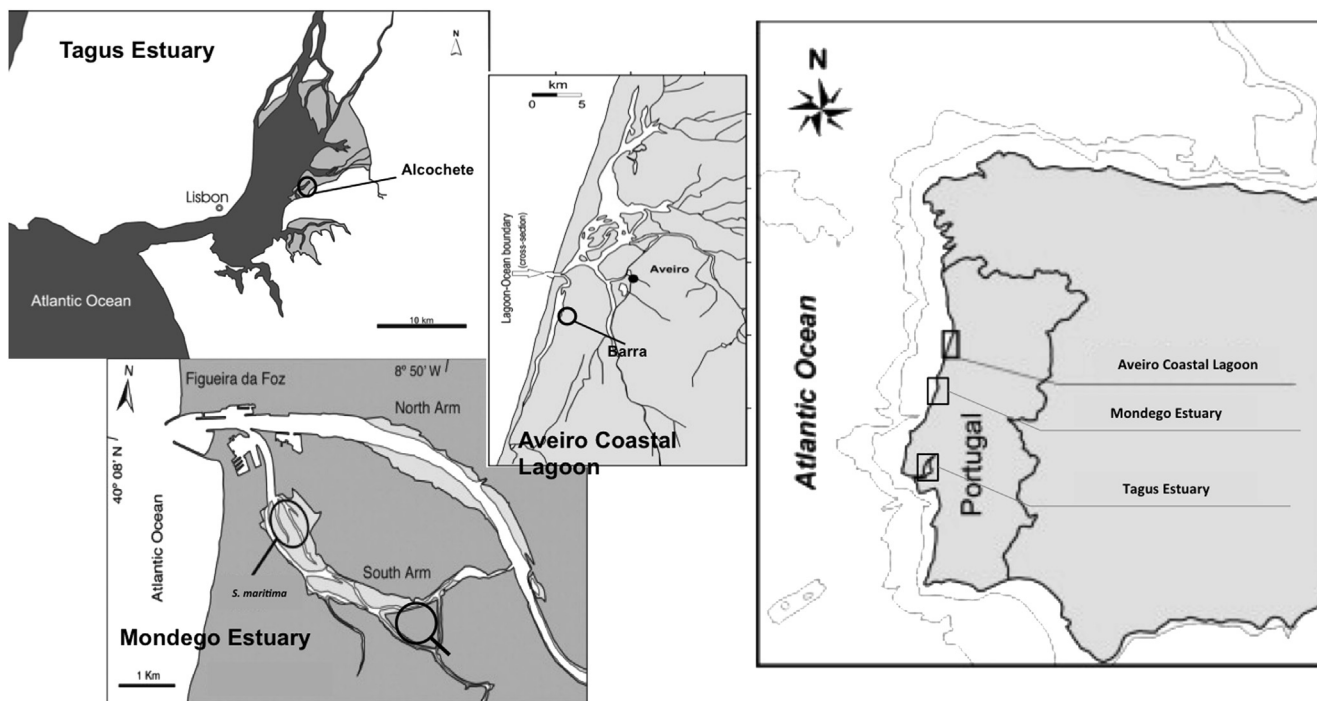


Fig. 1. Field sampling locations in the three considered estuaries.

in fluorescence. The maximal fluorescence level (F_M) in dark-adapted state was measured by a 0.8 s saturating pulse at $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$. The maximum photochemical efficiency was assessed as $(F_M - F_0)/F_M$. The same parameters were also measured in light-adapted leaves, being F_0' the minimum fluorescence, F_M' the maximum fluorescence and the operational photochemical efficiency. Rapid light curves (RLC) measurements, in dark-adapted leaves, were attained using the pre-programed LC1 protocol of the FluoroPen, consisting of a sequence of pulses from 0 to $500 \mu\text{mol m}^{-2} \text{s}^{-1}$. For this protocol the F_0 and F_M as well as the maximum photochemical efficiency were measured. Each Φ_{PSII} measurement was used to calculate the electron transport rate (ETR) through photosystem II using the following equation: $\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.5$, where PAR is the actinic photosynthetically active radiation generated by the FluoroPen and 0.5 assumes that the photons absorbed are equally partitioned between PSII and PSI (Genty et al., 1989). Without knowledge of the actual amount of light being absorbed, fluorescence measurements can only be used as an approximation for electron transport (Beer et al., 1998a, 1998b; Runcie and Durako, 2004). 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 in order to quantify the characteristic parameters, α and ETR_{max} (Platt et al., 1980). The initial slope of the RLC (α) is a measure of the light harvesting efficiency of photosynthesis and the asymptote of the curve, the maximum rate of photosynthesis (ETR_{max}), is a measure of the capacity of the photosystems to utilize the absorbed light energy (Marshall et al., 2000). The onset of light saturation (E_k) was calculated as the ratio between ETR_{max} and α .

1.5. Pigment analysis by Gauss peak spectra (GPS) method

Leaves for pigment analysis were freeze-dried in the dark during 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 ultra-sound bath for 2 min. Extraction occurred at -20°C during 24 h in the dark to prevent pigment degradation. After extraction, samples were centrifuged at 4000 rpm for 15 min at 4°C . For pigment analysis the GPS method was used (Kupper et al., 2007). Samples were scanned in a dual beam spectrophotometer from 350 nm to 750 nm at 0.5 nm steps. The absorbance spectrum was introduced in the GPS fitting library, using Sigmaplot Software.

The employment of this library allowed to identify and quantify Chlorophyll *a* (MgChl *a*), Chlorophyll *b* (MgChl *b*), Pheophytin *a* (Pheo *a*), Antheraxanthin (Anthera), β -carotene, Lutein, Violoxanthin (Viola) and Zeaxanthin (Zea). In order to better evaluate the light harvesting and photo-protection mechanisms the De-Epoxidation State (DES) was calculated as:

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

1.6. Statistical analysis

Due to the lack of normality and homogeneity, the statistical analysis of the data was based in non-parametric tests. Spearman correlations were computed using Statistica 10 Software. In order to understand the interaction and the effect of the abiotic factor (sediment physico-chemistry and climate) a Principal Component Analysis (PCA) and a Similarity Percentage test (SIMPER) were performed using Primer 6 software (Clarke and Gorley, 2006).

2. Results

2.1. Climatic environment

Observing the light environment of the three studied transitional systems (Table 1) it is possible to observe a decrease in both the maximum radiation and the daily average radiation towards higher latitudes. Concomitantly, the same can be observed for all the variables relative to the air temperature with the exception of the thermal amplitude. For the later, it is possible to assess that in winter all the systems present rather similar thermal amplitudes around the 20°C , while in the remaining seasons Tagus and Mondego showed high thermal amplitudes around 30°C (Table 1). In the Aveiro coastal lagoon the difference between the maximum and minimum temperatures is smaller. Gathering the information from the air temperature and radiation it was possible to compute the Potential Evapotranspiration on a seasonal basis (Table 1). The results show that this variable had its highest values during the summer, with a decrease towards winter. Spatially Mondego estuary and Aveiro coastal lagoon exhibited similar values, while the Potential Evapotranspiration for Tagus estuary was rather low, when compared with the previous ones. Once again, similarities were observed between Tagus and Mondego estuaries where the

Table 1
Climatic and environmental data for the three studied estuaries, available from snirh.inag.pt.

	Radiation ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)		Air temperature ($^\circ\text{C}$)				Air relative humidity (%)	Precipitation (mm/day)		Potential evapotranspiration
	Average midday maximum	Average	Max	Min	Average	Thermal amplitude	Average	Average maximum	Average	Average
Tagus Estuary										
Spring	1007 \pm 158	195 \pm 44	40.9	-5.1	15.2 \pm 2.2	31.3 \pm 4.7	71.1 \pm 3.2	21.8 \pm 3.2	0.09 \pm 0.03	6.7 \pm 0.2
Summer	981 \pm 53	247 \pm 19	42.7	-9.3	21.8 \pm 1.1	29.9 \pm 0.9	63.7 \pm 1.9	7.8 \pm 5.6	0.01 \pm 0.01	9.9 \pm 1.1
Autumn	908 \pm 262	125 \pm 44	42	-0.1	17.4 \pm 3.9	30.7 \pm 5.6	72.8 \pm 6.1	28.0 \pm 11.2	0.1 \pm 0.05	4.6 \pm 0.4
Winter	795 \pm 382	82 \pm 21	35	-9.1	10.6 \pm 0.4	21.1 \pm 4.3	80.3 \pm 1.6	22.4 \pm 8.5	0.1 \pm 0.0	2.3 \pm 0.1
Mondego Estuary										
Spring	924 \pm 106	183 \pm 39	34.5	-3	14.1 \pm 2.1	31.5 \pm 2.5	76.3 \pm 1.2	17.9 \pm 4.9	0.08 \pm 0.02	14.1 \pm 0.9
Summer	983 \pm 57	229 \pm 12	39.5	-3.8	20.3 \pm 0.6	35.7 \pm 4.0	75.3 \pm 0.6	18.5 \pm 18.2	0.03 \pm 0.01	20.2 \pm 2.3
Autumn	710 \pm 134	125 \pm 30	36.7	-3	15.7 \pm 3.7	33.7 \pm 3.4	79.5 \pm 2.4	21.5 \pm 4.3	0.12 \pm 0.06	15.7 \pm 1.4
Winter	809 \pm 299	97 \pm 22	23.1	-3.8	9.3 \pm 0.4	19.3 \pm 2.7	83.7 \pm 2.4	18.5 \pm 8.6	0.12 \pm 0.01	10.0 \pm 1.6
Aveiro Coastal Lagoon										
Spring	925 \pm 130	173 \pm 43	30.6	3.2	13.8 \pm 1.4	27.4 \pm 1.2	92.9 \pm 4.4	16.6 \pm 7.0	0.10 \pm 0.03	13.8 \pm 0.9
Summer	954 \pm 47	239 \pm 16	31.9	10.7	17.7 \pm 0.2	21.2 \pm 4.2	99.1 \pm 0.6	22.0 \pm 10.7	0.05 \pm 0.01	17.7 \pm 1.1
Autumn	648 \pm 161	118 \pm 52	32.5	1.7	15.9 \pm 2.4	30.8 \pm 1.6	95.4 \pm 2.3	18.2 \pm 2.9	0.16 \pm 0.07	15.9 \pm 1.3
Winter	501 \pm 108	71 \pm 21	21.1	-1.1	10.7 \pm 0.5	20 \pm 1.8	92.8 \pm 1.3	14.4 \pm 1.7	0.15 \pm 0.01	11.4 \pm 0.8

air relative humidity was around 70–80%, while in Aveiro coastal lagoon the average seasonal relative humidity was always above 90%. Regarding the precipitation data, calculated from a 10-year time series, it showed very similar values among the three monitored systems (Table 1).

2.2. Sediment physical-chemistry

Regarding sediment water content (Fig. 2) it is recognized that it increases towards the wet seasons, with the sediments collected at Aveiro coastal lagoon showing the highest percentages of water content, while Tagus estuary salt marsh sediments showed the lowest water contents. As for the sediment organic matter content (Fig. 2), the opposite trend was observed, with Tagus estuary having the highest percentages of organic matter (with maximum values in spring and autumn) followed by the rhizosediments collected at Aveiro coastal lagoon (with maximum values in the summer).

Mondego estuary sediments showed a rather constant value of organic matter content, during the studied period. Pore water salinity (Fig. 2) from Tagus and Mondego estuaries showed a seasonal variation, increasing from spring towards the summer dropping back in the winter season. As for Aveiro coastal lagoon sediments, it was found that although it had some fluctuations, there were no significant variations among the seasons. As for sediment total N content (Fig. 2), it was observed that, with the exception of Tagus sediments, there is a generalized trend of increasing N content from spring towards summer, dropping in the autumn and again increasing during winter. Sediment pH was relatively stable among seasons and marshes (Fig. 2).

2.3. Biomass production

For the biomass production it was found that the three analysed ecotypes have different aboveground biomass production seasonal

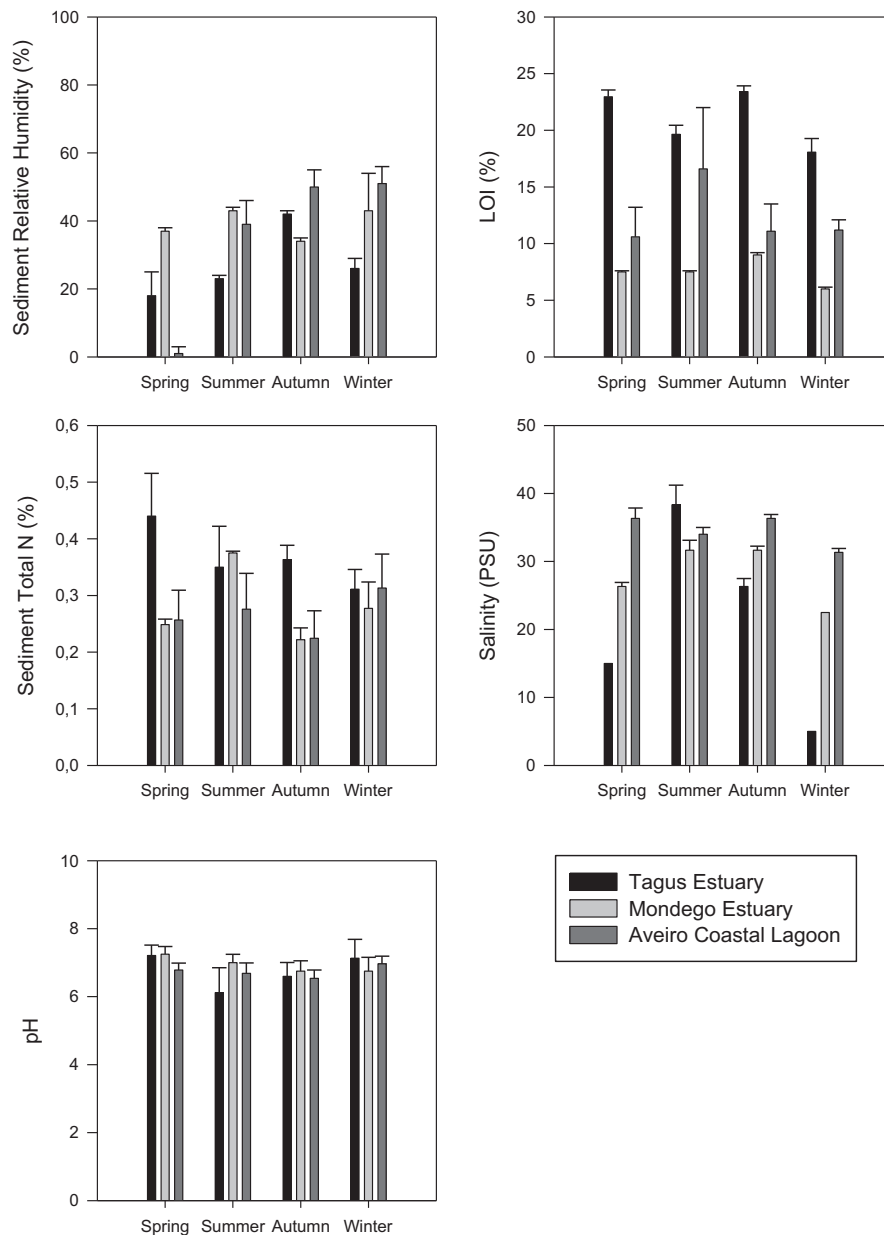


Fig. 2. *Spartina maritima* sediment physico-chemical characteristics seasonal variation in the three studied systems ($n = 6$, average \pm standard deviation).

patterns (Fig. 3). While Tagus *Spartina maritima* showed two peaks of aboveground biomass in spring and autumn, Mondego ecotype showed only one peak of biomass production during the studied period. As for Aveiro ecotype, it did not show any evident seasonal pattern. Regarding root biomass production, only the Mondego ecotype followed the same pattern as for the aboveground biomass. Tagus estuary ecotype showed a belowground biomass peak during summer, while the Aveiro coastal lagoon population of *S. maritima* showed constantly high belowground biomasses throughout all the year, with the exception of the autumn (Fig. 3). The same can be observed when considering total biomass production, due to the high contribution of the belowground biomass for this value, as reinforced by the R/S ratio.

2.4. PSII photochemistry

Considering the operational PSII quantum efficiency (light adapted state), both the Tagus and Mondego ecotypes showed a peak of operational PSII activity during spring, slowly decreasing towards autumn, where it abruptly decreases until its minimum value in winter (Fig. 4). Contrarily, Aveiro ecotype, showed a marked increase from spring towards winter, where the PSII operational efficiency had its maximum. Considering the maximum PSII efficiency (dark adapted state), these patterns are not so evident, although the same seasonal trend can be observed in all three ecotypes (Fig. 4). Regarding the light harvesting efficiency given by α (Fig. 4), it is possible to observe that both Tagus and

Mondego ecotypes showed constant α values from spring to autumn and an absence of light harvesting ability during the winter season. As for the Aveiro ecotype, it was possible to observe an increase of the light harvesting ability from spring towards its maximum in the summer, followed by a small decrease until the winter and spring seasons.

2.5. Pigment profile

Both chlorophylls showed similar seasonal patterns within each analysed ecotype (Fig. 5). Tagus estuary *Spartina maritima* showed a peak of both these pigments at the end of the growing season (autumn), while the Mondego population exhibited its chlorophyll peak during summer. Regarding the Aveiro coastal lagoon ecotype, it showed its chlorophyll maximum concentration during spring. Considering the Chl a/b ratio (Fig. 6) both the Tagus and Mondego populations exhibited the same trend, ratio values increasing towards summer, followed by a decrease to the levels observed in spring. Aveiro individuals showed an inverse seasonal pattern with lower values during summer, and very high Chl a/b values in the remaining seasons. Regarding the carotenoids (Fig. 5), β -carotene, it showed very constant values along all the year in Tagus and Mondego populations, decreasing greatly only during winter. The highest concentrations of β -carotene were found in the Aveiro ecotype. In this population, β -carotene showed constant concentrations during the warm seasons, decreasing in the autumn and increasing back during winter. The remaining analysed pigments

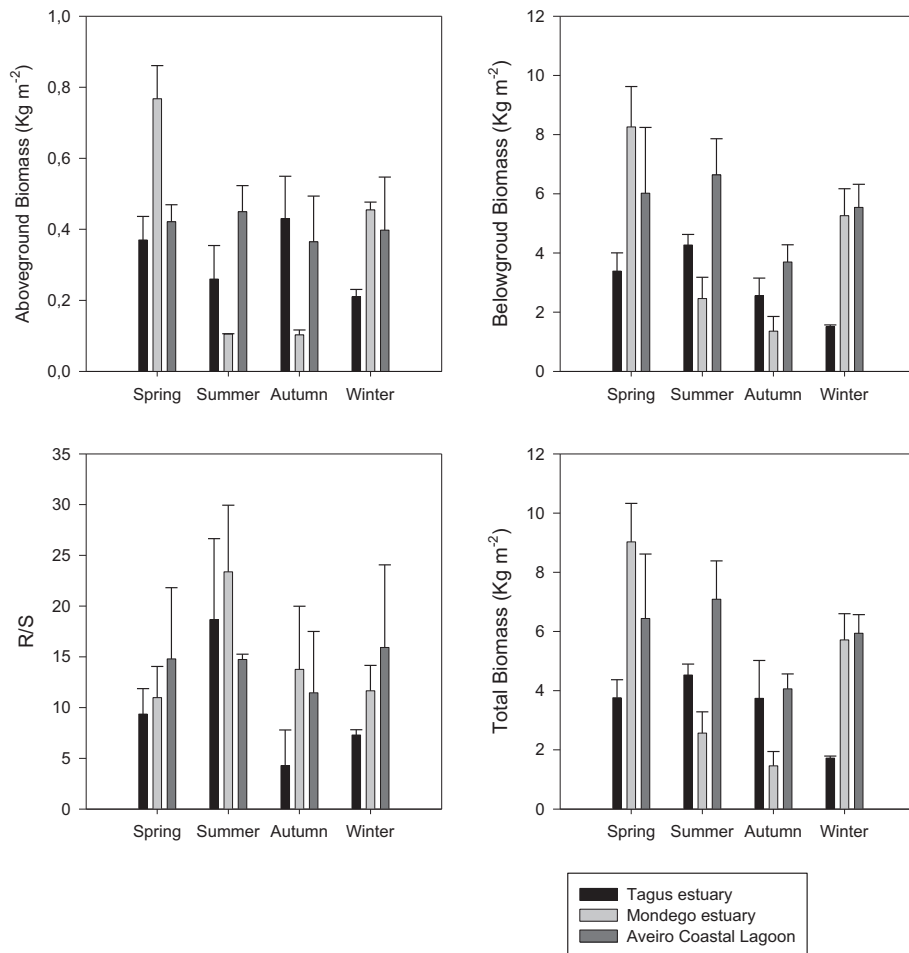


Fig. 3. *Spartina maritima* biomass production and R/S ratio seasonal variation in the three studied systems (n = 5, average ± standard deviation).

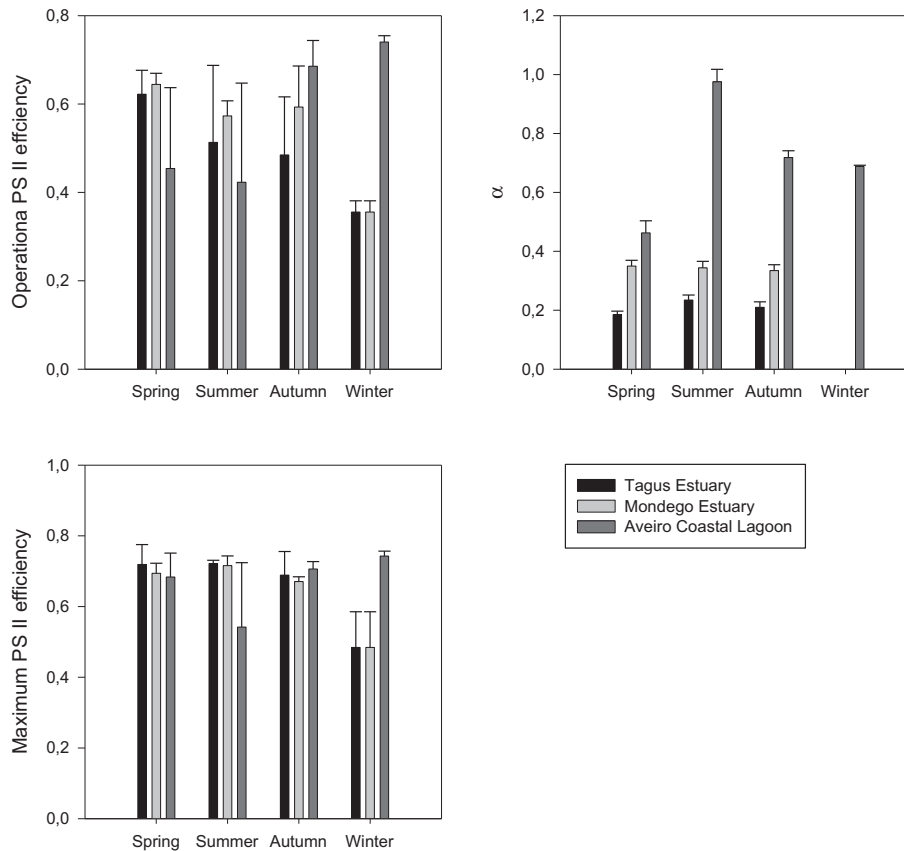


Fig. 4. PSII photochemical parameters seasonal variation in the three studied systems ($n = 6$, average \pm standard deviation).

integrate the xanthophyll cycle, thus its seasonal interpretation acquires a more accurate biological sense observing not their concentration, but the DES index, due to its inter-correlated variations (Fig. 6). At this point it was observed that the population from Tagus estuary showed constantly higher values, only decreasing in summer. Mondego and Aveiro ecotypes showed lower values decreasing from spring towards the winter, although Mondego individuals DES index was higher than that verified in the leaves collected at Aveiro coastal lagoon.

2.6. Abiotic factors versus photosynthetic traits

Considering the overall correlation coefficients between the photo-biological variables previously described and the climatic and environmental data (Table 2), some important interactions were observed. Biomass production is mostly associated with the thermal environment to which the population is subjected as well as the air relative humidity. For the PSII photochemistry it could be observed that the maximum efficiency and some of the associated parameters are mostly dependent on the sediment nitrogen concentration and humidity (as indirect measure of water availability), but also with the average air temperature and thermal amplitude (Table 2). On the other hand the relative air humidity appear to have a negative effect on the photochemical parameters. If considering only the operational PSII efficiency and associated parameters, there is a strong positive correlation with the sediment pore water salinity, while the average precipitation seems to have the opposite effect on these parameters. Again, and if considering the light harvesting efficiency (α), the pore water salinity, air relative humidity and minimum temperature are the parameters that more influence. As for the pigment profile, both Chl a and b are

similarly correlated with the potential evapotranspiration but negatively with the average precipitation. Pore water salinity and radiation also correlated positively with Chl a concentration in leaves. As for Chl b, the average radiation at midday appear as another environmental factor influencing positively this pigment concentration. On the other hand air relative humidity was negatively correlated to Chl b (Table 2). Considering the DES correlation with the abiotic factors was found that the potential evapotranspiration, radiation at midday, max air temperature and sediment N pool showed highly-positive correlations with this index (Table 2). Pore water salinity, sediment relative water content, air relative humidity, minimum temperature and average precipitation correlated negatively with the DES index. As for the Chl a/b ratio, it seems to be negatively influenced by the maximum air temperature, thermal amplitude and average monthly precipitation, but also by the sediment pH. On the other hand, pore water salinity along with air relative humidity and minimum monthly temperature was positively correlated.

Crossing the PCA (Figs. 7 and 8) results with the SIMPER analysis, showed an evident separation of the samples collected in Aveiro coastal lagoon, highly associated to their photosynthetic efficiency parameters but also with the sediment pore water salinity. SIMPER analysis showed that Aveiro samples similarity is mostly due to the importance that the climatic variables have upon these samples photo-biological characteristics (average $d^2 = 0$). Another smaller but also evident group, is formed by the winter samples collected at both Tagus and Mondego estuaries. The formation of this group is mostly associated to the sediment average pH and mean monthly precipitation, but also photo-biologically by the antheraxanthin concentration and high DES index. It was also possible to see that concomitantly to what was described above,

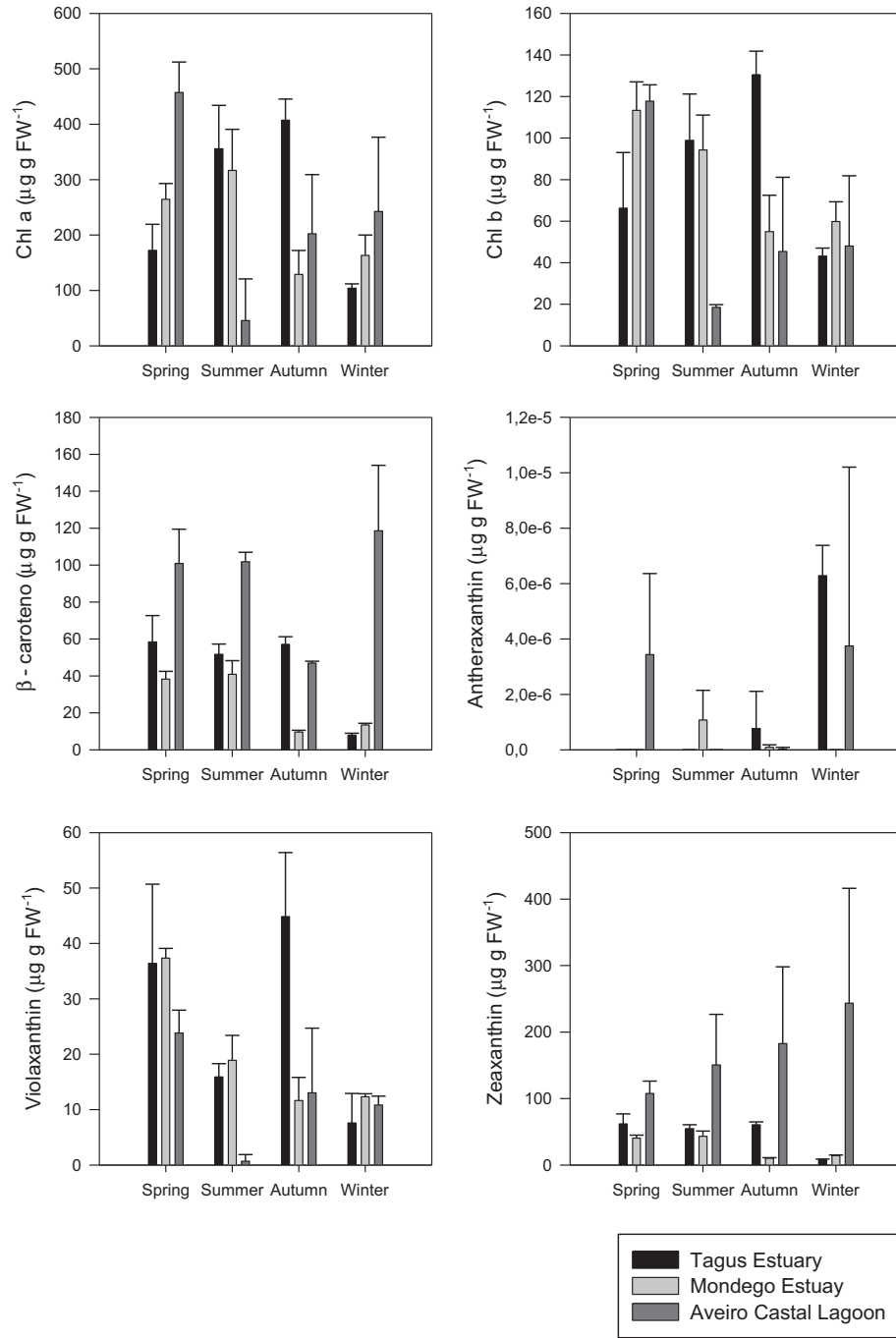


Fig. 5. Leaf chlorophyll and carotenoid concentrations seasonal variation in the three studied systems ($n = 6$, average \pm standard deviation).

this group is also influenced negatively by the lack of PSII light harvesting efficiencies and low associated parameters. The similarity analysis showed that this group was separated from the remaining Tagus and Mondego samples (average $d^2 = 98$) due to the influence of the potential evapotranspiration, average air temperature and radiation on the photosynthetic efficiencies, affecting particularly the light adapted maximum and variable fluorescences. Finally, a third group can be evidenced gathering all samples from both Tagus and Mondego estuaries from spring to autumn, mostly influenced by air thermal characteristics, maximum average midday radiation, but also by the sediment N concentration. On the other hand it is also observed that these samples share similar characteristics in chlorophylls and some

carotenoids (β -carotene and violaxanthin) concentrations. These last parameters showed the highest contribution to the high similarity observed in this group (average $d^2 = 41.6$).

3. Discussion

Observing the climatic data from the latitudinal gradient going from Tagus estuary to Aveiro coastal lagoon, there can be evidenced two distinct environments, more markedly in terms of air temperature. There is an evident relationship between biomass production and air temperature, as previously described (Giurgevich and Dunn, 1979; Drake, 1989; Nobel, 1991), as consequence of a C4-photosynthetic mechanism. Although it could be expected that

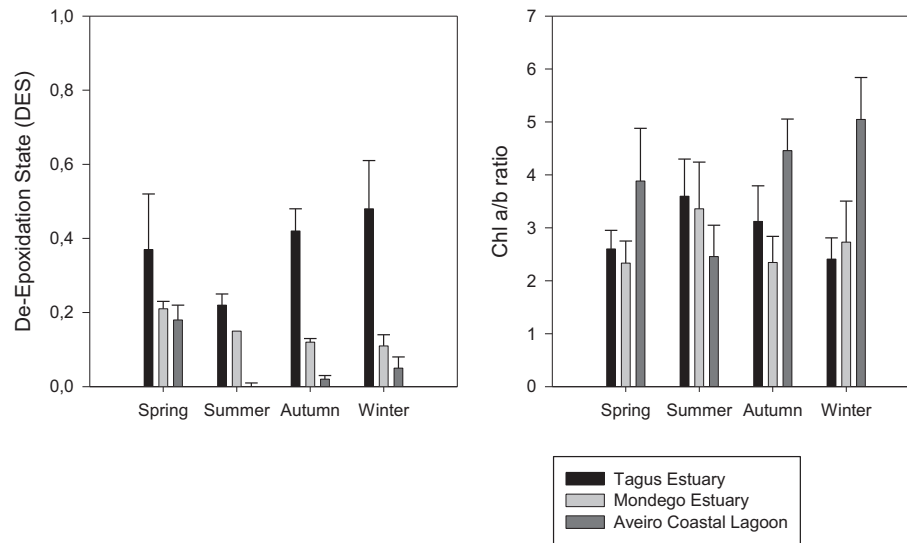


Fig. 6. De-epoxidation state (DES) and Chl a/b ratio seasonal pattern in the three studied systems ($n = 6$, average \pm standard deviation).

salinity could be a driving function for plant biomass production, it did not appear directly related to this parameter, as seen before for *Spartina alterniflora* (Cunha et al., 2005). Furthermore, although pore water salinity did not show any relationship with root biomass, this could be masked by precipitation, since this showed an evident correlation with root biomass. Both these influences are more evident if the PSII light-harvesting mechanism itself is analyzed. At this level there is an evident interaction between pore water salinity, precipitation and air humidity with the PSII efficiencies of *Spartina maritima*. From the data it is evident that salinity, at least under the observed range of salinities, affects positively PSII photochemistry. Belonging to a genus of obligatory halophytes (Koyro and Huchzermeyer, 2004), an increase of precipitation accompanied by a normal increase in air relative humidity would lead to a normal decrease in pore water salinity by dilution, thus affecting the halophytic PSII efficiencies of *S. maritima* (Mateos-Naranjo et al., 2010). Temperature can both act directly increasing the photosynthetic mechanism (and thus PSII efficiency) at least at temperatures below 35 °C (Cunha et al., 2005) or by increasing pore water salinity due to evaporation, again favoring

PSII photochemistry (Mateos-Naranjo et al., 2010). Furthermore, data points to an increase in the light harvesting capacity with the increase of the minimum air temperature, reinforcing once again the important role of the thermal environment (Gray and Mogg, 2001). Sediment N concentration appeared as another evident factor affecting *S. maritima* PSII efficiencies, in particular the dark-adapted state corresponding to the maximum activity achievable. This is in accordance with previous studies that found similar data for other species from the genus *Spartina* (Madrid et al., 2012). In this study it was found that an increase in sediment N allowed *S. maritima* to incorporate higher amounts of N in its leaves, which correlated with high PSII maximum quantum yields. An elevated value of this parameter is indicative of an efficient and healthy photosynthetic PSII reaction center (Bilger et al., 1995; Maxwell and Johnson, 2000; Quigg et al., 2008). Similar studies (Mendelssohn and Postek, 1982; Pennings et al., 2002), suggested that *S. alterniflora* could absorb nitrogen as it becomes available in sediments under a wide range of environmental conditions. Another important fact that modulates PSII quantum efficiencies is obviously the light environment. When under light stress

Table 2

Spearman correlation coefficients between the environmental and biological data (* $p < 0.05$).

	N	pH	Sal	Water content	ET	Radiation	Average midday rad	% Hum	Average temp	Min temp	Max temp	Thermal amplitude	Max precipitation	Average precipitation
Aereal Biomass	-0.05	0.16	-0.12	0.14	-0.05	0.01	0.05	0.15	-0.23	0.33*	-0.31	-0.25	-0.11	0.02
Root Biomass	-0.26	0.01	0.29	0.12	0.11	0.19	0.11	0.34*	-0.13	0.36*	-0.52*	-0.34*	-0.52*	-0.14
F_0	0.27	-0.03	0.20	0.20	0.27	0.25	0.03	-0.33*	0.47*	-0.05	0.32	0.34*	-0.29	-0.20
F_M	0.34*	0.02	0.11	0.28	0.17	0.14	-0.01	-0.34*	0.39*	-0.04	0.30	0.38*	-0.25	-0.09
F_V	0.38*	0.04	0.06	0.29	0.12	0.08	-0.03	-0.37*	0.34*	-0.09	0.31	0.38*	-0.26	-0.05
QY	0.43*	0.18	-0.06	0.34*	0.06	0.01	-0.01	-0.33	0.24	-0.09	0.26	0.47*	-0.19	0.05
F_0'	-0.04	-0.06	0.26	0.09	0.09	0.11	-0.05	-0.07	0.19	0.08	0.05	0.23	-0.34*	0.06
F_M'	-0.09	-0.01	0.41*	0.13	0.07	0.09	-0.12	0.03	0.18	0.17	-0.05	0.24	-0.52*	0.13
F_V'	-0.06	0.01	0.44*	0.11	0.08	0.10	-0.10	0.04	0.17	0.17	-0.07	0.22	-0.58*	0.12
QY'	-0.05	-0.04	0.57*	0.14	0.05	0.06	-0.15	0.17	0.16	0.27	-0.17	0.08	-0.66*	0.15
α	-0.16	-0.29	0.58*	0.22	0.04	0.10	-0.10	0.51*	0.28	0.7*	-0.26	-0.11	-0.11	0.22
MgChl a	0.09	-0.11	0.41*	-0.05	0.38*	0.35*	0.33	-0.23	0.33	0.22	0.15	0.07	-0.31	-0.35*
MgChl b	0.07	0.02	0.15	-0.13	0.37*	0.33	0.34*	-0.42*	0.23	0.06	0.29	0.24	-0.15	-0.38*
Antheraxanthin	0.16	0.04	0.05	-0.05	-0.06	-0.12	0.01	0.07	-0.07	0.10	-0.06	0.02	-0.03	0.09
β carotene	0.22	-0.13	0.41*	0.07	0.09	0.09	0.11	0.06	0.15	0.38*	-0.10	-0.07	-0.38*	0.10
Violaxanthin	0.16	0.19	-0.01	-0.05	0.38*	0.33*	0.38*	-0.43*	0.22	0.09	0.33*	0.35*	-0.02	-0.36*
Zeaxanthin	0.04	-0.25	0.53*	0.22	0.08	0.10	0.01	0.35*	0.24	0.58*	-0.24	-0.13	-0.31	0.14
DES	0.50*	0.14	-0.36*	-0.40*	0.34*	0.29	0.49*	-0.83*	0.15	-0.76*	0.65*	0.24	0.07	-0.45*
Chl a/Chl b	-0.03	-0.34*	0.69*	0.31	0.04	0.06	-0.09	0.50*	0.28	0.52*	-0.36*	-0.35*	-0.39*	0.08

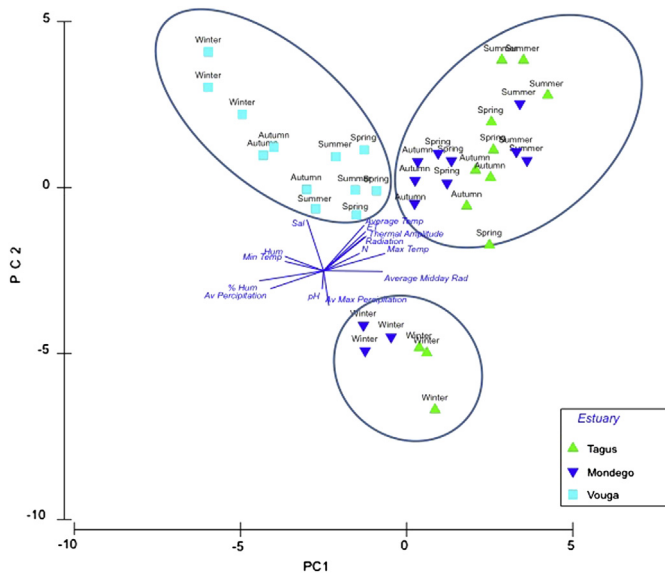


Fig. 7. PCA ordination of all samples according to the climatic and environmental data.

conditions, *S. maritima* xanthophyll cycle is activated as a protective measure, as confirmed by the increase in the *DES* index (Demmig-Adams and Adams, 1992). In colder seasons (autumn and winter) there is normally higher air humidity, lower temperatures, higher precipitation and consequently lower average midday radiation (mostly due higher cloud presence). Consequently, in these seasons the average light that reaches the photosystems decreases, reducing the probability of PSII light stress during these seasons, as it was verified observing the correlation between the *DES* index and these climatic variables. Along with light stress, also thermal stresses are responsible for triggering the xanthophyll cycle (Demmig-Adams and Adams, 1992). Similarly, increasing stress can also decrease the Chl a/b ratio, favoring the Chl b production as stress feedback instead of Chl a, the principal light harvesting pigment (Demmig-Adams and Adams, 1992). Although these physiological traits are in the basis of the differences observed between populations, after considering also the environmental

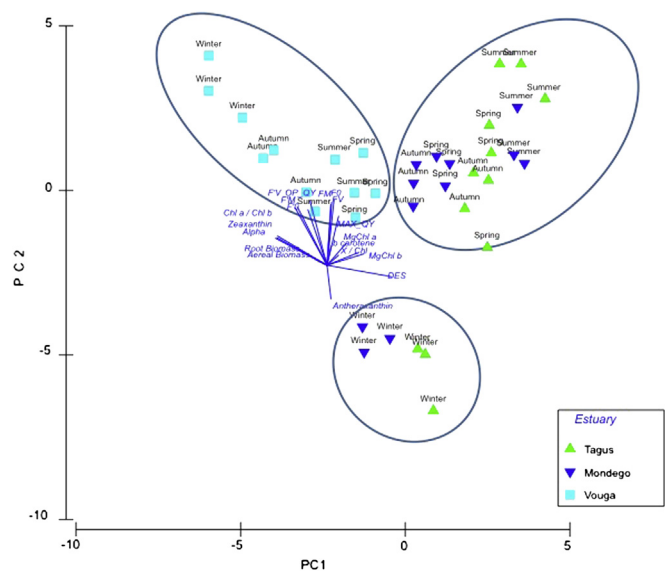


Fig. 8. PCA ordination of all samples according to the photosynthetic traits data.

data these differences acquire a new meaning describing the PSII efficiencies of *S. maritima* at different latitudinal environments. Three distinct groups of individuals are evident not only spatially but also seasonally. Although generally, the climatic variables explain most of the observed variations in *S. maritima* photobiology, overlooking the different groups assembled by the PCA, they could also explain most of the dissimilarities between samples. One of the more evident differences was the gathering of all Aveiro coastal lagoon samples. The basis of this similarity were the climatic variables, contributing to a large proximity among samples harvested at this transitional system. Considering these climatic variables, the climate at this latitude is more stable than in the central region of Portugal, with lower amplitudes and variations between seasons. Considering the photo-biological traits, namely the light harvesting capacity, which is inversely correlated with respiration (Taiz and Zeiger, 2002), it was found to be rather high and constant during almost all year in the individuals studied in Aveiro coastal lagoon. *S. maritima* has a C4 photosynthetic mechanism, which among other characteristics is temperature dependent, in particular daytime maximum temperature (Taiz and Zeiger, 2002). Furthermore, the respiratory mechanism is temperature dependent. In Aveiro coastal lagoon marshes there are no large variations of temperature over the year, neither maximum or in terms of thermal amplitude. Furthermore, the thermal range verified at this latitude is in agreement with the preferential thermal range for C4 species (Taiz and Zeiger, 2002), favoring the photosynthetic ability. The high dependency of this group with the light environment is reinforced by the low *DES* index and low antheraxanthin concentrations, supporting the elevated light harvesting capacities found in *S. maritima* from the Aveiro coastal lagoon. At this latitude it was found that the average maximum midday *PAR* was always lower than in the remaining systems, which along with the low energy dissipation (low *DES*), support the high efficiency of these individuals for light harvesting at these light intensities, without undergoing photo-inhibitory processes (Demmig-Adams and Adams, 1992). On the other hand, the individuals collected at Tagus and Mondego salt marshes during the winter season, showed high *DES*, although the light intensity during this season was the lowest. These high *DES* values are in agreement with recent data from other C4-halophyte from the Mondego estuary, *Scirpus maritimus* (Duarte et al., 2012). Due to the very high light intensities verified during the spring-summer period and the low light values verified during the winter, this halophyte, suffers from chronic photo-inhibition. The same can be attributed to *S. maritima* individuals located at Tagus and Mondego estuaries. On the other hand, it was found that the high precipitation observed during this season and consequent decrease in salinity and pH increase, together with a decrease in the average minimum temperature, are the principal driving factors for the formation of this group of samples in the PCA. This group is mostly constituted by individuals with very low photosystem II efficiencies and light harvesting capacities, as well as high *DES* index. All these climatic factors during winter lead to a drift of the environmental conditions, away from the optimal for *S. maritima*. The dilution of pore water salinity is adverse to an obligatory halophyte such as *S. maritima* (Koyro and Huchzermeyer, 2004), having a consequent decrease in the efficiency of the photosystem II (Naidoo et al., 2012). Additionally, the decrease in the average minimum temperature is also an adverse factor for the C4 photosynthesis, which has its lower optimum at 8–10 °C (Taiz and Zeiger, 2002). Previous studies (Long, 1983) suggest that there is a reversible reduction in the CO₂ assimilation capacity, that along with the lack of an alternative pathway for the utilization of light-generated reducing power, may be on the basis of the chilling-dependent photo-inhibition of most of the C4 species in particular, from the genus *Spartina*. Some specific climatic

conditions can be defined for the warmer seasons (spring, summer and autumn first months), in particular an increase in average and maximum temperature and lower humidities with consequent higher evapotranspiration and higher light intensities. These climatic characteristics, allied with higher N concentrations in the sediments, provided the ideal conditions for chlorophyll production and increase in the light-adapted photochemical processes. As nitrogen is a structural element of chlorophyll and protein molecules, it becomes understandable that its sediment concentration affects the formation of chloroplasts and accumulation of chlorophyll in them (Daughtry et al., 2000; Tucker, 2004). Also the increase in the light intensities and number of light hours per day can also explain the higher amounts of chlorophyll detected during these seasons, as a mechanism for increasing the light harvest potential of the Mondego and Tagus individuals.

4. Conclusions

Climate driven events and conditions are well known drivers of plant species distribution. During this work, it became evident that within the same species, the climatic conditions can also have important roles modulating the photo-biological mechanisms. *Spartina maritima* populations from different latitudes showed differences in both biomass production and photo-biological traits, seasonal patterns and intensities. Furthermore, as *S. maritima* is one of the more abundant halophytes colonizing the Portuguese salt marshes, these modulatory mechanisms acquire increased importance. Air temperature and humidity as well as light environment proved to be some of the most important drivers of variability in the photo-biological traits among *S. maritima* populations. Considering a global change in the climate, along with the large abundance of this halophytic species, it becomes of major importance understand the key modulators of *S. maritima* photo-biology, for a better understanding of how climate change will affect their metabolism and thus the ecosystem services provided to the estuarine system.

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