Bioinvasion by Spartina Patens Alters Sediment Biogeochemical Functioning European Salt Marshes

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Abstract

Bioinvasions pose undeniable threats and trigger changes in salt marsh ecosystem functioning. In Mediterranean and Atlantic marshes, the invasion by *S. patens* contributed to added competitive pressure to endemic middle-upper marsh species such as *H. portulacoides*. The introduction of a new and aggressive non-indigenous species (NIS) is a game-changer for the marsh biogeochemical functioning. In the present study we explored effects of this bioinvasion on an array of different extracellular enzymatic activities (EEA) of salt marsh sediments colonized by NIS and endemic plant species and its impacts on the biogeochemical functioning of a large estuarine ecosystem. In *H. portulacoides* there was an evident control of the extracellular enzymatic activities (EEA) by the sediment physic-chemical traits, but these relationships were weaker and fewer in number when compared to the EEA and the sediment abiotic traits in the sediments colonized by *S. patens*. This indicates a prevalence of a plant effect over abiotic traits in controlling EEA. Disruption in biogeochemical functioning was evident in the sediments colonized by *S. patens*, with clear enzymatic activity peaks during spring and summer and with significantly higher values of several EEA during autumn and winter. These led to an acceleration of necromass decomposition processes and thus to reduced marsh storage and remediation capacity. *Spartina patens* also showed increased phosphatase, urease, and protease activity contributing to increased release of the inorganic phosphorous and nitrogen (ammonia) into the system, with possible consequences for its eutrophication status. Higher activity of carbon-based substrates decomposition allied to an increase in dehydrogenase activity (proxy to microbial abundance and respiration), will lead to an inevitable decrease in the carbon sink capacity of the sediments and reduction of the blue carbon storage ecosystem service. The sulfatase activity in the *S. patens* rhizosediments also showed a significant decrease during autumn, leading to reduced production of sulfate, sulfides and increased metal bioavailability. All these changes amount to a loss of the sink capacity of the system, in terms of eutrophication reduction through organic nitrogen and phosphorous retention, carbon sink due to the acceleration of the decomposition processes and decreased metal remediation capacity. Thus, the present work highlights the need to control this invasive species not only to prevent potential reductions in floristic diversity but also to prevent the loss of key biogeochemical services, that can impact the entire estuarine system.

Introduction

Salt marshes are among the most productive ecosystems on the planet, as key areas not only for wildlife habitat but also providing important functions and services, such as erosion and flood control, water quality maintenance (via assimilation and filtration), carbon sequestration, storm protection(Adams 2020; Duarte et al. 2021). These ecosystem services are provided by the halophyte vegetation that colonizes estuarine habitats. These plants can cope with the harsh physic-chemical environment and are resilient to submersion, increased salinity, low oxygen rhizosphere concentrations and high thermal amplitudes (Duarte et al. 2013a, 2014a, 2015a). Importantly, these plants also produce large amounts of biomass (Caçador et al. 2009; Duarte et al. 2010), sequestering considerable amounts of carbon and nutrients and acting as carbon sinks (Duarte et al. 2014b, 2021). Nevertheless, these plants also generate large

amounts of detritus (Duarte et al. 2010) that supply the surrounding food webs (Fonseca et al. 2019), but these detritus are also stored in the sediments as necromass, prone to microbial decomposition (Duarte et al. 2008, 2009; Freitas et al. 2014), a key process for biogeochemical cycling in estuarine systems. These microbial-mediated processes are influenced by various factors, including abiotic features (Duarte et al. 2008, 2014a), anthropogenic impacts (Duarte et al. 2013b), and vegetation type (Duarte et al. 2012, 2013b), and thus any change at this level will have impacts on the system biogeochemical functioning, namely in terms of biomass recycling and nutrient regeneration (Caçador et al. 2016). These decomposition processes are mediated by the activity of microbial extracellular enzymes, especially active in the rhizosphere of these halophyte species, that promote microbial activity through rhizosphere oxygenation and carbon substrate supply (Duarte et al. 2008). Several enzymes are present in these rhizosediments, aiming to decompose different necromass components, being enrolled in the key biogeochemical cycles of carbon (e.g., peroxidases, phenol oxidases and *N*- acetylglucosaminidase and b-glucosidases), nitrogen (e.g., proteases and urease), phosphorous (e.g., phosphatases) and sulfur (e.g., sulfatases), on which the whole estuarine ecosystem functioning is dependent (Duarte et al. 2008; Freitas et al. 2014; Caçador et al. 2016). Thus, the evaluation of these enzymatic biogeochemical cycling processes can provide insights into the ecosystem necromass recycling functions (Duarte et al. 2012, 2013b).

Beyond the well-known anthropogenic impacts to which these ecosystems are exposed (e.g. pollution, land claiming, eutrophication), nowadays Mediterranean salt marshes are also prone to a new stressor: non-indigenous species (NIS) bioinvasions (Duarte et al. 2015a; Baumel et al. 2016; Duarte et al. 2018b). More specifically, salt marsh invasion by NIS *Spartina* species (Baumel et al. 2016; Duarte et al. 2018b) has been reported to have significant impacts on saltmarsh ecosystems, both in terms of floristic biodiversity losses (Duarte et al. 2018b) and in terms of changes in the services provided by these ecosystems, such as its natural remediation capacity (Paredes-Páliz et al. 2018; Human et al. 2020). *Spartina patens* has been reported in several salt marshes along the Mediterranean and Atlantic coasts (Baumel et al. 2016). This species has a high tolerance to salinity (Duarte et al. 2015b), thermal extreme events (Duarte et al. 2016), and a tremendous tolerance to anthropogenic stressors (Duarte et al. 2018a; Cruz de Carvalho et al. 2020), being capable of colonizing the entire upper-middle marsh elevation gradient, making it a formidable competitor against native flora (Duarte et al. 2015a). The *Spartina* genus (also known as cordgrasses) is one of the most successful halophytes, being present in a wide range of latitudes across the globe, highlighting the plasticity and adaptative capacity of these species (Bortolus et al. 2019; Borges et al. 2021).

Due to its aggressive colonization, mostly due to its high photosynthetic rates and large biomass production (Duarte et al. 2015a), colonization by *Spartina patens* of the middle-upper areas of Mediterranean and neighboring Atlantic saltmarshes will inevitably displace the endemic *Halimione portulacoides*, the prevalent species present in the region (Caçador et al. 2013; Borges et al. 2021; Duarte and Caçador 2021). Therefore, it is key to evaluate how the NIS *S. patens* is potentially changing the biogeochemical cycling processes in salt marshes. Hence, the present work aims to depict the effects of this bioinvasion on the extracellular enzymatic activities (EEA) of salt marsh sediments where this NIS

occurs in comparison with the EEA in the rhizosediments colonized by the endemic *H. portulacoides* and evaluate its impacts on the biogeochemical functioning of a large estuarine ecosystem.

Material And Methods

Sample collection

Sampling occurred in April 2012 (Spring), August 2012 (Summer), November 2012 (Autumn) and January 2013 (Winter) in the Hortas salt marsh (Tejo Estuary, Portugal, Figure 1). The Hortas salt marsh is a young undisturbed salt marsh located within the Tejo Estuary Natural Reserve and thus provides an anthropogenic interference-free context (Duarte et al. 2015a). Sediment cores were collected in pure stands of *S. patens* and neighbouring pure stands *of H. portulacoides*. Each pure stand was considered as an experimental unit (replicate). Five stands were considered for all samplings and all five stands from each species were chosen considering their similar position along the marsh to minimize elevation-driven differences. PVC tubular cores were used to collect sediment samples and sliced in the field. The depth between 5 and 8 cm depth was always used as in previous studies this was found to be the layer with higher microbial activity and root density (Duarte et al. 2008, 2009). Sliced sediment samples were placed in refrigerated bags and transported to the laboratory, where they were frozen at -20°C until analysis.

Rhizosediment physic-chemical characterization

Sediment relative water content (RWC) was determined by drying sediment samples at 60°C until constant weight. Organic matter was determined by the loss on ignition (LOI) method, by burning 1 g of airdried sediment at 550°C for 3 h. Values of pH were measured in the field in freshly sliced sediment samples using a HANNA pH/mV (HI 9025) probe. Carbon and nitrogen content (%) were determined in dried pulverized sediment samples using a Flash EA 1112 Series elemental analyzer coupled online via Finningan conflo III interface to a Thermo delta V S mass spectrometer.

Rhizosediment extracellular enzymatic activities

All enzymatic determinations were carried out with colorimetric methods and the absorbances were read on a TECAN Absorbance Microplate Reader (SPECTRA Rainbow). Dehydrogenase activity (DH) was determined using the triphenyltetrazolium chloride (TTC) method according to Kazemi et al. (2017), immediately after sampling upon arrival to the laboratory. Briefly, approximately 5 g of fresh sediment were incubated with 5 ml of TTC solution (1%). Samples without the substrate were also prepared with 5 ml Tris-HCl buffer (100 mM) instead of the TTC solution. Incubation was performed at 30°C for 24 h. After incubation, 40 ml of acetone were added to each tube to stop the reaction and extract the formed coloured compound (Triphenyltetrazolium formazan, TPF). The tubes were shaken and kept in the dark for 2 h and centrifuged at 14,000 x g for 15 minutes, at 4°C. The clear supernatant absorbance was read at 546 nm and compared with a standard curve for TPF. Phenol oxidase (FOX), peroxidase (POX), *N*-acetylglucosaminidase (NACET), phosphatase (PHO), b-glucosidase (GLU) and sulfatase (SULF) were assayed according to Ravit et al. (2003) with minor modifications as described in Duarte et al. (2008) and

Freitas et al. (2014). Briefly, 75 ml of sodium acetate buffer (pH 5) was added to 5 g of fresh sediment and mixed for 1 min to obtain the sediment slurry. The substrates (5 mM) used were p-nitrophenyl-N-acetyl-d-glucosaminide, p-nitrophenyl-phosphate, p-nitrophenyl-glucoside and p-nitrophenyl-sulphate, respectively, for *N*-acetylglucosaminidase, phosphatase, b-glucosidase and sulfatase. Two millilitres of each substrate were added to 2 ml of slurry and incubated at 30°C with gentle agitation for 30 min (phosphatase), 60 min (sulfatase and b-glucosidase) and 2 h (*N*-acetylglucosaminidase). After incubation, samples were centrifuged at 6.530 x g for 15 min at 4°C, and 0.2 ml of 1 N NaOH was added to stop the reaction and reveal the p-nitrophenol (pNP) formed. The absorbance of the supernatant was read at 410 nm. The activity was expressed as mg of pNP released per gram sediment dry weight per hour. Phenol oxidase and peroxidase were assayed using 5 mM L-DOPA (l-3,4-dihydroxyphenylalanine) as substrate. Two millilitres were added to 2 ml of slurry (adding 0.1 ml of 0.3% H_2O_2 for peroxidase assay) and were incubated for 60 min for both enzymes. After incubation, samples were centrifuged at 6.530 x g for 15 min at 4°C. The absorbance of the supernatant was read at 460 nm and the absorbance of phenol oxidase was subtracted from the absorbance of total peroxidase to obtain the real value for peroxidase activity alone. The activity is expressed as mmol L-DOPA oxidized per gram sediment dry weight per hour. Protease (PROT) activity was assayed according to Ladd et al. (1976). Briefly, 1 g of fresh sediment was incubated with 5 ml of Tris (tris-hydroxymethyl-aminomethane) buffer (0.05 M, pH 8.1) and a 2% (w/v) casein solution, for 2 h at 50°C. After incubation, the reaction was stopped with 1 ml of trichloroacetic acid 17.5% (w/v) and centrifuged at 14,690 x g for 15 min, at 4°C. For photometric analysis, 1 ml of supernatant was added to 1 ml of Folin–Ciocalteu's phenol reagent (0.2 N) and 2.5 ml alkali reagent, and left to stand for 90 min. The absorbance was measured at 700 nm and compared with the calibration curve for tyrosine. The activity was expressed as mg tyrosine equivalents per gram sediment dry weight per hour. Urease (URE) activity was assayed according to Aşkın and Kızılkaya (2006). Briefly, approximately 2 grams of sediment were incubated with 3.75 ml of citrate buffer (50 mM, pH 6.7) and 5 ml of urea 10% (w/v). Samples without the substrate were also prepared to subtract the citrate-extractable ammonia. The incubation was made at 37°C for 3 h. After this period the samples were centrifuged at 4000 × g for 15 min, at 4 ◦C. One ml of supernatant was diluted to a final volume of 10 ml with distilled water. This solution was used for ammonia determination using the iodophenol-blue method (Aminot et al. 1997). Ammonia concentrations were read at 630 nm and urease activity expressed as mmol NH_4 formed per gram of sediment fresh weight per hour.

Statistical analysis

The seasonal patterns of the sediment physic-chemical features and extracellular enzymatic activities were already evaluated and discussed in previous papers (Duarte et al. 2008, 2009, 2015a), and thus the statistical analysis of the present work focused only on a season by season comparison between the variables measured in the sediments colonized by *H. portulacoides* and *S. patens*. All statistical analyses were computed in R-studio (2021.09.0 Build 351). Differences among rhizosediments colonized by each of the surveyed species were evaluated through Kruskal–Wallis tests, performed using the 'ggsignif' package. Principal Component Analysis was performed using the 'ggfortify' package. Spearman

correlations were attained using the 'corrplot' package. A statistical significance level at $p < 0.05$ was considered in all tests.

Results

Rhizosediment physic-chemical characterization

Regarding the sediment relative water content (Figure 2, RWC), significant differences between the sediments of the two analyzed species were only observed in winter, with the rhizosediments colonized by *S. patens* presenting lower RWC values. On the other hand, the pH values (Figure 2) of the sediments colonized by this NIS showed higher values than the ones measured in *H. portulacoides* rhizosediments during the spring season. In terms of rhizosediment organic matter content (here evaluated through the loss on ignition, LOI), these were found to be substantially higher in the area colonized by *H. portulacoides* during spring, summer and winter, when compared to *S. patens* rhizosediments (Figure 2). Rhizosediment total carbon and nitrogen contents were found to be higher during all seasons in the sediments colonized by the NIS *S. patens* when compared to *H. portulacoides* rhizosediments (Figure 2). Analyzing the C/N ratio (Figure 2), the opposite trend was observed, with the sediments colonized by *H. portulacoides* showing higher values of this ratio during all seasons, in comparison with *S. patens* rhizosediments.

Rhizosediment extracellular enzymatic activities

Regarding the extracellular enzymatic activities (EEA) of the analyzed rhizosediments, several significant differences could be observed (Figure 3). Dehydrogenase EEA (DH, Figure 3) differed between rhizosediments of the two surveyed species only during Summer, with the rhizosediments colonized by NIS *S. patens* presenting significantly lower values of activity of this enzyme. On the other hand, Peroxidase EEA (POX, Figure 3), showed significantly higher activity levels during winter in the rhizosediments colonized by *S. patens*, when compared to the sediments from the native congener. During summer and autumn, phenol oxidase (FOX, Figure 3), was more active in the rhizosediments colonized by *S. patens*, in comparison to the ones colonized by *H. portulacoides*. During winter the inverse trend was observed. Regarding phosphatase activity (PHO, Figure 3), it was significantly higher in the rhizosediments colonized by *S. patens* when compared to the sediments collected in *H. portulacoides* monospecific stands. The hydrolase β-glucosidase (GLU, Figure 3) was more active during summer and winter sampling in the sediments colonized by *S. patens*. This same trend was found for *N*-acetylglucosaminidase (NACET, Figure 3), with this enzyme also showing higher activities in the sediments colonized by *S. patens* during spring. Analyzing sulfatase EEA (SULF, Figure 3), it was more active in the rhizosediments colonized by the endemic *H. portulacoides* during the autumn season when compared to the rhizosediments colonized by the NIS *S. patens*. During this season, protease activity (PROT, Figure 3) also showed higher activity levels in the rhizosediments colonized by *S. patens*, in comparison to its endemic congener. Finally, urease (URE, Figure 3) was more active during all seasons in the rhizosediments colonized by the NIS *S. patens*.

Rhizosediment biogeochemical profiles

The relationships between the extracellular enzymatic activities and the sediment physic-chemical traits in the rhizosediments colonized by *H. portulacoides* (Figure 4A) and by *S. patens* are highlighted in Figure 4A-B, respectively. In the rhizosediments colonized by *H. portulacoides*, all enzymes, except for protease and urease, showed significant correlations with the analyzed physic-chemical traits. Specifically, the relative water content was negatively correlated with dehydrogenase (DH), peroxidase (POX) and phenol oxidase (FOX) activities. The two latter enzymes also showed a significantly negative correlation with the sediment pH, total carbon and nitrogen. Sediment organic matter showed a positive and significant correlation with dehydrogenase (DH), peroxidase (POX) and phenol oxidase (FOX) activities, whilst phosphatase (PHO), b-glucosidase (GLU), *N*-acetylglucosaminidase (NACET) and protease (PROT), showed strong negative correlations with the rhizosediment organic matter (LOI). Moreover, sulfatase activity also showed a positive strong correlation with sediment RWC and total nitrogen, and an inverse significant correlation with the rhizosediment C/N ratio. Analyzing these same trends between enzymatic and physic-chemical traits in *S. patens* rhizosediments, it was possible to observe that there were fewer and weaker significant correlations (Figure 4B). Sediment water content (RWC) showed an inverse significant correlation with peroxidase (POX), phosphatase (PHO) and sulfatase (SULF) activities, whilst dehydrogenase (DH) activity was enhanced by RWC. Sediment pH showed very similar relationships with the analyzed EEAs, being positively correlated with dehydrogenase (DH) and protease activities, and negatively correlated with peroxidase (POX), phosphatase (PHO), b-glucosidase (GLU), *N*-acetylglucosaminidase (NACET) and sulfatase (SULF) activities. None of the evaluated EEAs showed any significant correlation with *S. patens* rhizosediment organic matter (LOI). Sediment total carbon was significantly correlated with dehydrogenase (DH) activity and inversely correlated with the peroxidase (POX), phosphatase (PHO) and sulfatase (SULF) activities. *Spartina patens* rhizosediment total N and C/N ratio showed a negative correlation with dehydrogenase (DH) activity. Additionally, the sediment C/N ratio showed a strong positive correlation with phosphatase (PHO), *N*-acetylglucosaminidase (NACET) and sulfatase (SULF) activities.

Analyzing both sediments physic-chemical and enzymatic traits as multivariate biogeochemical profiles (Figure 5), using a principal component analysis (PCA), several sample clusters were observed. The first PCA biplot showed a clear separation between the sediments colonized by the endemic *H. portulacoides* and the NIS *S. patens*, with the latter associated with the majority of the EEAs analyzed in this study (Figure 5A). On the other hand, *H. portulacoides* biogeochemical profiles appear highly related to sediment dehydrogenase (DH) activity, relative water (RWC) and organic matter (LOI) contents (Figure 5A). Analyzing the second biplot which also accounts for seasonal variation, a clear separation of rhizosediments by the colonizing species is also evident, but several seasonal clusters are also observable (Figure 5B). The seasonal clusters formed by the samples collected from *H. portulacoides* rhizosphere had a lower dispersion (higher similarity) when compared to the seasonal clusters formed by the samples collected from *S. patens* rhizosphere, in all periods considered. The

exception to this is the cluster formed by *S. patens* rhizosediment samples collected during winter, which showed a higher degree of similarity among them.

Discussion

Bioinvasions trigger undeniable changes in ecosystem functioning of estuarine and coastal ecosystems(Carlton 1996; Chakraborty 2019). Salt marshes are no exception, with *Spartina* bioinvasions occurring all over the globe, leading to significant changes in the wetland systems, namely at the floristic biodiversity level (Daehler and Strong 1996; Zhong-Yi et al. 2004; Li et al. 2009; Baumel et al. 2016; Duarte et al. 2018b), and with consequent effects at the ecosystem services level (e.g., Human et al. 2020). In Mediterranean and Atlantic marshes, invasion by *S. patens* has been putting middle-upper marsh endemic species such as *H. portulacoides* under increased competitive pressure. Spartina species are characterized by high biomass production due to their highly efficient C4 photosynthetic mechanism, and strong rhizomatous radicular system that allows them to efficiently capture nutrients (Cheng et al. 2006). But in the Tejo estuary, *H. portulacoides*, a C3 shrub plant, is the most abundant species in the middle-upper marsh (Duarte et al. 2013c). But as *S. patens* generate fewer necromass (48-52% of the total biomass produced) (Vera et al. 2009) when compared to the endemic *H. portulacoides* (Caçador et al. 2009; Duarte et al. 2010), bioinvasions by *S. patens* decrease the necromass input to the system and inevitably affecting the decomposition and biogeochemical cycling process occurring in the salt marsh sediments. This biogeochemical cycling is known to be modulated by the colonizing species (Costa et al. 2007; Duarte et al. 2008, 2009) along the salt marsh floristic inventory. The introduction of a new and aggressive NIS is a game-changer for the marsh biogeochemical functioning. This is demonstrated when analyzing the relationship between the EEA profiles and the physic-chemical characteristics in the sediments colonized by both species. While in *H. portulacoides* there is an evident control of the EEA by the sediment physico-chemical traits (high number of significant correlations observed), these relationships are weaker and in fewer number when comparing the EEA and the sediment abiotic traits in the sediments colonized by *S. patens*. This indicates a prevalence of the plant effect over the abiotic traits in controlling the extracellular enzymatic activities. In fact, the highly active photosynthetic mechanism present in the C4 *S. patens*, allied to a dense rhizospheric system, promotes oxygen input to the sediments but also the exudation of low molecular weight organic acids (LMWOA), known to stimulate the rhizosphere activity, namely at the microbial level (Duarte et al. 2009, 2011).

Mediterranean salt marsh necromass production has its peak at the end of the growing season (late summer and autumn), concomitant with the peak extracellular enzymatic activity normally observed in the rhizosediments of the endemic halophyte species (Duarte et al. 2008, 2009, 2010; Caçador et al. 2009). This allows the system to recycle the organic input from the necromass plants, into inorganic forms bioavailable for the primary producers to incorporate into their biomass in the next growing season (Duarte et al. 2008; Freitas et al. 2014). When observing the enzymatic activity peaks in the sediments colonized by *S. patens*, the onset of biogeochemical functioning disruption is evident. In the sediments colonized by this NIS, the activity peaks occurred during spring and summer (observed e.g. for *N*-acetylglucosaminidase, urease, phenol oxidase and b-glucosidase activities), evidencing a temporal

disruption compared to the seasonal pattern associated with the endemic species. Moreover, during the cold seasons (autumn and winter) several extracellular enzymatic activities showed significantly higher values in *S. patens* rhizosediments. If on one hand, there is a temporal disruption of the biogeochemical recycling processes, on the other, there is a very active decomposition system occurring during the cold seasons. This completely shifts the marsh biogeochemical functioning. Salt marshes are considered efficient organic matter sinks, retaining large amounts of the so-called blue carbon, nitrogen and phosphorous (Caçador et al. 2016; Geraldi et al. 2019; Duarte et al. 2021), not only due to their high productivity but also due to their low decomposition rates, especially when compared to other terrestrial ecosystems (Pereira et al. 2007). This allows marshes to retain, in the form of recalcitrant organic matter, large amounts of necromass, storing high amounts of carbon and reducing the eutrophication loading of the estuarine systems where they are present (Caçador et al. 2004; Duarte et al. 2021). This extracellular enzymatic activity observed in the sediments colonized by *S. patens* during the warmer seasons (spring and summer) alongside the comparatively higher activity assessed during the cold seasons, has as an ultimate consequence the acceleration of the necromass decomposition processes and thus a reduced marsh storage and remediation capacity (Duarte et al. 2008).

Analyzing the extracellular enzymatic activity profiles, the abovementioned facts acquire added importance when associated with each of the biogeochemical cycles for which these ecosystems play a key role. Observing *S. patens* rhizosediments, phosphatase activity peaks during winter, with values far above the ones observed in the sediments colonized by the endemic *H. portulacoides*, points to an enhanced release of the inorganic phosphorous into the system, with potential consequences in the eutrophication degree of the estuary. Previous studies have shown there is also a clear relationship between the phosphatase activity and the inorganic phosphorous generation in the vegetated marsh rhizosediments, with vegetated sediments acting as phosphorus sinks, with the recycling of the organic forms of this element being modulated by the plant species present (Freitas et al. 2014). Thus, this change in colonization promoted by *S. patens* has potential consequences on the ecosystem inorganic phosphorous load. In line with this, it could also be observed a higher urease activity year-round in the sediments colonized by the NIS, as well as a peak of proteasic activity during autumn. Urease and protease act in the hydrolysis of organic to inorganic nitrogen, the former using urea-type substrates and the latter simple peptidic substrates (Caravaca et al. 2005). These enzymes produce inorganic nitrogen forms, namely ammonia, one of the key compounds, that when in elevated concentrations is responsible for eutrophication in estuarine systems (Domingues et al. 2011). Moreover, this is not the preferred form of inorganic nitrogen assimilable by phytoplankton (Domingues et al. 2011), nor by halophytic vegetation (Stewart et al. 1972), thus no phytoremediation process will filter this increase ammonia concentration.

Impacts on carbon biogeochemical cycling is also a matter of concern in sediments colonized by *S. patens*. The two oxidoreductases (POX and FOX) showed higher activities in the sediments colonized by the NIS, in more than one of the sampling seasons, along with high values of b-glucosamidase and N-acetylglucosaminidase. All these enzymes actively participate in the decomposition of carbon-based substrates, namely, phenolic compounds (e.g., originated from plant lignin decomposition), carbohydrates and chitin exoskeletons (Freeman et al. 2004; Duarte et al. 2008). The increase in these

enzymes activities increases the availability of carbon-based respiratory substrates, that end in carbon release from the sediments in the form of CO_2 from bacterial respiration (Alonso-Sáez et al. 2008). Moreover, these carbon decomposition activity peaks in *S. patens* rhizosediments were concomitant with an increase in the dehydrogenase activity. Dehydrogenase activity is considered to be a proxy of microbial biomass (Kelley et al. 2011). The higher activity of carbon-based substrates decomposition activity allied to this increase in dehydrogenase activity reinforces the need for carbon use for microbial heterotrophic growth and thus a correspondent increase in respiration, with an inevitable decrease in the carbon sink capacity of the sediments and reduction of the blue carbon storage ecosystem service (Duarte et al. 2021). Although sulfur storage in salt marshes is not among the most recognized ecosystem services provided by these habitats, changes in its biogeochemistry have tremendous implications in other key services such as metal remediation (Duarte et al. 2008). Previous studies (e.g., van Hullebusch et al. 2005) indicate that high sulfatase activity promotes the conversion of the sulfate produced by this enzyme into sulfides by sulfate-reducing bacteria (SRB). The sulfides establish chemically stable bonds with heavy metals, maintaining these elements in a reduced form (residual fraction) for extended periods of time (Tabak et al. 2005), thus reducing the heavy metal bioavailable fraction. Previous studies focusing on the metal speciation in the rhizosediments of the NIS *S. patens* showed that these metals are present in higher proportions in the more bioavailable chemical fractions (lower residual fraction) (Human et al. 2020). This is aligns with our present findings. In Autumn, the period of typically higher organic matter recycling activity (Duarte et al. 2008, 2009), the sulfatase activity in the *S. patens* rhizosediments showed a significant decrease. This would lead to reduced production of sulfate, and consequently lower production in sulfides to reduce the metals present in these sediments into more stable forms (residual fraction) (Tabak et al. 2005; van Hullebusch et al. 2005; Duarte et al. 2008).

Conclusion

The threats of invasive species to salt marshes ecosystems are not limited to aboveground space competition processes but also to belowground processes, namely at the biogeochemical cycling level. The expansion of *S. patens* in Mediterranean marshes has led to the displacement of the endemic *H. portulacoides*. With this, there is an evident shift in the sediment's extracellular enzymatic activities, as well as a significant increase in several enzyme activities, all key processes to recycle organic matter. These biogeochemical profiles appear to be rather controlled by the plant more than by the sediment physic-chemical environment. All these changes lead to a loss of sink capacity of the system, namely in terms of eutrophication reduction and organic nitrogen and phosphorous retention, blue carbon sink due to the acceleration of decomposition processes, and decreased metal remediation capacity allied to reduction in the production of sulfate. Therefore, the present work highlights the need to control this invasive species, not only to prevent potential floristic diversity losses but also to prevent losses of key biogeochemical services, that can affect the entire estuarine system.

Declarations

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Data Availability

Not applicable

References

1. Adams JB (2020) Salt marsh at the tip of Africa: Patterns, processes and changes in response to climate change. Estuarine, Coastal and Shelf Science 237:106650. https://doi.org/10.1016/j.ecss.2020.106650
2. Alonso-Sáez L, Vázquez-Domínguez E, Cardelús C, et al (2008) Factors Controlling the Year-Round Variability in Carbon Flux Through Bacteria in a Coastal Marine System. Ecosystems 11:397–409. https://doi.org/10.1007/s10021-008-9129-0
3. Aminot A, Kirkwood DS, Kérouel R (1997) Determination of ammonia in seawater by the indophenol-blue method: Evaluation of the ICES NUTS I/C 5 questionnaire. Marine Chemistry 56:59–75. https://doi.org/10.1016/S0304-4203(96)00080-1
4. Aşkın T, Kızılkaya R (2006) Assessing spatial variability of soil enzyme activities in pasture topsoils using geostatistics. European Journal of Soil Biology 42:230–237. https://doi.org/10.1016/j.ejsobi.2006.02.002
5. Baumel A, Rousseau-Gueutin M, Sapienza-Bianchi C, et al (2016) Spartina versicolor Fabre: Another case of Spartina trans-Atlantic introduction? Biol Invasions 18:2123–2135. https://doi.org/10.1007/s10530-016-1128-z
6. Borges FO, Santos CP, Paula JR, et al (2021) Invasion and Extirpation Potential of Native and Invasive Spartina Species Under Climate Change. Frontiers in Marine Science 8:1157. https://doi.org/10.3389/fmars.2021.696333
7. Bortolus A, Adam P, Adams JBBJBJBJB, et al (2019) Supporting Spartina: Interdisciplinary perspective shows Spartina as a distinct solid genus. Ecology 100:1–6. https://doi.org/10.1002/ecy.2863
8. Caçador I, Caetano M, Duarte B, Vale C (2009) Stock and losses of trace metals from salt marsh plants. Marine Environmental Research 67:75–82. https://doi.org/10.1016/j.marenvres.2008.11.004
9. Caçador I, Costa ALAL, Vale C (2004) Carbon storage in tagus salt marsh sediments. Water, Air, and Soil Pollution: Focus 4:701–714. https://doi.org/10.1023/B:WAFO.0000028388.84544.ce
10. Caçador I, Duarte B, Marques JC, Sleimi N (2016) Carbon Mitigation: A Salt Marsh Ecosystem Service in Times of Change. In: Khan MA, Ozturk M, Gul B, Ahmed MZ (eds) Halophytes for Food Security in

Dry Lands. Academic Press, San Diego, pp 83–110

11. Caçador I, Neto JM, Duarte B, et al (2013) Development of an Angiosperm Quality Assessment Index (AQuA-Index) for ecological quality evaluation of Portuguese water bodies—A multi-metric approach. Ecological Indicators 25:141–148. https://doi.org/10.1016/j.ecolind.2012.09.021
12. Caravaca F, Alguacil MM, Torres P, Roldán A (2005) Plant type mediates rhizospheric microbial activities and soil aggregation in a semiarid Mediterranean salt marsh. Geoderma 124:375–382. https://doi.org/10.1016/j.geoderma.2004.05.010
13. Carlton JT (1996) Marine Bioinvasions: The Alteration of Marine Ecosystems by Nonindigenous Species. Oceanography 9:36–43
14. Chakraborty SK (2019) Bioinvasion and Environmental Perturbation: Synergistic Impact on Coastal–Mangrove Ecosystems of West Bengal, India. In: Makowski C, Finkl CW (eds) Impacts of Invasive Species on Coastal Environments: Coasts in Crisis. Springer International Publishing, Cham, pp 171–245
15. Cheng X, Luo Y, Chen J, et al (2006) Short-term C4 plant Spartina alterniflora invasions change the soil carbon in C3 plant-dominated tidal wetlands on a growing estuarine Island. Soil Biology and Biochemistry 38:3380–3386. https://doi.org/10.1016/j.soilbio.2006.05.016
16. Costa AL, Paixão SM, Caçador I, Carolino M (2007) CLPP and EEA profiles of microbial communities in salt marsh sediments. Journal of Soils and Sediments 7:418–425. https://doi.org/10.1065/jss2007.02.211
17. Cruz de Carvalho R, Feijão E, Kletschkus E, et al (2020) Halophyte bio-optical phenotyping: A multivariate photochemical pressure index (Multi-PPI) to classify salt marsh anthropogenic pressures levels. Ecological Indicators 119:106816. https://doi.org/10.1016/j.ecolind.2020.106816
18. Daehler CC, Strong DR (1996) Status, prediction and prevention of introduced cordgrass Spartina spp. invasions in Pacific estuaries, USA. Biological Conservation 78:51–58. https://doi.org/10.1016/0006-3207(96)00017-1
19. Domingues RB, Barbosa AB, Sommer U, Galvão HM (2011) Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication. Aquat Sci 73:331–343. https://doi.org/10.1007/s00027-011-0180-0
20. Duarte B, Almeida PRR, Caçador I (2009) Spartina maritima (cordgrass) rhizosediment extracellular enzymatic activity and its role in organic matter decomposition processes and metal speciation. Marine Ecology 30:65–73. https://doi.org/10.1111/j.1439-0485.2009.00326.x
21. Duarte B, Baeta A, Rousseau-Gueutin M, et al (2015a) A tale of two spartinas: Climatic, photobiological and isotopic insights on the fitness of non-indigenous versus native species. Estuarine, Coastal and Shelf Science 167:178–190. https://doi.org/10.1016/j.ecss.2015.06.015
22. Duarte B, Caçador I (2021) Iberian Halophytes as Agroecological Solutions for Degraded Lands and Biosaline Agriculture. Sustainability 13:1005. https://doi.org/10.3390/su13021005
23. Duarte B, Caetano M, Almeida PR, et al (2010) Accumulation and biological cycling of heavy metal in four salt marsh species, from Tagus estuary (Portugal). Environmental Pollution 158:1661–1668.

https://doi.org/10.1016/j.envpol.2009.12.004

24. Duarte B, Carreiras J, Caçador I (2021) Climate Change Impacts on Salt Marsh Blue Carbon, Nitrogen and Phosphorous Stocks and Ecosystem Services. Applied Sciences 11:1969. https://doi.org/10.3390/app11041969
25. Duarte B, Carreiras J, Pérez-Romero JA, et al (2018a) Halophyte fatty acids as biomarkers of anthropogenic-driven contamination in Mediterranean marshes: Sentinel species survey and development of an integrated biomarker response (IBR) index. Ecological Indicators 87:86–96. https://doi.org/10.1016/j.ecolind.2017.12.050
26. Duarte B, Couto T, Freitas J, et al (2013a) Abiotic modulation of Spartina maritima photobiology in different latitudinal populations. Estuarine, Coastal and Shelf Science 130:127–137. https://doi.org/10.1016/j.ecss.2013.02.008
27. Duarte B, Freitas J, Caçador I (2011) The role of organic acids in assisted phytoremediation processes of salt marsh sediments. Hydrobiologia 674:169–177. https://doi.org/10.1007/s10750-011-0731-3
28. Duarte B, Freitas J, Caçador I (2012) Sediment microbial activities and physic-chemistry as progress indicators of salt marsh restoration processes. Ecological Indicators 19:231–239. https://doi.org/10.1016/j.ecolind.2011.07.014
29. Duarte B, Freitas J, Couto T, et al (2013b) New multi-metric Salt Marsh Sediment Microbial Index (SSMI) application to salt marsh sediments ecological status assessment. Ecological Indicators 29:390–397. https://doi.org/10.1016/j.ecolind.2013.01.008
30. Duarte B, Freitas J, Valentim J, et al (2014a) Abiotic control modelling of salt marsh sediments respiratory CO2 fluxes: application to increasing temperature scenarios. Ecological Indicators 46:110–118. https://doi.org/10.1016/j.ecolind.2014.06.018
31. Duarte B, Marques JC, Caçador I (2016) Ecophysiological response of native and invasive Spartina species to extreme temperature events in Mediterranean marshes. Biol Invasions 18:2189–2205. https://doi.org/10.1007/s10530-015-0958-4
32. Duarte B, Mateos-Naranjo E, Goméz SR, et al (2018b) Cordgrass Invasions in Mediterranean Marshes: Past, Present and Future. In: Queiroz AI, Pooley S (eds) Histories of Bioinvasions in the Mediterranean. Springer International Publishing, Cham, pp 171–193
33. Duarte B, Reboreda R, Caçador I (2008) Seasonal variation of extracellular enzymatic activity (EEA) and its influence on metal speciation in a polluted salt marsh. Chemosphere 73:1056–1063. https://doi.org/10.1016/j.chemosphere.2008.07.072
34. Duarte B, Santos D, Marques JC, Caçador I (2015b) Ecophysiological constraints of two invasive plant species under a saline gradient: Halophytes versus glycophytes. Estuarine, Coastal and Shelf Science 167:154–165. https://doi.org/10.1016/j.ecss.2015.04.007
35. Duarte B, Santos D, Marques JC, Caçador I (2013c) Ecophysiological adaptations of two halophytes to salt stress: Photosynthesis, PS II photochemistry and anti-oxidant feedback – Implications for

resilience in climate change. Plant Physiology and Biochemistry 67:178–188. https://doi.org/10.1016/j.plaphy.2013.03.004

36. Duarte B, Valentim JM, Dias JM, et al (2014b) Modelling sea level rise (SLR) impacts on salt marsh detrital outwelling C and N exports from an estuarine coastal lagoon to the ocean (Ria de Aveiro, Portugal). Ecological Modelling 289:36–44. https://doi.org/10.1016/j.ecolmodel.2014.06.020
37. Fonseca VF, França S, Duarte B, et al (2019) Spatial Variation in Mercury Bioaccumulation and Magnification in a Temperate Estuarine Food Web. Front Mar Sci 6:117. https://doi.org/10.3389/fmars.2019.00117
38. Freeman C, Ostle NJ, Fenner N, Kang H (2004) A regulatory role for phenol oxidase during decomposition in peatlands. Soil Biology and Biochemistry 36:1663–1667. https://doi.org/10.1016/j.soilbio.2004.07.012
39. Freitas J, Duarte B, Caçador I (2014) Biogeochemical drivers of phosphatase activity in salt marsh sediments. Journal of Sea Research 93:57–62. https://doi.org/10.1016/j.seares.2014.04.002
40. Geraldi NR, Ortega A, Serrano O, et al (2019) Fingerprinting Blue Carbon: Rationale and Tools to Determine the Source of Organic Carbon in Marine Depositional Environments. Frontiers in Marine Science 6:1–9. https://doi.org/10.3389/fmars.2019.00263
41. Human LRD, Feijão E, Cruz de Carvalho R, et al (2020) Mediterranean salt marsh sediment metal speciation and bioavailability changes induced by the spreading of non-indigenous Spartina patens. Estuarine, Coastal and Shelf Science 243:106921. https://doi.org/10.1016/j.ecss.2020.106921
42. Kazemi K, Zhang B, Lye LM (2017) Assessment of Microbial Communities and Their Relationship with Enzymatic Activity during Composting. World Journal of Engineering and Technology 5:93–102. https://doi.org/10.4236/wjet.2017.53B011
43. Kelley AM, Fay PA, Polley HW, et al (2011) Atmospheric CO2 and soil extracellular enzyme activity: a meta-analysis and CO2 gradient experiment. Ecosphere 2:art96. https://doi.org/10.1890/ES11-00117.1
44. Ladd JN, Brisbane PG, Butler JHA, Amato M (1976) Studies on soil fumigation—III: Effects on enzyme activities, bacterial numbers and extractable ninhydrin reactive compounds. Soil Biology and Biochemistry 8:255–260. https://doi.org/10.1016/0038-0717(76)90053-5
45. Li B, Liao C, Zhang X, et al (2009) Spartina alterniflora invasions in the Yangtze River estuary, China: An overview of current status and ecosystem effects. Ecological Engineering 35:511–520. https://doi.org/10.1016/j.ecoleng.2008.05.013
46. Paredes-Páliz K, Rodríguez-Vázquez R, Duarte B, et al (2018) Investigating the mechanisms underlying phytoprotection by plant growth-promoting rhizobacteria in Spartina densiflora under metal stress. Plant Biology 20:497–506. https://doi.org/10.1111/plb.12693
47. Pereira P, Caçador I, Vale C, et al (2007) Decomposition of belowground litter and metal dynamics in salt marshes (Tagus Estuary, Portugal). Science of the Total Environment 380:93–101. https://doi.org/10.1016/j.scitotenv.2007.01.056

48. Ravit B, Ehrenfeld JG, Haggblom MM (2003) A comparison of sediment microbial communities associated withPhragmites australis andSpartina alterniflora in two brackish wetlands of New Jersey. Estuaries 26:465–474. https://doi.org/10.1007/BF02823723
49. Stewart GR, Lee JA, Orebamjo TO (1972) NITROGEN METABOLISM OF HALOPHYTES. New Phytologist 71:263–267. https://doi.org/10.1111/j.1469-8137.1972.tb04072.x
50. Tabak HH, Lens P, van Hullebusch ED, Dejonghe W (2005) Developments in Bioremediation of Soils and Sediments Polluted with Metals and Radionuclides – 1. Microbial Processes and Mechanisms Affecting Bioremediation of Metal Contamination and Influencing Metal Toxicity and Transport. Rev Environ Sci Biotechnol 4:115–156. https://doi.org/10.1007/s11157-005-2169-4
51. van Hullebusch ED, Lens PNL, Tabak HH (2005) Developments in Bioremediation of Soils and Sediments Polluted with Metals and Radionuclides. 3. Influence of Chemical Speciation and Bioavailability on Contaminants Immobilization/Mobilization Bio-processes. Rev Environ Sci Biotechnol 4:185–212. https://doi.org/10.1007/s11157-005-2948-y
52. Vera F, Gutiérrez JL, Ribeiro PD (2009) Aerial and detritus production of the cordgrass Spartina densiflora in a southwestern Atlantic salt marsh. Botany 87:482–491. https://doi.org/10.1139/B09-017
53. Zhong-Yi C, Bo LI, Jia-Kuan C (2004) Ecological consequences and management of Spartina spp. invasions in coastal ecosystems. Biodiversity Science 12:280. https://doi.org/10.17520/biods.2004034

Figures

Figure 1

Map of the location of the sampling site of the rhizosediments colonized by the endemic *H. portulacoides* and the non-indigenous species (NIS) *S. patens*, in the Hortas salt marsh (Alcochete) in the Tejo estuary.

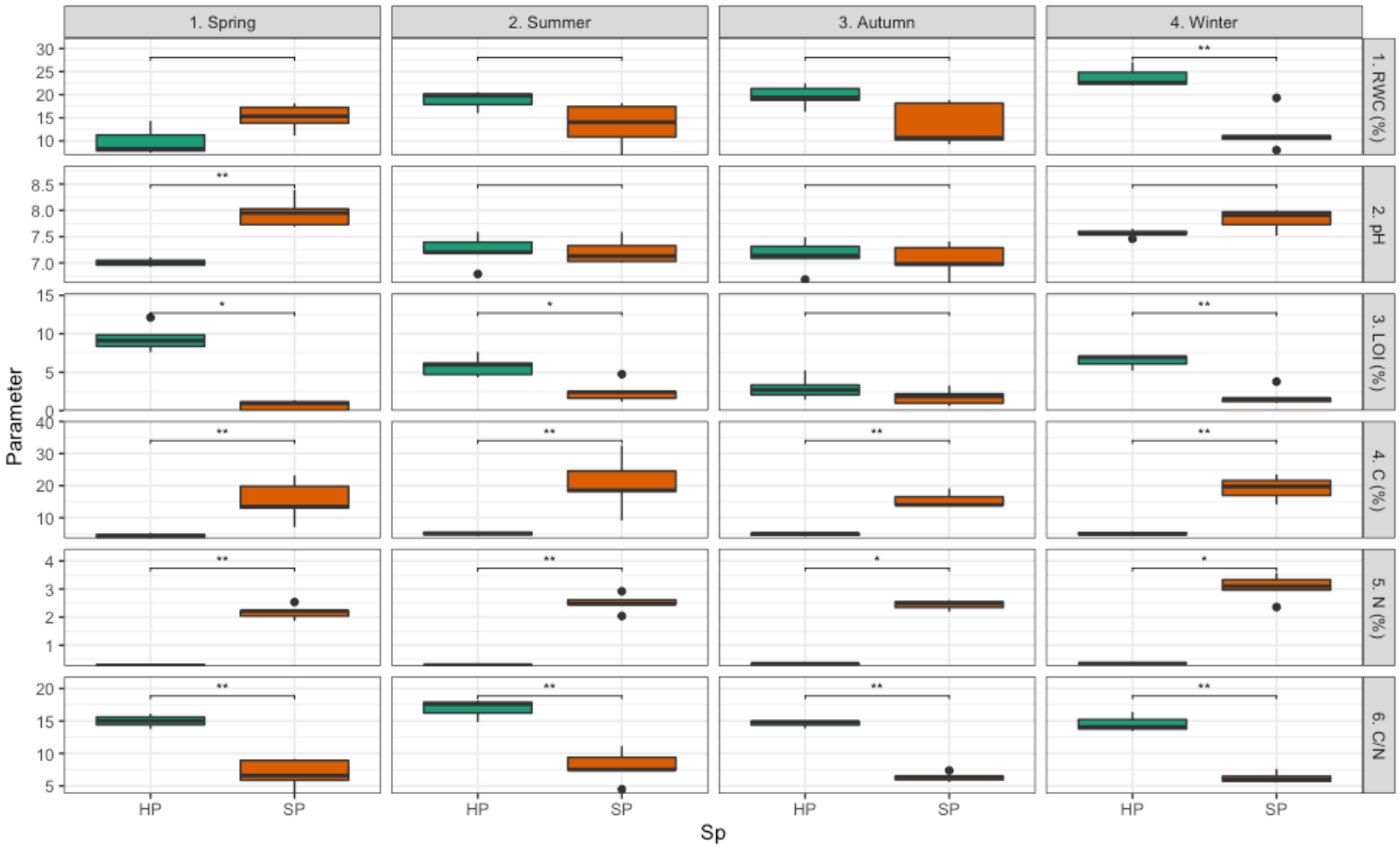

Figure 2

Physic-chemical characteristics (RWC, relative water content; LOI, organic matter express as loss on ignition; C, total carbon content; N, total nitrogen content; C/N, carbon to nitrogen ratio) of the rhizosediments colonized by the endemic *H. portulacoides* (HP) and the NIS *S. patens* (SP), across seasons (values are given as mean and standard deviation of 5 replicates per species per season). Significant differences between seasons sites are represented by asterisks (Kruskal–Wallis test, followed by a posterior multiple comparisons, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 3

Extracellular Enzymatic Activities (EEA) (DEH, dehydrogenase; POX, peroxidase; FOX, phenol oxidase; PHO, acid phosphatase; GLU, b-glucosidase; NACET, *N*-acetylglucosaminidase; SULF, sulfatase; PROT, protease; URE, urease) of the rhizosediments colonized by the endemic *H. portulacoides* (HP) and the NIS *S. patens* (SP), along a seasonal gradient (values are given as mean and standard deviation of 5 replicates per species per season). Significant differences between seasons sites are represented by asterisks (Kruskal–Wallis test, followed by a posterior multiple comparisons, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

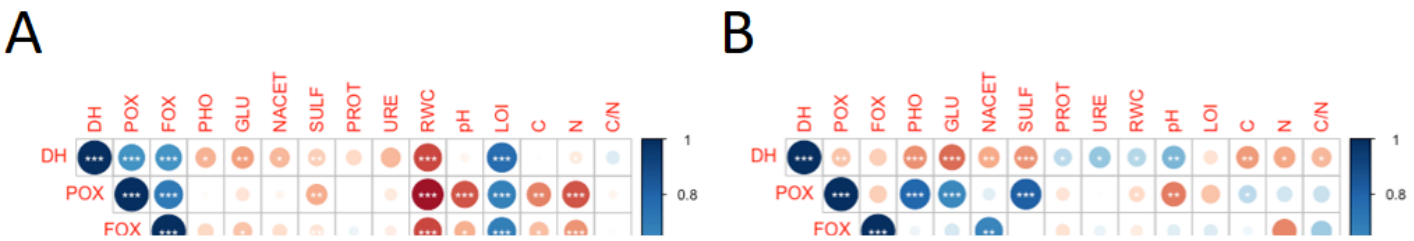

Figure 4

Spearman correlation coefficients (r) correlograms, between the sediment Extracellular Enzymatic Activities (EEA) (DEH, dehydrogenase; POX, peroxidase; FOX, phenol oxidase; PHO, acid phosphatase; GLU, b-glucosidase; NACET, *N*-acetylglucosaminidase; SULF, sulfatase; PROT, protease; URE, urease) and physic-chemical traits (RWC, relative water content; pH; LOI, organic matter content; C, total carbon content; N, total nitrogen content and C/N ratio) of the rhizosediments colonized by the endemic *H. portulacoides* (A) and the NIS *S. patens* (B), considering samples collected across all sampling times. Significant correlation coefficients are represented by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

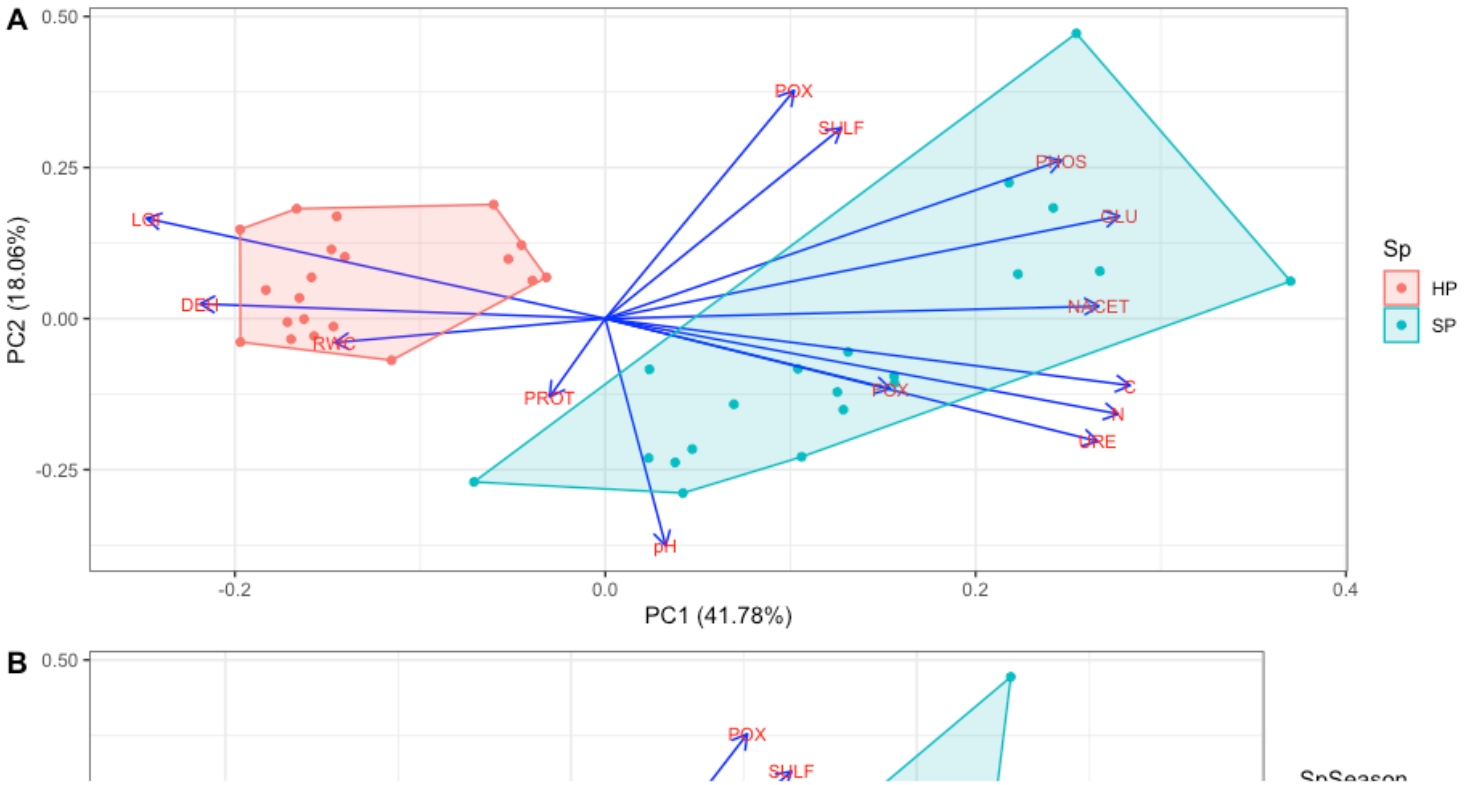

Figure 5

Principal Component Analysis (PCA) of the Extracellular Enzymatic Activities (EEA) (DEH, dehydrogenase; POX, peroxidase; FOX, phenol oxidase; PHO, acid phosphatase; GLU, b-glucosidase; NACET, *N*-acetylglucosaminidase; SULF, sulfatase; PROT, protease; URE, urease) and physic-chemical traits (RWC, relative water content; pH; LOI, organic matter content; C, total carbon content; N, total nitrogen content) of the rhizosediments colonized by the endemic *H. portulacoides* and the NIS *S. patens*, grouped by species (A) and by species and seasonal period (B). Shaded polygons represent the area covered by a group of dispersed points delimited by its furthest points.