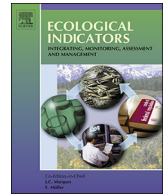




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Original Articles

Interactive effects of co-occurring anthropogenic stressors on the seagrass, *Zostera noltei*Raquel Vieira^a, Airam Martin^a, Aschwin H. Engelen^b, Mads S. Thomsen^c, Francisco Arenas^{a,*}^a Aquatic Ecology & Evolution Group, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Nova Terminal Cruzeiros Porto Leixões, 4450-208 Matosinhos, Portugal^b Biogeographical Ecology and Evolution, Center of Marine Sciences (CCMAR), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal^c Marine Ecology Research Group and Centre for Integrative Ecology, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

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ABSTRACT

Coastal ecosystems are subjected to multiple co-occurring anthropogenic stressors which potentially interact to produce complex impacts on the structure and functioning of biological communities. Seagrass meadows are among the most rapidly declining coastal habitats on Earth. In particular, high nutrient loadings, enhanced sedimentation and competition from blooming seaweeds, like the invasive red alga *Gracilaria vermiculophylla*, are claimed to be associated with this decline.

In this study, we tested for individual and potential interactive impacts on the intertidal seagrass *Zostera noltei* of these three stressors using a factorial field experiment. We measured seagrass shoots density and biomass (both above and below ground seagrass biomass) as proxies of seagrass physical condition. We also examined changes in fauna assemblages. The study suggested that sediment loading had the most detrimental impacts on health of the seagrass meadows. The effect of seaweed addition was negative and denso-dependent. Deleterious effects of nutrient enrichment were less evident. Non-additive interactions were also noticeable for some of the structural traits measured. Particularly, nutrient enrichment changed the effect of the other stressors for some of the responses measured. The effect of the treatments was also perceptible in the associated fauna assemblages. The ubiquitous grazer *Peringia ulvae* was more abundant in treatments with sediment loading, but those sediment effects in the density of the small grazer were modulated by nutrient and seaweed addition levels. Finally, we suggest that more studies should test for synergistic effects of co-occurring stressors to better understand the direction and intensity of changes triggered by human activities in natural ecosystems.

1. Introduction

On shallow soft-sediment sea beds, from tropical to cold-water temperate oceans, seagrasses are the main ‘foundation’ species (Hemminga and Duarte, 2000). Seagrass meadows are highly productive, biologically diverse and economically valuable habitats. They provide functions such as nutrient recycling, stabilization of sediment, habitat provision to a variety of organisms and nursery ground for economically important animals (e.g. Costanza et al., 1997; Hemminga and Duarte, 2000; Heck et al., 2003). However, seagrasses are one of the most rapidly declining coastal ecosystems on Earth. Globally the annual rate of loss of seagrasses is up to 7% (Waycott et al., 2009), generating negative effects on ecosystem services such as commercial fisheries, nutrient cycling, sediment stabilization and carbon sequestration (Waycott et al., 2009; Short and Wyllie-Echeverria, 1996).

While there are natural threats to seagrass meadows such as

overgrazing, storms or diseases, seagrass loss is particularly associated with eutrophication (Airoldi and Beck, 2007), one of the most significant human-induced stressor to coastal ecosystems (Worm and Lotze, 2006). The main cause of coastal eutrophication is the high input of nitrogen and phosphorus from agricultural fertilizers and wastewater discharges which flow into coastal systems (Nixon, 1995). High nutrient levels can support excessive growth of phytoplankton, epiphytic algae and macroalgae (Liu et al., 2009; Hauxwell et al., 2001), and thereby increase competition for light and nutrient, decrease oxygen levels, and ultimately smother seagrass leaves (Hauxwell et al., 2001; Cabaço et al., 2007).

In addition, construction of artificial coastal structures (e.g. harbours, docks and breakwaters), beach stabilization, dredging and excess siltation from changes in land catchments, increase sediment loads, even resulting in local burial events of seagrass after storms and major run-off events often causing seagrass meadows loss (Cabaço et al.,

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2008). All these effects may result in dramatic shifts in coastal ecosystems from seagrass to seaweed dominated assemblages (Sfriso et al., 1992; Martins et al., 2001), with potential major consequences on water quality and ecosystem functioning (Valiela et al., 1997; Dolbeth et al., 2003).

In fact, seaweeds have increasingly been implicated in the destruction of seagrass beds, particularly where eutrophication is high or where invasive seaweeds have been introduced (Thomsen et al., 2012a). Invasive seaweeds are a significant threat to seagrasses in estuaries and coastal lagoons, which are among the most invaded ecosystems on the planet and where invasive seaweeds have been shown to have dramatic effects on the structure and functioning of numerous coastal ecosystem (Williams and Smith, 2007). For example, increasing seaweed abundances typically result in decreased seagrass summer production with a subsequent decrease in recruit of new shoots (Cunha and Almeida, 2009; Williams, 2007)

Given the global decline of seagrass meadows, unravelling which stress factors drive changes is key to develop conservation and management strategies (McKenzie et al., 2014). Importantly, experimental studies that combine multiple stressors allow researchers and managers to identify possible additive or synergistic effects that cannot be identified from single-stress experiments. To test for possible interactive effects of invasive seaweed, sediment loading and nutrient enrichment on seagrass beds, we carried out a 3-factorial experiment in a healthy *Zostera noltei* Hornemann bed in the Ria de Aveiro lagoon, Portugal. Ria de Aveiro is a temperate coastal lagoon located along the Atlantic Ocean on Portugal. This lagoon has extensive intertidal flats and salt marshes with large meadows of *Zostera. noltei* and seasonal beds of the introduced seaweed *Gracilaria vermiculophylla* (Ohmi) Papenfuss.

Our initial hypothesis was that these stressors (nutrient enrichment, sediment loading and invasive seaweed occurrence) interact with non-additive effects on seagrass meadows.

2. Material and methods

2.1. Study area

Ria de Aveiro is a shallow well-mixed coastal lagoon on the Northwestern coast of Portugal (40°38'N, 08°45'W) connected to the sea by a single channel (Fig. 1). The lagoon is 45 km long with a maximum width of 10 km, and covers approximately 83 km² of wetland at high spring tide and 66 km² at low spring tide (Abrantes et al., 2006; Dias and Fernandes, 2006). Characterized as a mesotidal system with predominantly semi-diurnal tides, the Ria de Aveiro has a mean tidal range of circa 2.0 m (0.6 m neap to < 3.2 m spring tides), corresponding to a maximum and a minimum water level of 3.5 and 0.3 m, respectively (Dias et al., 2000). Tidal currents cause high levels of re-suspension during ebb and flood periods, but mainly at spring tides (Lopes et al., 2001). The Ria de Aveiro was declared as an official LTER (Long Term Ecological Research) site in 2011, further ecological information of this lagoon is available at the website <https://www.lterportugal.net/ria-de-aveiro>.

The experimental study was carried out on an intertidal sandy and mudflat at the Ovar Channel – São Jacinto (40°47'35"N; 08°39'49"W, Fig. 1). These flats are dominated by *Zostera noltei*, with smaller patches of drifting algae, in particular green *Ulva* spp. and *Gracilaria vermiculophylla*.

Zostera noltei is a small seagrass species occurring in the intertidal and shallow subtidal areas of the Northern and Western Europe, Mediterranean Sea and North–West Africa (den Hartog, 1970). In the Ria de Aveiro, *Z. noltei* covers up to ca. 0.5 km² and plays an important role in the lagoon productivity (Cunha et al., 2013). Seagrass decline in this estuary has been also attributed to anthropogenic disturbances with large impacts on the dynamics of Ria de Aveiro lagoon (Cunha et al., 2013). Among those disturbances, sediment loading is probably the most cited driver of seagrass decline (Azevedo et al., 2013).

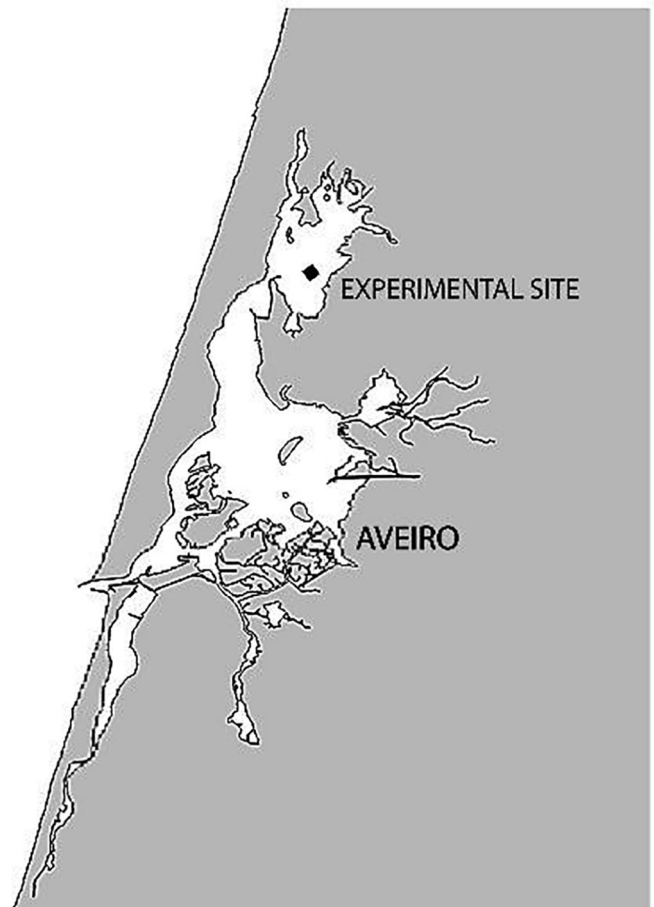


Fig. 1. Ria de Aveiro lagoon, Portugal (insert Portugal). The experiment was carried out at the area indicated by a small diamond, placed in the North of ria de Aveiro, at the Ovar Chanel (40°47'35"N; 08°39'49"W).

Gracilaria vermiculophylla is a coarsely branched red alga from the Northwest Pacific Ocean including Japan and East Asia (Ruess, 2005). With a cartilaginous cylindrical structure and growing up to 50 cm long, this species modifies the local abiotic environment (e.g., sedimentation, anoxia, light levels) (Ramus et al., 2017; Davout et al., 2017) and provides habitat for numerous sessile and mobile species (Thomsen et al., 2010; Byers et al., 2012). *G. vermiculophylla* has spread to shallow-water estuaries and coastal lagoons along the coastlines of the East Pacific, the West and East Atlantic, and the Mediterranean Sea, making it one of the world's most successful marine invasive species (Kim et al., 2010; Sfriso et al., 2010). *G. vermiculophylla* is common on bare sediments (Nejrup and Pedersen, 2010; Sfriso et al., 2012) as well as salt marshes (Thomsen et al., 2009), seagrass meadows (Cacabelos et al., 2012; Hernández et al., 2012), fucoid seaweed beds (Weinberger et al., 2008; Hammann et al., 2013) and Polychaeta and bivalves' reefs (Thomsen et al., 2010). The species is the dominant macrophyte in the Ria de Aveiro, where the mud and fine sand flats that characterize this sheltered soft-bottom lagoon offer an ideal site for its establishment (Abreu et al., 2011).

2.2. Experimental design

The field experiment was done between June and October in 2012. Sixty 50 × 50 cm experimental plots (0.25 m² plot area) separated by at least 5 m were tagged with plastic spikes along two parallel transects in a natural *Zostera noltei* bed. Once tagged, plots were randomly assigned to the manipulative treatments included in the orthogonal design: 2 nutrient enrichment levels, 2 sediment addition levels and 3 seaweed

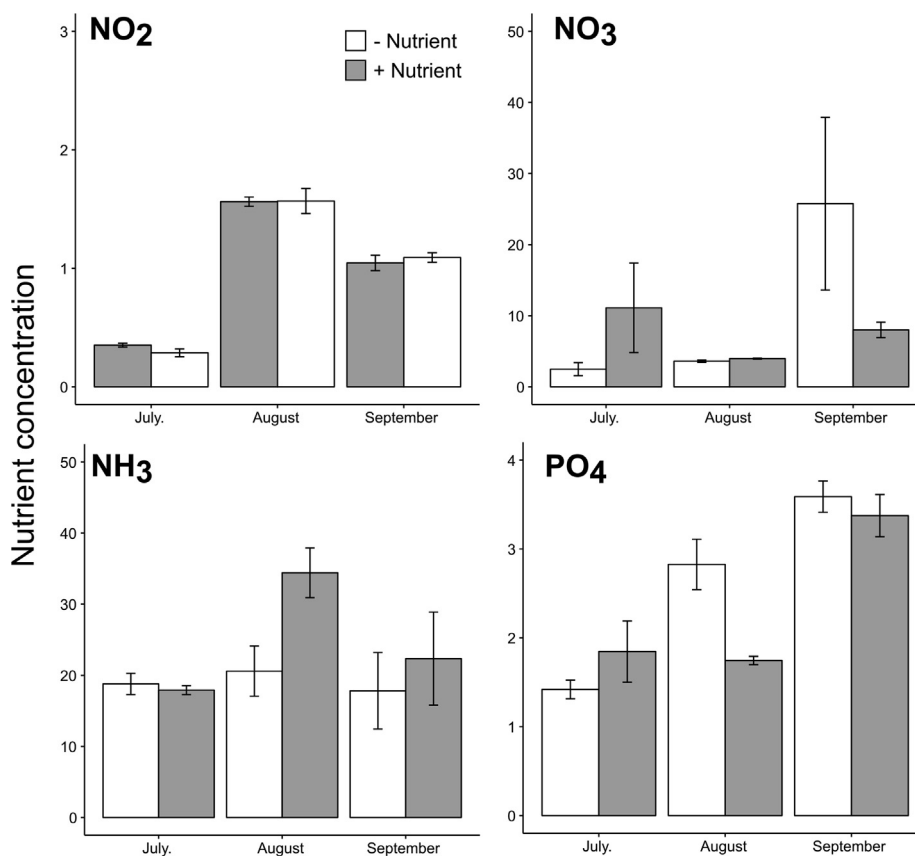


Fig. 2. Seasonal variation of nutrient mean concentration ($\mu\text{mol l}^{-1}$) (nitrite, nitrate, ammonium and phosphate) of surface water for treatment with nutrient addition and control plots. Bars denote standard deviation error ($n = 3$).

addition levels, ($n = 5$ replicates for each of the 12 treatment combinations).

Nutrient enrichment was applied to half the plots using slow release fertilizer pellets (Osmocote Exact® Standard). Fertilizer pellets (250 g m^{-2}) were added in small mesh bags with 1 mm mesh size and consisted of $15 + 3.9 + 9.1 + 1.5 \text{ Mg}$, corresponding to 7% nitrate N and 8% ammonia N, 9% P_2O_5 , 11% K_2O and 2.5% MgO, plus microelements (Worm and Sommer, 2000). Each experimental plot had two mesh bags (125 g each) placed on opposite corners and anchored to the substrate with cable ties and a plastic spike. To cancel out experimental artefacts, control mesh bags filled with sand were placed in the control plots (-N). Regarding the sediment addition treatment, sediment was collected from the same site, sieved through a 1 mm mesh and added to half the plots as 6500 g m^{-2} of sand. This amount of sand corresponded to a sediment layer of approx. 2 cm depth covering the whole plot. Finally, seaweed addition treatment included two different levels and consisted in the addition of 1500 gm^{-2} of fresh biomass of *Gracilaria vermiculophylla* (low intensity) and 3000 gm^{-2} (high intensity). Seaweed were added on top of the seagrass leaves, and fixed in each plot with 5 u-shaped metal pegs flushed in the sediment surface (the same number of pegs were added to control plots to avoid artefacts). These seaweed biomass levels are commonly observed in seagrasses (Cardoso et al., 2004; Thomsen et al., 2010; Thomsen, 2010). Seaweed were also collected in areas nearby the São Jacinto salt-marsh.

On those plots randomly selected and which combined the three treatments, the treatments were always applied in the same order: first, nutrient mesh bags were attached, then the seaweed *G. vermiculophylla* fronds were spread uniformly on the plots, and finally the sediment was added. All treatments were applied to plots selected randomly. Nutrient and control mesh bags were replaced monthly, whereas seaweeds and sediment treatments were re-applied after 2 months (most sediments and *Gracilaria* had disappeared from the plots after two months,

authors' per. obs.).

In addition, we collected monthly water samples monthly, at ca. 20 cm depth when plots were submerged on a rising tide. Each month, 10 plots (5 control and 5 nutrient enriched) were sampled by collecting three 10 ml samples of water and stored in a cool-box with ice. In the laboratory samples were frozen at -20°C until further analysis. The concentrations of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) were measured using a colorimetric auto-analyzer (Skalar SAN Plus Segmented Flow Analyser), with Skalar methods M461-318 (EPA 353.2) and M503-555R (Standard Method IP-450), and validating the analytical procedures by reference to samples containing known concentrations of each nutrient.

At the end of the experiment, a core (15 cm inner diameter) was collected from each plot center, down to a depth of 15 cm. In the field the cores were sieved through a 1 mm sieve and retained seagrass, macroalgae and large macroinvertebrates were stored in plastic bags. Once at the laboratory, we counted seagrass shoots and measured biomass of macrophytes (after drying at 50°C for 48 h) in fractions: i) seagrass above ground biomass (stems and leaves), ii) seagrass below ground biomass (roots and rhizomes), iii) the seaweed *Ulva* and iv) the seaweed *Gracilaria vermiculophylla*. We also examined the fauna of the sediment and identify the organisms up to large taxonomic groups. Since the mud snail (*Peringia ulvae*, Pennant 1777) was the most abundant species in the corers (> 98% of individuals belonged to this species), we examined closely the effect of the experimental treatments in this species.

2.3. Data analysis

We used three-way factorial ANOVA to test for effect of nutrients, sediment and seaweed (all fixed factors) on seagrass shoot density, seagrass total biomass, seagrass above ground biomass, seagrass below

ground biomass, *Ulva* spp. biomass and *Peringia ulvae* density. Significant effects were followed by Student-Newman-Keuls (SNK) post hoc tests to determinate differences between treatments. Multivariate analysis of fauna assemblages was done using PERMANOVA (Anderson, 2001) with the same design as in the ANOVA described above.

To evaluate the effect of nutrient addition, NH₃ and PO₄ in water samples were analysed with 2-way ANOVA, testing for effects of fertilization (fixed effect) and sampling date (random effect).

In all the analyses, homogeneity of variance was examined using Cochran tests and when required data was log-transformed.

3. Results

3.1. Experimental treatments: Nutrient enrichment and *Gracilaria vermiculophylla* addition

The temporal patterns of nutrient concentration are presented in Fig. 2. We did not found any obvious increase of the nutrient concentrations on those plots with the fertilizer bags. Large differences were found among dates and in the case of Nitrite and Phosphate, the interactions Treatment × Date were significant, (Table 3).

Regarding the treatment of *Gracilaria vermiculophylla* addition, we did find higher biomass of *Gracilaria vermiculophylla* in the plots where it was added but differences were not significant (ANOVA, $p > 0.05$ for the *Gracilaria* addition treatment) probably due to the large variability among corers. On average, control plots had a 0.8 ± 0.3 mg DW corer⁻¹, intermediate *G. vermiculophylla* treatments 18.1 ± 15 mg DW corer⁻¹ and more intense addition treatment, 30.1 ± 19 mg DW corer⁻¹.

3.2. Effects of different treatments in *Zostera noltei* communities.

Experimental treatments did affect the structure of *Zostera* beds, but the impact was different for the various structural proxies measured.

In the case of the biomass of the seagrass, no significant effects of any of the experimental treatments were found for the below biomass (i.e. *Zostera noltei* roots, results not shown), but on the above seagrass biomass (stem and leaves) significant effects were detected for the sediment loading treatment ($F_1 = 12.60$ $p < 0.05$) (Table 1). Thus, higher values of seagrass above biomass were found in the controls than in those plots where sediment was added (Fig. 3). Concerning total *Zostera noltei* biomass (i.e. above + below ground biomass), the effects were more complex. Thus, we found significant effects for the treatment macroalgae addition ($F_{2,48} = 6.57$ $p < 0.05$) and for the interaction Nutrient × Sediment ($F_{1,48} = 4.50$ $p < 0.05$) (Table 1, Fig. 4). In the first case, the addition of the seaweed *Gracilaria vermiculophylla* reduced the total biomass of *Zostera noltei* but only at the highest biomass treatments. For the interaction Nutrient enrichment × Sediment loading (Fig. 5), the effects of sediment were stronger and significant on those plots where no nutrient addition was also implemented. When nutrients were added the effects of sedimentation were partially

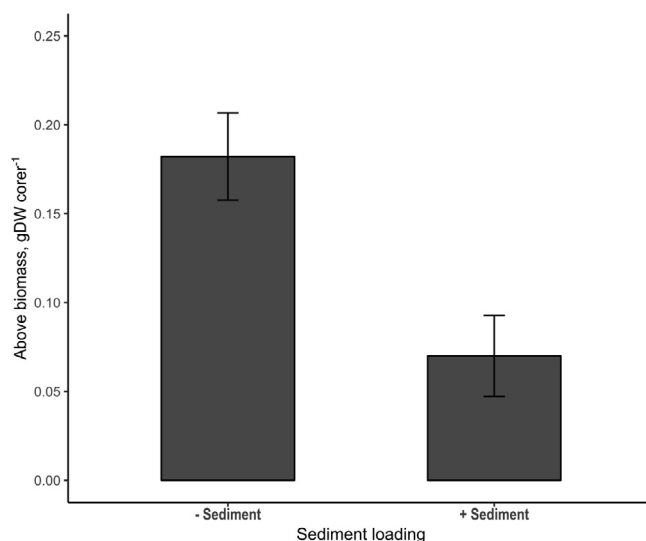


Fig. 3. Mean (± SE) above ground biomass of *Zostera noltei* (g DW corer⁻¹) for sediment loading treatment. Bars with different letter denote significant differences in a posteriori SNK test ($p < 0.05$).

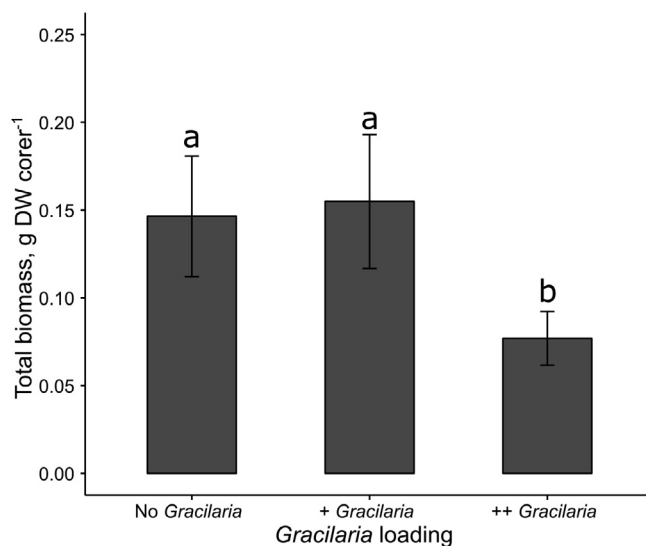


Fig. 4. Mean (± SE) total biomass of *Zostera noltei* (g DW) for *Gracilaria* loading treatment. Macroalgae addition levels were: “No *Gracilaria*” Control treatment; “+ *Gracilaria*” addition of 1500 gm^{-2} of *Gracilaria*; “++ *Gracilaria*” addition of 3000 gm^{-2} of *Gracilaria*. Bars with different letters denote significant differences in a posteriori SNK test ($p < 0.05$).

Table 1

Results of three-way ANOVA for the four *Zostera noltei* predictors (above, total biomass and shoot density) and *Ulva* sp. dry weight biomass for different treatments in Ria de Aveiro. Significant values at $p = 0.05$ indicated in bold.

Source	df	Above biomass		Total biomass		Shoot density		<i>Ulva</i> sp.	
		F	P	F	P	F	P	F	P
Gracilaria (Grac)	2	2.45	0.097	6.57	0.003	0.03	0.967	0.88	0.421
Nutrient (Nut)	1	0.49	0.488	0.17	0.684	0.03	0.870	1.95	0.169
Sediment (Sed)	1	12.60	0.001	23.62	0.000	3.12	0.083	1.37	0.247
Grac × Nut	2	1.71	0.191	2.56	0.087	0.20	0.821	0.99	0.377
Grac × Sed	2	1.89	0.162	3.08	0.055	1.53	0.227	0.97	0.385
Nut × Sed	1	3.38	0.072	4.50	0.039	0.28	0.600	1.21	0.277
Grac × Nut × Sed	2	0.64	0.533	0.02	0.982	3.41	0.041	1.08	0.346
Res	48								

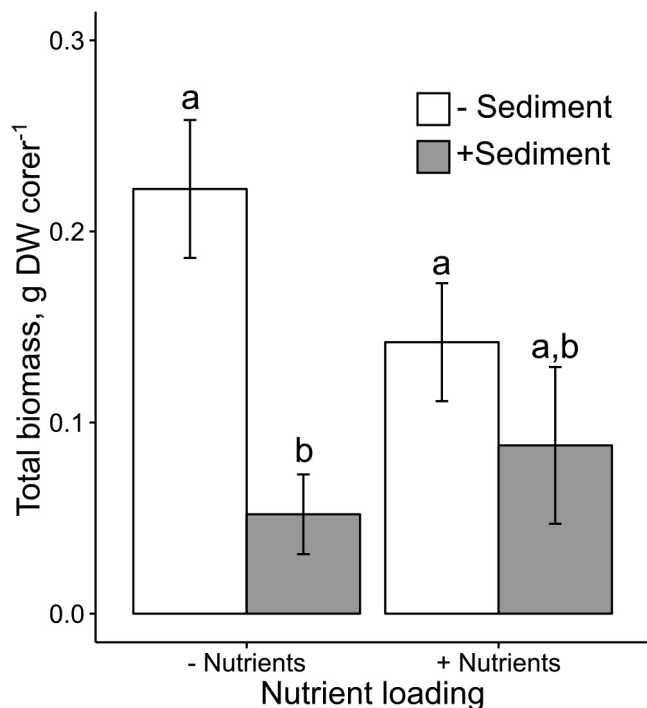


Fig. 5. Mean (\pm SE) total biomass of *Zostera noltei* (g DW) for the combined effects of Nutrient and Sediment addition. Bars with different letters denote significant differences in a posteriori SNK test ($p < 0.05$).

compensated and no significant differences were found.

Concerning shoot density, sediment addition had an overall negative impact on shoot density but in the analysis this driver was marginally not significant ($F_{2,48} = 3.12$ $p = 0.08$). Also, the significant interaction Seaweed \times Nutrient \times Sediment addition ($F_{2,48} = 3.41$ $p < 0.05$) denotes the existence of synergistic effects for some of the treatments (Table 1, Fig. 6). In fact, the effect found was a clear

Table 2

Results of three-way ANOVA for the density of *Peringia ulvae* from the different treatments in our experiment. Significant values at $p = 0.05$ indicated in bold.

Source	df	MS	F	P
Gracilaria (Grac)	2	8062.72	6.47	0.003
Nutrient (Nu)	1	8166.67	6.55	0.014
Sediment (Se)	1	25461.60	20.44	0.000
Grac \times Nut	2	1421.62	1.14	0.328
Grac \times Se	2	7419.95	5.96	0.005
Nut \times Se	1	12499.27	10.03	0.003
Grac \times Nut \times Se	2	578.52	0.46	0.631
Res	48			

reduction on the density of shoots as result of the sediment treatment occurring only on plots with nutrient addition and mid treatment of *Gracilaria vermiculophylla* addition.

We also examined the potential increase of blooming forming seaweeds (namely *Ulva* spp.) in the experimental plots but we found no significant effects for any of the treatments (Table 1).

Regarding the effects on the fauna, permutational multivariable anova (PERMANOVA) detected significant effects on fauna assemblages of the *Gracilaria vermiculophylla* addition and Sediment loading (Permanova, Pseudo- $F_{2,48}$: 3.35 and $p < 0.01$ for the treatment of *G. vermiculophylla* addition and Pseudo- $F_{2,48}$: 3.56 and $p < 0.05$ for the sediment loading). Those changes were mostly consequence of changes in the abundance of two species: the bivalve *Scrobicularia* sp. (results not shown) and the mud-snail *Peringia ulvae*, since the overall abundance of other taxa was very low. A closer look to the changes in the highly abundant *P. ulvae* revealed some interactive effects of the treatments. First, the jointly effect of sediment and nutrients enrichment was not additive (Nutrient \times Sediment, $F_{1,48} = 10.03$ $p < 0.05$; Table 2, Fig. 7A). Nutrient induced a clear increase in the density of the snail but only in the treatment of sediment addition. Also a second positive synergistic effect was found among macroalgal and sediment addition, with the highest densities of *P. ulvae* found on those treatments with medium level of seaweed biomass and sediment addition (Mac \times Sed, $F_{2,48} = 5.96$ $p < 0.05$; Table 2, Fig. 7B).

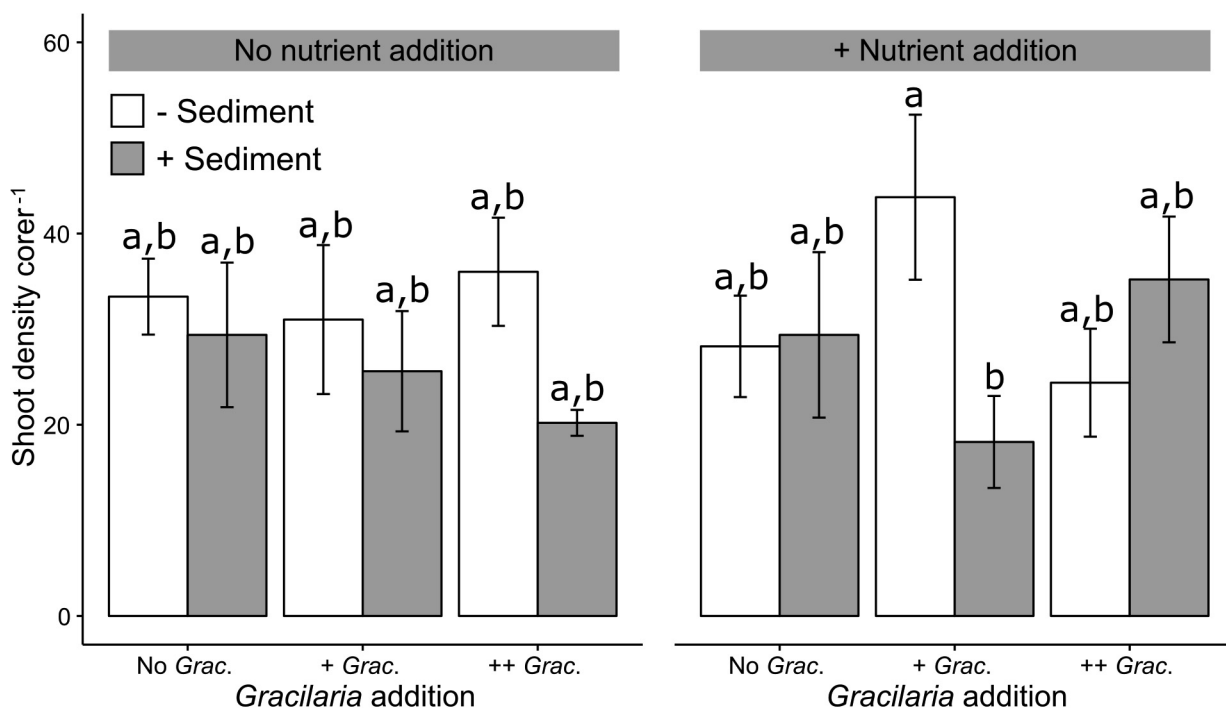


Fig. 6. Mean (\pm SE) shoot density of *Zostera noltei* (shoot number corer⁻¹) for the combined effects of Nutrient, Sediment and seaweed *Gracilaria* addition. Bars with different letters denote significant differences in a posteriori SNK test ($p < 0.05$).

Table 3
ANOVA for nutrient concentration in the water column, for control and treatment plots (n = 5). Significant values in bold.

Source	DF	NO ₂		NO ₃		NH ₃		PO ₄	
		F	P	F	P	F	P	F	P
Treatment (Tr)	1	0.99	0.424	2.94	0.228	1.35	0.365	3.93	0.185
Date (Dt)	2	65.89	0.000	4.21	0.027	2.34	0.118	24.98	0.000
Tr × Dt	2	114.23	0.000	0.76	0.479	2.24	0.128	5.83	0.008
Res	24								

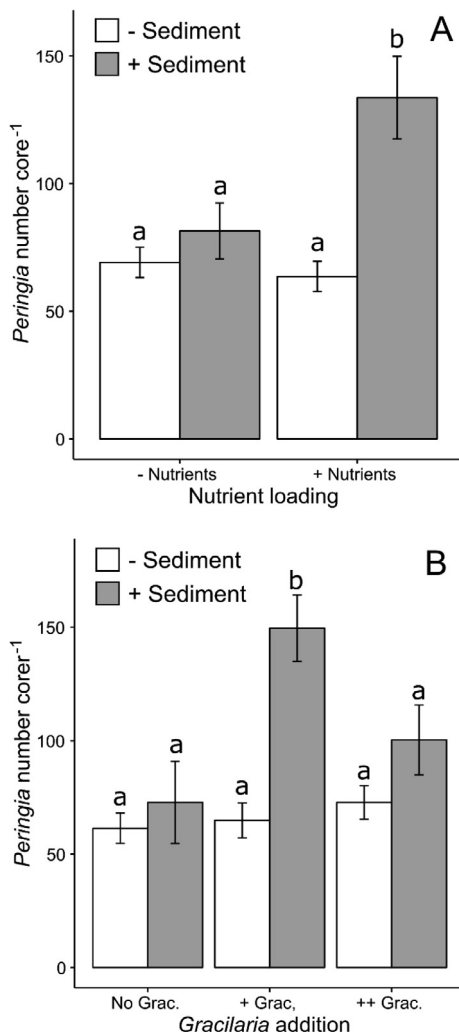


Fig. 7. A: Abundance of *Peringia ulvae* from the interaction Nutrients × Sediment, B: Abundance of *Peringia ulvae* from the interaction *Gracilaria* × Sediment. Bars denote standard deviation error (n = 5). Bars sharing the same letter indicate no significant differences.

4. Discussion

Our study examined simultaneously the combined effects of some of the most important threats to seagrass habitats (Grech et al., 2012) in a *Zostera noltei* meadow in the Ria de Aveiro lagoon, Portugal. Results confirmed the negative impacts of some of the stressors tested and the interactions between them. Experimental sediment load seemed to have the most detrimental impacts in the seagrass beds. The effect of seaweed addition was also negative and because we tested three different levels, we were able to detect density dependent effects. Finally, the negative effect of nutrient enrichment was less obvious. Furthermore, our findings showed that the non-additive interactions were frequent and varied with stressor intensity and among response variables.

Sediment loading was by far the single stressor with the most deleterious impact in our experiment. Sediment affected leaves growth and ultimately induced necrosis, thus the treatment reduced above ground biomass around 60% and overall seagrass biomass by 50%. Negative effects were also reported in the shoot density with a reduction in density of 20% approx., but differences were marginally not significant, probably because of the relative short duration of the experiment. Negative effects of sediment loading are widely reported in seagrass meadows (see Cabaço et al., 2008 for a review). In Ria de Aveiro, Silva et al. (2004) suggest that siltation due to the remobilization of sandy sediment as the potential major cause of seagrass decline in the last decades. Sediments can bury seagrass leaves and thereby remove light for photosynthesis and smother tissues through abrasion, reduce oxygen levels in sediments, and increase turbidity through re-suspension. These effects can be difficult to separate (Ralph et al., 2006), but effects associated with the burial of shoots (and in particular removal of light) are likely key reasons for the negative impact on *Zostera noltei*. Cabaço and Santos (2007) found 50% shoot mortality after 8 weeks of burial with 2 cm sand and 100% mortality when the burial depth was > 8 cm. Mortality in our case was smaller, probably because our experimental approach was different and unlike the study cited above, no devices were used to keep the sediment in place. The high hydrodynamism of the Ria probably also re-suspended and reduced the amount of sediment.

As in our experiment, the negative effect of *G. vermiculophylla* on seagrasses have been widely documented in literature, particularly when occurring in high abundances (Hauxwell et al., 2001; Thomsen et al., 2012a,b). Previous experiments testing for impacts of *Gracilaria* on the larger congeneric *Zostera marina* showed minor effects on this species (Martinez-Luscher and Holmer, 2010; Hoeffle et al., 2011; Thomsen et al., 2013). In the case of *Z. noltei*, the deleterious effects were density-dependent. Thus, *Gracilaria vermiculophylla* only reduced the total biomass of seagrass in the highest abundance treatment. Also seagrass shoot density was affected by this invasive species only at the mid intensity invasion treatment and when the other two stressors were also applied. The open spatial structure of the fronds of *G. vermiculophylla* at low abundance is likely to allow water movement, light penetration and high oxygen levels (Hoeffle et al., 2011). Also, when emerged during low tide, the seaweed could protect *Zostera* shoots from desiccation and heat related stress. Conversely, when very abundant, unattached seaweeds shade small seagrasses and seedlings, and reduce gas exchange negatively affecting growth (Thomsen et al., 2012a,b). Indeed, Hauxwell et al. (2001) reported that seaweed canopies, even at relatively low densities may reduce incident irradiance by > 95%. Thus the primary cause of eelgrass loss associated with the occurrence of macroalgal blooms is light reduction by macroalgae canopy (Hauxwell et al., 2001). The genus *Gracilaria* is reported all around the world as a genus of opportunistic blooming species responding rapidly to nutrient enrichment (e.g. Thornber et al., 2008; Piñón-Gimate et al., 2009; Newton and Thornber, 2012; Nelson et al., 2015). We also found some complex synergies like the 3-factorial interaction between *Gracilaria*, nutrients and sediments on shoot density. More specifically, although sediment burial had an overall negative effect on shoot density, this negative effect was only significant at low *Gracilaria* biomass and with nutrient enrichment. Perhaps the response of the abundant mud

snail *Peringia ulvae* were partially responsible for these results. *P. ulvae* was facilitated by sediment addition under nutrient enrichment conditions and by low seaweed levels. *P. ulvae* feeds by grazing and/or by swallowing sediment particles and is able to make active habitat selection, for example to avoid polluted sediments (Araújo et al., 2016a). *P. ulvae* were probably attracted by favourable environmental conditions created by the combination of sand and seaweeds addition and also by potential highest food availability in form of biofilms or periphyton (Mauffret et al., 2009) although it can also consumes epiphytes, eelgrass shoots and *Gracilaria* fronds. In sum, higher grazing intensity by *P. ulvae* in treatments with sediment and nutrient enrichment or sediment enrichment together with intermediate levels of seaweed addition could create patterns of shoots density similar to those found in our experiment (Araújo et al., 2016b).

Nutrient effects were always mediated by other stressors and were never significant as a single source of variability for any of the responses measured, except in the case of *Peringia ulvae* abundance. Negative effects of excessive nutrient conditions on seagrass meadows have been widely reported in literature, however this effect is dependent on where the enrichment happens. For example, additions of inorganic nutrients to sediments generally stimulate seagrass growth, suggesting nutrient limitation of plant production (Hughes et al., 2004). Silva et al. (2009) found that in Ria de Aveiro, the sediments with *Zostera noltii* appear to act as a large reservoir of N and P by accumulating greater concentrations of fine sediment (silt and clay) and organic matter when compared with the coarser sediment covered with macroalgae.

Water column enrichments, which are frequently linked with increase epiphyte biomass, have strong negative effects on seagrass biomass. The proposed mechanism would be the shading effects by epiphytic algae that would decrease the photosynthetic output of seagrass leaves (Zimmerman, 2010). In our case, we enriched the water column using slow release fertilisers but we did not observe any significant increase on epiphyte biomass. Background nutrient levels in the area of the experiment (Ovar channel) are highly variable in time and space (Cunha and Almeida, 2009), which probably reduced our ability to detect the enrichment and ameliorated the potential impact of our treatment.

Also, top-down control by grazers (e.g. *P. ulvae*) may hinder the proliferation of epiphytes, reducing or fully cancelling the potential negative effect of nutrient enrichment. This process have been described by the “mutualistic mesograzers model” (sensu Duffy, Hughes and Moksnes, 2013). It is a frequent mechanisms in communities dominated by *Zostera* and *Fucus* of the North Atlantic, where grazers – by selectively grazing on epiphytes and fast-growing ephemeral algae – reduce the competition for light or nutrients, facilitating the growth of seagrasses (Reynolds et al., 2014).

Seagrass ecology and management strategies have often focused most on water quality, specifically turbidity and nutrient loading (Fraser and Kendrick, 2017). Species interact in the ecosystems, thus examining the effects of global or local stressors requires not only scrutinizing into the vulnerability of single species to those stressors but also examining how interactions among species are modified. Particularly consumer effects may lead disproportionate changes in assemblages. These effects have been sometimes named as biotic multipliers (Zarnetske et al., 2012) and should be further explored in order to understand the direction and intensity of the changes triggered directly or indirectly by human activities. For example, several of the studies discussed above from temperate seagrass beds found that experimental fertilization had little effect on epiphytic algae, whereas experimental mesograzers reduction had stronger effects than fertilization (Heck et al., 2000; Hughes et al., 2004; Spivak et al., 2009).

In conclusion in our experiment, we found that sediment loading, seaweeds and nutrient enrichment had deleterious impacts on *Zostera noltii* meadows but the relative importance varies among stressors. Sediment was the prevalent driver of seagrass decline in our

experimental plots, which agrees with observational studies done in Ria de Aveiro (Azevedo et al., 2013; Silva et al., 2004). But the study also unravel some potential synergistic effects among stressors that may create complex responses. It is worthy to further investigate the interactions between small mesograzers like *Peringia ulvae* and environmental drivers. It is probable that experimental stressors also affect the seagrass through influencing the biota in the meadow, these indirect effects will be difficult to tease out as they usually require large experiments.

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