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The role of seagrass meadows in the coastal trapping of litter

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ABSTRACT

The accelerated discard and mismanagement of human-made products are resulting in the continued input of litter into the oceans. Models and field observations show how floating litter can accumulate in remote areas throughout the global ocean, but far less is known about the non-floating litter fraction. Seagrass meadows play an important role in the sediment and natural-debris dynamics, and likely also in the storage and processing of non-floating litter. In this work, non-floating litter was studied across six *Posidonia oceanica* meadows. Litter accumulated mainly around the landside edge of the meadow. The outer margin of the edge predominantly trapped macro-litter, whilst microplastics accumulated mainly along the inner margin. On average, macro-litter concentrations increased 3-fold after heavy rainfall. Retention of non-floating litter by coastal meadows facilitates the recurrent landward-seaward conveyance of the easily-transportable litter (mainly plastic items) and its fragmentation before it is buried or transferred to deeper areas.

1. Introduction

Seagrasses flank substantial oceanic shoreline areas, providing essential ecosystem services and benefits (Campagne et al., 2015; Duarte, 2000). It has been reported how seagrass meadows can absorb significant amounts of CO_2 (Deyanova et al., 2017), improve water quality (de los Santos et al., 2020), serve as refuge and breeding grounds for invertebrates and fish (Whitfield, 2017), mitigate coastal erosion by wave action (Ondiviela et al., 2014), or produce and capture sediments for stabilization of the seabed and beaches (Gacia et al., 2003). In a current global scenario of increasing litter pollution (Cózar et al., 2014; Jambeck et al., 2015; Morales-Caselles et al., 2021), this portfolio of ecosystem services has been extended to include the ability to trap marine litter (Cozzolino et al., 2020; de los Santos et al., 2021; Sánchez-Vidal et al., 2021). Trapping litter in shallow coastal areas prevents further dispersal and facilitates its recovery, especially when these litter accumulations are washed up back to the shoreline (Sánchez-Vidal et al., 2021).

The entrapment of litter in seagrass meadows has been inferred from the finding of significant amounts of plastic fragments intertwined in seagrass remains stranded on Mediterranean beaches, particularly within accumulations of leaves (known as 'banquettes') and aegagropilae of *Posidionia oceanica* (Sánchez-Vidal et al., 2021). In another study, Huang et al. (2020) compared benthic microplastic inside and outside seagrass meadows. They found that microplastic concentrations in seagrass beds were between 2.1 and 2.9 higher than concentrations in non-vegetated areas. Seagrass canopies are well known to reduce water flow in their surroundings (Fonseca et al., 1983), promoting deposition and reducing resuspension of sedimentary particles within the meadow (Gacia and Duarte, 2001; Hendriks et al., 2008). However, there are still few studies which have elucidated the role of seagrasses in the dynamics of coastal litter (i.e. Cozzolino et al., 2020; de los Santos et al., 2021; Huang et al., 2020; Jones et al., 2020; Sánchez-Vidal et al., 2021).

In the present work, we sampled different litter fractions extending from the micro to the macro, along transects extending from the outside to the inside of *P. oceanica* meadows, and before and after heavy rainfall events. *Posidonia oceanica* is one of the main habitat-forming infralittoral species in the Mediterranean Sea (e.g. Ballesteros et al., 2007; Bermejo et al., 2013), while torrential rains can flush large litter loads from land into the sea within short time periods (González-Fernández et al., 2021). Given its semi-enclosed nature, the Mediterranean basin is one of the main plastic accumulation zones in the global ocean (Cózar et al., 2015), and *P. oceanica* is considered an excellent particle trap due to its high

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structural complexity, large leaf size and meadow density (Folkard, 2005; Marbà et al., 2014). A priori, the capture of litter by the Mediterranean meadows of *P. oceanica* should be of great significance given their widespread distribution within nearshore waters in the basin.

On the other hand, the trapping of litter in seagrass meadows could compromise other ecological services provided by the same habitat, which is acknowledged within European legislation as a priority one in need of strict protection (Code 1120, *Posidonion oceanicae;* Directive 92/43/EEC). The accumulation of litter in meadows can alter the physico-chemical and biological conditions in this environment. Plastic bags and bioplastics, for instance, decrease oxygen levels and temperature in seagrass bed, affect growth and clonal architecture and might potentially change the competitive intensity between seagrass species and macroalgal ones (Balestri et al., 2017; Menicagli et al., 2021). Also, the presence of litter and microplastics in meadows implies a risk of incorporation into the seagrass food web of such fractions (e.g. Karlsson et al., 2017). In this study, we aim to test if *P. oceanica* meadows act as a barrier to the further advection of marine litter (macro-litter and microplastic) into deeper waters and to evaluate the potential effect of heavy rain episodes on the abundance and composition of non-floating litter.

2. Material and methods

2.1. Study site and sampling design

A total of 6 meadows of *P. oceanica* (Table S1) were sampled by SCUBA diving at a depth between 4 and 7 m, covering a seafloor band from 40 and 90 m from the coastline. Three of them were located at the western end of the Mediterranean Sea in Andalusia (southern Spain): Puerto Aguadulce (PD, 36°48′50.82″N and 2°33′51.03″W), Puerto Roquetas (PR, 36°45′36.60″N and 2°36′18.30″W) and Rambla del Cura (RC, 36°46′22.28″N and 2°36′1.18″W), whereas the other three were located within the central Mediterranean Sea, along the east coast of Malta: Exiles Bay (EB, 35°54′57.52″N and 14°29′45.90″E), Sliema (WCDC, 35°54′48.55″N and 14°30′28.31″E) and Bahar iċ-Ċagħaq (BIC, 35°56′55.91″N and 14°26′51.21″E). The height of the meadows ranged between 15 and 122 cm, being maximum in BIC and minimum in WCDC. The percentage of cover of *P. oceanica* varied between 20% and 100%. The highest mean cover was found in BIC, while the lowest mean cover was found in EB. Regarding the waste managed in the surveyed areas, it is estimated that poorly-managed waste accounts for 2% of the total waste generated in Spain, and 8% of the waste generated in Malta, with such a percentage that ranging from 2% to 69% for countries in the Mediterranean region (Jambeck et al., 2015). Appropriate permissions were sought prior to sampling, as the study involved a protected marine species (*P. oceanica*) and sampling was performed in protected Nature 2000 network sites (e.g. within the MT105 marine Natura 2000 site in Malta).

The three Andalusian sites (‘PD’, ‘PR’ and ‘RC’) are located in the agricultural region called Campo de Dalías. The Campo de Dalías has a greenhouse cultivation area of 207.69 km^2 (Junta, 2015). From May to October, the region receives around 400,000 tourists, of which half are concentrated in the months of July and August (“INE. Instituto Nacional de Estadística,” 2021). It is divided into two main hydrological basins, the larger one (ca. 2500 km^2) drains 16 km from our sites while the smaller one (ca. 300 km^2) drains close to ‘RC’ site. The flow in these basins is torrential, with intermittent rivers and wadis that are activated by heavy rains (Jager and Vogt, 2007). The wadis often accumulate litter for long periods before being washed out to sea in torrential rainfall events (unpublished personal observations).

Malta has an area of just 316 km^2 and is one of the most densely-populated island nations in the world. The resident population is approximately half a million and it receives almost 1.5 million tourists a year, mainly between May and October (Government services and information, 2021; Graham and Dennis, 2010). The population is mainly clustered along the east coast. The three sampled Malta sites (‘EB’, ‘WCDC’ y ‘BIC’) were located along this coast, in a mixed resort/residential area characterized by high levels of recreational activity (Graham and Dennis, 2010) and active recreational fishing (Agius Darmanin and Vella, 2019). The agricultural activity is relatively low and is focused along the coastline opposite our sampling sites (MSDEC-Government of malta, 2018). Malta has ca. 100 km of sizeable watercourses and ca. 200 km of minor ones. Rain typically falls around October and March. The annual average rainfall is under 500 mm (Haslam, 1997), making the island a semi-arid one, although torrential rains are relatively common.

Fieldwork was conducted between July and December 2019. All six meadows were sampled in the summer period, after relatively long periods without significant rainfall (before heavy rain). Later, four of the meadows, two from eastern Andalusia (i.e. PR and RC) and two from Malta (i.e. EB and WCDC), were re-sampled following heavy rainfall periods and likely pulses of land-sourced litter entering the sea (González-Fernández et al., 2021). In Andalusia, the heavy rains after the summer period was in October 2019, while in Malta the rainy season occurred during November. Before and just after the occurrence of the heavy rainfall events, the spatial distribution of micro- and macro-litter was assessed along a gradient of distances to shore, from the outer sandy zone to the core of the *P. oceanica* meadows.

2.1.1. Experiment 1: spatial distribution of marine litter

To study the possible effects of *P. oceanica* canopy on the distribution and composition of non-floating litter, micro to macro- litter were sampled in gradients perpendicular to the coastline from outside to inside the meadows. For macro-litter (>0.6 cm), strip transects were placed parallel to the edge of the meadow, three towards land (between 0 and 5 m; 5 and 10 m; and 10 and 15 m from the edge) and another three towards the interior of the meadow (between 0 and 5 m; 5 and 10 m; and 10 and 15 m from the edge). Each strip transect was 50 m long and 5 m width. Henceforth, each transect will be denoted by its mean distance to the edge, positive numbers refer to transects inside the meadow (i.e., 2.5; 7.5; 12.5 m) and the negative ones to transects outside the meadows (i.e., −2.5; −7.5; −12.5 m). All litter items larger than 0.5 cm found in the transects were collected for subsequent quantification and characterization. Regarding microplastics, samples were only collected at the three meadows in Andalucía (i.e. PD, PR and RC) before the rainy season.

2.1.2. Experiment 2: effect of rainfall in the distribution of macro-litter

In order to assess the effects of rainfall events in the distribution of macro-litter in the proximity of *P. oceanica* meadows, four meadows (PR and RC in Andalusia, EB and WCDC in Malta) were re-sampled just after a period of heavy rainfall (>100 mm in 3 days) (Zoomash Ltd, 2021; TWC, 2021), and compared with the previous scenario (Experiment 1). We used the same sampling method than in Experiment 1, based on strip transects (50 m long and 5 m width) parallel to the meadow edge, but this time we were not able to resample the inner transects at 7.5 and 12.5 m from the edge due to the time needed to collect the abundant litter found in some strips.

2.2. Sample collection and processing

2.2.1. Macro-litter

In each transect, all macro-litter items larger than 0.5 cm were collected manually, placed in a net and transported to the laboratory for processing. Here, macro-litter items were counted, photographed with a high-resolution camera (NIKON, 118 pixel/mm), measured from calibrated photographs taken and using ImageJ software (https://imagej.nih.gov/ij/) and weighed with a 0.1 g precision balance. The density was determined according to the material of which each item was composed (Ashby and Jones, 2012; Besednjak, 2009). In terms of shape, they were classified into ‘Lines items’ which refers to thin and flexible filaments (e.g., thread, twine, rope, string, tangle); 'Laminars items' which refers to flat and thin pieces (e.g., bag, piece of bag, wrapper, towelette, cloth, solid sheet); 'Hollows items' which refer to pieces that are not compact or solid because they have a space without matter inside (e.g., bottle, can, hollow tube, glass, stopper); 'Solids items' which are those formed by a solid mass without hollows inside (e.g., rubber ball, boat fragment, metal structure, fishing sinker).

Subsequently, based on density, size and shape of the litter items, they were classified in different categories according to how easy these are to transport, namely, very high mobility (VH), high mobility (H), medium mobility (M), low mobility (L) or very low mobility (VL) (Table 1).

2.2.2. *Micro-litter*

Three sediment samples were collected in each transect (at −12.5, −7.5, −2.5, 2.5, 7.5 and 12.5 m from the meadow edge) by scuba diving. Each sample consisted in a portion of sediment (approx. 400 cm^2) comprising the first 2 cm depth of sediment, which was collected using a sampling jar and a handle scraper (Graham and Thompson, 2009). Samples were transported to the laboratory in a coolbox where they were kept at 4 °C until microplastic extraction.

Table 1

Classification of macro-litter into five categories according to how easy they are to transport (VH: very high mobility, H: high mobility, M: medium mobility, L: low mobility and VL: very low mobility), See methods for details.

Shape	Density (Kg/m^3) / Weight (Kg)	<1000	(1000-1500]	(1500-2000]	(2000-3000]	(>3000]
LINES ITEMS	<0.005	VH	VH	H	M	M
	[0.005-0.05)	VH	H	H	M	M
	[0.05-0.25)	H	M	M	L	L
	[0.25-1)	M	L	L	VL	VL
	[1-2)	L	L	L	VL	VL
	[2-5)	VL	VL	VL	VL	VL
	≥5	VL	VL	VL	VL	VL
LAMINARS ITEMS	<0.005	VH	VH	H	M	M
	[0.005-0.05)	VH	H	H	M	M
	[0.05-0.25)	H	H	M	L	L
	[0.25-1)	M	M	L	L	VL
	[1-2)	L	L	L	L	VL
	[2-5)	VL	VL	VL	VL	VL
	≥5	VL	VL	VL	VL	VL
HOLLOWS ITEMS	<0.005	VH	VH	H	M	M
	[0.005-0.05)	H	H	M	M	M
	[0.05-0.25)	H	H	M	M	L
	[0.25-1)	M	M	L	L	VL
	[1-2)	M	M	L	L	VL
	[2-5)	L	L	VL	VL	VL
	≥5	VL	VL	VL	VL	VL
SOLIDS ITEMS	<0.005	VH	H	M	M	M
	[0.005-0.05)	H	M	M	M	M
	[0.05-0.25)	M	M	L	L	L
	[0.25-1)	L	L	L	VL	VL
	[1-2)	L	L	VL	VL	VL
	[2-5)	VL	VL	VL	VL	VL
	≥5	VL	VL	VL	VL	VL

Before the extraction, each sample was digested in H_2O_2 (10%) during 18 h in a fume hood covered with aluminum foil to remove organic matter. When the reaction stops, the sediment was washed with ultra-pure water, and then rinsed through a metal sieve with a mesh size of 63 μm. Subsequently the sediment was dried at 40 °C for at least 24 h. Microplastic extraction was performed from 100 g of oxidized and dried sediment (approx. 40 cm^2). During the extraction, an open petri dish was placed in the working area as a negative control and no micro-plastic contamination was detected. Microplastics were extracted following standard procedures as described in Frias et al. (2018). A saturated NaCl solution (1.2 g cm^{-3}) was chosen for separation. The solution was filtered through a 20 μm Whatman filter to avoid any source of contamination. The column prototype proposed by (Coppock et al., 2017) was used to separate suspended micro-plastic from settled sediment components after shaking during 5 min and settling for 1 h. Each sample was split in 2 subsamples of similar weight (i.e. 50 g) and each subsample analyzed in a column. The columns were thoroughly washed with pure millipore water before use and a blank was run every 3 samples to check for contamination and did not indicate any source of potential contamination.

The column supernatant was sieved at 63 μm using stainless steel sieves. Next, the particles retained on the filter were resuspended in a crystallizer with NaCl solution, and the microplastic was carefully picked from the water surface with the aid of a dissecting stereomicroscope. Any debris that was of unnatural appearance was washed with deionized water and dried at room temperature. Then, the items were counted and photographed for their measurement by the same technique used for macro-litter. Then, the items were dried and weighed in a digital balance (Nahita 5041/200). Fibers were not included in this study. Likewise, only items between 100 and 5000 μm were accounted for.

Finally, all items used for this study were chemically identified using a Perkin Elmer Spectrum 100 FT-IR Spectrometer with a diamond crystal ATR accessory. This spectroscopy allowed the identification of the polymer composition of each item based on IR absorption bands that represent the presence or absence of specific functional groups in the material. The spectral range analyzed was from 4000 to 600 cm^{-1}, with a 4 cm^{-1} resolution and 4 accumulations. All items included in this work were tested as micro-plastics.

2.3. *Statistical analysis*

2.3.1. *Effect of seagrass meadow on macro-litter distribution*

To study the spatial distribution of the macro-litter abundance along a horizontal gradient in the edge between seagrass meadows and sandy bottoms, a Generalized Linear Mixed Model (GLMM) was performed. Two fixed factors, "Area" (2 levels; "inside" and "outside" the meadow) and "Distance to the edge" (3 levels; 12.5, 7.5 and 2.5 m), and a random factor, Site (6 levels), were considered. Before the analysis, the macro-litter abundance was standardized by site to reduce local effects in litter distribution patterns (e.g. differences in litter abundances between meadows, anomalous litter loadings) and obtain a clearer insight in common patterns. Standardized data of macro-litter abundance complied with normality and homoscedasticity assumptions according to Shapiro-Wilks and Levene's tests. A post hoc Tukey's test was used to compare levels of any factor or combinations of factors when an effect was significant.

A multivariate three-way permutational analysis of variance (PERMANOVA) was performed to test the macro-litter composition (Anderson and Walsh, 2013), with "Area" (2 levels as above) and "Distance to the edge" (3 levels as above) as fixed factors. In addition, a third random factor “Site” (6 levels; PD, PR, RC, BIC, EB and WCDC) was considered. A distance based test for homogeneity of multivariable dispersion (PERMDISP; Anderson and Walsh, 2013) and a Principal Component Analysis (PCA) were performed to interpret and visualize data patterns. To identify the categories of macro-litter that most contributed to the differences among the different levels of significant fixed factors, a similarity percentage analysis (SIMPER; Clarke and Gorley, 2006) was carried out. All these analyses were based on euclidean distances between transects calculated from the five categories of macro-litter defined in Table 1, being the abundances for each category previously standardized per site.

2.3.2. Effect of seagrass meadow on micro-plastic distribution

The distribution of micro-plastic abundance along the gradient of distances from the meadow edge, towards the unvegetated seafloor and the inner meadow, was tested by a Scheirer Ray Hare non-parametric test (Sokal and Rohlf, 1995). The factor considered for this analysis were “Site” (4 levels) and “Position” (6 levels), the latter one resulting from the combination between the different levels of “Area” (2 levels as above) and “Distance to the edge” (3 levels as above). Before the analysis, micro-plastic abundance was standardized by location to reduce local effects in micro-plastic distribution patterns. Scheirer Ray Hare was used instead a traditional ANOVA, because standardized data of micro-plastic abundance did not comply with normality and homoscedasticity assumptions even after different transformations. A post hoc Dunn test (Dunn, 1961) was used to compare between the different positions.

2.3.3. Effect of rainfall on the distribution of macro-litter

The effect of the heavy rainfall events in the abundance of macro-litter across the seagrass meadows was tested by GLMM, considering “Position” (4 levels; 12.5, 7.5, 2.5 m outside and 2.5 m inside the meadow) as a fixed factor and “Site” (6 levels) as a random factor. The difference in macro-litter abundance (item/m^2) before and after the heavy rainfall period was the variable of interest. Macro-litter abundances were standardized by site. A post hoc Tukey’s test was used to compare levels of the factor when an effect was significant. A Student’s *t*-test for each position was used to evaluate the statistical significance of the potential increases in macro-litter abundance after the rains (Zar, 1996).

A multivariable three-way permutational analysis of variance (PERMANOVA) was performed to assess the effect of the rainfall on macro-litter composition (Anderson and Walsh, 2013). The three factors considered were: “Time” (2 levels; before and after the torrential rains) and “Position” (4 levels as above), which were computed as fixed factors, and Site (4 levels; PR, RC, EB and WCDC), which was considered as random factor. The interaction between “Site” and “Time” was pooled (see designs lack replication in Anderson et al., 2008 for further statistical rationale). A distance-based test for homogeneity of multivariable dispersion (PERMDISP; Anderson et al., 2008) and a Principal Component Analysis (PCA) were performed to interpret and visualize data patterns. To identify the categories of macro-litter that most contributed to the differences between the different levels of significant factors, an analysis of species contribution to similarity (SIMPER; Clarke and Gorley, 2006) was carried out. All these analyses were based on euclidean distances between transects calculated from the five categories of macro-litter, being the abundances for each category previously standardized per site.

All statistical analyses were performed with the R free software (R Development Core Team, 2020; “lme4” and “rcompanion” packages were used for the performance of GLMM and Scheirer Ray Hare tests, respectively) and with PERMANOVA+ add-on PRIMER 6 (Plymouth Routines in Multivariate Ecological Research) software. Significance level was set up $p < 0.05$ probability, and when necessary were based on 9999 permutations.

3. Results

3.1. Overall microplastic and macro-litter characteristics

The macro-litter items recorded consisted of 80% plastic and 20% non-plastic items. Non-plastic materials included glass, metal, wood and paper (40%, 30%, 20% and 10%, respectively). Laminated items dominated (70%), for both plastics and non-plastics (Fig. S1). Overall, the size-relative abundance distribution between sizes was similar for both plastic and non-plastic items (Fig. S2). The most abundant macro-litter items were those smaller than 50 cm in maximum length, for both plastic and non-plastic items (90–100%). Differences in the relative abundances of size classes before and after heavy rainfall phenomena were small (Fig. S2), although a slight increase in the abundance of litter sizes from 10 to 50 cm was observed following heavy rains.

Before the heavy rains, the average maximum length of litter items in the vegetated area tended to be larger than in the non-vegetated area. After heavy rains, the mean and median maximum item length were similar between the different positions considered. The only exception was observed at the PR site, where larger items were found in the non-vegetated zone (Fig. S3).

With respect to microplastics, a predominance of sizes between 1 and 2.5 mm of maximum length was observed (56.25%), followed by those from 2.5 to 5 mm (31.25%) and from 0.5 to1 mm (12.5%). At the PD site, items between 1 and 2.5 mm were predominant ($>$ 80%). At the PR site, items within 1–2.5 and 2.5–5 mm size classes accounted for almost 80% of the items, while half of the recorded items ranged between 2.5 and 5 mm (Fig. S4). No clear pattern in the distribution of litter sizes was observed between the different sampling stations positioned along a gradient from the meadow edge. The recorded average maximum length of microplastic was approximately 2.5 mm (Fig. S5).

3.2. Effect of seagrass meadow on macro-litter distribution

At all the sampled sites, a greater amount of macro-litter was detected along the 5-m outer strip of the landside edge of the meadow (i.e. -2.5 m), except at BIC where the amount of litter was very small and only macro-litter was found outside the meadow at the −7.5 m strip from the edge (Fig. 1a). The GLMM revealed that the two fixed factors assessed, ‘Area’ and ‘Distance to the edge’ of the seagrass meadow, and the interaction between them had a significant effect on the distribution of standardized macro-litter abundances (Table 2). The distribution of standardized macro-litter abundances followed a similar pattern inside and outside the meadow (Fig. 1b), with the maximum abundances being measured close to the edge (i.e. 2.5 m) and the minimal in the farthest strip transect (i.e. 12.5 m). However, this pattern was more evident outside the meadow. Overall, the highest standardized macro-litter abundances were found outside the meadow in the strip transect closest to the edge (0.99 ± 0.02 items/m^2), and the lowest in the strip farthest outside the meadow (0.05 ± 0.06 item/m^2).

Regarding macro-litter composition, the PERMANOVA results indicated that the composition differed significantly with the distance from the meadow edge, stressing an interaction between the distance to the edge and the area (Table 3). No significant effects were observed for the factors “Site” and “Area”, or other interactions different to that between “Distance” and “Area”. The PERMDISP analysis showed significant differences in the multivariate dispersion between transects (p-value $<$ 0.001), with the lowest multivariate dispersion in the farthest strip outside the meadow (table S2). The pairwise comparisons between the six different positions resulting from the interaction between the factors “Distance to the edge” and “Area” indicated that the outer margin of the meadow edge had the most differentiated composition, diverging from all others. The litter composition in the inner edge was also different from other strip transects outside the meadow but not from those within the meadow (table S3). Overall, the multivariate dispersion was lower for positions outside than inside the meadow. Despite the observed

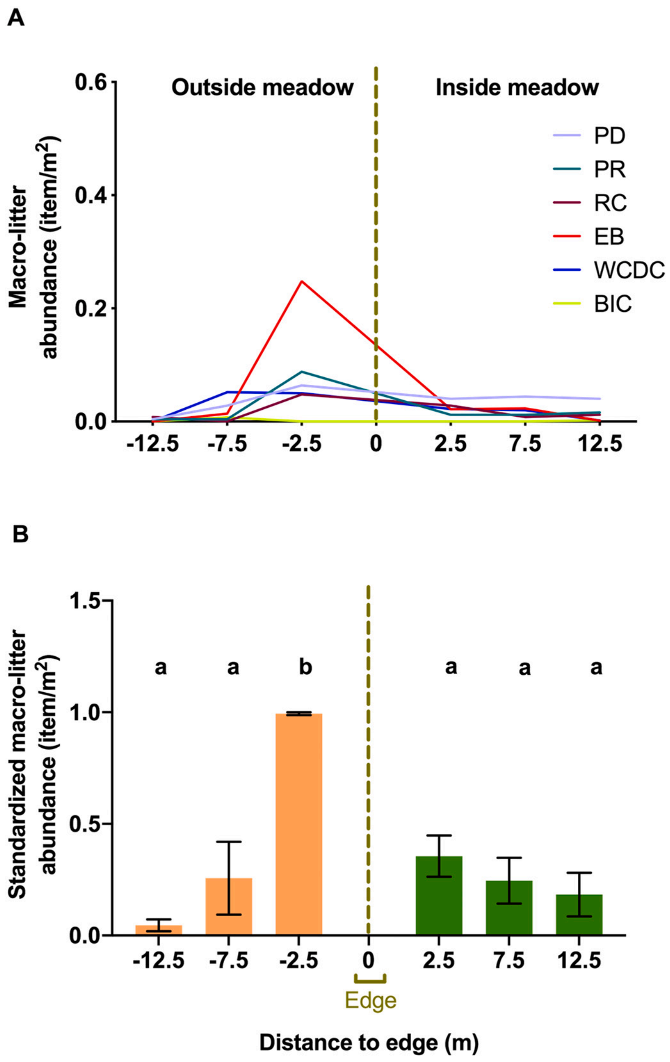

Fig. 1. (a) Macro-litter abundance (item/m^2) in relation to the distance to edge (m) in each site (PD, PR, RC, BIC, EB, WCDC). (b) Mean (± SE) standardized concentration outside (orange) and inside (green) of the meadow in relation to the distance to edge (m). Letters denote differences between transects in the pairwise analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results of GLMM analysis testing the effects of the factors "Area" (Inside and Outside of the meadow) and "Distance to edge" (12.5, 7.5 and 2.5 m of the meadow's edge). Df: Degrees of freedom; MS: Mean Square.**

ANOVA	Df	MS	F value
Area	1	0.263	6.49*
Distance	2	1.022	25.19***
Area × Distance	2	0.509	12.56***

*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

differences in multivariate dispersion between positions, the PCA supported the existence of differences in macro-litter composition (Fig. 2). Different groupings were observed as a function of area and distance from the edge. Most of the samples taken outside the meadow are grouped in the upper part of the graph (red, orange and yellow), while the samples taken inside the meadow were more evenly distributed in the upper and lower parts (greens colors). It should be noted that the differences were more pronounced as we approached the meadow edge (light green vs. yellow). The outer edge of the meadow (yellow) was located in the upper left part of the graph and the inner edge (light green) in the middle part at the top and bottom of the graph. Overall, the differences in macro-litter composition were due to a greater abundance of higher mobility items (i.e. H and VH categories) outside the seagrass meadow than inside. Conversely, macro-litter less susceptible to be transported by weak currents and waves (i.e. L and VL categories) were more frequently found inside the meadow or just at the outer edge of the meadow. Items classified as having intermediate mobility (i.e. M category) are between the two groups mentioned above (Table S4, Fig. S6).

Table 3
Results of the PERMANOVA analysis testing the effects of the factors "Site" (PD; Puerto Aguadulce, PR; Puerto de Roquetas, RC; Rambla del Cura, BIC; Bahar iq cahac, EB; Exiles Bay, WCDC; Water Colour diving center), "Area" (Inside and Outside of the meadow) and "Distance to edge" (12.5, 7.5 and 2.5 m of the meadow's edge) in the composition of macro-litter according to how easy is to transport it. Df: Degrees of freedom; MS: Mean Square.***

PERMANOVA	Df	MS	Pseudo-F
Site	5	0.486	1.003
Area	1	1.134	2.186
Distance	2	3.177	6.255**
Site × Area	5	0.519	1.071
Site × Distance	10	0.508	1.048
Area × Distance	2	1.436	2.963*
Residual	10	0.485	

* p-value < 0.05.
** p-value < 0.01.
*** p-value < 0.001.

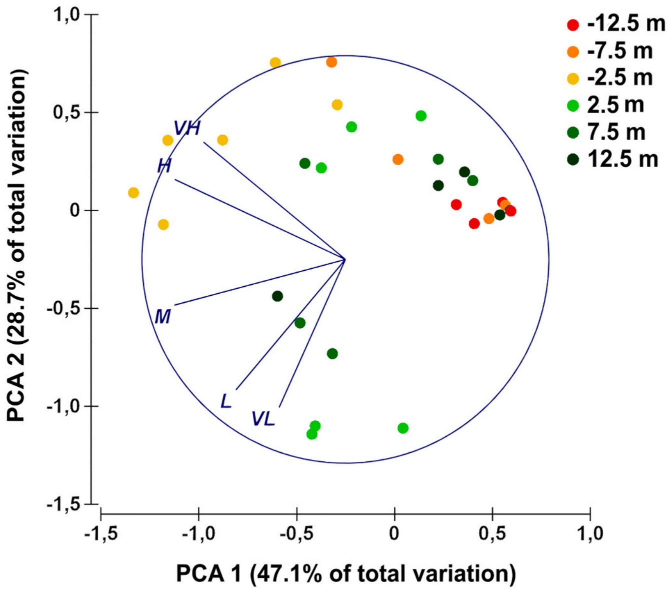

Fig. 2. Principal component analysis based on standardized abundances of litter types (VH, H, M, L and VL) at different transects, outside (−12.5, −7.5 and − 2.5 m) and inside (12.5, 7.5 and 2.5 m) of the meadow's edge.

3.3. *Effect of seagrass meadow on micro-plastic distribution*

The concentration of micro-plastics was higher inside than outside of the meadow (Fig. 3a). The 2.5 m strip transect showed the highest micro-plastic concentrations. The Scheirer Ray Hare test only rendered a significant effect for the factor 'Distance to the edge' in the distribution of standardized micro-plastic abundances ($H = 13.661$, $df = 5$, p-value $= 0.018$). No significant effects were observed for the factor "Site", or for the interaction between the two assessed factors (i.e. "Site" and

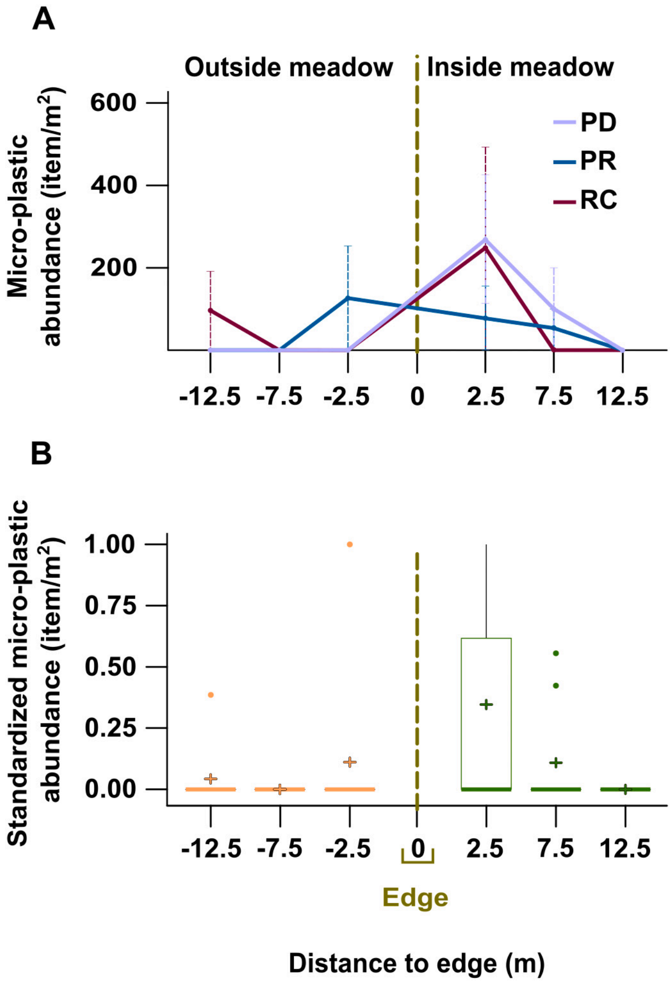

Fig. 3. a) Mean (± SE) of micro-plastic abundance (item/m^2) versus distance to edge (m) in each site from Andalusia before the heavy rains (PD, PR, RC). (b) Standardized abundance (items/m^2) of the three sites from Andalusia before the heavy rain period, outside (orange) and inside (green) the meadow. The bold horizontal lines correspond to the median and crosses indicate the means. The upper end of the column is the 75th percentile and the lower end is the 25th percentile. The dots are outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

"Position"; *p*-values >0.05). The distribution of standardized micro-plastic abundances followed a similar pattern as macro-litter (Fig. 3b), with the highest abundances measured in the strip adjacent to the edge (2.5 m). In this case, the accumulation of micro-plastic was mainly found in the inner strip of the edge (0–0.63 standardized items/m^2, 25th and 75th percentile, respectively).

3.4. *Effect of rainfall on the distribution of macro-litter*

The macro-litter densities were higher after the heavy rain period in most of the transects at all the sampling sites. This increase was accentuated especially at the edge of the meadow, in particular inside the meadow (Fig. 4a&b). The *t*-tests revealed significant accumulations (the difference in the standardized abundance of macro-litter after minus before heavy rain period) in this strip (p-value = 0.022). In the cases of 2.5 and 7.5 m strip transects outside the meadow, marginal differences were observed (t-test; p-values < 0.1). No significant accumulations were found in the farthest transect outside the meadow. The GLMM analysis indicated significant differences in macro-litter accumulation between positions ($F_{3,12} = 5.844$, p-value = 0.011). The inner margin of the meadow edge showed the highest standardized accumulation, whilst the farthest strip transects outside the meadow showed the lowest accumulation (Fig. 4c).

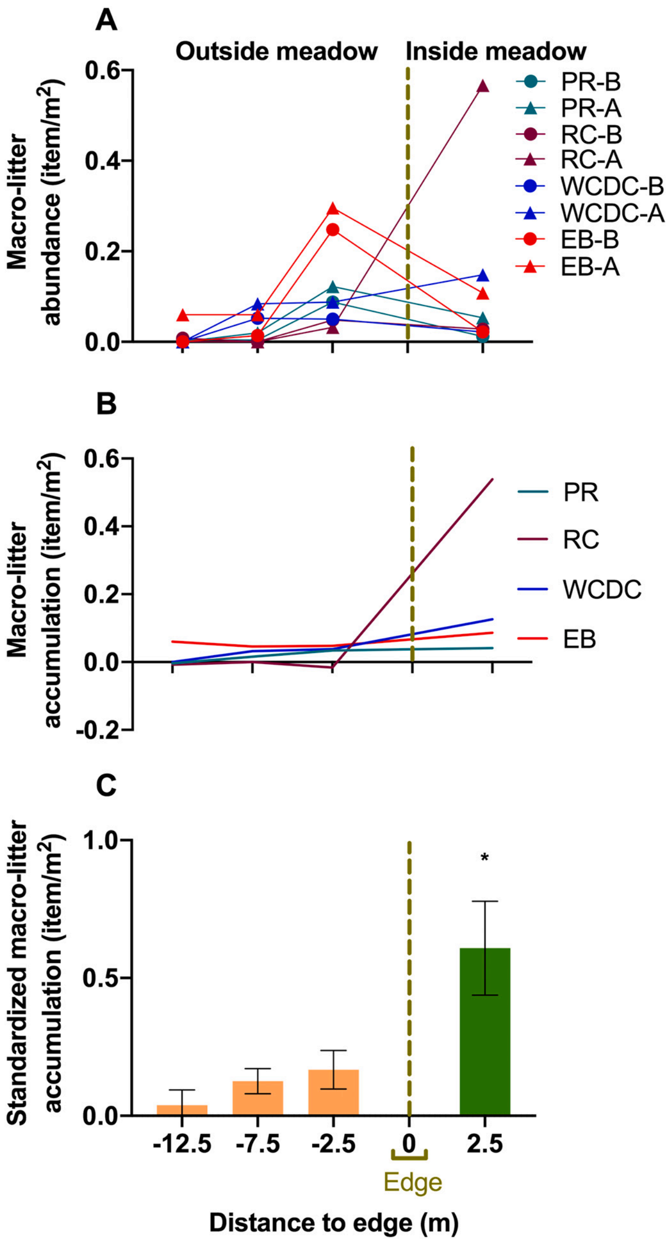

Fig. 4. **(a)** Abundance (items/m^2) versus distance to edge (m) after (triangle) and before (circle) heavy rain events in four sampling sites (PR, RC, EB, WCDC). **(b)** Macro-litter accumulation (abundance after minus before heavy rains) along distance to edge per site (PR, RC, EB, WCDC). **(c)** Mean ± SE of standardized macro-litter accumulation (items/m^2) after minus before heavy rains. Strip transects outside and inside of the meadow are shown in orange and green bars, respectively. The asterisk indicated a significant accumulation of litter after the heavy rains. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The PERMANOVA analysis indicated that the factors "Distance" and "Time" significantly affected the composition of the macro-litter (Table 4). A significant interaction between both factors was also identified. No significant effects were observed for the factor "Site" but there was a small difference in the interaction between "Site" and "Distance" because one of the categories (i.e. VL) was absent in a sampling site. The PERMDISP analysis indicated significant differences in the multivariate dispersion between transects before and after the torrential rain (PERMDISP; p-value = 0.007) (Table S5). The pairwise

Table 4

Results of PERMANOVA analysis testing the effects of the random factor "Site" (PR; Puerto de Roquetas, RC; Rambla del Cura, EB; Exiles Bay, WCDC; Water Colour diving center), and the fixed factors "Time" (before and after the heavy rain period) and "Distance to edge" (12.5, 7.5 and 2.5 m of the meadow's edge) in the composition of macro-litter. Df: Degrees of freedom; MS: Mean Square.

PERMANOVA	df	MS	Ps-F
Site	3	0.305	1.247
Time	1	1.990	8.129***
Distance	3	2.668	6.025***
Site × Distance	9	0.443	1.809*
Time × Distance	3	0.988	4.038**
Residual	12	0.245	

* p-value < 0.05.
** p-value < 0.01.
*** p-value < 0.001.

PERMANOVA revealed that the composition of the two transects close to the meadow edge (i.e. 2.5 m inside and outside the meadow) were the most differentiated and the most affected by the heavy rain period, especially into the meadow (Table S6). Before the rains, the outer margin of the meadow edge was the most different. After the heavy rain period, the two transects closer to the edge were the most different. No differences in macro-litter composition were found between strip transects at 7.5 and 12.5 m, while the composition varied significantly between the two periods considered in both strips located closest to the edge. Table S7 shows the percentage of contribution of each type of macro-litter to the differences between positions and periods, and the fig. S7 visualizes these differences.

The PCA supported the above-described differences in macro-litter composition (Fig. 5). Groupings were observed in relation to the sampling period, area and distance from the edge. Most of the samples taken after a heavy rain period were grouped at the left and the center side of the graph (cool colors, blues and purple), while the samples collected before the heavy rain were located at the right part of the graph (warm colors: red, orange and yellow). In addition, differences were observed among strips, mainly around the edge. After heavy rain, most of the samples in the outer margin of the edge were placed diagonally across the center of the graph (strong blue squares), whereas most of the samples collected in the inner margin of the edge were grouped at the upper left corner (light blue circle). Before heavy rain, most of the samples collected at the outer margin of the edge were distributed diagonally across the center of the graph (orange square) and those collected at the inner margin were grouped on the right side of the graph (yellow circles). The abundance of items of all categories increased on average 2.7 times after heavy rain. The differences in composition were related to a greater abundance of 'H', 'M' and 'L' categories on the inner margin of the edge, and greater abundance of items more susceptible to be transported by currents (i.e. VH category) in the outer margin after heavy rain. No clear pattern is observed for the 'VL' category. Items with very low mobility stayed in the same position as in Fig. 4. Table 2 in the supplementary material shows the percentage of contribution of each type of macro-litter per transect and sampling period, both before and after the heavy rain.

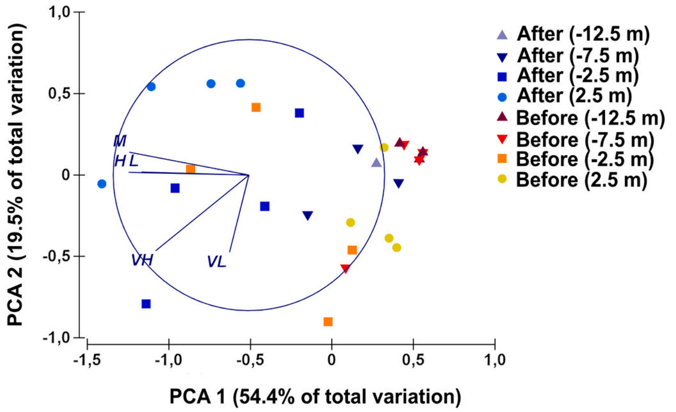

Fig. 5. Principal component analysis based on standardized abundances of litter types (VH, H, M, L and VL) at different transects, outside (−12.5, −7.5 and − 2.5 m of the meadows's edge) and inside of the meadow (2.5 m of the meadows's edge), after and before the heavy rains.

4. Discussion

A number of recent studies have investigated the role of subtidal seagrass beds as litter traps. Huang et al. (2020) report that microplastic concentrations in seagrass meadows dominated by *Enhalus acodoides* were between 2.1 and 2.9 higher than concentrations in non-vegetated areas. Jones et al. (2020) found significantly higher abundances of microplastics in sediments within *Zostera marina* seagrass meadows than in non-vegetated areas surrounding the meadows. They also reported microplastic particles in all the seagrass leaf samples collected within the same study. In contrast, data from Cozzolino et al., (2020) showed no differences between vegetated (subtidal seagrass meadows) and unvegetated areas in terms of microplastic abundances within the sediment, although they found evidence of microplastic adhesion to the canopies of seagrasses. In another study, de los Santos et al., 2021 found that microplastic particles were trapped within *Z. marina* canopies in a laboratory experiment, but the same observation was not replicated in the field. Only one study (Cozzolino et al., 2020) has quantified the impact of seagrass meadows (mixed seagrass meadows represented by both *Cymodocea nodosa* and *Z. marina* in this case) on macroplastic trapping rates, finding no significant differences between the rates registered for subtidal seagrass and for unvegetated areas. However, all these studies did not account for the small-scale variability of micro- or macro-litter across the meadows.

The sampling approach used here to study micro- and macro-litter abundances in six Mediterranean *P. oceanica* meadows allows us to infer in broad terms litter accumulation patterns for nearshore seafloor areas. Even though a detailed phenological study of the sampled *P. oceanica* meadows was not conducted in the current study, the values for the height (ranging between 15 cm and 122 cm) and seabed percentage cover (ranging between 20% and 100%) of the same meadows fall within corresponding average values cited in literature. With respect to the degree of waste management in the surveyed coast, it is estimated that poorly-managed waste accounts for 2% of the total waste generated in Spain, and 8% of the waste generated in Malta, a percentage that ranges between 2% and 69% for countries in the Mediterranean region (Jambeck et al., 2015). Therefore, we could state that the present study is representative of Mediterranean seagrass meadows exposed to a medium to high level of terrestrial waste management.

Results demonstrate the ability of seagrass meadows to trap land-sourced litter, with the landside edge just outside the same meadows being the main area of macro-litter accumulation. Areas away from the edge, both inside and outside the meadow, recorded lower litter-concentration values than the meadow edge. Macro-litter concentrations increased by 2.7 times, on average, after episodes of heavy rainfall. Litter was mainly deposited along the inner and outer margins of the meadow edge. Storm waters trigger the transport of large loads of mismanaged litter from land to the sea (González-Fernández et al., 2021), part of which has been shown to be retained by subtidal seagrass beds.

Easily-transportable litter, mainly plastic items, including bags and packaging, sanitary wipes or fragments of these items ('H', 'VH', Fig. 2) dominated the outer margin of the meadow. The inner margin of the meadow was dominated by less mobile macro-litter ('L', 'VL'). Interestingly, the observed pattern involving the highest concentrations of microplastics being recorded within the sediment of the inner margin of the meadows was consistent with that reported for sediment particles and nutrients (Adhitya et al., 2016; Fonseca et al., 1983, 1982).

According to these studies, the reduction of current flow velocity within the meadow facilitates sedimentation rates and hinders the resuspension of particles (Terrados and Duarte, 2000). Sánchez-Vidal et al. (2021) proposed that some of the micro-plastics deposited on *P. oceanica* vegetation could be subsequently stranded on beaches by waves and currents during winter storms and eventually transported back to seagrass meadows by coastal runoff or through aeolian deposition.

On the basis of our results and those contributed by other recent studies (Chubarenko and Stepanova, 2017; Sánchez-Vidal et al., 2021), we hereby outline a mechanism for the dynamics of non-floating litter trapped in seagrass meadows. While the continued input of litter from the land to the ocean might result in a long-term trend of litter accumulation on the seafloor, the nearshore distribution of non-floating litter, either on an annual or on the scale of a few years, could be considered to be in a state of dynamic equilibrium (Fig. 6). The distribution of litter recorded during our summer sampling, after periods without heavy rainfall and swell, can be considered as representing the equilibrium scenario, whereas heavy rainfall episodes act as steady-state disturbances by flushing out considerable litter loads within short time periods. The equilibrium would be restored by waves and tides, which recurrently bring litter ashore and drag it back to the coastal zone, to be eventually buried, fragmented or exported to deeper areas.

According to our results, a fraction of the land-sourced litter transported by runoff into nearshore waters is deposited along the edge of the seagrass meadows, as result of the so-called meadow edge effect (González-Ortiz et al., 2014). Part of this litter remains accumulated along the outer edge of the meadow, whilst another component penetrates the first few meters of the meadow (Gacia et al., 1999; Peralta et al., 2008), raising the litter concentration within the inner margin of the meadow edge (Fig. 4).

After litter input, swell, tides and bottom currents drive the litter dynamics in and around the meadows, as is the case for sediment particles (Adhitya et al., 2016; Fonseca et al., 1982; González-Ortiz et al., 2014). Waves are able to move the most easily-transportable macro-litter fractions (Fig. 6) as well as micro-plastics towards the outer edge of the meadow (based on Figs. 1 and 2), and towards the beaches (Chubarenko and Stepanova, 2017; Sánchez-Vidal et al., 2021). Macroplastic in transit to the beach can break down through mechanical processes (Efimova et al., 2018; Brouzet et al., 2021), while beached plastic is exposed to accelerated photodegradation processes (Andrady, 2011). In contrast, the heaviest litter items mostly remain within the core and inner margin of the meadow's edge (Fig. 2). Only the most energetic of storms are able to carry large and heavy litter items to the beach (Chubarenko and Stepanova, 2017).

Following the above-described succession of scenarios following an episode of heavy rainfall, litter appears to repeatedly migrate up and down within the coastal zone, with the support of subtidal seagrass beds. The present study focused on *P. oceanica*; however, most of the canopy-forming seagrass species are capable of influencing hydrodynamic conditions on site (Lara et al., 2016) and thus likely also to play a role in the storage and processing of litter. It is also important to note that extreme rainfall episodes occur across the globe, and it is projected that these will increase due to climate change (Lehmann et al., 2015; Masson-Delmotte et al., 2021; Program (U.S.), 2007). An increase in the frequency of extreme rainfall events increases the potential for litter to spill into coastal waters and to pose an additional pressure on existing stormwater management systems (Axelsson and van Sebille, 2017). Moreover, torrential rains are a frequent meteorological event in the Mediterranean basin (Pastor et al., 2001). Evidence suggests that Mediterranean coastlines are vulnerable to an increase in the frequency of flooding, especially during the autumn season (Bevacqua et al., 2020, Diez et al., 2013). Recently, the severe storm witnessed in the western Mediterranean during the second week of September 2019 resulted in a record-breaking flood (known as 'cold drop') and in disaster-scale infrastructural damage in southeastern Spain, surpassing previous historical records for rainfall counts (Caballero et al., 2019).

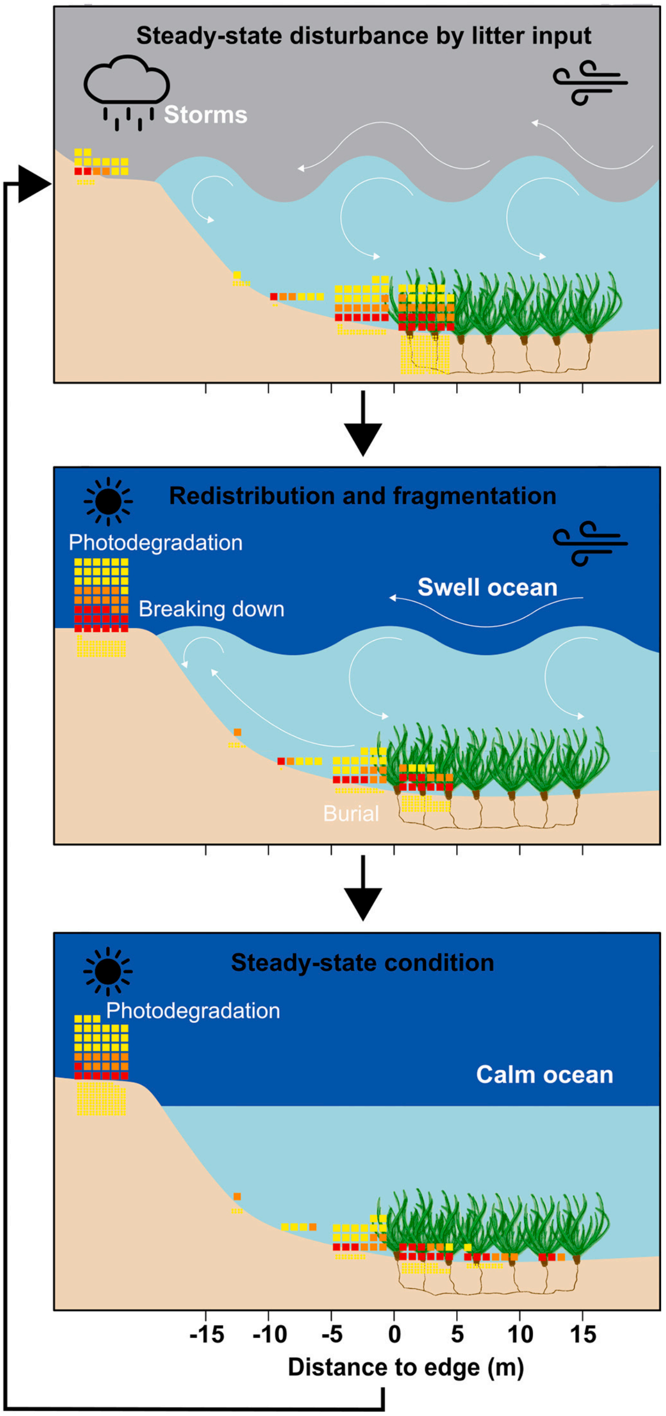

Fig. 6. Proposed succession of scenarios of non-floating litter distribution following an event of heavy rainfall event and litter input from land Macro- and micro-litter is shown with large squares and small circles, respectively. Red, orange and yellow squares indicate heavy ('VL' and 'L'), medium ('M'), and light ('VH' and 'H') macro-litter, respectively. Each square and circle correspond to relative units of concentration. Microplastics in the 'Steady-state disturbance by litter input' scenario are assumed to behave similarly to light macro-litter. The relative amounts of macro- and micro in the "Redistribution and fragmentation state" scenario is assumed as the midpoint between 'Steady-state disturbance by litter input' and 'Steady-state condition' scenarios, while beach litter results from balance the missing litter on the seafloor at the 'Steady-state condition'. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The forecasted increase in the frequency of intense storm phenomena which transfer litter from land to coastal waters, coupled with the high value of ecosystem services provided by *P. oceanica* call for further investigations into the influence of seagrass meadows on nearshore litter dynamics. Seagrass meadows and their ecomorphology could potentially be considered as an important driver for litter sequestration. Likewise, the increasing inputs of litter into the ocean pose an unprecedented threat to these ecosystems (Balestri et al., 2017; Menicagli et al., 2021). In particular, the fact that litter accumulation mainly happens at the edges of the meadows could have drastic consequences on seagrass-associated biota considering the critical role of this transitional ('ecotone') zone (El Zrelli et al., 2020; Gillanders, 2006). Phytoplankton and organic matter in suspension sediment in larger quantities at these seagrass edges/margins than within the inner part of the meadow itself (Carroll and Peterson, 2013; González-Ortiz et al., 2014). Many species (e.g. suspension-feeders) using this organic matter as a food source show higher abundances and growth rates at the edges rather than within the same seagrass meadow (Bologna and Heck, 1999; Carroll and Peterson, 2013; Irlandi and Peterson, 1991), which in turn makes it an ideal feeding ground for predators (Smith et al., 2011). On the other hand, seagrass meadows can also act as a refuge by reducing predation pressure (Peterson et al., 2001), since their edges provide a suitable zone for "food risk trade-off" for many seagrass-associated prey species, such as filter feeders or small fish (Carroll and Peterson, 2013; Peterson et al., 2001; Smith et al., 2011). The accumulation of litter in seagrass meadows, and particularly along their landside edge, may imply its incorporation into the seagrass food web as already occurs in other coastal habitats, through filtering (Karlsson et al., 2017), grazing (Sawalman et al., 2021) and predation (Ory et al., 2017). Thus, increased knowledge is required to inform the formulation of effective management measures and policies to address the ecological challenge posed by litter to Mediterranean coastal ecosystems.

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Credit authorship contribution statement

T Navarrete-Fernández: original idea, conceptualization, project administration, field work, methodology, investigation, formal analysis, visualization, writing – original draft, writing – review & editing, R Bermejo: formal analysis, visualization data curation and writing, I Hernández: review and writing, A Deidun: field work, review and writing, M Andreu-Cazenave: field work and review, JI González-Gordillo: review, A Cózar: funding acquisition, conceptualization, methodology, review, editing and writing. All authors approved the final submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.113299.

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