Plastics and sedimentation foster the spread of a non-native macroalga in seagrass meadows

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HIGHLIGHTS

- Plastics altered seagrass architecture and prevented vertical rhizome growth.
- Plastics and sedimentation reduced seagrass-invasive alga competition.
- Plastics may increase seagrass vulnerability to invasive algae and sedimentation.

GRAPHICAL ABSTRACT
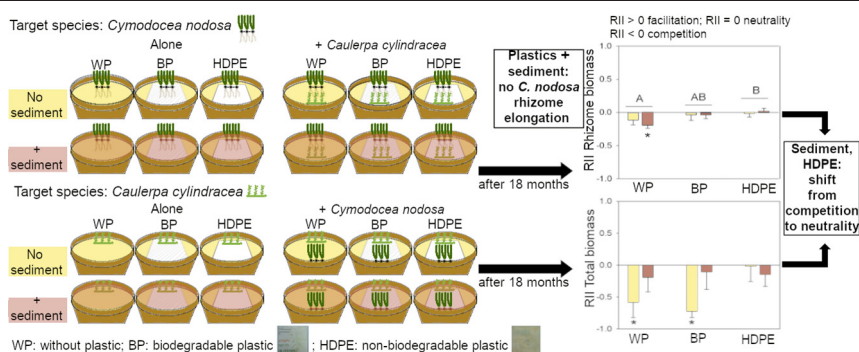
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ABSTRACT

Plastics are found in marine environments worldwide, and their effects on macrophytes (seagrasses and macroalgae) colonizing sandy bottoms are still poorly known. Seagrass meadows are valuable but declining ecosystems due to local and global-change related stressors, including sediment disturbance and introduced macroalgae. Understanding whether plastics pose a further threat to seagrasses is critically important. In two simultaneous additive experiments performed in an aquaculture tank, we examined the individual and combined effects of macroplastics (non-biodegradable high-density polyethylene and biodegradable starch-based) and sedimentation (no and repeated sedimentation) on the performance (in terms of biomass and architectural variables) of a native Mediterranean seagrass (*Cymodocea nodosa*) and an introduced macroalga (*Caulerpa cylindracea*), and on the intensity of their interactions. Macroplastics were still present in sediments after 18 months. *Cymodocea nodosa* produced a greater biomass and longer horizontal rhizome internodes forming clones with more spaced shoots probably to escape from plastics. Plastics prevented *C. nodosa* to react to sedimentation by increasing vertical rhizome growth. Under *C. cylindracea* invasion, *C. nodosa* allocated more biomass to roots, particularly to fine roots. In the presence of *C. nodosa*, *C. cylindracea* performance was reduced. High-density polyethylene (HDPE) plastic and sedimentation shifted species interactions from competitive to neutral. These results suggest that both HDPE and biodegradable starch-based macroplastics, if deposited on marine bottoms, could make seagrasses vulnerable to sedimentation and reduce plant cover within meadows. HDPE plastic and sedimentation could contribute to the decline of seagrass habitats by facilitating the spread of non-native macroalgae within meadows. Overall, the study highlights the urgent need to implement more effective post-marketing management actions to prevent a further entering of plastics in natural environments in the future, as well as to establish to conservation measures specifically tailored to protect seagrass habitats from plastic pollution.

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1. Introduction

Introduction of plastic waste in the oceans is an emerging threat to coastal and marine environments globally (Law, 2017; Cozzolino et al., 2020; Lau et al., 2020). Numerous studies have shown the detrimental effects of plastics floating on the sea surface or submerged in the water column on a variety of marine organisms and ecosystems (Cole et al., 2013; Foekema et al., 2013; Bergmann et al., 2015). However, floating plastics can sink in the oceans because of fouling by organisms and adherence of sediment particles (Lobelle and Cunliffe, 2011). Some recent studies have shown the accumulation of plastics in marine bottoms, especially in those colonized by macrophytes such as seagrasses and macroalgae (Cozzolino et al., 2020; Huang et al., 2020). The presence of plastics in marine sediments can create "novel" micro-habitats for macrophytes by changing local abiotic conditions (e.g., pH, temperature and redox condition), microbial community composition and biogeochemical cycling processes (Green et al., 2015; Balestri et al., 2017; Seeley et al., 2020). Yet, the threats posed by these novel habitats on macrophytes and the possible cascading effects on their associated ecosystems need to be evaluated (Bonanno and Orlando-Bonaca, 2020). The only study addressing this issue has focused on the effect of a type of starch-based biodegradable and compostable plastic bag, which has recently been introduced into the market to reduce plastic pollution, on two co-occurring native seagrasses (Balestri et al., 2017). Results of this study showed that the bag can shift the interactions between the species from neutral to competitive by acting as a physical barrier and altering sediment quality (Balestri et al., 2017). Whether durable non-biodegradable plastics (such as high-density polyethylene, low-density polyethylene and polystyrene), which currently constitute a large fraction of plastics in oceans (Suaria et al., 2016; Avio et al., 2017), can have similar or even more detrimental effects on macrophytes is unknown. In addition, the possible interactive effects of plastics and other global change-related stressors that currently affect seagrass habitats remain to be explored. This issue is highly relevant as seagrasses are foundation species that deliver a wide range of valuable functions and services to coastal ecosystems and human being, but many species are experiencing a widespread decline due to multiple anthropogenic stressors and global change (Orth et al., 2006; Barbier et al., 2011; Cullen-Unsworth and Unsworth, 2013; de los Santos et al., 2019).

One of the factors contributing to determine seagrass decline is the introduction and spread of non-native algae, such as those belonging to the genus Caulerpa, Codium and Sargassum (Williams, 2007; Williams and Smith, 2007; Martínez-Lüscher and Holmer, 2010; de los Santos et al., 2019). These macroalgae can negatively affect native seagrasses by competing for basic resources (i.e., light and space) and altering sediment deposition and biogeochemical characteristics (Piazzi et al., 2007; Deudero et al., 2011; Drouin et al., 2012; Matijević et al., 2013; Ceccherelli et al., 2014), resulting in biodiversity loss and changes in ecosystem functioning (Piazzi et al., 2007; Bulleri et al., 2010; Tuya et al., 2014). However, the success of the invasion of seagrass meadows by these species depends on various factors, including biotic resistance derived from interspecific competition for resources between the seagrass and the introduced species, environmental conditions and the conservation status of meadows at the time of invasion (Bulleri et al., 2010; Deudero et al., 2011; Piazzi et al., 2016; Balestri et al., 2018). Well-preserved meadows are generally resistant to algal invasion, although some macroalgae can penetrate slowly intact seagrass meadows (Ceccherelli et al., 2000; Deudero et al., 2011). Disturbance events, such as sudden deposition of sediments due to storms or anthropogenic impacts (i.e., beach nourishment and discharging of dredged material) can reduce seagrass cover and thus increase the chance of meadow invasion (Duarte et al., 1997; Manzanera et al., 1998; Mills and Fonseca, 2003; Tuya et al., 2013). The critical burial threshold level and the sedimentation duration tolerated by seagrasses vary greatly among species

depending on their size and architectural traits (Duarte et al., 1997; Cabaço et al., 2008). Species with large size or that produce vertical rhizomes (e.g., Cymodocea, Thalassia, Thalassodendron) have generally a greater tolerance to sudden burial as they are able to relocate leaf-producing meristems close to sediment surface (Marbà and Duarte, 1994; Cabaço et al., 2008; Balestri and Lardicci, 2014a). However, repeated sedimentation events of low or moderate intensity can reduce the concentration of pore-water oxygen and increase the content of toxic sulfur compounds in the root zone, leading to high plant mortality rates (Manzanera et al., 1998; Mills and Fonseca, 2003; Borum et al., 2005; Larkum et al., 2006). In contrast, introduced opportunistic species such as those belonging to genus Caulerpa can benefit from sediment deposition resulting in increased competitive strength (Glasby et al., 2005; Piazzi et al., 2005). Plastic pollution, sediment disturbance and non-native algae introduction will very likely continue to affect seagrass habitats in the future (Williams, 2007; Dolch and Reise, 2010; Lau et al., 2020). Therefore, assessing the joint effects of plastic litter and sedimentation on the outcome of interactions among non-native algae and seagrasses is critically important.

In this study, we explored the individual and the combined effects of macroplastics and repeated sedimentation events on the performance of a native seagrass and a non-native macroalga when grown alone and in competition with each other for 18 months in mesocosms. A traditional non-biodegradable plastic bag made of high-density polyethylene (HDPE) and a biodegradable/compostable bag were chosen for testing; this latter was included as the global production of biodegradable plastics is predicted to greatly increase and reach the level of non-biodegradable ones in the next decades (EU Parliament, 2018; European bioplastics, 2018). However, the potential risks associated to their dispersion in marine environments have still not systematically investigated. As native seagrass model we chose Cymodocea nodosa (Ucria) Ascherson, and as non-native alga model we selected Caulerpa cylindracea Sonder. Cymodocea nodosa is a fast-growing clonal species that forms monospecific and mixed meadows along Mediterranean and Atlantic coasts (Short et al., 2007). The species tolerates moderate sediment deposition rates and sudden burial events less than 7 cm (Marbà and Duarte, 1994). Caulerpa cylindracea is a marine Chlorophyta introduced into the Mediterranean Sea in 1990 from south-western Australia that has severely altered the structure of native communities and local sediment conditions (Ceccherelli and Campo, 2002; Piazzi et al., 2005; Piazzi et al., 2007; Bulleri et al., 2010). This rhizophytic alga spreads mainly vegetatively by fragmentation and can establish within C. nodosa meadows (Ceccherelli and Campo, 2002). Experimental evidence indicates that C. cylindracea can tolerate sedimentation (Piazzi et al., 2005), but the critical burial threshold level has still not determined. The aim of this study was two-fold. First, we aimed to evaluate how HDPE and biodegradable plastic bags affected the performance of the target species. We hypothesized that the impact of HDPE bag on plants would be larger than that of the biodegradable bag. This is because HDPE plastics can persist intact in marine environments for years (Napper and Thompson, 2019; Chamas et al., 2020) while biodegradable plastics can lose gradually mechanical resistance and degrade completely within months (O'Brine and Thompson, 2010; Napper and Thompson, 2019). Second, we aimed to assess whether plastics and sedimentation events of moderate intensity facilitate the spread of the macroalga in the seagrass habitat. We hypothesized that C. cylindracea would take advantage of the presence of plastics and sedimentation since studies have shown that this alga can cope with stressful conditions and benefit from increased sediment deposition (Piazzi et al., 2005; Piazzi et al., 2007).

2. Materials and methods

2.1. Plastic material and plant collection

Non-biodegradable bags were made of high-density polyethylene (HDPE). Biodegradable/compostable bags (BP) were made of starch

and vinyl-alcohol copolymers (Mater-Bi®) and complied with the European standard EN13432:2000. Apical plagiotropic rhizomes of *C. nodosa* were collected in a shallow meadow near to Rosignano Solvay (Italy, Ligurian Sea, 43°22′55.66″N 10°26′7.05″E) at 0.5 m depth at the end of April 2018. The rhizomes were cut into fragments, approximately 6.5 cm long with 3–4 shoots and apical meristem, that have proven to have the ability to regenerate and grow (Balestri and Lardicci, 2012). Similar-sized vegetative propagules of *C. cylindracea* (approximately 8 cm stolon length with 1–3 fronds) were collected at 0.5 m depth in the *C. nodosa* meadow in June 2018.

2.2. Experimental design and set-up

Two simultaneous additive experiments were conducted. In one experiment, we examined the individual and combined effects of plastic, sedimentation, and invasion by the non-native macroalga *C. cylindracea* on the performance of the native seagrass *C. nodosa*. In the other experiment, we evaluated the individual and combined effects of plastic, sedimentation, and *C. nodosa* presence on the performance of *C. cylindracea*. The experiments were conducted at the INVE Aquaculture Research Centre of Rosignano Solvay in 2018 and 2019 and covered two entire growing seasons of the two species (Caye and Meinesz, 1985; Klein and Verlaque, 2008). Experiments were performed in mesocosms placed into an outdoor aquaculture tank, filled with natural seawater continuously pumped from the sea, and equipped following a protocol previously established for successfully growing seagrasses (Balestri and Lardicci, 2006; Balestri and Lardicci, 2012). The water level within the tank was maintained constant (1.20 m) during the whole experimental period. The temperature of the seawater flowing in the aquaculture tank ranged from 10 to 27 °C (Fig. S1), the pH was 8–8.2, and the salinity varied between 37.8 and 38.1 over the experimental period. The mesocosms consisted of 20 cm diameter and 20 cm height pots (Nuova Pasquini & Bini, Italy) partially filled with natural sand (<0.6–1 mm, <0.01% organic content) previously homogenized by hand. In each mesocosm, a slow-release fertilizer (Pluscote Garden 16% N, 8% P, 16% K) was added to the sediment (1.5 g l^{-1} of substrate) before the start of the experiments (Balestri and Lardicci, 2012).

Each experiment was set up as a full factorial orthogonal design with 12 combinations (3×2×2) of the following factors; Plastic: without plastic (WP), biodegradable/compostable bag (BP), non-biodegradable bag (HDPE); Neighbour: no hetero-specific, hetero-specific presence; Sedimentation: no sedimentation (ND), sedimentation (D). In the experiment with *C. nodosa* as the target, the hetero-specific was *Caulerpa cylindracea* (Cau). In the experiment with *C. cylindracea* as the target, the hetero-specific was *Cymodocea nodosa* (Cym). There were seven replicates for each treatment combination for a total of 84 mesocosms per experiment (Fig. 1). Before the start of the experiments (January 2018), HDPE and BP bags were immersed in seawater inside the tank to experience natural weathering. After three months (April 2018), they were retrieved and cut into pieces with a size (12 × 12 cm) in the range of that of plastic macro-fragments found in coastal vegetated habitats (Cozzolino et al., 2020). In the mesocosms assigned to the plastic presence treatments, a single piece of bag (either HDPE or BP) was placed in the centre of the mesocosm, covered with a layer of 5 cm of fresh sediment and left undisturbed for 20 days. In the mesocosms assigned to WP treatment, only the sediment was added. Then, collected fragments of *C. nodosa* were planted in all the mesocosms (one fragment per mesocosm) assigned to the experiment with *C. nodosa* as the target (84) and in half of the mesocosms assigned to the experiment with *C. cylindracea* as the target (42). In this latter experiment, *C. nodosa* fragments served as the neighbour. The remaining mesocosms assigned to the experiment with *C. cylindracea* as the target (42) were left unplanted until the start of the experiments. Mesocosms were left undisturbed for four weeks to allow the establishment of plants. We considered as established those fragments that had produced at least one shoot during this period.

In the experiment with *C. nodosa* as the target (Fig. 1), propagules of *C. cylindracea* were planted in half of the mesocosms (one propagule per mesocosm) colonized by *C. nodosa* (42 mesocosms) to simulate *C. cylindracea* invasion. These mesocosms were assigned to the heterospecific presence treatment (Cau). In the other half of the mesocosms (42) colonized by *C. nodosa*, no *C. cylindracea* propagule was added and hence they contained only *C. nodosa*. These mesocosms were assigned to the no heterospecific treatment (NoCau). In the experiment with *C. cylindracea* as the target (Fig. 1), one *C. cylindracea* propagule was planted in the mesocosms containing only sediment (42 mesocosms), and they were assigned to the no heterospecific treatment (NoCym). One *C. cylindracea* propagule was also planted in the remaining mesocosms (42) containing *C. nodosa*, and they were assigned to the treatment heterospecific presence (Cym). In the mesocosms containing both the target species and the heterospecific, the distance between plants was approximately 10 cm. To mimic prolonged (press) repeated burial events, plants assigned to the sedimentation treatment were covered with a sediment layer 3 cm thick on two occasions during the experimental period, June 2018 and June 2019. This simulated a burial depth of approximately 40% relative to plant height of *C. nodosa* (7.8 ± 0.1 cm, mean ± SE) considered as moderate for this species (Tuya et al., 2013), and of approximately 120% relative to frond height of *C. cylindracea* (2.49 ± 0.04 cm) before sediment addition. Mesocosms assigned to no sedimentation were left undisturbed (no sediment was added). Mesocosms were randomly interspersed throughout the tank and weekly reallocated at random during the experiment to minimize possible position effects. Plants were checked weekly for the whole duration of the experimental period.

2.3. Measurements

The number of alive shoots of *C. nodosa* and the number of fronds of *C. cylindracea* in each mesocosm was counted monthly during the experimental period. At the end of the second growing season of the two species (October 2019), all plants and plastic materials were harvested, transported to the laboratory, and washed with tap water to remove sand. For *C. nodosa*, three architectural variables were measured; the total length of plagiotropic (horizontal) rhizomes, the mean spacer length between connected ramets (internodal length) which are considered as a proxy for the ability of clonal plants to spread in horizontal space, and the mean length of orthotropic (vertical) rhizomes which is considered as a proxy for the ability of plants to cope with sediment deposition. *Cymodocea nodosa* plants were then separated into shoots, rhizomes and roots. Roots were further divided in coarse roots (root >1 mm in diameter) and fine roots (root <1 mm in diameter; Freschet and Roumet, 2017). Fine roots have a greater surface to volume ratio and are produced to increase nutrient uptake efficiency (*i.e.*, uptake per unit root biomass) while coarse roots are mainly involved in maintaining flux of oxygen to the roots from the photosynthetic structures (Duarte et al., 1998). For *C. cylindracea*, the number of fronds of each plant was counted, and the average maximum frond height was calculated measuring the two highest fronds. Total stolon length was measured by summing the length of all stolon branches produced by each plant. For both the study species, plant materials were weighted after being dried at 70 °C for at least 72 h to determine biomass. Total root biomass was calculated by summing the biomasses of coarse roots and fine roots. The root to shoot biomass ratio of *C. nodosa* was also calculated as it is considered as an important indicator of changes in relative resource allocation in response to surrounding abiotic and biotic conditions (nutrient/water/space limitation and neighbour presence; Poorter et al., 2012).

To examine how plastic and sedimentation affect the intensity of plant-plant interactions, the relative interaction intensity index (RII; Armas et al., 2004) based on the biomass of shoots, total root and rhizome in the experiment with *C. nodosa* as the target, and on frond
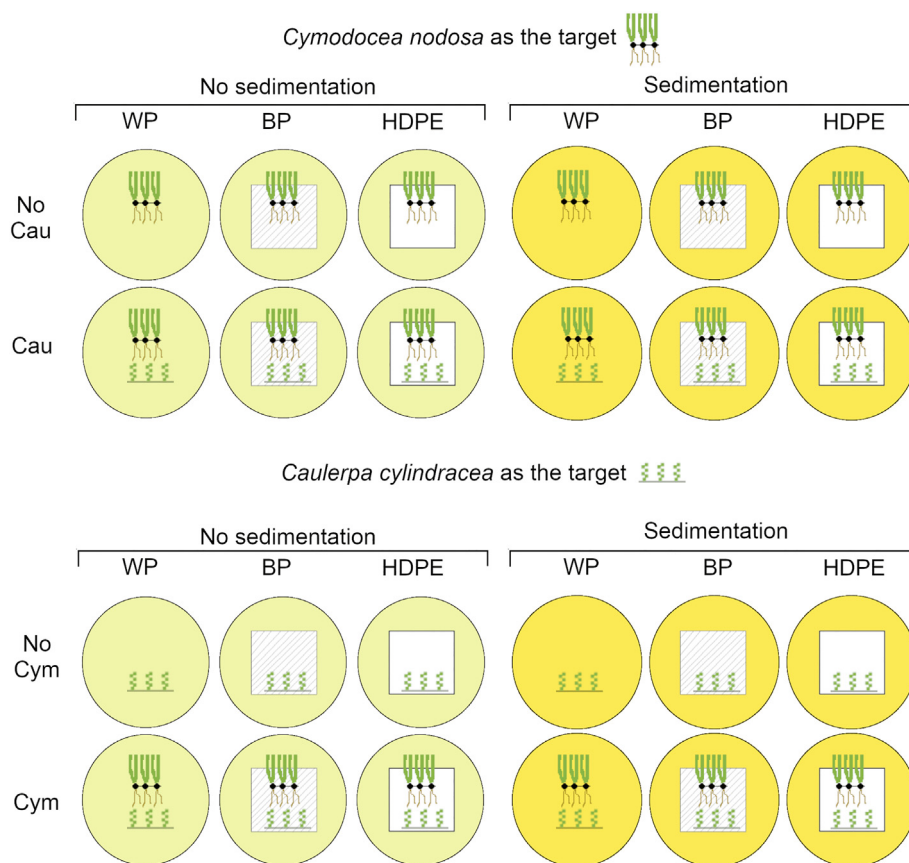
Fig. 1. Experimental design. Target plants (either *Cymodocea nodosa* or *Caulerpa cylindracea*) were grown in mesocosms containing no plastic (WP), biodegradable/compostable plastic (BP, dashed square), and non-biodegradable plastic (HDPE, white square) under no sedimentation and under sedimentation in the presence of a hetero-specific neighbour (with *C. cylindracea*- Cau for *C. nodosa* as the target or with *C. nodosa*- Cym for *C. cylindracea* as the target) and in the absence of a hetero-specific neighbour (without *C. cylindracea*- No Cau for *C. nodosa* as the target or without *C. nodosa*-No Cym for *C. cylindracea* as the target). Each experimental combination was replicated seven times.

number, stolon length and total biomass in the experiment with *C. cylindracea* as the target, was calculated for each treatment as follows

$$RII = \frac{B_W - B_0}{B_W + B_0}$$

where B_W is the target performance with a neighbour and B_0 is the target performance grown as isolated plant. In this study, B_0 values were calculated as the average performance of the target species when grown alone separately for each combination of the levels of the factors Plastic and Sedimentation. The values of RII are −1 for competitive exclusion, 0 for neutral interaction and +1 for obligate facilitation (Armas et al., 2004). Finally, for each experiment a sample ($n = 20$) of pieces of each of the two plastic types retrieved from mesocosms were observed immediately after collection under a stereomicroscope (Leica WILD M3C, Germany) to check for changes in plastic appearance (the presence of crazing and perforations and fragmentation) which are considered as first visual effects of initial plastic degradation (Shah et al., 2008; Gewert et al., 2015).

2.4. Statistical analysis

Three-way ANOVAs were performed on the initial number of *C. nodosa* shoots and *C. cylindracea* fronds of individuals planted in each mesocosm to check for possible differences in the size of plants assigned to the different treatments. Three-way ANOVAs were also carried out on the number of *C. nodosa* shoots and *C. cylindracea* fronds produced by plants before the first sediment addition (in June 2018) to check for possible differences among plants assigned

to the different levels of the factor Sedimentation. For each species as the target, a three-way permutational multivariate analysis of variance (PERMANOVA) on plant variables was carried out to assess the effects of treatments at the whole plant level. Before the analysis, data were normalized by subtracting the mean and dividing by the standard deviation for that variable from each entry of a single variable as the variables were not on comparable measurement scales (Clarke and Gorley, 2006). For *C. nodosa*, the PERMANOVA was performed on all plant variables while for *C. cylindracea* stolon length was excluded as it was correlated with the other metrics. PERMANOVA analyses were computed using the Euclidean distance matrices and 9999 permutations of the residuals under a reduced model, and pair-wise comparisons were done when differences were detected. Statistically significant terms were also checked for differences in multivariate group dispersion through permutational analysis of multivariate dispersion (PERMDISP). Separate three-way ANOVAs were performed to identify which variables were influenced by the experimental factors. PERMANOVA and ANOVA analyses on *C. cylindracea* growth variables were carried out on five replicates per treatment due to the death of some plants. Student-Newman-Keuls (SNK) tests were used to identify differences among levels of significant factors. Two-way ANOVAs were also conducted to examine the effect of Plastic and Sedimentation on RIIs. To test whether RII values calculated in all level combinations of the factors Plastic and Sedimentation (WP, HDPE and BP under ND or D) significantly differed from zero, two-sided single mean t-tests were separately performed.

Prior to ANOVAs, data were checked for normality and homogeneity of variances by Shapiro-Wilk test and Cochran' C test, respectively, and transformed to meet assumptions when necessary (data

transformations are reported in ANOVA tables). For *C. nodosa* orthotropic rhizome length and for *C. cylindracea* total biomass and RIIs, no transformation was effective in removing the non-normal error distribution, and ANOVAs were performed on untransformed data as ANOVA is robust to departures from the normality assumption when samples are balanced and there are many treatments (Underwood, 1997). RII data for stolon length and total biomass of *C. cylindracea* were analyzed by t single mean test even if they were approximately normally distributed as this test is robust to violations of normality for $n \geq 5$. Statistical significance was set at $\alpha = 0.05$. ANOVA analyses (GAD package) and *t*-tests were conducted using R software (v. 3.5.1; Team, Rs. RStudio, 2015), and PERMANOVA and PERMDISP analyses were carried out using PRIMER v6 (Primer-E Ltd., Plymouth) with PERMANOVA add-on software (Clarke and Gorley, 2006; Anderson et al., 2008).

3. Results

3.1. Effects of plastic, sedimentation and non-native macroalga on Cymodocea nodosa as the target

Before the start of the experiment, no significant differences in initial size of *C. nodosa* fragments among treatments were detected (Table 1). All *C. nodosa* plants were successfully established and still alive at the end of the experiment. Before the first sediment addition, plants

assigned to the different sedimentation levels had similar shoot number (Table 1). The number of shoots increased during the experimental period (Fig. S2) following the typical seasonal growth pattern of the species in the Mediterranean. PERMANOVA analysis on *C. nodosa* variables measured at the end of the experiment detected significant effects of the factors Plastic, Neighbor (*C. cylindracea*), Sedimentation, as well as Plastic x Sedimentation at whole plant level (Table S1). There was a significant effect of the main factors Plastic and Sedimentation on shoot biomass (Table 1). Plants grown with HDPE had on average a larger shoot biomass compared to those grown with BP or WP, regardless of sedimentation and *C. cylindracea* treatments (Table 1; Fig. 2a). Plants had on average a greater shoot biomass when grown under sedimentation than under no sedimentation, irrespective of plastic and *C. cylindracea* treatments (Table 1; Fig. 2a). A significant Plastic x Sedimentation effect was found on rhizome biomass, fine and coarse root biomass, root to shoot biomass ratio, plagiotropic rhizome length and orthotropic rhizome length (Table 1). Regardless of *C. cylindracea* treatments, rhizome biomass (Fig. 2b), fine root biomass (Fig. 2c), coarse root biomass (Fig. 2d), root to shoot biomass ratio (Fig. 2e), and plagiotropic rhizome length (Fig. 2f) were significantly greater in plants grown with HDPE or BP than without plastic but only under no sedimentation. Orthotropic rhizome length was significantly greater in plants grown with BP than without plastic under no sedimentation, irrespective of *C. cylindracea* presence/absence (Fig. 2g). Averaged across *C. cylindracea* treatments, fine root biomass (Fig. 2c), coarse root

Table 1
Summary of 3-way ANOVAs testing the effect of plastic (no plastic *vs.* biodegradable/compostable *vs.* non-biodegradable plastic bag macro-fragment), sedimentation (no sedimentation *vs.* sedimentation) and neighbour (presence *vs.* absence of *Caulerpa cylindracea*) on variables of *Cymodocea nodosa* as the target.

Source	df	Initial shoot number (April)		Shoot number (June)		Shoot biomass		Rhizome biomass		Fine root biomass	
		F	p	F	p	F	p	F	p	F	p
Plastic (P)	2	0.96	0.385	3.85	**0.025**	6.59	**0.002**	8.44	**<0.001**	7.32	**0.001**
Sediment (S)	1	0.20	0.653	0.82	0.366	7.73	**0.006**	0.55	0.458	0.03	0.850
Neighbour (N)	1	3.25	0.075	0.01	0.897	0.30	0.581	0.15	0.691	5.54	**0.021**
P × S	2	0.66	0.519	0.11	0.888	1.44	0.241	4.74	**0.011**	4.83	**0.010**
P × N	2	0.05	0.950	0.62	0.538	0.49	0.610	1.24	0.294	1.27	0.287
S × N	1	0.20	0.653	0.82	0.366	0.00	0.986	0.01	0.911	0.02	0.874
P × S × N	2	0.35	0.701	0.72	0.487	1.87	0.161	0.26	0.769	2.80	0.066
Residual	72										
Transformation				Log(x)							
Shapiro test		$p < 0.05$		$p < 0.05$		$p = 0.186$		$p = 0.460$		$p = 0.189$	
Cochran's test		$p = 3.54$		$p = 1.165$		$p = 0.365$		$p = 0.082$		$p = 0.347$	
SNK test				WP < BP=HDPE		WP=BP < HDPE ND < D		ND:WP < BP < HDPE HDPE:D < ND		ND:WP < BP=HDPE WP:ND < D HDPE:D < ND NoCau<Cau	

Source	df	Coarse root biomass		Root shoot ratio		Plagiotropic rhizome length		Orthotropic rhizome length		Spacer length	
		F	p	F	p	F	p	F	p	F	p
Plastic (P)	2	5.99	**0.003**	4.75	**<0.011**	10.17	**<0.011**	1.01	0.369	5.60	**0.005**
Sediment (S)	1	0.44	0.505	16.57	**0.001**	0.29	0.589	0.00	0.968	2.63	0.109
Neighbour (N)	1	1.23	0.270	16.81	**0.001**	0.71	0.399	2.67	0.106	1.03	0.312
P × S	2	4.06	**0.021**	4.36	**0.016**	3.83	**0.026**	4.81	**0.010**	1.28	0.283
P × N	2	1.50	0.228	1.09	0.340	0.48	0.618	1.91	0.154	0.12	0.879
S × N	1	0.05	0.814	0.06	0.800	0.19	0.662	0.33	0.566	0.11	0.731
P × S × N	2	1.17	0.313	0.09	0.908	0.53	0.589	0.24	0.780	0.82	0.440
Residual	72										
Transformation						Sqrt(x)				Log(x)	
Shapiro test		$p = 0.163$		$p = 0.394$		$p = 0.105$		$p < 0.05$		$p = 0.111$	
Cochran's test		$p = 0.359$		$p = 0.962$		$p = 0.600$		$p = 0.248$		$p = 0.828$	
SNK test		ND: WP < BP < HDPE WP:ND < D		NoCau<Cau ND: WP < BP < HDPE HDPE, BP:D < ND		ND: WP < BP < HDPE HDPE:D < ND		ND:WP < BP WP:ND < D		WP < BP=HDPE	

Data transformations and results of SNK tests are reported. Bold values indicate significance at $p < 0.05$. $n = 7$.
WP: without plastic; BP: biodegradable/compostable plastic; HDPE: non-biodegradable plastic; NoCau: *C. nodosa* grown without *C. cylindracea*; Cau: *C. nodosa* grown with *C. cylindracea*; ND: no sedimentation; D: sedimentation.
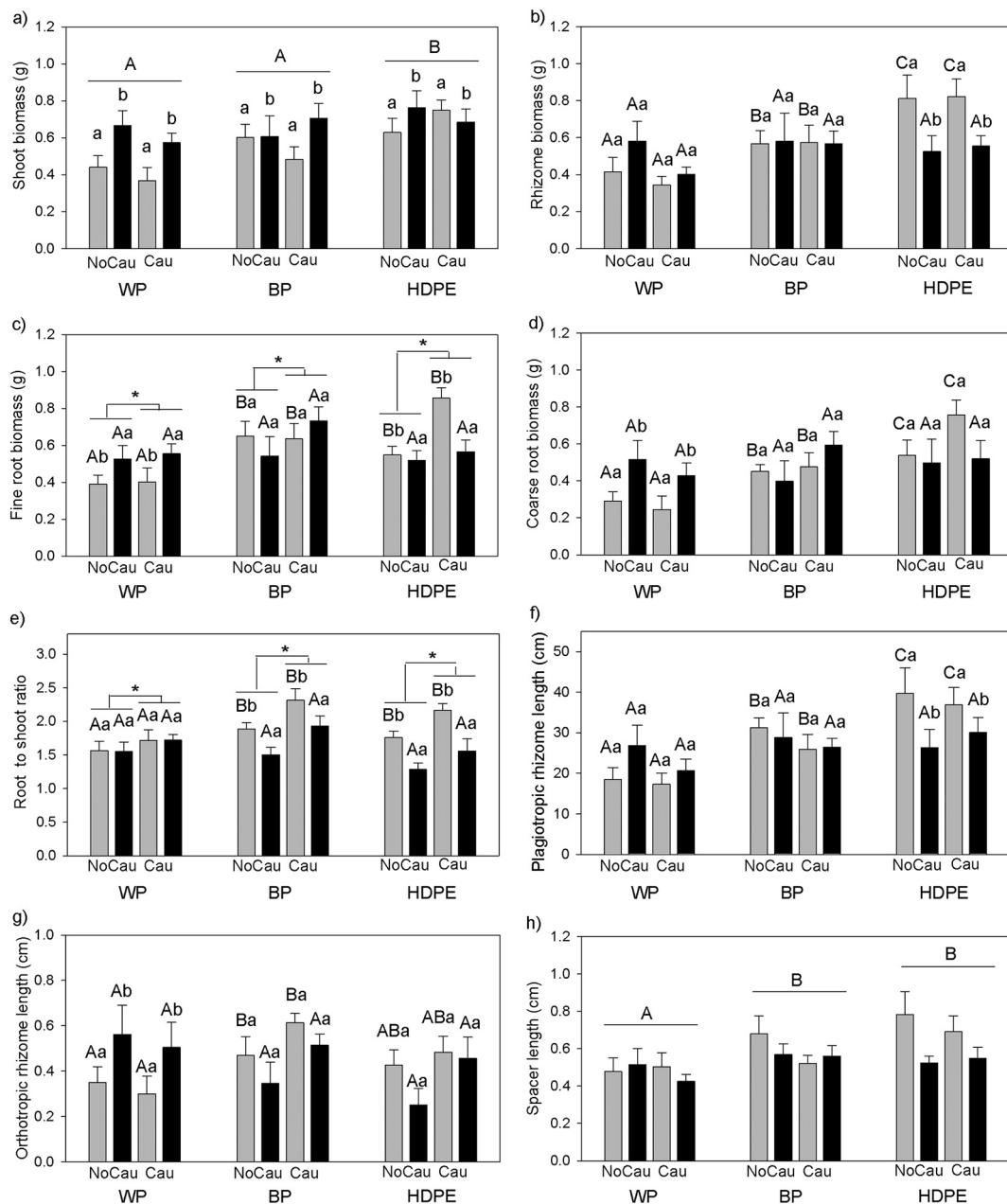
Fig. 2. Shoot biomass (a), rhizome biomass (b), fine root biomass (c), coarse root biomass (d), root to shoot biomass ratio (e), plagiotropic rhizome length (f), mean orthotropic rhizome length (g), and mean spacer length of plagiotropic rhizome (h) of *Cymodocea nodosa* as the target grown without *Caulerpa cylindracea* (NoCau) or with *Caulerpa cylindracea* (Cau) as the neighbour on substrate without plastic (WP), with biodegradable/compostable plastic (BP) or non-biodegradable plastic (HDPE) bag macro-fragments under no sedimentation (grey bars) or with sedimentation (black bars). Letters above bars report the outcome of SNK test comparing treatments. Mean ± SE, n = 7.

biomass (Fig. 2d), and orthotropic rhizome length (Fig. 2g) of plants grown without plastic were greater under sedimentation than under no sedimentation (Table 1). Instead, averaged across *C. cylindracea* treatments the rhizome biomass (Fig. 2b), fine root biomass (Fig. 2c), and plagiotropic rhizome length (Fig. 2f) of plants under no sedimentation were greater than those under sedimentation in the presence of HDPE (Table 1). In plants grown with HDPE or BP, a significantly lower root to shoot biomass ratio was found under sedimentation than under no sedimentation (Table 1; Fig. 2e). On average, this ratio was greater in *C. nodosa* plants grown in the presence of *C. cylindracea* than alone, regardless of the other factors (Table 1; Fig. 2e). Averaged across plastic and sedimentation treatments, fine root biomass was greater in plants grown with than without *C. cylindracea* (Table 1; Fig. 2c). Plants grown with HDPE or BP showed a greater internodal

length than those without plastic independently of the other factors (Table 1; Fig. 2h). A significant effect of the factor Plastic x Sedimentation was detected on RII based on shoot and root biomass (Table 2). Under no sedimentation, RII values on shoot and root biomass were greater for plants grown with HDPE than BP or without plastic, and the RII on shoot biomass for plants grown with BP was greater under sedimentation than under no sedimentation (Table 2; Fig. 3a, b). RII based on rhizome biomass were significantly smaller for plants grown without plastic than with HDPE (Table 2; Fig. 3c). The RII on root biomass of plants grown under sedimentation and with BP and those of plants grown under no sedimentation with HDPE were positive and significantly different from zero indicating a facilitative interaction (Table 2; Fig. 3b). Instead, the RII based on rhizome biomass of plants grown under sedimentation and without plastic was negative and

Table 2

Summary of (a) 2-way ANOVAs for the effect of plastic (no plastic vs. biodegradable/compostable vs. non-biodegradable plastic bag macro-fragment) and sedimentation (no sedimentation vs. sedimentation) on the relative interaction intensity index (RII) based on plant variables of *Cymodocea nodosa* as the target and *Caulerpa cylindracea* as the neighbour; and (b) one-sample mean t-test for significant departures from zero (neutral interaction) of RIIs.

(a)		RII shoot		RII root		RII rhizome	
Source	df	F	p	F	p	F	p
Plastic (P)	2	1.87	0.167	2.75	0.077	3.40	**0.044**
Sediment (S)	1	0.41	0.522	0.00	0.927	0.09	0.763
P × S	2	3.90	**0.029**	3.51	**0.040**	0.36	0.699
Residual	36						
Transformation				exp			
Shapiro test		p = 0.476		p = 0.283		p = 0.270	
Cochran's test		p = 0.155		p = 0.144		p = 0.458	
SNK test		ND:		ND:		WP < HDPE	
		WP=BP < HDPE		WP=BP < HDPE			
		BP:ND < D					

(b)		RII shoot		RII root		RII rhizome	
	df	t	p	t	p	t	p
No sedimentation							
WP	6	−1.55	0.170	−0.85	0.424	−1.68	0.143
BP	6	−2.08	0.082	−0.37	0.723	−0.43	0.678
HDPE	6	1.45	0.195	4.79	**0.003**	−0.24	0.817
Sedimentation							
WP	6	−1.87	0.110	−0.86	0.422	−4.21	**0.005**
BP	6	1.04	0.338	3.25	**0.017**	−0.55	0.596
HDPE	6	−1.19	0.276*	−0.09	0.929*	0.25	0.804

RIIs were based on shoot biomass, total root biomass, and rhizome biomass of *C. nodosa*, and they were calculated for all treatment combinations (n = 7). Data transformations and results of SNK tests are reported. Bold values indicate significance at p < 0.05. * denotes data approximately normally distributed. WP: without plastic; BP: biodegradable/compostable plastic; HDPE: non-biodegradable plastic; ND: no sedimentation; D: sedimentation.

significantly different from zero indicating a competitive interaction (Table 2, Fig. 3c). At the end of the experiment, all plastics were still present in sediments, and only BP plastic showed visual signs of deterioration such as holes (5.1 ± 1.1) due to the perforation of *C. nodosa* roots (Fig. S3).

3.2. Effects of plastic, sedimentation and native seagrass on *Caulerpa cylindracea* as the target

Before the start of the experiment, no significant differences in initial size of *C. cylindracea* propagules among treatments were detected (Table S2). All *C. cylindracea* propagules established a new plant in mesocosms. Before the first sediment addition, *C. cylindracea* plants assigned to the two sedimentation levels had a similar frond number (Table S2). The production of fronds during the experimental period (Fig. S4) showed the typical growth pattern of the species in the North Mediterranean with no frond in winter. In the presence of *C. nodosa*, two plants established with BP and two plants established without plastic (WP) died during the experiment. PERMANOVA analysis on *C. cylindracea* variables detected a significant effect of Neighbour (*C. nodosa*) and Plastic x Sedimentation at whole plant level (Table S3). Frond number (Fig. 4a), stolon length (Fig. 4c), and total biomass (Fig. 4d) of alive *C. cylindracea* plants were on average significantly lower in plants grown with than without *C. nodosa*, irrespective of the other investigated factors (Table 3). Frond height was affected by the factor Sedimentation x Neighbour (*C. nodosa*). Under no sedimentation, *C. cylindracea* fronds were shorter in the presence than in the absence of *C. nodosa*, regardless of plastic treatments. In the presence of the seagrass, *C. cylindracea* fronds were on average shorter under no sedimentation than under sedimentation, irrespective of plastic treatments (Table 3; Fig. 4b). A marginally significant effect of the factor Plastic x Sedimentation was also found on total biomass (Table 3). No significant differences among treatments were detected on RIIs based on
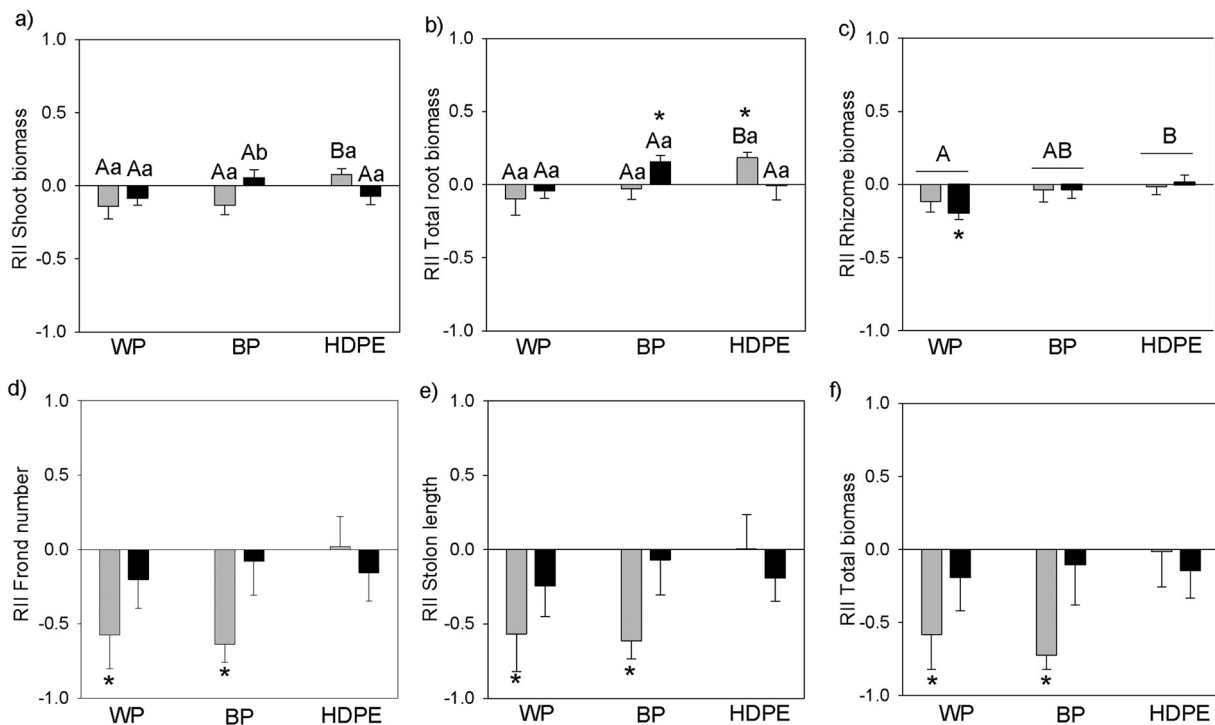

Fig. 3. Relative index of the intensity of interaction (RII) between *Cymodocea nodosa* as the target and *Caulerpa cylindracea* as the neighbour (upper panels) and between *C. cylindracea* as the target and *C. nodosa* as the neighbour (lower panels) grown on substrate without plastic (WP), with biodegradable/compostable plastic (BP) or non-biodegradable plastic (HDPE) bag macro-fragments under no sedimentation (grey bars) or with sedimentation (black bars). Indices were based on shoot biomass (a), total root biomass (b), and rhizome biomass (c) for *C. nodosa* as the target and on frond number (d), stolon length (e), and total biomass (f) for *C. cylindracea* as the target. Letters above bars report the outcome of SNK test comparing treatments, and * denotes values significant different from zero. Mean ± SE, n = 7.
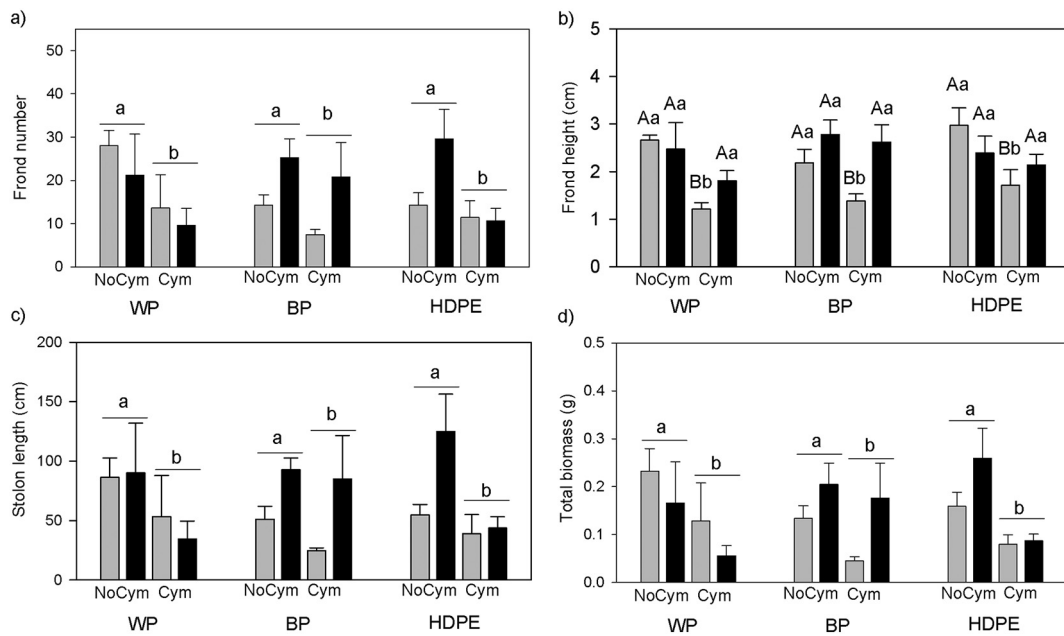
Fig. 4. Frond number (a), frond height (b), stolon length (c), and total biomass (d) of *Caulerpa cylindracea* as the target when grown without *Cymodocea nodosa* (NoCym) or with *Cymodocea nodosa* (Cym) as the neighbour on substrate without plastic (WP), with biodegradable/compostable plastic (BP) or non-biodegradable plastic (HDPE) bag macro-fragments, under no sedimentation (grey bars) or with sedimentation (black bars). Letters above bars report the outcome of SNK test comparing treatments. Mean ± SE, $n = 5$.

C. cylindracea variables (Table 4). RIIs on frond number (Fig. 3d), stolon length (Fig. 3e), and total biomass (Fig. 3f) of plants grown under no sedimentation and without plastic or with BP were negative and significantly different from zero indicating competitive interactions (Table 4). By contrast, under sedimentation and/or in the presence of HDPE the RIIs on these variables did not significantly differ from zero suggesting neutral interactions between species (Fig. 3d, e, f). Even in this experiment, all plastics were still present in sediments, and only BP plastic showed holes (3.7 ± 0.7 holes) due to the perforation of *C. nodosa* roots.

4. Discussion

Seagrass meadows are key ecosystems playing a global role in coastal protection, climate change mitigation and biodiversity maintenance (Barbier et al., 2011; Cullen-Unsworth and Unsworth, 2013), but they are currently experiencing the negative effects of a plethora

of anthropogenic disturbances (Orth et al., 2006; Barbier et al., 2011; Cullen-Unsworth and Unsworth, 2013; de los Santos et al., 2019).

Our study shows that macrofragments of HDPE and BP plastic bags buried in marine sediments affected the clonal architecture and growth of *C. nodosa*. This species produced longer internodes in response to the presence of these plastics, showing a more guerrilla-like oriented growth form with widely dispersed ramets. Plants also produced a greater shoot biomass with HDPE than with BP bags, supporting our first hypothesis that these two types of plastic differentially affected *C. nodosa* performance. However, for some *C. nodosa* variables (biomass of rhizome and fine and coarse roots, root to shoot ratio and plagiotropic rhizome length), the effects of the factors Plastic and Sedimentation depended each from other. Specifically, both plastics increased the mean values of these variables but only under no sedimentation. On average, the effects of HDPE were larger than those of BP, suggesting that plants invested more resources to cope with HDPE than BP. Since both HDPE and BP bags were still present in sediments at the end of the

Table 3
Summary of 3-way ANOVAs testing the effect of plastic (no plastic *vs.* biodegradable/compostable *vs.* non-biodegradable plastic bag macro-fragment), sedimentation (no sedimentation *vs.* sedimentation) and neighbour (presence *vs.* absence of *Cymodocea nodosa*) on variables of *Caulerpa cylindracea* as the target.

Source	df	Frond number		Frond height		Stolon length		Total biomass	
		F	p	F	p	F	p	F	p
Plastic (P)	2	0.03	0.970	0.80	0.451	0.11	0.888	0.01	0.980
Sediment (S)	1	1.45	0.234	3.87	0.054	3.13	0.082	0.98	0.326
Neighbour (N)	1	11.13	**0.001**	18.42	**<0.001**	9.67	**0.003**	11.52	**0.001**
P × S	2	2.93	0.062	2.77	0.072	2.02	0.142	3.14	0.051
P × N	2	0.36	0.693	0.87	0.424	0.31	0.734	0.48	0.619
S × N	1	0.01	0.910	5.23	**0.026**	0.07	0.785	0.04	0.826
P × S × N	2	0.99	0.377	0.09	0.912	0.45	0.637	0.60	0.548
Residual	48								
Transformation		Sqrt(x)				Sqrt(x)			
Shapiro test		$p = 0.101$		$p = 0.410$		$p = 0.053$		$p < 0.05$	
Cochran's test		$p = 0.111$		$p = 0.070$		$p = 0.091$		$p = 0.138$	
SNK test		Cym < NoCym		ND:Cym < NoCym		Cym < NoCym		Cym < NoCym	
				Cym:ND < D				BP:ND < D	

Data transformations and results of SNK tests are reported. Bold values indicate significance at $p < 0.05$. $n = 5$.
WP: without plastic; BP: biodegradable/compostable plastic; HDPE: non-biodegradable plastic; NoCym: *C. cylindracea* grown without *C. nodosa*; Cym: *C. cylindracea* grown with *C. nodosa*; ND: no sedimentation; D: sedimentation.

Table 4
Summary of (a) 2-way ANOVAs for the effect of plastic (no plastic *vs.* biodegradable/compostable *vs.* non-biodegradable plastic bag macro-fragment) and sedimentation (no sedimentation *vs.* sedimentation) on the relative interaction intensity index (RII) based on plant variables of *Caulerpa cylindracea* as the target and *Cymodocea nodosa* as the neighbour; and (b) one-sample mean t-tests for significant departures from zero (neutral interaction) of RIIs.

(a)		RII frond number		RII stolon		RII total biomass	
Source	df	F	p	F	p	F	p
Plastic (P)	2	2.28	0.116	1.80	0.178	2.03	0.145
Sediment (S)	1	3.42	0.072	2.49	0.123	3.79	0.059
P x S	2	2.64	0.084	2.39	0.105	2.16	0.129
Residual	36						
Shapiro test		p < 0.05		p < 0.05		p < 0.05	
Cochran's test		p = 1.459		p = 0.995		p = 0.852	

(b)		RII frond number		RII stolon		RII total biomass	
	df	t	p	t	p	t	p
No sedimentation							
WP	6	−3.02	**0.023**	−2.67	**0.036***	−2.89	**0.027***
BP	6	−6.21	**<0.001**	−5.96	**<0.001***	−8.84	**<0.001**
HDPE	6	0.11	0.910	0.01	0.988	−0.07	0.941
Sedimentation							
WP	6	−1.25	0.255	−1.42	0.204	−0.99	0.359
BP	6	−0.42	0.685	−0.34	0.738	−0.46	0.657
HDPE	6	−0.97	0.369	−1.47	0.190	−0.91	0.396

RIIs were based on based on frond number, stolon length, and total biomass of *C. cylindracea*, and they were calculated for all treatment combinations ($n = 7$). Bold values indicate significance at $p < 0.05$. * denotes data approximately normally distributed. WP: without plastic; BP: biodegradable/compostable plastic; HDPE: non-biodegradable plastic.

experiment, the observed change in *C. nodosa* growth form could be a strategy to escape from these plastics. Studies have shown that clonal plants can adjust their growth form according to local environmental conditions to escape from stressful or unfavorable conditions (Ye et al., 2006; Puijalon et al., 2008; Chen et al., 2011). The larger effects of HDPE than BP observed here on *C. nodosa* variables could be related to the different degree of resistance of these plastics to mechanical stresses. Indeed, *C. nodosa* roots were able to perforate only BP bags. This is consistent with the less mechanical resistance showed by starch-based biodegradable polymers, in terms of tensile strength, than HDPE (10.47 ± 1.23 MPa *vs.* 20.61 ± 1.87 MPa, Napper and Thompson, 2019). The persistence of HDPE bags reflects the recalcitrant nature of the polymer HDPE to biodegradation processes, and it is consistent with the durability of this material when buried in coastal dunes (Menicagli et al., 2019a; Menicagli et al., 2020) and in marine sediments (Nauendorf et al., 2016). However, conversely to previous studies on the effects of HDPE bags on clonal plants inhabiting coastal dunes (Menicagli et al., 2020), in the present study HDPE did not affect plant survival. The persistence of BP found in our study was consistent with results of previous studies showing that this material degrades slowly in marine sediments (Accinelli et al., 2012; Nauendorf et al., 2016; Balestri et al., 2017). Instead, it is not in agreement with its shorter persistence in coastal dunes (Menicagli et al., 2019a; Menicagli et al., 2020). This discrepancy in BP plastic behaviour could be due to the lower temperatures reached in marine habitats than in coastal dunes. Indeed, it is known that the degradation of plastic *via* thermal and photo-oxidation reactions markedly increases at higher ambient temperatures, and it is faster on beaches than on seafloor (Andrady, 2015). In addition, the composition of microbial communities living on plastics deposited on seafloor and beach might substantially differ (De Tender et al., 2015). However, the stimulatory effects of BP bags on *C. nodosa* root production observed here under no sedimentation was in agreement with those previously observed in dune plants (Menicagli et al., 2020) and *C. nodosa* (Balestri et al., 2017) grown with a different type of starch-based biodegradable bags. A larger biomass investment in roots

is considered as a seagrass strategy for maintaining an adequate flow of oxygen from photosynthetic organs to roots and acquiring nutrients from marine sediments (Duarte et al., 1998; Larkum et al., 2006). Experimental evidence indicates that both biodegradable and non-biodegradable plastics once buried in marine sediments can reduce pore-water oxygen concentration and gaseous sediment-water exchanges (Green et al., 2015). A reduction of sediment pore-water oxygen concentration caused by a starch-based biodegradable bag was already observed in a previous experiment on *C. nodosa* grown in the same culture condition used in the present study (Balestri et al., 2017). Further studies should be conducted to better investigate the effects of plastics on physical/chemical proprieties of marine sediments, as well as the possible release of phytotoxic chemical compounds from plastics into sediments (Hermabessiere et al., 2017; Balestri et al., 2019; Menicagli et al., 2019b).

Interestingly, both plastics prevented *C. nodosa* from reacting to sedimentation by increasing orthotropic rhizome growth. HDPE plastic also reduced rhizome biomass and plagiotropic rhizome length in plants grown under sedimentation. These responses to sedimentation could be probably due to the depletion of rhizome reserves used to escape from plastics. Indeed, seagrasses can deplete stored carbohydrate reserves to respond to physical disturbances, and they require time to recover and build new energy reserves to cope with the next disturbance (Owen et al., 2004; Balestri and Lardicci, 2014b). Vertical rhizome elongation and horizontal expansion are two typical strategies evolved by *C. nodosa* to counteract sand burial (Marbà and Duarte, 1994; Duarte et al., 1997; Cabaço et al., 2008; Balestri and Lardicci, 2014a). Since in natural environments *C. nodosa* can experience more frequent and intense sediment depositions (Marbà and Duarte, 1995) than those mimicked in our study, the effects of plastic on this species could be greater than those observed here.

Our study also shows that HDPE plastic and sedimentation influenced the intensity of interspecific interactions between *C. nodosa* and *C. cylindracea*. This result partially supports our second hypothesis which predicts that *C. cylindracea* would suffer less from competition by *C. nodosa* in the presence of plastics and sedimentation. Indeed, under no sedimentation and without plastic, the performance of *C. cylindracea* was strongly reduced due to the competition by *C. cylindracea*, as shown by negative RII values based on all investigated variables. In contrast, the performance of *C. nodosa* was not affected by the introduction of *C. cylindracea*, as indicated by neutral values of RII on all plant variables. These results suggest that the seagrass could impose a resistance to the spread of the macroalga under undisturbed conditions. This is in agreement with previous studies reporting a low establishment success of *C. cylindracea* in native and healthy macrophyte communities (Bulleri et al., 2010; Balestri et al., 2018; Gribben et al., 2018) due to unfavorable environmental conditions (*i.e.*, low light availability and mechanical sweeping of the substratum by shoots) underneath the canopy (Bernardeau-Esteller et al., 2015; Marín-Guirao et al., 2015; Bulleri et al., 2017). BP plastic had similar effects to those detected for plants grown without plastic on the intensity of species interactions (negative RII for *C. cylindracea* as the target and neutral RII for *C. nodosa* as the target). Instead, HDPE reduced the competitive effect of *C. nodosa* on *C. cylindracea* observed without plastic, as suggested by the neutral values of RII on all *C. cylindracea* variables. On the other hand, HDPE increased *C. nodosa* biomass investment in roots, mainly to fine roots. Fine roots are involved in substrate resource acquisition and in sustaining the growth of fast-growing seagrasses with greater nutrient demands per unit biomass (Duarte et al., 1998). Since rhizophytic algae like *C. cylindracea* can acquire nutrients from sediment porewater (Chisholm et al., 1996), the proliferation of roots observed here in *C. nodosa* when in competition with *C. cylindracea* could be a strategy for enhancing belowground resource capture.

Without plastic, sedimentation canceled out the competitive effects of *C. nodosa* on *C. cylindracea* observed under no sedimentation, as suggested by the shift of RII on all *C. cylindracea* variables from negative to

neutral values. On the other hand, sedimentation shifted RII on *C. nodosa* rhizome biomass from neutral to negative. These results suggest that sedimentation could favor *C. cylindracea* by reducing the resistance of *C. nodosa*. With BP, sedimentation counteracted the competitive effects of *C. nodosa* on *C. cylindracea* observed under no sedimentation, as indicated by the shift of RII on all *C. cylindracea* variables from negative to neutral. Instead, sedimentation shifted the RII on *C. nodosa* root biomass from neutral to positive, and this could be a response of the seagrass to competition by the alga for belowground resources. The effect of HDPE on the intensity of species interactions (*i.e.*, neutral interaction) did not vary between sedimentation conditions for *C. cylindracea* as the target. The effect of HDPE on the intensity of interactions (*i.e.*, neutral interaction) did not vary between sedimentation conditions also for *C. nodosa* as the target, except that for roots (for this variable, RII shifted from positive under no sedimentation to neutral under sedimentation). This suggests that HDPE in combination with sedimentation prevented *C. nodosa* to compete with *C. cylindracea* for belowground resources. The shift from competitive to neutral interactions observed with sedimentation and HDPE is in agreement with the stress-gradient hypothesis, which predicts that decreased competition or positive interactions (*i.e.*, facilitation) are more likely to occur at increasing levels of stress (Bertness and Callaway, 1994). Overall, the findings of this study suggest that HDPE could facilitate the spread of the macroalga in *C. nodosa* meadows by reducing the competitive effect of the seagrass, regardless of sedimentation conditions. They also provide further support to the hypothesis that the establishment and spread of non-native macroalgae within vegetated stands relies upon disturbances (Bulleri et al., 2010; Katsanevakis et al., 2010; Ceccherelli et al., 2014).

5. Conclusion

Plastic in marine environments is an emerging issue, and large amounts of this pollutant end up on vegetated bottoms. Seagrass meadows are valuable but declining ecosystems due to global-change related stressors, including sediment disturbance and introduced macroalgae. Our study shows that both HDPE and biodegradable starch-based plastics can persist in marine sediments of temperate regions for at least 18 months. These plastics can alter the architecture of the seagrass *C. nodosa,* and the effects of HDPE were greater than those of biodegradable plastic. This alteration leads to the formation of clones with more spaced shoots that could allow the infiltration of the non-native macroalga *C. cylindracea* in meadows. HDPE and sedimentation can also affect the intensity of interactions between the two species and facilitate the macroalgae spread in seagrass meadows. These findings supported our hypotheses that the impact of HDPE bag on *C. nodosa* would be larger than that of the biodegradable one and that *C. cylindracea* could take advantage of the presence of plastics and sedimentation. Interestingly, our findings also indicate that plastics are not inert materials from a biological/ecological point of view as they can interact with environmental stressors in influencing marine vegetation. Research efforts evaluating the real impact of plastic litter on coastal ecosystems should consider the possible interactions of this pollutant with multiple biotic and abiotic factors. To date, specific conservation policies have not been established to protect seagrass meadows from plastic pollution. Our study highlights the need of implementing effective post-marketing management strategies to prevent the entering of massive amounts of both non-biodegradable and so-called eco-friendly biodegradable plastics in natural environments, including marine areas colonized by seagrasses, in the future.

CRediT authorship contribution statement

Virginia Menicagli: Conceptualization, Formal analysis, Investigation, Visualization, Writing- original draft. **Elena Balestri:** Conceptualization, Formal analysis, Investigation, Visualization, Supervision, Writing-review & editing. **Flavia Vallerini:** Formal analysis, Investigation, Visualization. **Davide De Battisti:** Formal analysis, Investigation, Visualization. **Claudio Lardicci:** Conceptualization, Investigation, Supervision, Writing-review & editing, Funding acquisition.

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.scitotenv.2020.143812.

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