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Abstract

Plastic pollution is a global issue involving all environments, including coastal dunes which are among the most valuable habitats worldwide. Vegetation plays a fundamental role in dune formation and maintenance, and it is subjected to macro-plastic entanglement. Yet the effects of plastics on dune plant communities and dune system stability are unknow. To fill this knowledge gap, we investigated the impact of macro-plastics of non-biodegradable bags and compostable starch-based bags deposited in foredunes on seedling emergence, morphology and biomass production of three ecologically relevant dune species Thinopyrum junceum, Ammophila arenaria and Glaucium flavum. Non-biodegradable bags are a large component of beach litter. Compostable bags have recently been introduced to reduce plastic pollution, but their behavior on natural habitats is poorly known. We hypothesized that bags would affect seedling emergence and development, and that the magnitude of their effects would vary due to different material behavior and species sensitivity. Both virgin bags and bags exposed to beach conditions were used to examine whether weathering would reduce or exacerbate bag effects. Both bag types reduced seedling emergence percentage irrespectively of weathering and species. Virgin MB bags also delayed the emergence of A. arenaria seedlings. Six months after emergence, T. junceum seedlings from MB and PE bags had lower aboveground biomass, and those from PE bags had also altered root system compared to controls. Few A. arenaria seedlings from PE bags survived, and those emerged from MB ones were smaller than controls. No G. flavum seedlings survived regardless of treatments. These findings showed that not only traditional bags, but also compostable ones are a threat to foredunes. They also highlight the need of informing people and managers about the impact of the incorrect disposal of compostable bags and of establishing more appropriate beach cleaning practices on dune ecosystem health.

Keywords	Coastal environment; dune plants; ecology; plastic pollution; seedlings
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Dear Editor,

My co-authors (Virginia Menicagli, Flavia Vallerini, Alberto Castelli and Claudio Lardicci) and I are pleased to submit the enclosed manuscript “Adverse effects of beached plastic litter on the establishment of coastal dune vegetation: first experimental evidences” as Research Article in Environmental Pollution.

The manuscript focuses on plastic litter and its effects on sexual recruitment and growth of dune plants which play fundamental role in structuring and maintaining dune systems. The impact of plastics on dune vegetation has been neglected up to date. Previous studies have investigated the composition and distribution of plastics on beaches and dunes and their effects on biota. However, increasing evidence indicate that plastics can influence sand physical/chemical properties and can release in sand plasticizers and other additives included during manufacture that are toxic to a variety of organism, including plants. In addition, macro-fragments can entangle plant organs and seeds.

Our study is the first that experimentally examined the effects of plastics deposited in foredune on the establishment and growth of plants. Specifically, in an eight-months field seed sowing experiment we investigated the impact of macro-fragments of two types of shopping bags, a non-biodegradable bag (that is common in beach litter) and a compostable starch-based bag (recently marketed to reduce plastic pollution), on seedling emergence, morphology and biomass production of three ecologically relevant species *Thinopyrum junceum*, *Ammophila arenaria* and *Glaucium flavum.* Both virgin bags and bags exposed to beach conditions were used to examine whether weathering would reduce or exacerbate bag effects on plants. The response of plants grown with bags were compared with that of plants grown without bags (controls).

The results show that both types of bags once buried in sand reduced the percentage of seedling emergence in all the investigated species. In addition, they caused morphological and growth alterations both at below- and aboveground level. *Thinopyrum junceum* was more sensitive to non-biodegradable bag while *A. arenaria* was sensitive to both the bag types. In general, weathering minimized the impact of bags on plants. Overall, the results suggest that plastic litter is an actual threat to coastal dune vegetation and hence to dune stability and ecosystem health.

The manuscript fits the Aims and Scope of the Journal, as it relates for the first time the global issue of environmental pollutants (macro-plastics) to the recruitment success and growth of coastal dune plants, providing new insights into the interaction between plastics and dune environment, as well as into its effect on dune ecosystem health. The results highlight the need of informing people and producers about the impact of their incorrect disposal on natural ecosystems to prevent further dune damage. Management actions, such as those involving the burial of beach wrack in foredunes and the use of mechanical wrack removal during cleaning operations (Poeta et al., 2014) should be avoided in order to preserve the ecological functions and services of coastal dune habitats.

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On behalf of all co-authors,
Yours sincerely,
the corresponding author Elena Balestri

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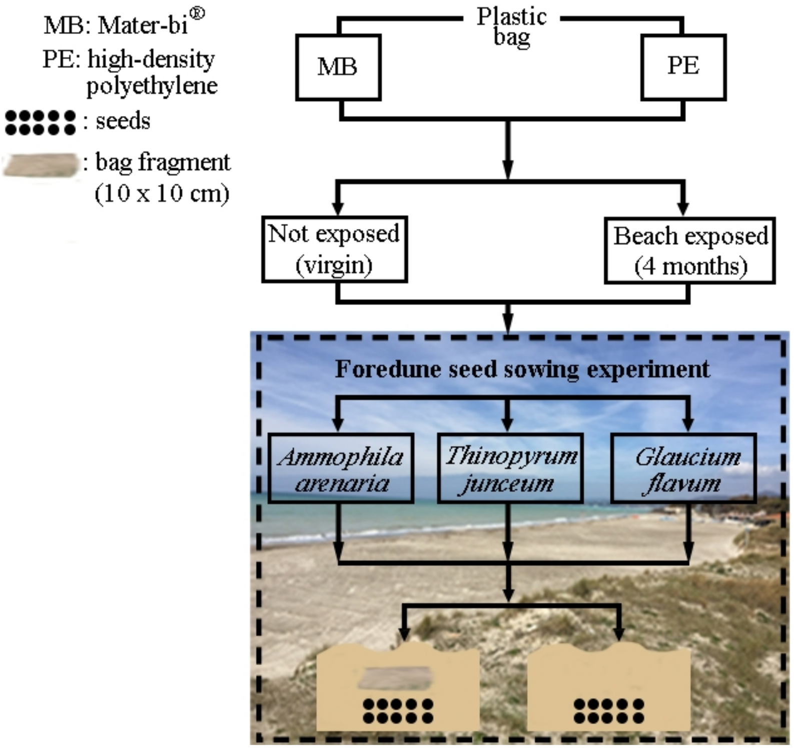
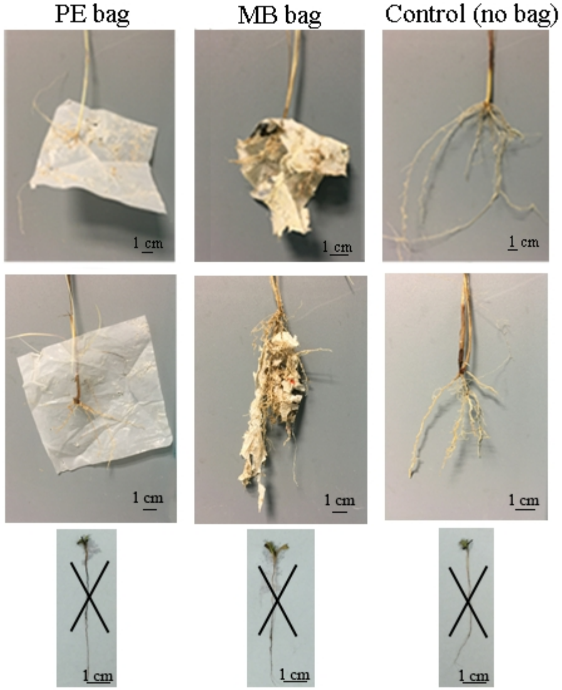
- Shopping bags are a consistent fraction of plastic litter deposited in beaches.
- Effects of beached bags on dune plant recruitment from seed have been neglected.
- Non-biodegradable and compostable bags reduced the emergence of three dune plants.
- Bags differentially affected seedling growth and morphology of the study species.
- Bag effects could translate in changes in plant communities and dune structure.



Seedlings (six months after emergence)



Ammophila arenaria

Seedling emergence (%): PE = MB < C

Mean emergence time: not exposed bag PE = C < MB

Seedling performance: MB ≠ C

PE: few survived seedlings

Thinopyrum junceum

Seedling emergence (%): PE = MB < C

Mean emergence time: PE = MB = C

Seedling performance: not exposed bag PE = MB ≠ C

PE: not exposed bag ≠ exposed bag

Glaucium flavum

Seedling emergence (%): PE = MB < C

No survived seedling

Adverse effects of beached plastic litter on the establishment of coastal dune vegetation: first experimental evidences

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Keywords

Coastal environment; dune plants; ecology; plastic pollution; seedlings

Capsule

Plastic litter negatively affect the development of coastal dune vegetation by drastically reducing seedling emergence and growth.

Declaration of interest: none

1. Introduction

Plastic pollution is a global ecological problem affecting all marine and terrestrial environments (Barnes et al., 2009; Lavers and Bond, 2017; Machado et al., 2018a). Due to their location at the interface between the terrestrial and the marine habitat, beaches and sand dunes are final receptors of plastics waste and may represent hot-spots of plastic pollution (Calvão et al., 2013; Poeta et al., 2014; UNEP, 2015; Poeta et al., 2017; Ceccarini et al., 2018; Rangel-Buitrago et al., 2018). Coastal dunes are structured by a relatively low number of plant species adapted to cope with stressful conditions (Maun, 2009), and they are one of the most valuable but threatened habitats worldwide (Martìnez et al., 2004; Defeo et al., 2009; Barbier et al., 2011; Doody 2013; Drius et al. 2019). Over the last decade, increasing efforts have been made to examine the composition and abundance of plastics deposited in beaches and dunes (Kordella et al., 2013; Poeta et al., 2014; Lee and Sanders, 2015; Munari et al., 2016; Moreira et al., 2016; Alshawafi et al., 2017; Munari et al., 2017; Šilc et al., 2018). Available data indicate that beached plastic fragments can alter physical sediment properties, including temperature and permeability, with potential consequences for beach biota (Carson et al., 2011). However, plastic items accumulated in embryonic and mobile dunes (Poeta et al., 2014; de Francesco et al., 2018) can interact with vegetation through direct mechanisms, such the entanglement of plant organs and seeds (Poeta et al., 2017; Šilc et al., 2018), and indirect mechanisms, including altered sand structure, nutrient mobilization and release of plasticizers and additives (León et al., 2019), similarly to that observed for plastic films used in agriculture (Steinmetz et al., 2016; Machado et al., 2018a; Qi et al., 2018; Rillig et al., 2019). However, no information is available on the actual impact of the deposition of plastics in dunes on plant development.

Plastic bags made of conventional non-biodegradable polymers are a common component (17-23%, up to 1 bag per linear meter) of the plastic litter deposited in beaches and dunes (Munari et al., 2016; Alshawafi et al., 2017; Pasternak et al., 2017; Schmuck et al., 2017; Šilc et al., 2018), and they are among the most harmful plastic litter items to marine biota (Hardesty et al., 2015). Most of these bags derives from terrestrial sources via rivers or marine-based activities, but some of them are accidentally or intentionally discarded on beaches by beach-goers (Ryan et al., 2009; Accinelli et al., 2012). Due to the durability and adverse impacts on natural environments and biota, non-biodegradable bags have recently been banned in many countries over the world (Xanthos and Walker, 2017). Biodegradable and compostable bags have been introduced as eco-friendly substitute of conventional ones, but all of these bags are intended to be properly disposed in industrial or home compost facilities at the end of their life (EN 13432, 2000). Increasing evidence indicates that once entered coastal environments these bags degrade slowly and represent a new emerging threat for organisms and habitats (Accinelli et al., 2012; Tosin et al., 2012; Green et al., 2015; Balestri et al., 2017; Nazareth et al., 2018). In addition, recent laboratory studies have shown that when in contact with water, macro-fragments derived from non-biodegradable bags and compostable bags can release plasticizers and additives incorporated during manufacture in the surrounding environment (Bejgarn et al., 2015; Balestri et al., 2019). The leachates generated from these bags have found to be toxic to terrestrial plants such as *Lepidium sativum* L. a species used in standard phytotoxicity tests (Balestri et al., 2019). Since the persistence and the integrity of coastal dunes depend on preserving vegetation (Acosta et al. 2007; Drius et al., 2013), understanding the effects of beached plastic bags on early life history stages that are most critical for dune plants (Balestri and Cinelli, 2004; Maun, 2009; Rajaniemi and Barrett, 2018), it is fundamental and may help to guide more appropriate management actions.

The aim of the present study was to evaluate the impact of the deposition of two types of shopping bags, a non-biodegradable bag and a compostable starch-based bag, in foredunes on plant populations and communities. We hypothesized that these bags would affect the emergence and development of dune plant seedlings, but the magnitude of their effects would differ due to different bag composition and behavior in nature, and plant species sensitivity. To test these hypotheses, a field seed sowing experiment was performed using as models three plant species widely distributed along Mediterranean and Atlantic foredunes, *Thinopyrum junceum* (L.) Á. Love, *Ammophila arenaria* (L.) Link and *Glaucium flavum* Crantz, that differ in their ecological role (Acosta et al., 2007; van Puijenbroek et al., 2017). The emergence, morphology and biomass production of seedlings in experimentally bag polluted and not polluted plots (controls) were examined eight months after seed sowing. Both new bags and bags exposed to beach conditions were assessed to investigate whether weathering would reduce or exacerbate bag effects on plants.

2. Materials and methods

2.1. Plant material collection and bag types

Thinopyrum junceum is a monocotyledon rhizomatous pioneer species that contributes to the formation of embryonic shifting dunes (Drius et al., 2016; van Puijenbroek et al., 2017). *Ammophila arenaria* is also a monocotyledon rhizomatous species, and it is involved in the formation of mobile shifting dunes (Hesp, 2002; van Puijenbroek et al., 2017). Both these species are clonal and can propagate through sexually reproduction and clonal growth (Harris and Davy, 1986; Del Vecchio et al., 2013; Hilton et al., 2019). *Glaucium flavum* is a dicotyledon non-clonal herb (Scott, 1983) common along coastal dunes (Thanos, 1989; Walmsley and Davy, 1997; Cambrollè et al., 2015).

Seeds of *T. junceum*, *A. arenaria* and *G. flavum* were collected from natural plant populations along the dune system of Rosignano Solvay (43°21’06.11’’N 10°27’21.64’’E - Italy). Seeds of each species were separately placed in clean glass jars and stored outdoor in darkness until the start of the experiment. Before sowing, the viability of seeds was checked through a pressure test (Borza et al., 2007), and non-viable seeds were discarded.

Two type of plastic bags were used in this experiment: high-density polyethylene (PE) bag and Mater-bi® bag (MB). High-density polyethylene is one the most abundant polymers found on beach litter (Corcoran et al., 2009; Munari et al., 2017; Ceccarini et al., 2018). Mater-bi® is based on starch and vinyl-alcohol copolymers (Sforzini et al., 2016) and is certified as compostable in accordance with the main European and international standards (EN13432, 2000). This certification guarantees the complete degradation of MB bags in industrial composting and the absence of any phytotoxic effect of compost. However, the behavior and degradation rate of MB bags in dune habitats is unknown. All the bags were purchased from Italian retailers and belong to the same lots used in a previous laboratory experiment on effects of bag leachates on higher plants (Balestri et al., 2019).

2.2. Set up and design of seed sowing experiment

To investigate the effect of the two different types of plastic bags on recruitment and seedling development, a seed sowing experiment was set up in a foredune area of Rosignano Solvay. During the experimental period, air temperature varied from 4.7 to 29.8 °C and total precipitation was 574.2 mm. Before the experiment (October 2017), half of bags (hereafter referred to as not exposed or virgin bags, NE) was left in laboratory at a temperature of 22 °C (± 1 SE) while the other half (hereafter referred to as exposed bags, E) was placed on the embryo dune for 4 months to allow bags experiencing weathering before their burial in the seed banks. All bags were then cut into fragments of approximately 10 x 10 cm with scissor, a size within the range of that of macro-plastics found on beaches (van Cauwenberghe et al., 2013; Williams et al., 2016; Prevenios et al., 2018). The average weight of MB bag fragments was 0.286 g (± 0.001) and that of PE ones was 0.241 g (± 0.001). Bag thickness was about 25 μm.

In February 2018, 25 cm x 25 cm plots (tens of centimeters each from other) were randomly established in the study area and the sand within each plot was removed. Ten seeds of one of the selected species were sown at 6-7 cm depth within each plot, which correspond to the depth of viable seeds found in foredunes (Hilton et al., 2019). Then, a fragment of PE or MB bag, either not exposed or beach exposed, was placed on seeds and covered with sand previously sieved through a

0.5 mm mesh-sieve to remove extraneous seeds and materials. Additional plots in which seeds were sown and covered with sand as treated ones, but no bag fragment was added over, were also established and used as controls. The experiment thus followed a full factorial design with three factors, plastic bag (fixed, three levels: PE bag, MB bag and no bag or control), bag exposure (fixed, two levels: no exposure and beach exposure) and plant species (fixed, three levels: *T. junceum*, *A. arenaria* and *G. flavum*). There were eight replicates per each treatment combination. In total there were 144 plots.

Plots were regularly inspected over the experimental period, and the number of new emerged seedlings of each species within each plot was recorded until the end of the emergence period. The total percentages of seedling emergence and mortality were computed for each species and treatment, and the mean emergence time (MET) was calculated as

$$t = \sum_{i=1}^{k} n_i t_i / \sum_{i=1}^{k} n_i ,$$

where t_i was the time elapsed from seed sowing to the i^{th} day of observation; n_i was the number of newly emerged seedlings in the day i, and k was the day in which the last seedlings emerged. At the end of the experiment (October 2018), plots were excavated, and seedlings were carefully extracted from sand and transported to the laboratory for morphological measurements (number and length of adventitious roots, number of culms and plant height for *T. junceum* and *A. arenaria*, and length of the primary root and rosette diameter for *G. flavum*). The length of adventurous roots was computed by averaging the length of three intact roots of each plant. Plants were then separated into above-and below-ground organs, dried at 70 °C and weighted to determine the above- and belowground biomass. The below- to aboveground biomass ratio was also computed as considered as an indicator of plant investment into allocation of below- or aboveground resources (Poorter et al., 2012, 2015). Since a different number of seedlings were available at the end of the experiment for each species, the analyses of morphological and growth data were conducted on a sample of five seedlings selected at random among those survived for each species. Only very few seedlings of *G. flavum* emerged from sand, and hence MET and morphological and growth data were not included in the analyses. Finally, all bag fragments in plots were extracted from sand, dried and weighted to calculate weight loss relative to initial value, as this variable is considered a qualitative indicator of plastic degradation (Accinelli et al., 2012; Tosin et al., 2012).

2.2. Statistical analysis

Separate three-way analyses of variance (ANOVA) were conducted to examine the effects of treatments (plastic bag, exposure and species) on the percentage of emergence, the mean emergence time and percentage of mortality of seedlings. Due to the low number of seedlings of *A. arenaria* emerged from plots in the presence of exposed and not exposed PE bags, as well as in the presence of not exposed MB bags, separate analyses on morphological and growth variables were conducted for each species. Two-way permutational multivariate analysis of variance (PERMANOVA) was performed on morphological and growth data, and biomass ratio of *T. junceum* to investigate the overall effect of treatments on seedling performance. Since significant treatment effects were detected in PERMANOVA, separate two-way ANOVAs were performed on each variable. One-way PERMANOVA was conducted only on morphological data of *A. arenaria* seedlings to examine the effects of the presence of beach exposed MB on seedling performance, as biomass data were highly correlated (> 0.95) with other morphological variables. Since PERMANOVA revealed significant treatment effects, separate one-way ANOVAs were conducted on each seedling variable. Three-ways ANOVA was also performed on the percentage of bag weight loss to compare the effects of treatments (plastic, exposure and species) on the extent of plastic degradation after burial in the experimental seed bank. A Student-Newman-Keuls (SNK) test was applied to perform post hoc comparisons among levels of significant terms.

PERMANOVA analyses were carried out on the Euclidean distance of previously normalized data using 9999 permutations of the residuals under a reduced model for *T. junceum* and using unrestricted permutation of raw data for *A. arenaria* (Anderson et al., 2008). Statistically significant terms ($p < 0.05$) were checked for differences in multivariate group dispersion through permutational analysis of multivariate dispersion (PERMDISP). Prior to ANOVA analyses, data were checked for normality and homogeneity of variance using Shapiro-Wilk test and Cochran's *C* test, respectively, and transformed when necessary to meet ANOVA assumptions. Residuals of data of percentage of emergence, number of roots for *T. junceum* and number of culms for *T. junceum* and *A. arenaria* were not normally distributed, but ANOVA analysis was performed anyway as it is quite robust to violation of this assumption (Underwood, 1997). PERMANOVA analyses were conducted using PERMANOVA + for PRIMER 6 statistical software (Anderson et al., 2008; Clarke and Gorley, 2006) while ANOVA analyses by using GMAV version 5.0 for Windows (Underwood and Chapman, 1998).

3. Results

Seedlings started to emerge from sand about four weeks after seed sowing and ceased to emerge two months later. The presence of a plastic bag fragment over seeds, irrespectively of the type and the exposure of bag, significantly reduced the percentage of emergence of seedlings for all the investigated species (Table 1; Fig. 1). *Thinopyrum junceum* showed a significant higher percentage of seedling emergence compared to the other two species, and *G. flavum* exhibited the lowest values (Table 1; Fig. 1). No *G. flavum* seedling emerged from MB bag plots. No difference in the mean seedling emergence time was detected among treatments for *T. junceum*. Seedlings of *A. arenaria* from plots with not exposed MB bag emerged significantly later compared to those from plots with PE bags and controls (Table 1; Fig. 1). They also emerged later than seedlings of *T. junceum* from seeds exposed to the same MB treatment (Table 1).

Multivariate PERMANOVA analysis on all examined variables of *T. junceum* detected a significant effect of the main factors, plastic bag and exposure, and of their interaction (Table 2). ANOVA analysis on the number of roots revealed a significant effect of the interaction between the two main factors (Table 2). Seedlings emerged from MB bag plots, irrespectively of bag weathering, had a similar number of roots to that of controls. Instead, seedlings emerged from virgin PE bags had a significantly lower number of roots than controls while the opposite was found for those grown with beach exposed PE bags (Table 2; Fig. 2). The length of roots of seedling grown with MB bags was similar to that of controls and significantly greater than that of seedlings grown with PE bags, irrespectively of weathering (Table 2; Fig. 2). The presence of both PE or MB bags significantly reduced seedling height and aboveground biomass as compared to controls. No significant difference was observed in the number of culms, belowground biomass and biomass ratio among treatments (Table 2; Fig. 2).

Multivariate PERMANOVA analysis on all examined seedling variables of *A. arenaria* detected significant differences between seedlings grown in the presence of beach exposed MB bags and controls (Table 3). Seedlings grown with MB bags had a significantly lower number and shorter roots, and their height was significantly lower than those of controls (Table 3; Fig. 3). In addition, they showed significantly smaller below- and aboveground biomasses compared to controls (Table 3; Fig. 3). The number of culms and the biomass ratio were not significantly affected by the investigated factors (Table 3; Fig. 3). No statistically significant effect was detected on percentage of seedling mortality among treatments (Table 1). However, only very few emerged seedlings of *A. arenaria* survived with PE bags (Fig. 1). No *G. flavum* seedling survived to the end of the experiment, regardless of treatments. All plastic fragments were still in place and showed holes and breakages on their surface caused by the perforation of emerged seedlings. Irrespectively of weathering and species, MB bags exhibited a significant higher percentage of weight loss relative to their initial value (14.5 % ± 2) compared to PE bags (5.2 % ± 0.8; Table 4).

4. Discussion

Macro-plastics pollution in coastal environments is a serious global issue, and its potential consequences on marine biota, habitats and human health are well described. The present study is the first aimed at assessing the effects of the occurrence of macro-plastics, both not biodegradable and compostable, in coastal sand dunes on the recruitment by seed and the early growth of plants inhabiting these environments.

The results of the study partly supported our first hypothesis, i.e. plastic bags would influence seedlings emergence, and that the magnitude of their effects would vary according to different bag behavior and plant species sensitivity. Indeed, for all the examined species the number of seedlings emerged from plots with buried bag macro-fragments, irrespectively of bag type and weathering, was lower compared to that of controls. Since all bag fragments placed over seeds were not degraded at the end of the experimental period, the observed reduction in the percentage of seedling emergence could be due to the mechanical resistance of bag films as previously observed for seedlings of a crop species grown in the presence of plastic fragments (Liu et al., 2014). In fact, the coleoptile (or the hypocotyl) of seedlings had to break and penetrate the plastic film to move upward and emerge from sand. In addition, alterations of the physical/chemical characteristics of the micro-environment, for example oxygen, temperature, moisture, sand texture and gas diffusion rate induced by bag presence might have played a role. The percentage of emergence of *T. junceum* and *A. arenaria* seedlings observed in control plots was relatively high and similar to that reported for these species in other studies (Harris and Davy, 1986; Van der Putten, 1990; Del Vecchio et al., 2013). Instead, the percentage of seedling emergence of *G. flavum* was lower than that previously recorded (Thanos, 1989; Walmsley and Davy, 1997), and the high seedling mortality prevented us to assess the effects of bags on plants. The observed low emergence capability of this species was probably due to the imposed sowing depth, as burial of seeds deeper than 0.5 cm has found to inhibit seed germination of *G. flavum* (Harris and Davy, 1986; Thanos, 1989; Maun, 2009). Our results also showed that MB bags delayed the time of seedling emergence of *A. arenaria* but this effect was consistent only for virgin bags. This suggests that *A. arenaria* seedlings are more sensitive than *T. junceum* ones to chemical compounds migrated from MB bag to the surrounding environment (Lithner et al., 2012; Li et al., 2016; Balestri et al., 2019). Overall, these findings indicate that the burial of MB bags in coastal dunes can adversely affect seedling emergence as well as that of PE bags.

Our prediction concerning the differential influence of the two bag types on seedling development in the study species was partly confirmed. Indeed, the performance of *T. junceum* seedlings was influenced by PE bags regardless of weathering while that of *A. arenaria* by exposed MB bags. However, only a limited number of *A. arenaria* seedlings emerged from plots with PE bags and not exposed MB bags, thus this species seemed to be vulnerable to both types of bag. As concerning root development of *T. junceum*, root length decreased regardless of weathering. Instead, root number decreased with virgin bags and increased with exposed ones. This latter response could be due to the mitigation of the inhibitory growth effect of additives released from exposed bags during weathering (Balestri et al. 2019). Indeed, previous studies have shown that these compounds can either inhibit (at high concentration) or stimulate (at low concentration) root development in crops (Staples et al., 2010; Li et al., 2018). Instead, the low number, and the reduced length and biomass of roots observed in *A. arenaria* seedlings grown with exposed MB bags could be explained by changes in soil properties and rhizosphere bacteria community (Bandopadhyay et al., 2018; Machado et al., 2018b; Rilling et al., 2019) beneath bag fragments. Previous studies on crop species have found that soil property changes associated to the presence of biodegradable plastic films (mulches) in soils could be responsible for below- and aboveground biomass reduction (Qi et al., 2018). The production of smaller root systems may not allow plants to efficiently uptake nutrients and water from sand in coastal dunes. This could explain the reduced plant height and aboveground biomass observed in *T. junceum* seedling grown in the presence of both MB and PE bags as well as in *A. arenaria* seedlings with MB bags. These findings are in agreement with results of recent studies on mulches made of biodegradable plastics that have revealed adverse effects of this material on agroecosystem and soil health (Steinmetz et al., 2016; Qi et al., 2018; Sintim et al., 2019).

5. Conclusions

Results of this study demonstrate that the occurrence of plastic bags in coastal dunes can reduce the success of sexual recruitment and the growth of newly established seedlings, hindering the formation of a well-developed vegetation cover that is critical to maintain dune system integrity. Thus, plastic bags represent a further threat to these important but vulnerable habitats. Importantly, the results of this study indicate that even new generation of bags made of biodegradable and compostable polymers can affect plants similarly to, or even more than, traditional ones made of non-biodegradable polymers. As compostable bags are currently proposed as an environmental solution to plastic pollution and their production will increase in the future (UNEP, 2015), it is essential informing people and producers about the impact of their incorrect disposal on natural ecosystems to prevent further dune damage. Management actions, such as those involving the burial of beach wrack in foredunes and the use of mechanical wrack removal during cleaning operations (Poeta et al., 2014) should be avoided in order to preserve the ecological functions and services of coastal dune habitats.

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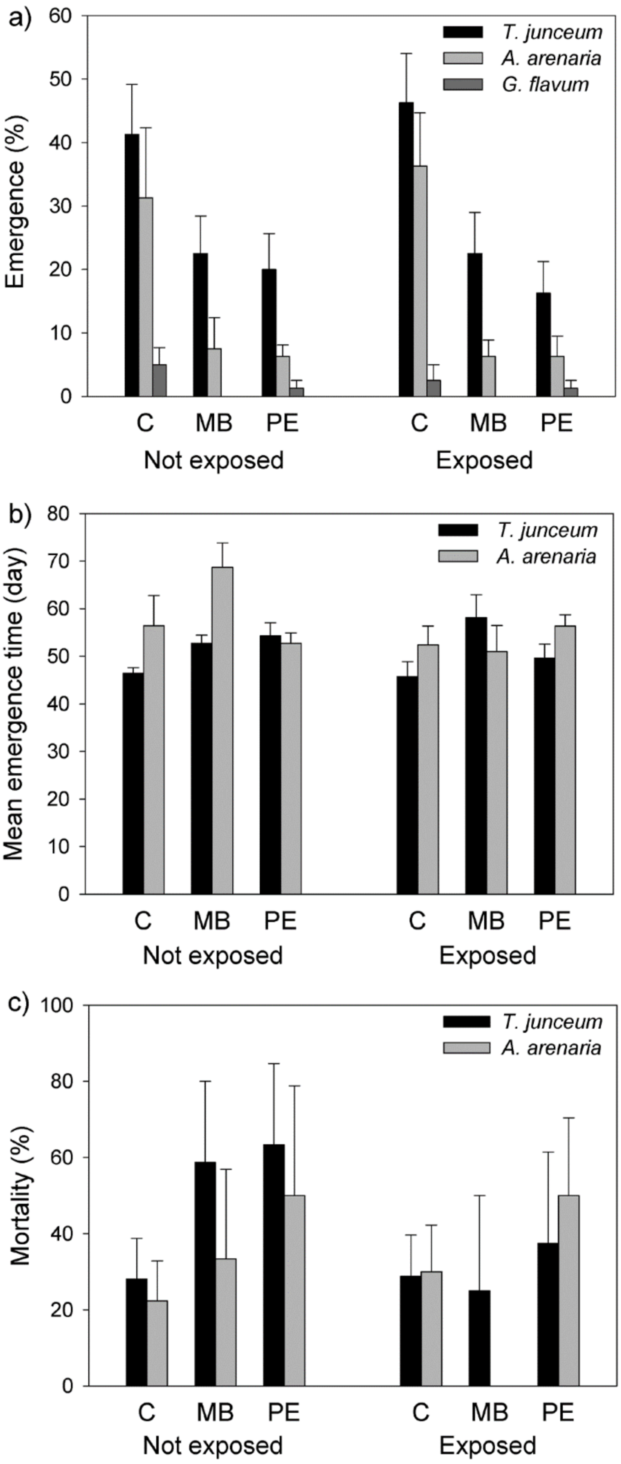

Fig. 1. Percentage of emergence (a), mean emergence time (b) and percentage of mortality (c) of seedlings of the study dune plants emerged from control plots without bags (C) and plots with not exposed and beach exposed Mater-bi® (MB) and high-density polyethylene (PE) bag fragments. Data are means ± SE.

Single-column fitting image

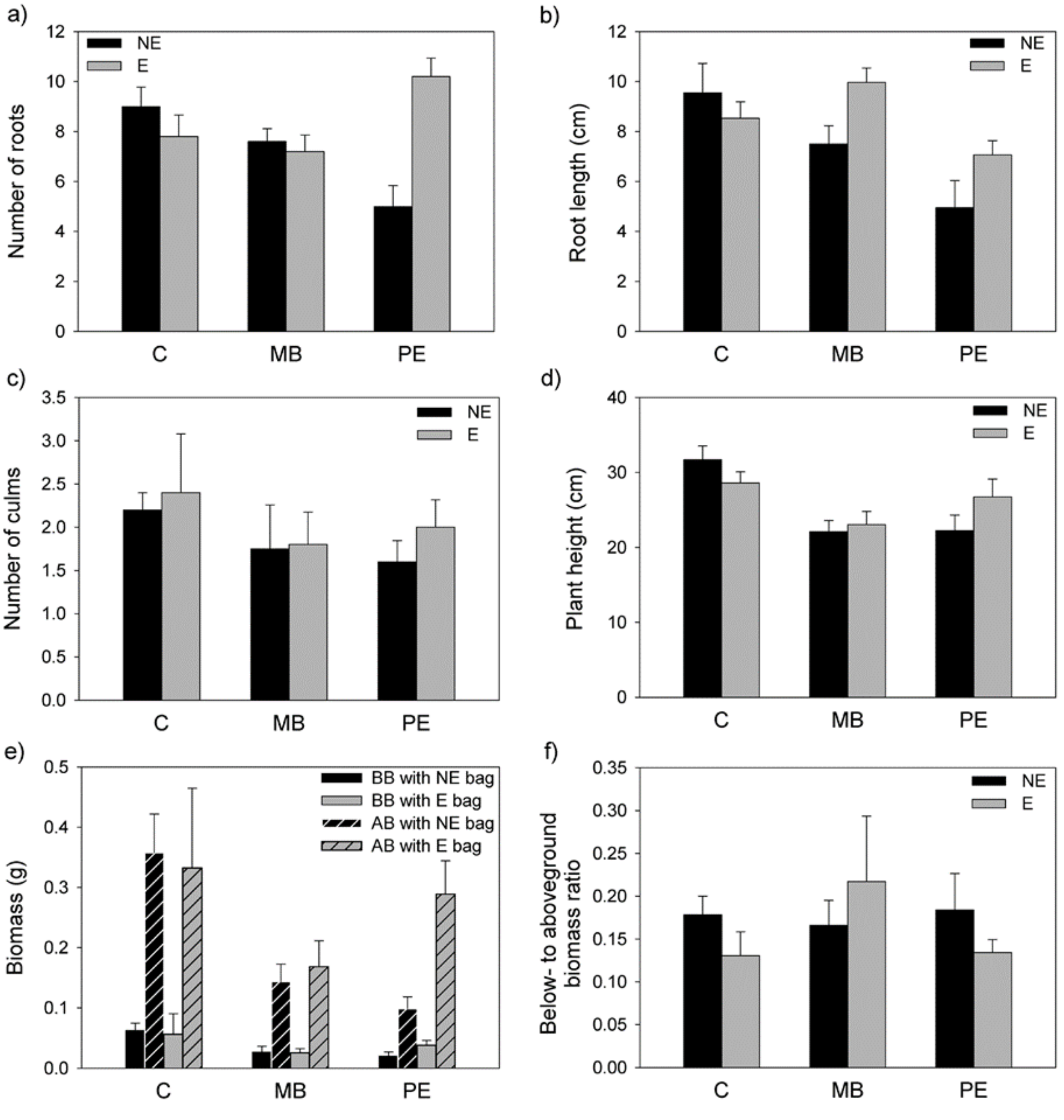

Fig. 2. Morphological and growth variables of *Thinopyrum junceum* seedlings emerged from control plots without bags (C) and plots with not exposed (NE) and beach exposed (E) Mater-bi® (MB) and high-density polyethylene (PE) bag fragments. Data are means ± SE. BB: belowground biomass. AB: aboveground biomass.

2-column fitting image

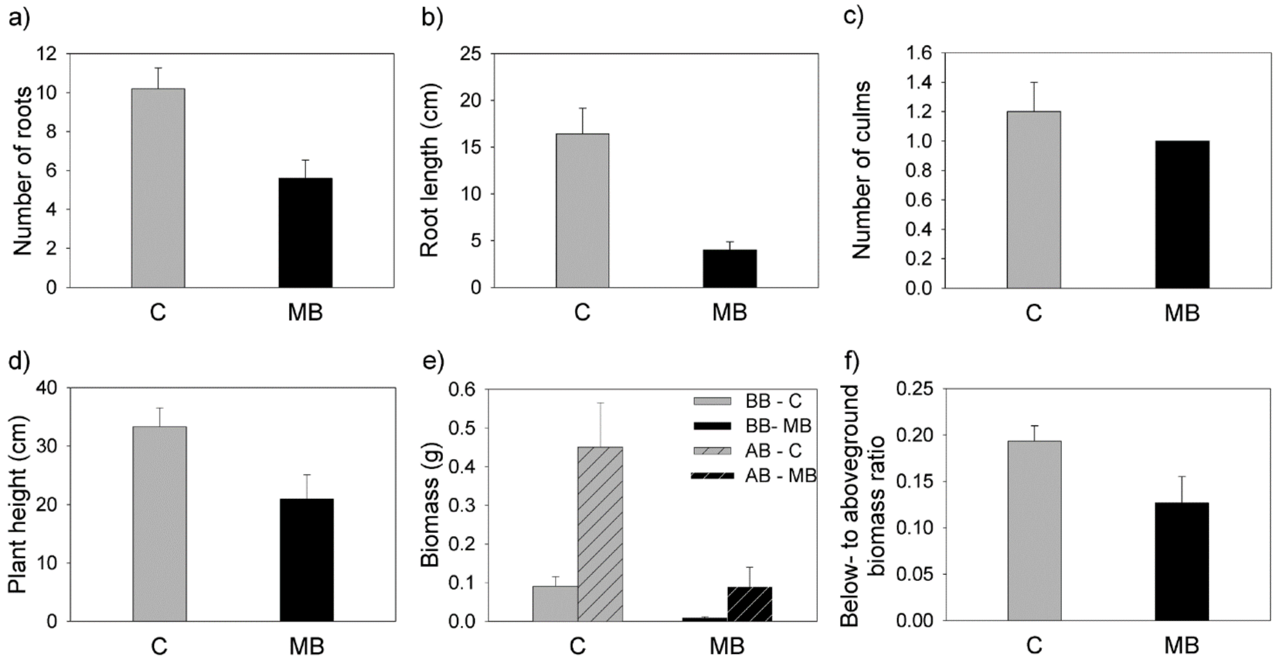

Fig. 3. Morphological and growth variables of *Ammophila arenaria* seedlings emerged from control plots without bags (C) and plots with beach exposed Mater-bi® (MB) and high-density polyethylene (PE) bag fragments. Data are means ± SE. BB: belowground biomass. AB: aboveground biomass.

2-column fitting image

Table 1

Results of ANOVA analysis on the percentage of emergence, mean emergence time and percentage of mortality of seedlings of *Thinopyrum junceum* and *Ammophila arenaria* grown in the absence and in the presence of not exposed and beach exposed Mater-bi®(MB) and high-density polyethylene (PE) bags. Significant results are in bold, and results of SNK tests are showed.

		Emergence (%)			Mean emergence time		Mortality (%)	
Source	df	F	p	df	F	p	F	p
Plastic (P)	2	15.75	**<0.001**	2	3.80	**0.031**	1.76	0.187
Exposure (E)	1	0.03	0.861	1	1.88	0.178	1.63	0.210
Species (S)	2	63.79	**<0.001**	1	5.33	**0.026**	0.71	0.406
P x E	2	0.39	0.675	2	0.55	0.579	0.97	0.388
P x S	4	1.91	0.113	2	0.59	0.558	0.52	0.599
E x S	2	0.34	0.710	1	1.87	0.180	0.25	0.618
P x E x S	4	0.65	0.629	2	4.28	**0.021**	0.12	0.888
Residual	126			36				
SNK test		MB = PE < C T > A > G			NE, A: PE = C < MB MB, A: E < NE MB, NE: T < A			

C: absence of plastic bag; or control MB: presence of Mater-bi® bag, PE: presence of high-density polyethylene bag, NE: not exposed bag, E: beach exposed bag, T: *T. junceum*, A: *A. arenaria*, G: *G. flavum*

Table 2

Results of multivariate PERMANOVA (a) and ANOVA (b) analyses on morphological and growth variables of *Thinopyrum junceum* seedlings grown in the absence (C) and in the presence of not exposed (NE) and beach exposed (E) Mater-bi®(MB) and high-density polyethylene (PE) plastic bags. Significant results are in bold, and results of SNK tests are showed.

a) PERMANOVA

Source	df	Pseudo-F	p
Plastic (P)	2	3.21	**0.006**
Exposure (E)	1	1.14	0.326
P x E	2	2.33	**0.041**
Residual	24		
Transformation		Sqrt(x)	
Pair-wise test		NE: C ≠ MB = PE; PE: NE ≠ E	

b) ANOVA

		Root number		Root length		Number of culms	
Source	df	F	p	F	p	F	p
Plastic (P)	2	1.02	0.374	7.96	**0.002**	0.94	0.406
Exposure (E)	1	3.95	0.058	3.02	0.095	0.00	0.975
P x E	2	11.12	**<0.001**	2.64	0.092	0.74	0.488
Residual	24						
Transformation		None		None		Ln(x)	
SNK test		NE: C = MB > PE E: C = MB < PE PE: NE < E		C = MB > PE			

		Plant height		Below biomass		Above biomass		Biomass ratio	
Source	df	F	p	F	p	F	p	F	p
Plastic (P)	2	8.90	**0.001**	2.35	0.116	4.54	**0.021**	0.21	0.811
Exposure (E)	1	0.26	0.611	0.13	0.717	1.29	0.267	0.66	0.423
P x E	2	2.10	0.145	2.21	0.132	2.96	0.071	0.51	0.607
Residual	24								
Transformation		None		Ln(x)		Ln(x)		Ln(x)	
SNK test		MB = PE < C				MB = PE < C			

Table 3

Results of multivariate PERMANOVA (a) and ANOVA (b) analyses on morphological and growth variables of *Ammophila arenaria* seedlings grown in the absence (C) and in the presence of beach exposed Mater-bi®(MB) plastic bags. Significant results are in bold, and results of SNK tests are showed.

a) PERMANOVA

Source	df	Pseudo-F	p
Plastic (P)	1	6.86	**0.015**
Residual	8		
Transformation		Sqrt(x)	
Pairwise test		C ≠ MB	

b) ANOVA

		Root number		Root length		Number of culms	
Source	df	F	p	F	p	F	p
Plastic (P)	1	10.58	**0.011**	19.13	**0.002**	1.00	0.346
Residual	8						
Transformation		None		Ln(x)		None	
SNK test		C > MB		C > MB			

		Plant height		Below biomass		Above biomass		Biomass ratio	
Source	df	F	p	F	p	F	p	F	p
Plastic (P)	1	5.46	**0.047**	13.46	**0.006**	8.32	**0.020**	4.07	0.078
Residual	8								
Transformation		None		Ln(x)		None		None	
SNK test		C > MB		C > MB		C > MB			

Table 4

Results of ANOVA analysis on the percentage of weight loss of not exposed and beach exposed Mater-bi® (MB) and high-density polyethylene (PE) bags buried in sand above seeds of *Thinopyrum junceum*, *Ammophila arenaria* and *Glaucium flavum*. Significant results are in bold, and results of SNK test are showed.

		Weight loss (%)	
Source	df	F	p
Plastic (P)	1	14.30	**<0.001**
Exposure (E)	1	2.88	0.093
Species (S)	2	0.09	0.914
P x E	1	0.51	0.477
P x S	2	0.30	0.739
E x S	2	0.24	0.785
P x E x S	2	0.61	0.544
Residual	84		
Transformation		Ln (x+1)	
SNK test		MB > PE	