Exposure of coastal dune vegetation to plastic bag leachates: A neglected impact of plastic litter

Virginia Menicagli, Elena Balestri, Claudio Lardicci

PII: S0048-9697(19)32286-7
DOI: https://doi.org/10.1016/j.scitotenv.2019.05.245
Reference: STOTEN 32406

To appear in: *Science of the Total Environment*

Received date: 20 March 2019
Revised date: 15 May 2019
Accepted date: 17 May 2019

Please cite this article as: V. Menicagli, E. Balestri and C. Lardicci, Exposure of coastal dune vegetation to plastic bag leachates: A neglected impact of plastic litter, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.05.245

Exposure of coastal dune vegetation to plastic bag leachates: a neglected impact of plastic litter

Virginia Menicagli[1], Elena Balestri[1]*, Claudio Lardicci[1]

[1] Department of Biology, University of Pisa, via Derna 1, 56126, Pisa, Italy

*Corresponding author

elena.balestri@unipi.it

University of Pisa, via Derna 1, 56126 Pisa, Italy

Keywords:

Compostable bag; *Glaucium flavum*; Polyethylene bag; Seed germination; Seedling growth; *Thinopyrum junceum*

Highlights.

- Dune systems are hot-spots of accumulation of plastic bags.
- Effects of plastic bag leaching by water on dune plants are largely ignored.
- Leachates from not biodegradable and biodegradable bags alter water quality.
- Leachates differentially influence germination and early growth of two dune plants.
- Leachates can affect plant population and community dynamics of dune habitats.

1. Introduction

Coastal dunes consist of a strip of sand located at the interface between the terrestrial and the marine environment, and they result from the dynamic interaction among vegetation, sand and aeolian processes (Fenu et al., 2013; van Puijenbroek et al., 2017). Coastal dunes are considered among the most valuable habitats in the world providing key ecological functions and services, such as coastal protection, erosion control and carbon sequestration (Barbier et al., 2011; Drius et al., 2016; Martìnez et al., 2004). However, they are currently threatened by global climate changes, like sea level rise and increase of high intensity storms and anthropogenic activities, such as excessive resource exploitation and coastal development (Balestri and Lardicci, 2013; Barbier et al., 2011; Frosini et al., 2012). Moreover, sand beaches and dunes are hot-spots of plastic accumulation (Ceccarini et al., 2018; Poeta et al., 2014; Poeta et al., 2017; Rangel-Buitrago et al., 2018).

Plastic pollution is a global environmental problem, and many studies have dealt with the adverse effects of plastic debris on human health and terrestrial and aquatic organisms (Fossi et al., 2018; Law, 2017; Rochman et al., 2015; Wilcox et al., 2018; Wright and Kelly, 2017). Recent studies have shown that plastic items entering dune systems can physically interact with vegetation (Poeta et al., 2017) and alter sand physical/chemical properties, such as temperature and permeability, and geochemical cycling of elements (Carson et al., 2011). Plastic items can also adsorb chemicals from the natural environment, like persistent organic pollutants, polycyclic aromatic hydrocarbon and metals (Mato et al., 2001; Rochman et al., 2014; Teuten et al., 2009), toxic for a variety of organisms (Balmer et al., 2015; Minkina et al., 2018; Zhang et al., 2017). When in contact with water, adsorbed chemicals and water-soluble additives included in the polymer matrix during the manufacturing process (Alam et al., 2018; Hermabessiere et al., 2017; Nazareth et al., 2018) can be released from plastics and migrate into the surrounding environment (Nakashima et al., 2012; Teuten et al., 2009). This phenomenon can also occur in dune habitats, especially during rainfall events. Under this circumstance, the water contaminated by plastics can leach into sand and eventually soak seeds and plant roots. Results of a recent study have shown that leachates from plastic bags, both not-biodegradable and biodegradable, affected the development of seedlings of a terrestrial species considered as indicator of phytotoxicity of a variety of chemicals (Balestri et al., 2019). However, no studies have investigated the impact of plastic leachates on dune vegetation. This information is essential for advancing our understanding of the environmental effects of plastics on coastal dune ecosystems.

In the present study, we focused on the effects of the leaching of shopper bags by water on the most vulnerable life history stages of dune plants, germination and early seedling growth (Balestri and Cinelli, 2004; Maun 2009; Rajaniemi and Barrett, 2018). Shopper bags made of conventional not biodegradable polymers such as high-density polyethylene (HDPE) are among the most common types of plastic item found along sandy shores and dunes (Alshawafi et al., 2017; Munari et al., 2016; Pasternak et al., 2017; Schmuck et al., 2017; Šilc et al., 2018). Most of these bags derives from land-based sources, such as runoff from rivers and wind-blown litter, or marine-based sources (maritime traffic), but some bags are deliberately or unintentionally discarded directly on beaches by beachgoers (Ryan et al., 2009). Recently, biodegradable and compostable bags have also been found in coastal environments (Balestri et al., 2017). These latter bags are considered as a valid eco-friendly alternative to conventional ones, but they are designed to be disposed in industrial or home compost facilities at the end of their life (EN 13432, 2000). Increasing evidences indicate that once entered natural environments compostable bags degrade slowly and can affect marine organisms and communities (Accinelli et al., 2012; Balestri et al., 2017; Green et al., 2015; Nazareth et al., 2018; Tosin et al., 2012). Predictions suggest that the number of compostable bags entering in the environment will greatly increase and even reach similar levels to that of conventional bags in the future (UNEP, 2015).

Here, we examined in laboratory whether (i) conventional and compostable bags would influence via leaching water chemical/physical variables relevant to dune plant development and (ii) the conditions experienced by bags before being deposited close to dune vegetation, i.e., seawater immersion (for those bags derived from land- and marine- based sources) or sand burial (for those bags deposited on beaches) would influence leachate quality. We then assessed whether bag leachates would differentially affect seed germinability, germination phenology and seedling growth of dune plant species. To this end, we used as models two species belonging to different taxonomic groups that often co-occur on coastal mobile dunes, the monocotyledon *Thinopyrum junceum* (L.) Á. Löve (common name, sand couch), previously known also as *Elymus farctus* (Viv.) Runemark ex Melderis or *Agropyron junceum* (L.) P. Beauv. (Rodwell, 2000), and the dicotyledon *Glaucium flavum* Crantz (Scott, 1963) commonly known as yellow horned poppy.

2. Material and methods

2.1. Preparation and chemical/physical analysis of bag leachates

This experiment tested whether plastic shopper bags would affect water quality via leaching and natural weathering (beach or marine exposure condition) would influence leachate quality. Two types of bags, a high-density polyethylene (PE) bag and a biodegradable and compostable bag made of Mater-bi®(MB), purchased from Italian retailers were used to produce leachates. Mater-bi® is constituted by starch and vinyl-alcohol copolymers (Sforzini et al., 2016) and certified for industrial composting (EN13432) and "home" composting scheme (OK Compost Home). This certification means that bags are capable to completely degrade in six months in industrial facilities and to be converted in compost devoid of any phytotoxic effects (EN 13432, 2000). All the bags employed in this study belong to the same lot of plastic bags used in a previous study on the effects of bag leachate on the bioindicator species *Lepidium sativum* L. (Balestri et al., 2019).

Before the leaching experiment (March 2018), the bags were divided in three groups, each consisting of both PE bags and MB bags, and randomly assigned to three different environmental exposure treatments, not-exposure, beach exposure and marine exposure. Not-exposed bags (NE) were left in laboratory at a temperature of 22 °C (± 1 SE) and they serve to test the effects of bag material itself on chemical/physical properties of the water. Beach-exposed (BE) and marine-exposed bags (ME) were placed in a Mediterranean coastal area at Rosignano Solvay (43°23'N 10°26' E, Livorno, Italy) for weathering over a period of 10 days (Fig. 1). This exposure period has been shown to be long enough to allow the initial degradation of plastics and the release of chemical compounds and additives from plastics into the surrounding environment (Bejgarn et al., 2015; Balestri et al., 2019; Hahladakis et al., 2018). Beach-exposed bags (BE) were individually laid out on the upper beach (average daily temperature of 7.1 ± 1.6 °C, total amount of precipitations of 60.8 mm and mean daylength of 11 hours) and partially covered with pebbles and sand to prevent their dispersion in the beach (Fig. 1). Marine-exposed bags were submersed in the sea (average seawater temperature of 13.7 ± 0.1 °C) at 0.5 m depth (Fig. 1). These bags were left to move freely in the water column and to prevent their dispersion in the sea were tied with a cotton thread fixed to stakes planted in the bottom. At the end of the exposure period, the bags were collected and transported to the laboratory for the leachate preparation.

Each plastic bag was cut into pieces of approximately 1 cm^2. For each type of bag, different amounts of pieces were placed into clean glass flasks to obtain liquid-to-solid (water-to-plastic) ratios of 100, 10 and 5, corresponding respectively to approximately 8.3×10^{-4}, 8.3×10^{-3} and 1.6×10^{-2} bag/mL, hereafter referred to as low (L), intermediate (I) and high (H) pollution degree. These ratios were chosen to mimic various degrees of bag pollution occurring in natural sandy shores (Alshawafi et al., 2017; Munari et al., 2016; Pasternak et al., 2017; Schmuck et al., 2017). To obtain bag leachates, sterilized deionized water with a pH value (6.40 ± 0.03) was used. This value is within the range of the rainwater pH values recorded in some coastal Mediterranean areas during the last decades (Herut et al., 2000; Loye-Pilot et al., 1986; Menz and Seip, 2004; Pieri et al., 2010). For each bag type, deionized water with no plastic material was used as control solution (no pollution). Flasks were placed in a culture chamber in darkness on an orbital shaker (95 rotations per minute) for 72 h (Bejgarn et al., 2015) at 24 ± 1 °C. The experiment followed a full factorial design with three factors, plastic (fixed, two levels: PE and MB), exposure (fixed, three levels: not-exposed bags, beach-exposed bags and marine-exposed bags) and pollution (fixed, four levels: control or no pollution, low, intermediate and high). There were three replicates per each treatment combination.

After the incubation period, plastic fragments in each flask were removed from the liquid phase by filtration using a nylon mesh (200 µm). To investigate whether the leaching from plastic bags altered the quality of water, a sample (30 mL) of filtered leachate obtained from each MB and PE flask was collected, and water chemical/physical variables (pH, oxidation-reduction potential, total dissolved solids and salinity) relevant to plant development (Husson, 2013; Isermann, 2005; Sykes and Wilson, 1989) were measured by a multiparameter meter (HI98194, Hanna Instruments). The remaining filtered leachate of each flask was used for the seed germination and seedling growth test.

2.2. Seed germination and seedling growth test

This experiment evaluated the potential effect of bag leachates on the seed germination process and early growth of *Thinopyrum junceum* and *Glaucium flavum*. Seeds were harvested from plants inhabiting Rosignano Solvay dune system in July 2017 (Fig. 1) and separately stored outdoor in clean glass jars in darkness until the setup of the germination test. In March 2018, seeds were visually examined under a stereomicroscope (Wild M3C, Leica) and subjected to pressure test with forceps. This test allowed us to distinguish between non-viable seeds, as those that collapse under gentle pressure, and viable seeds as those that remain firm after the pressure (Borza et al., 2007). Non-viable seeds were discarded.

Viable seeds were rinsed with sterile deionized water and sown in 9-cm Petri dishes (6 seeds per dish) containing sterilized natural sand (0.5 - 1 mm, < 0.01% organic matter content) previously moistened with 3 mL of one of the filtered leachates. Each dish was sealed with parafilm to prevent desiccation, and the dishes were randomly placed in a culture chamber in darkness at 15 ± 1 °C to simulate favorable natural condition for germination (Sykes and Wilson, 1989; Thanos et al., 1989). During the incubation period, seeds were daily checked by using low green light intensity to record the number of germinated seeds in each dish. A seed was considered to have germinated when the radicle length had reached at least 2 mm (Balestri and Cinelli, 2004; Luo et al., 2017). A visual evaluation of seedling developmental abnormalities (Chandler, 2008; ISTA, 2003) was also carried out. Germinated seeds were removed from each dish five days after their germination and stored in 70% ethanol for morphological measurements. The test was considered finished when no additional seeds germinated for at least three consecutive weeks. The experiment followed a full factorial design with four factors, plant species (fixed, two levels: *T. junceum* and *G. flavum*), plastic (fixed, two levels: PE and MB), exposure (fixed, three levels: not-exposed bags, beach-exposed bags and marine-exposed bags) and pollution (fixed, four levels: control or no pollution, low, intermediate and high). Four replicates were used for each treatment combination.

At the end of the experiment, for each dish the percentage of seed germination and the mean germination time, i.e., the time in days elapsed between seed sowing and germination, 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 the start of the experiment to the i^{th} day of observation; n_i was the number of germinated seeds in the day i, and k was the day in which the last germination event was observed (Ranal and De Santana, 2006). The percentage of abnormal seedlings was also calculated for each dish. For each treatment, a sample of the normal seedlings (n = 6) stored in ethanol was randomly chosen and each seedling was carefully placed on squared paper and photographed. The length of the radicle and the length of aboveground organ (hypocotyl for *G. flavum* and coleoptile for *T. junceum*) of each seedling was measured with an image analysis software (ImageJ 2, Rueden et al., 2017). The below- to aboveground organ length ratio for each seedling was also computed as considered as a valuable indicator of relative resource allocation (Yang et al., 2018).

2.3. Data analysis

Non-metric multidimensional scaling (MDS) based on the Euclidean distance was separately conducted for each bag type on chemical/physical variables of leachates (pH, oxidation-reduction potential, total dissolved solids and salinity) to visualize differences among samples of each treatment (exposure and pollution). Three-way permutational multivariate analysis of variance (PERMANOVA) was performed on chemical/physical data to examine the overall effect of treatments (plastic, exposure and pollution), followed by univariate three-way PERMANOVAs to test for differences between treatments for each individual variable.

Four-way multivariate PERMANOVAs were separately conducted on log (x+1) transformed germination data (percentage of germination and mean germination time) and on seedling growth data (radicle length, aboveground organ length and below- to aboveground organ length ratio) to investigate the effects of the different type of plastic bag, exposures and pollution on the performance of *T. junceum* and *G. flavum*. Then, separate analysis of variance (ANOVA) were conducted on each variable and on the percentage of abnormal seedlings. 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, and when significant effects were detected posteriori pair-wise comparisons using 9999 random permutations were conducted. In posteriori pairwise comparisons of chemical/physical variables there were not enough permutable units to get a reasonable test by permutation for some terms, thus p-values were obtained using a Monte Carlo random sample from the asymptotic permutation distribution (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 with Shapiro-Wilk test and Cochran's *C* test, respectively. Data of mean germination time (sqrt (x+1)), radicle length (ln x) and below- to aboveground length ratio (ln (x+1)) were transformed to meet ANOVA assumptions. Since the transformations applied to the percentage of germination and the percentage of abnormalities failed to remove the heterogeneity of variances, untransformed data were analyzed, and the results were considered robust if not significant (at $p > 0.05$) or significant at $p < 0.01$ to compensate for increased probability of type I error (Underwood, 1997). PERMANOVA analyses were conducted using PERMANOVA + for PRIMER 6 statistical software (Anderson et al., 2008; Clarke and Gorley, 2006) while ANOVA analyses were performed using GMAV version 5.0 for Windows (Underwood and Chapman, 1998).

3. Results

3.1. Chemical/physical analysis of leachates

Non-metric multidimensional scaling plots relative to chemical/physical variables of leachates obtained from PE and MB bags showed that control samples (no pollution) were largely overlapping, and they substantially differed from samples belonging to the plastic pollution treatments (Fig. 2). Significant effects of all the main factors and of their interactions were detected by multivariate and univariate PERMANOVA (Table 1). The pH of control solutions (no pollution) was significantly lower than that of PE leachates irrespectively of the type of exposure, and it was also lower than that of MB leachates from marine-exposed bags. Instead, the pH of controls was significantly higher than that of leachates from not-exposed and beach-exposed MB bags (Fig. 3 and Table 2). The impact of each type of bag on pH leachates increased with increasing pollution (Fig. 3 and Table 2). For the pH of leachates obtained from the intermediate and the high amount of

MB bags, as well as from the low and the intermediate amount of PE bags, there were significant differences among exposure conditions, and the lowest pH values were measured in the leachates from not-exposed bags (Fig. 3 and Table 2). The oxidation-reduction potential (ORP) of PE and MB bag leachates was lower compared to that of the control, except that for those from not-exposed MB bags, and it decreased with increasing pollution irrespectively of bag type (Fig. 3 and Table 2). A significant greater amount of total dissolved solids (TDS) was measured in plastic leachates compared to controls (Fig. 3 and Table 2). Except that for leachates from not-exposed MB bags, the amount of TDS significantly increased with increasing pollution, regardless of the type of bag and exposure (Fig. 3 and Table 2). The salinity of control solutions was similar to that of leachate from not-exposed MB bags but significantly lower compared to that of all the other leachates produced with the intermediate and the high amount of bag material (Fig. 3 and Table 2). Significant higher values of TDS and salinity were measured in leachates from marine-exposed bag than that from not-exposed and beach-exposed bags, irrespectively of plastic type and pollution level. For the leachates obtained from the intermediate and the high amount of bag material, there were significant differences in TDS and salinity values among exposure conditions, and the lower values were measured in the leachates from not-exposed bags (Fig. 3 and Table 2).

3.2. Seed germination and seeding growth test

Results of PERMANOVA on germination variables and of ANOVA on germination time revealed a significant effect of the main factors, species and pollution, and of the interaction among species, plastic, exposure and pollution (Tables 3 and 4). Seeds of *T. junceum* treated with leachates from the high amount of marine-exposed MB bags geminated later than control seeds (Figs. 4 and 5 and Table 5). Instead, seeds of *G. flavum* treated with bag leachates germinated earlier than control seeds irrespectively of bag type and exposure conditions, except that those soaked with the highest amount of not-exposed MB bags (Figs. 4 and 5 and Table 5). Results of ANOVAs also detected a significant effect of the interactions between the factors, species and exposure, and exposure and pollution, on the germination percentage (Table 4). A higher seed germination percentage was observed for *T. junceum* compared to *G. flavum* (Table 5). For both species, seeds treated with leachates from the low concentration of marine-exposed bags showed a higher germination percentage compared to control seeds regardless of plastic type (Fig. 4 and Table 5).

Some seedlings failed to produce the hypocotyl (for *G. flavum*) or the coleoptile (for *T. junceum*), and the percentage of these abnormalities was significantly higher in seedlings grown with leachates from the high amount of bag material, irrespectively of plastic type and exposure (Figs. 4 and 6 and Table 5). Multivariate PERMANOVA analysis on seedling growth variables detected a significant effect of the main factors, species, plastic and pollution, and of the interaction among all factors (Table 3). ANOVA analysis on radicle length revealed a significant effect of factors, species and pollution, and of the interaction among all the main factors (Table 6). *Thinopyrum junceum* seedlings grown with the leachate from the high amount of not-exposed MB bags showed a significant shorter radicle compared to control seedlings (Fig. 7 and Table 7). A shorter radicle length was also found in *G. flavum* seedlings grown with the leachate from the low amount of beach-exposed PE bags compared to control seedlings (Fig. 7 and Table 7). ANOVA performed on the length of aboveground organs detected a significant effect of all the main factors and of their interaction (Table 6). Seedlings of *G. flavum* grown with the leachate from the low amount of not-exposed PE bag had a significant longer hypocotyl compared to those belonging to control (Fig. 7 and Table 7). For below- to aboveground organ length ratio, a significant effect of the factors, species and pollution, and of the interaction among all the main factors was detected (Table 6). *Glaucium flavum* seedlings grown with the leachate from the intermediate amount of beach-exposed PE bags or with that from the high amount of marine-exposed PE bags showed a significantly higher radicle to hypocotyl ratio than that of control seedlings as a result of relatively greater radicle elongation (Fig. 7 and Table 7). A significantly higher radicle to hypocotyl ratio was also found for *G. flavum* seedlings grown with the intermediate or high amount of not-exposed MB leachates compared to control seedlings, but this was mainly due to relatively lower hypocotyl elongation (Fig. 7 and Table 7).

4. Discussion

Results of our seed germination experiment indicate that both conventional and compostable bags affected via leaching seed germination processes and seedling growth. The magnitude and direction of leachate effects on the germination phenology varied between the species and depended on type and amount of bag as well as on the environmental conditions experienced by the bag before being deposited close to seeds. In general, bag leachates advanced the timing of seed germination in *G. flavum.* This was likely due to the migration of chemical compounds from bags into the water phase, including bisphenol A (BPA) (Balestri et al., 2019), that could interfere with plant hormonal metabolism and signaling mimicking phytohormones. However, variations of water chemical/physical proprieties, especially salinity, could have played a role as these variables are known to interact with factors controlling germination and dormancy release (El‐Maarouf‐Bouteau and Bailly, 2008). Instead, the bag leachate produced from the high amount of marine-exposed MB bags delayed the timing of seed germination in *T. junceum.* This could be related to the presence of high amounts of salts in leachates. Indeed, previous studies have shown an increase of mean germination timing of *T. junceum* seeds exposed to high salt concentrations (El-Katony et al., 2015). On the other hand, the germinability of seeds of *G. flavum* and *T. junceum* was positively affected by leachates from low amount of marine-exposed PE and MB bags. Salinity is known as the major selective force in seed germination and seedling growth of dune plants (Maun, 2009). Generally, high salinity inhibits seed germination, however in species frequently exposed to immersion in seawater, such as *T. junceum*, moderate salt concentrations can increase seed germination percentage (Ungar, 1978; Woodell, 1985). Since the salinity of leachates from marine-exposed bags was higher than that of leachates from not-exposed bags and controls, their effects on the germination process can be attributed to the presence of salts deposited on the bags during submersion in the sea and migrated from bags into leachates.

Concerning seedling growth, *T. junceum* and *G. flavum* responded differentially to leachates from PE and MB bags. Indeed, *T. junceum* seedlings were affected only by the leachate produced from the high concentration of not-exposed MB bags, and the radicle was the most sensitive organ. Instead, *G. flavum* seedlings were influenced by leachates from PE bags, showing a relatively greater elongation of the radicle than hypocotyl with leachates from beach- and marine-exposed bags and a relative increase of hypocotyl length with the leachate from the low concentration of not-exposed bags compared to controls. These alterations could be due to basic water pH values, that are known to promote *G. flavum* growth (Scott, 1963), and to the biological activity of BPA present in the water phase (Balestri et al., 2019). Indeed, this additive at low concentration has been shown to promote plant growth (Li et al., 2018; Pan et al., 2013; Staples et al., 2010). Seedlings of *G. flavum* were also sensitive to the leachate from the high concentration not-exposed MB bags, showing a lower investment in hypocotyl growth relative to radicle compared to controls. This inhibitory effect could be attributed to the activity of chemical compounds migrated from bags as well as to water acid pH values that are known to adversely affect plant growth (Turner et al., 1988). However, regardless of bag type and exposure a consistent number of seedlings of both the species grown with leachates from the high concentration of bags exhibited a developmental abnormality. The occurrence of seedling abnormalities in sensitive plants is an indicator of phytotoxicity of a certain substance (Chandler, 2008; De Barro, 2008; ISTA, 2003; Mitchell et al., 1988). Here, the observed abnormality could be related to the direct effects by leachates toxicity or the presence in the leachates of compounds derived from bags such as plastic additives and non-intentionally added compounds, for example BPA and 1,6-dioxacyclododecane-7,12-dione, that are toxic to a variety of organisms and terrestrial plants (Balestri et al., 2019; Jyothi et al., 2014; Kennedy, 2002).

A number of environmental factors can influence coastal dunes and the establishment of plants, and among these factors, rainfall play a paramount role (Maun, 2009). Sandy soils of coastal dune may have low rainwater retention capacity but all soil moisture is readily available to plants (Maun 2009). Alterations of chemical/physical characteristics of sandy soil water can have considerable effects on plant development. Indeed, the pH and the ORP values of water can influence the mobility of nutrients and carbonate and their availability to plants (Husson, 2013; Maun, 2009; Sykes and Wilson, 1989). Results of our experiment simulating the leaching of conventional and compostable bags by rainwater on sand dunes demonstrate that this phenomenon can alter all the investigated chemical/physical variables of water, and the magnitude of these alterations varied with bag concentration and the environmental conditions experienced by bags. Generally, PE bags enhanced water pH while MB bags, except that those immersed in seawater, reduced it, confirming results of a previous studies on the characteristics of the leachates obtained with the same lots of plastic bags (Balestri et al., 2019). The basic pH of PE leachates could be related to the release of linear long-chain alkanes and alkenes from bags, while the acid pH of MB leachates could be due to the release of free butane 1,4-diol from bags and other compounds (Balestri et al., 2019) employed with corn starch for the preparation of Mater-bi® (Canellas et al., 2015). Instead, the slightly basic and neutral pH values of leachates obtained respectively from marine- and beach- exposed MB bags could be a result of the buffering effect of salts deposited on bags during weathering. The ORP values of the leachates from not-exposed PE and MB bags could be related to the changes of pH induced by these bags (Liu et al., 2009), while those of the leachates from exposed bags could be mainly a result of biological activities of microorganisms grown on bags during weathering. Lastly, the greater amount of total dissolved solids and the higher salinity in leachates from all exposed PE and MB bags compared to not-exposed ones could depend on the embrittlement of bags and the deposition of salts due to weathering.

In dune environments, shifts in germination phenology, alterations in biomass allocation to above-belowground organs and reduced plant vitality could have a considerable impact on plant population dynamics and structure of communities (Maun, 2009). For example, delayed seed germination of a species could result in changed competitive intra- and interspecific interactions. In addition, the production of short radicles could not guarantee an adequate uptake of nutrients and water to seedlings while short hypocotyls could make seedlings more vulnerable to sand burial (Balestri et al., 2012; Maun, 2009). Therefore, the reduction in seedling vitality and alterations of organ development, induced by the leaching of plastic bags inappropriately discarded in coastal environment, in dune engineer species such as *T. junceum* could have remarkable consequences for the building of dune systems, as it may result in lower sand accumulation and accretion rate.

5. Conclusion

The present study provides the first experimental evidence of the impact of plastic litter, and specifically of shopper bags, on dune plant establishment and early development. Our findings demonstrated that both conventional and compostable bags can interact with abiotic/biotic factors before entering vegetated dunes, and their leachates can interfere in complex ways with mechanisms that regulate germination, dormancy release and early growth. The presence of both types of bag on mobile dunes could locally affect sexual recruitment and the intensity of intra- and interspecific seedling interactions, and hence could have a consistent impact on dune community dynamics and structure. Overall, these findings indicate that the leaching of plastic bags should be considered as a further threat to coastal environments and associated ecosystems. Importantly, they suggest that more efforts should be paid in the future to avoid or limit the accidental or intentional dispersion of biodegradable and compostable bags in natural environments.

Acknowledgements

We sincerely thank Flavia Vallerini, Viviana Ligorini and Andrea Torre for their support in the laboratory experiment. This work is part of the PhD research project in Biology of Virginia Menicagli.

Funding

This work was supported by the University of Pisa (PRA and FA).

References

Accinelli, C., Saccà, M.L., Mencarelli, M., Vicari, A., 2012. Deterioration of bioplastic carrier bags in the environment and assessment of a new recycling alternative. Chemosphere. 89, 136-143. https://doi.org/10.1016/j.chemosphere.2012.05.028.

Alam, O., Billah, M., Yajie, D., 2018. Characteristics of plastic bags and their potential environmental hazards. Resour Conserv Recycl. 132, 121-129. https://doi.org/10.1016/j.resconrec.2018.01.037

Alshawafi, A., Analla, M., Alwashali, E., Aksissou, M., 2017. Assessment of marine debris on the coastal wetland of Martil in the North-East of Morocco. Mar Pollut Bull. 117, 302-310. https://doi.org/10.1016/j.marpolbul.2017.01.079

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK.

Balestri, E., Cinelli, F., 2004. Germination and early-seedling establishment capacity of *Pancratium maritimum* L. (Amaryllidaceae) on coastal dunes in the north-western Mediterranean. J Coast Res. 20, 761-770. https://doi.org/10.2112/1551-5036(2004)20[761:GAEECO]2.0.CO;2

Balestri, E., Lardicci, C., 2013. The impact of physical disturbance and increased sand burial on clonal growth and spatial colonization of *Sporobolus virginicus* in a coastal dune system. PLoS ONE. 8(8): e72598. https://doi:10.1371/journal.pone.0072598

Balestri¸ E., Menicagli, V., Vallerini, F., Lardicci C., 2017. Biodegradable plastic bags on the seafloor: A future threat for seagrass meadows? Sci Total Environ. 605-606, 755-763. https://doi.org/10.1016/j.scitotenv.2017.06.249

Balestri, E., Vallerini, F., Castelli, A., Lardicci, C., 2012. Application of plant growth regulators, a simple technique for improving the establishment success of plant cuttings in coastal dune restoration. Estuar Coast Shelf Sci. 99, 74-84. https://doi.org/ 10.1016/j.ecss.2011.12.017

Balestri, E., Menicagli, V., Ligorini, V., Fulignati, S., Raspolli Galletti, A.M., Lardicci, C., 2019. Phytotoxicity assessment of conventional and biodegradable plastic bags using seed germination test. Ecol Indic. 102, 569-580. https://doi.org/10.1016/j.ecolind.2019.03.005

Balmer, B.C., Ylitalo, G.M., McGeorge, L.E., Baugh, K.A., Boyd, D., Mullin, K.D., Rosel, P.E., Sinclair, Wells, R.S., Zolman, E.S., Schwacke, LH., 2015. Persistent organic pollutants (POPs) in blubber of common bottlenose dolphins (*Tursiops truncatus*) along the northern Gulf of Mexico coast, USA. Sci Tot Environ. 527-528, 306-312. http://dx.doi.org/10.1016/j.scitotenv.2015.05.016

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., Silliman, B., 2011. The value of estuarine and coastal ecosystem services. Ecol Monogr. 81, 169-193. https://doi.org/10.1890/10-1510.1

Bejgarn, S., MacLeod, M., Bogdal, C., Breitholtz, M., 2015. Toxicity of leachate from weathering plastics: an exploratory screening study with *Nitocra spinipes*. Chemosphere. 132, 114-119. https://doi.org/10.1016/j.chemosphere.2015.03.010

Borza, J.K., Westerman, P.R., Liebman, M., 2007. Comparing estimates of seed viability in three foxtail (Setaria) species using the imbibed seed crush test with and without additional tetrazolium testing. Weed Technology. 21, 518–522. http://dx.doi.org/10.1614/WT-06-110

Canellas, E., Vera, P., Nerin, C., 2015. UPLC-ESI-Q-TOF-MS(E) and GC-MS identification and quantification of non-intentionally added substances coming from biodegradable food packaging. Anal. Bioanal. Chem. 407, 6781–6790. https://doi.org/10.1007/s00216-015-8848-2

Carson, H.S, Colbert, S.L., Kaylor, M., McDermid, K.J., 2011. Small plastic debris changes water movement and heat transfer through beach sediments. Mar Pollut Bull. 62, 1708-1713. https://doi.org/10.1016/j.marpolbul.2011.05.032

Ceccarini, A., Corti, A., Erba, F., Modugno, F., La Nasa, J., Bianchi, S., Castelvetro, V., 2018. The hidden microplastics: new insights and figures from the thorough separation and characterization of microplastics and of their degradation by-products in coastal sediments. Environ Sci Technol. 52, 5634-5643. https://doi.org/10.1021/acs.est.8b01487

Chandler, J.W., 2008. Cotyledon organogenesis. J Exp Bot. 59, 2917-2931. https://doi.org/10.1093/jxb/ern167

Clarke, K.R., Gorley, R.N., 2006. *PRIMER* v6: User manual/tutorial. PRIMER-E: Plymouth.

De Barro, J., 2008. Understanding and managing the causes of abnormal seedlings in Lucerne. Rural Industries Research and Development Corporation.

Drius, M., Carranza, M.L., Stanisci, A., Jones, L., 2016. The role of Italian coastal dunes as carbon sinks and diversity sources. A multi-service perspective. Appl. Geogr. 75, 127–136. https://doi.org/10.1016/j.apgeog.2016.08.007

El‐Maarouf‐Bouteau, H., Bailly, C., 2008. Oxidative signaling in seed germination and dormancy. Plant Signal Behav. 3, 175–182.

El-Katony, T. M., Khedr, A. A., Soliman, N. G., 2015. Nutrients alleviate the deleterious effect of salinity on germination and early seedling growth of the psammophytic grass *Elymus farctus.* Botany. 93, 559-571. https://doi.org/10.1139/cjb-2015-0096

EN 13432, 2000. Packaging - Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging.

Fenu, G., Carboni, M., Acosta, A.T.R., Baccetta, G., 2013. Environmental factors influencing coastal vegetation pattern: new insights from the mediterranean basin. Folia Geobot. 48: 493-508. https://doi.org/10.1007/s12224-012-9141-1

Fossi, M.C., Baini, M., Panti, C., Baulch, S., 2018. Impacts of marine litter on cetaceans: A focus on plastic pollution, in: Fossi, M.C., Panti, C. Marine mammal ecotoxicology. Impacts of multiple stressors on population health. Academic press, pp. 147-184.

Frosini, S., Lardicci, C., Balestri, E., 2012. Global change and response of coastal dune plants to the combined effects of increased sand accretion (burial) and nutrient availability. PLoS ONE. 7: e47561. https://doi.org/10.1371/journal.pone.0047561

Green, D.S., Boots, B., Blockley, D.J., Rocha, C., Thompson, R.C., 2015. Impacts of discarded plastic bags on marine assemblages and ecosystem functioning. Environ Sci Technol. 49, 5380-5389. https://doi.org/10.1021/acs.est.5b00277.

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard. Mater. 344:179-199. https://doi.org/10.1016/j.jhazmat.2017.10.014

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere. 182, 781-793. https://doi.org/10.1016/j.chemosphere.2017.05.096

Herut, B., Starinsky, A., Katz, A., Rosenfeld, D., 2000. Relationship between the acidity and chemical composition of rainwater and climatological conditions along a transition zone between large deserts and Mediterranean climate, Israel. Atmos Environ. 34, 1281-1292. https://doi.org/10.1016/S1352-2310(99)00291-5

Husson, O., 2013. Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: a transdisciplinary overview pointing to integrative opportunities for agronomy. Plant Soil. 362, 389–417. https://doi.org/10.1007/s11104-012-1429-7

Isermann, M., 2005. Soil pH and species diversity in coastal dunes. Plant Ecol. 178, 111–120. https://doi.org/10.1007/s11258-004-2558-8

ISTA 2003. International rules for seed testing. Edition 2003, International seed testing association, Zurich, Switzerland.

Jyothi, G., Reddy, K.R.K., Reddy, K.R.N., 2014. Oleic acid: a mycoherbicide from *Cochliobolus* lunatus for management of *Echinochloa crusgalli* in paddy. JEBAS. 2, 405-409.

Kennedy, G.L. Jr., 2002. Toxicity of adipic acid. Drug Chem Toxicol. 25, 191-202.

Law, K.L., 2017. Plastics in the marine environment. Annu Rev Mar Sci. 9, 205–29. https://doi.org/10.1146/annurev-marine-010816-060409

Li, X., Wang, L., Wang, S., Yang, Q., Zhou, Q., Huang, X., 2018. A preliminary analysis of the effects of bisphenol A on the plant root growth via changes in endogenous plant hormones. Ecotoxicol Environ Saf. 150, 152-158. https://doi.org/10.1016/j.ecoenv.2017.12.031

Liu, X., Wang, J., Zhang, D., Li, Y., 2009. Grey relational analysis on the relation between marine environmental factors and oxidation-reduction potential. Chinese J Oceanol Limnol. 27, 583-586, https://doi.org/10.1007/s00343-009-9152-9

Loye-Pilot, M.D., Martin, J.M., Morelli, J., 1986. Influence of Saharan dust on the rain acidity and atmospheric input to the Mediterranean. Nature. 321, 427- 428.

Luo, Y., Liang, J., Zeng, G., Chen, M., Mo, D., Li, G., Zhang, D., 2018. Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. Waste Manag. 71, 109-114. https://doi.org/10.1016/j.wasman.2017.09.023

Martinez M.L., Psuty, N.P., Lubke, R.A., 2004. A Perspective on Coastal Dunes, in: Martínez, M.L., Psuty, N.P. (Eds.), Coastal dune - ecology and conservation. Springer, pp. 3-10.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ Sci Technol. *35*, 318–324. https://doi.org/10.1021/es0010498

Maun, M. A., 2009. The biology of coastal sand dunes. Oxford University Press, New York.

Menz, F.C., Seip, H.M., 2004. *Acid rain in Europe and the United States: an update. Environ Sci Policy. 7, 253–265. https://doi.org/* 10.1016/j.envsci.2004.05.005

Minkina, T., Fedorenko, g., Nevidomskaya, D., Fedorenko, A., Chaplygin, V., Mandzhieva, S., 2018. Morphological and anatomical changes of *Phragmites australis* Cav. due to the uptake and accumulation of heavy metals from polluted soils. Sci Tot Environ. 636, 392–401. https://doi.org/10.1016/j.scitotenv.2018.04.306

Mitchell, R.L., Burchett, M.D., Pulkownik, A., McCluskey, L., 1988. Effects of environmentally hazardous chemicals on the emergence and early growth of selected Australian plants. Plant Soil. 112, 195-199. https://doi.org/10.1007/BF02139995

Munari, C., Corbau, C., Simeoni, U., Mistri, M., 2016. Marine litter on Mediterranean shores: analysis of composition, spatial distribution and sources in north-western Adriatic beaches. Waste Manag. 49, 483-490. http://dx.doi.org/10.1016/j.wasman.2015.12.010

Nakashima, E., Isobe, A., Kako, S., Itai, T., Takahashi, S., 2012. Quantification of toxic metals derived from macroplastic litter on Ookushi beach, Japan. Environ Sci Technol. 46, 10099-10105. https://doi.org/10.1021/es301362g

Nazareth, M., Marques, M. R. C., Leite, M. C. A., Braga Castro, I., 2019. Commercial plastics claiming biodegradable status: Is this also accurate for marine environment? J Hazard Mater. 366, 714-722. https://doi.org/10.1016/j.jhazmat.2018.12.052

Pan, W.J., Xiong, C., Wu, Q.P., Liu, J.X., Liao, H.M., Chen, W., Liu, Y.S., Zheng, L., 2013. Effect of BPA on the germination, root development, seedling growth and leaf differentiation under different light conditions in *Arabidopsis thaliana*. Chemosphere. 93, 2585-2592. https://doi.org/10.1016/j.chemosphere.2013.09.081

Pasternak, G., Zviely, D., Ribic, C.A., Ariel, A., Spanier, E., 2017. Sources, composition and spatial distribution of marine debris along the Mediterranean coast of Israel. Mar Pollut Bull. 114, 1036-1045. https://doi.org/10.1016/j.marpolbul.2016.11.023

Pieri, L., Matzneller, P., Gaspari, N., Marotti, I., Dinelli, G., Rossi, P., 2010. Bulk Atmospheric Deposition in the Southern Po Valley (Northern Italy). Water Air Soil Pollut. 210, 155–169. https://doi.org/10.1007/s11270-009-0238-y

Poeta, G., Battisti, C., Acosta, A. T. R., 2014. Marine litter in Mediterranean sandy littoral: spatial distribution patterns along central Italy coastal dunes. Mar Pollut Bull. 89, 168-173. http://dx.doi.org/10.1016/j.marpolbul.2014.10.011

Poeta, G., Fanelli, G., Pietrelli, L., Acosta, A. T. R., Battisti, C., 2017. Plastisphere in action: evidence for an interaction between expanded polystyrene and dunal plants. Environ Sci Pollut Res. 24, 11856-11859. https://doi.org/10.1007/s11356-017-8887-7

van Puijenbroek, M.E.B., Teichmann, C., Meijdam, N., Oliveras, I., Berendse, F., Limpens, J., 2017. Does salt stress constrain spatial distribution of dune building grasses *Ammophila arenaria* and *Elytrichia juncea* on the beach? Ecol. Evol. 7, 7290–7303. https://doi.org/10.1002/ece3.3244

Rajaniemi, T. K., Barrett, D. T., 2018. Germination responses to abiotic stress shape species distributions on coastal dunes. Plant Ecol. 219, 1271-1282. https://doi.org/10.1007/s11258-018-0877-4(0123456789().,-volV()0123456789().,-volV)

Ranal, M. A., De Santana, D. C., 2006. How and why to measure the germination process? Revista Brasil Bot. 29, 1. https://dx.doi.org/10.1590/S0100-84042006000100002

Rangel-Buitrago, N., Castro-Barros, J. D., Gracia, A., Villamil Villadiego, J. D., Williams, A. T., 2018. Litter impacts on beach/dune systems along the Atlantico Department, the Caribbean coastline of Colombia. Mar Pollut Bull. 137, 35-44. https://doi.org/10.1016/j.marpolbul.2018.10.009

Rochman, C.M., Hentschel, B.T., Teh, S.J., 2014. Long-term sorption of metals is similar among plastic types: Implications for plastic debris in aquatic environments. PLoS ONE 9(1): e85433. https://doi.org/10.1371/journal.pone.0085433

Rochman, C.M., Tahir, A., Williams, W.L., Baxa, D.V., Lam, R., Miller, J.T., Then, F., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci Rep. 5, 14340. https://doi.org/10.1038/srep14340

Rodwell, J.S., 2000. British plant communities: Volume 5. Maritime communities and vegetation of open habitats. Lancaster University.

Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., Eliceiri, K.W., 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18:529, https://doi.org/10.1186/s12859-017-1934-z

Ryan, P.G., Moore, C.J., van Franeker, J.A., Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Phil Trans R Soc B. 364, 1999–2012. https://doi.org/10.1098/rstb.2008.0207

Schmuck, A.M., Lavers, J.L., Stuckenbrock, S., Sharp, P.B., Bond, A.L., 2017. Geophysical features influence the accumulation of beach debris on Caribbean islands. Mar Pollut Bull. 121, 45-51. https://doi.org/10.1016/j.marpolbul.2017.05.043

Scott, G. A. M., 1963. *Glaucium flavum* Crantz. J Ecol. 51, 743-754.

Sforzini, S., Oliveri, L., Chinaglia, S., Viarengo, A., 2016. Application of biotest for the determination of soil ecotoxicity after exposure to biodegradable plastics. Front Env Sci. 4: 68. https://doi.org/10.3389/fenvs.2016.00068

Šilc, U., Kuzmic, F., Cakovic, D., Stesevic, D., 2018. Beach litter along various sand dune habitats in the southern Adriatic (E Mediterranean). Mar Pollut Bull. 128, 353-360. https://doi.org/10.1016/j.marpolbul.2018.01.045

Staples, C., Friederich, U., Hall, T., Klečka, G., Mihaich, E., Ortego, L., Caspers, N., Hentges, S., 2010. Estimating potential risks to terrestrial invertebrates and plants exposed to bisphenol A in soil amended with activated sludge biosolids. Environ Toxicol Chem. 29, 467-475. https://doi.org/10.1002/etc.49

Sykes, M. T., Wilson, J. B., 1989. The effect of salinity on the growth of some New Zealand sand dune species. Acta Bot Neerl. 38, 173-182. https://doi.org/10.1111/j.1438-8677.1989.tb02040.x

Teuten, E. L., Saquing, J. M., Knappe, D. R. U., Barlaz, M. A., Jonsson, S., Bjorn, … Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Phil Trans R Soc B. 364, 2027-2045. https://doi.org/10.1098/rstb.2008.0284

Thanos, C. A., Georghiou, K., Skarou, F., 1989. *Glaucium flavum* seed germination- an ecophysiological approach. Ann Bot. 63, 121-130. https://doi.org/10.1093/oxfordjournals.aob.a087714

Tosin, M., Weber, M., Siotto, M., Lott, C., Degli Innocenti, F., 2012. Laboratory test methods to determine the degradation of plastics in marine environmental conditions. Front Microbiol. 3, 225. https://doi.org/10.3389/fmicb.2012.00225

Turner, G.D., Lau, R.R., Young, D.R., 1988. Effect of acidity on germination and seedling growth of *Paulownia tomentosa*. J Appl Ecol. 25, 561-567. https://doi.org/ 10.2307/2403844

Underwood, A.J., 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University press. https://doi.org/10.1017/CBO9780511806407

Underwood, A. J., Chapman, M. G., 1998. GMAV, Version 5.0 for Windows.

UNEP, 2015. Biodegradable plastics and marine litter. Misconceptions, concerns and impacts on marine environments. United Nations Environment Programme (UNEP), Nairobi (2015).

Ungar, I.A., 1978. Halophyte seed germination. Botanical Review. 44, 233–264. *JSTOR*, www.jstor.org/stable/4353933.

Wilcox, C., Puckridge, M., Schuyler, Q.A., Townsend, K., Hardesty, B.D., 2018. A quantitative analysis linking sea turtle mortality and plastic debris ingestion. Sci Rep. 8, 12536. https://doi.org/10.1038/s41598-018-30038-z

Woodell, S. R. J., 1985. Salinity and seed germination patterns in coastal plants. Vegetatio. 61, 223-229.

Wright, S.L., Kelly, F.J., 2017. Plastic and human health: A micro issue? Environ. Sci. Technol. 51, 6634−6647. https://doi.org/ 10.1021/acs.est.7b00423

Yang, Y., Dou, Y., An, S., Zhu, Z., 2018. Abiotic and biotic factors modulate plant biomass and root/shoot (R/S) ratios in grassland on the Loess Plateau, China. Sci Tot Environ. 636, 621–631. https://doi.org/10.1016/j.scitotenv.2018.04.260

Zhang, C., Feng, Y., Liu, Y., Chang, H., Li, Z., Xue, J., 2017. Uptake and translocation of organic pollutants in plants: a review. J Integr Agric. 16, 1659-1668. https://doi.org/10.1016/S2095-3119(16)61590-3

Caption list

Fig. 1. Coastal area where high-density polyethylene and Mater-bi bags were placed for weathering (a) and plants of *T. junceum* and *G. flavum* growing in this area (b). Collocation of beach-exposed bags (c) and marine-exposed bags (d) in the area for weathering.

single column fitting image

Color image only in online version

Fig. 2. Non-metric multidimensional scaling ordination (MDS) of chemical/physical characteristics of leachates from obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE (a) and MB (b) bags simulating different degrees of pollution. Arrows indicate increasing gradient of bag pollution.

2-column fitting image

Color image only in online version

Fig. 3. Chemical/physical variables (pH, oxidation-reduction potential, total dissolved solids and salinity) of leachates obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE and MB bags simulating different degrees of pollution. Data are mean ± SE, n = 3.

2- column fitting image

Fig. 4. Percentage of germination and mean germination time of seeds and percentage of abnormal seedlings of *T. junceum* and *G. flavum* grown with leachates obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE (a, b, c) and MB (d, e, f) bags simulating different degrees of pollution. Data are mean ± SE, n = 4.

2-column fitting image

Fig. 5. The time spread of germination and the mean germination time of *T. junceum* (green) and *G. flavum* (red) seeds treated with leachates from various bag pollution degrees (—— high — — intermediate —··— low and ········ no pollution).

2-column fitting image

Color image in online version and grayscale image in printed version

Fig. 6. Normal and abnormal seedlings lacking aboveground organs of *T. junceum* (a, b) and *G. flavum* (c, d) grown with bag leachates.

Single column-fitting image

Color image in online version and grayscale image in printed version

Fig. 7. Radicle length, aboveground organ length, and below- to aboveground ratio of seedlings grown with leachates obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE (a, b, c) and MB (d, e, f) bags simulating different degrees of pollution. Data are mean ± SE, n = 6.

2-column fitting image

Table 1

Results of multivariate (a) and univariate (b) PERMANOVA analyses on pH, oxidation-reduction potential (ORP), total dissolved solids (TDS) and salinity of leachates obtained from different amounts of not-exposed, beach- or marine-exposed MB or PE bags simulating different degrees of pollution. Significant results are in bold.

a) Multivariate analysis

Source	d.f.	Pseudo-F	p
Plastic (P)	1	365.13	**<0.001**
Exposure (E)	2	437.78	**<0.001**
Pollution (Po)	3	287.24	**<0.001**
P x E	2	103.51	**<0.001**
P x Po	3	106.34	**<0.001**
E x Po	6	137.22	**<0.001**
P x E x Po	6	45.68	**<0.001**
Residual	48		

b) Univariate analysis

		pH		ORP		TDS		Salinity	
Source	d.f.	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p
Plastic (P)	1	365.13	**<0.001**	11.19	**<0.001**	274.11	**<0.001**	266.24	**<0.001**
Exposure (E)	2	437.78	**<0.001**	7.53	**<0.001**	2004.5	**<0.001**	1743.2	**<0.001**
Pollution (Po)	3	287.24	**<0.001**	14.61	**<0.001**	827.22	**<0.001**	732.12	**<0.001**
P x E	2	103.51	**<0.001**	6.62	**<0.001**	295.4	**<0.001**	286.46	**<0.001**
P x Po	3	106.34	**<0.001**	9.06	**<0.001**	99.27	**<0.001**	102.09	**<0.001**
E x Po	6	137.22	**<0.001**	4.06	**<0.001**	709.22	**<0.001**	641.99	**<0.001**
P x E x Po	6	45.62	**<0.001**	4.04	**<0.001**	101.82	**<0.001**	105.35	**<0.001**
Residual	48								

Table 2

Results of *a posteriori* pair-wise comparison test performed on the statistically significant term Plastic x Exposure x Pollution in the PERMANOVA on pH, oxidation-reduction potential (ORP), total dissolved solids (TDS) and salinity data measured in bag leachates.

pH	**MB ≠ PE**:	NE (I, H) - BE, ME (L, I, H)		
	NE ≠ BE ≠ ME:	PE (L, I) - MB (I, H)	**NE = BE ≠ ME**:	PE (H) - MB (L)
	C ≠ H ≠ I ≠ L:	PE (NE, BE)	**C ≠ L ≠ I = H**:	PE (ME)
	C = L ≠ I = H:	MB (NE)	**C = L ≠ I ≠ H**:	MB (BE)
	C ≠ L = I = H:	MB (ME)		
ORP	**MB ≠ PE**:	NE (I, H) - BE (L, I, H) - ME (H)		
	NE ≠ BE ≠ ME:	PE (L, I, H) - MB (I)	**NE = BE ≠ ME**:	MB (L)
	NE ≠ BE = ME:	MB (H)		
	C ≠ L ≠ I ≠ H:	PE (NE, BE, ME) - MB (ME)	**C ≠ L ≠ I = H**:	MB(BE)
TDS	**MB ≠ PE**:	NE (I) - BE (L) - ME (L, I, H)		
	NE ≠ BE ≠ ME:	PE, MB (I, H)	**NE = BE ≠ ME**:	PE, MB (L)
	C ≠ L ≠ I ≠ H:	PE (ME) - MB (BE, ME)	**C ≠ L ≠ I = H**:	PE (NE, BE)
	C ≠ L = I = H:	MB (NE)		
Salinity	**MB ≠ PE:**	NE (H) - ME (L, I, H)		
	NE ≠ BE ≠ ME:	PE, MB (I, H)	**NE = BE ≠ ME**:	PE, MB (L)
	C ≠ L ≠ I ≠ H:	PE, MB (ME)	**C = L ≠ I = H**:	PE (NE, BE)
	C = L ≠ I ≠ H:	MB (BE)		

H: high pollution, I: intermediate pollution, L: low pollution, C: control or no pollution, NE: not-exposed bag, BE: beach-exposed bag, ME: marine-exposed (ME), MB: Mater-bi®, PE: high-density polyethylene (PE).

Table 3

Results of multivariate PERMANOVA analyses performed on (a) germination (percentage of germination and mean germination time) and (b) growth variables (radicle length, aboveground organ length and below- to aboveground ratio) of *T. junceum* and *G. flavum* seedlings grown with leachates obtained from different amounts of not-exposed, beach- or marine-exposed MB or PE bags simulating different degrees of pollution. Significant results are in bold.

	a) Germination variables			b) Growth variables		
Source	d.f.	Pseudo-F	p	d.f.	Pseudo-F	p
Species (S)	1	257.82	**<0.001**	1	48.00	**<0.001**
Plastic (P)	1	1.00	0.347	1	5.87	**0.004**
Exposure (E)	2	1.12	0.336	2	1.94	0.108
Pollution (Po)	3	3.53	**0.003**	3	4.21	**0.001**
S x P	1	1.80	0.164	1	4.77	**0.010**
S x E	2	3.66	**0.009**	2	1.09	0.348
S x Po	3	5.28	**0.003**	3	2.27	**0.039**
P x E	2	2.64	**0.049**	2	1.88	0.114
P x Po	3	0.82	0.530	3	1.31	0.250
E x Po	6	1.54	0.132	6	1.19	0.282
S x P x E	2	0.78	0.495	2	4.45	**0.002**
S x P x Po	3	0.48	0.786	3	0.36	0.896
S x E x Po	6	1.58	0.113	6	2.04	**0.025**
P x E x Po	6	0.92	0.508	6	1.15	0.311
S x P x E x Po	6	2.05	**0.034**	6	3.21	**<0.001**
Residual	144			240		

Table 4

Results of ANOVA analyses performed on the percentage of germination, mean germination time and percentage of abnormal seedlings of *T. junceum* and *G. flavum* treated with leachates obtained from different amounts of not-exposed, beach- or marine-exposed MB or PE bags simulating different degrees of pollution. Significant results are in bold.

		Total germination (%)		Mean germination time		Abnormalities (%)	
Source	d.f.	F	p	F	p	F	p
Species (S)	1	252.90	**<0.001**	980.21	**<0.001**	4.37	**0.038**
Plastic (P)	1	2.48	0.117	0.92	0.339	1.01	0.317
Exposure (E)	2	0.02	0.975	2.81	0.063	0.79	0.457
Pollution (Po)	3	2.45	0.065	14.88	**<0.001**	4.48	**0.004**
S x P	1	4.86	**0.029**	2.33	0.129	0.21	0.646
S x E	2	5.16	**0.006**	4.86	**0.009**	2.63	0.075
S x Po	3	0.50	0.680	28.61	**<0.001**	2.34	0.076
P x E	2	1.81	0.167	9.10	**<0.001**	0.28	0.752
P x Po	3	1.60	0.191	0.49	0.692	0.43	0.732
E x Po	6	3.20	**0.005**	1.48	0.190	0.33	0.920
S x P x E	2	1.07	0.347	0.44	0.642	0.60	0.548
S x P x Po	3	0.91	0.438	1.78	0.154	0.09	0.965
S x E x Po	6	2.46	**0.026**	2.58	**0.021**	1.32	0.251
P x E x Po	6	0.74	0.621	0.56	0.764	0.20	0.977
S x P x E x Po	6	2.42	**0.029**	3.15	**0.006**	1.23	0.294
Residual	144						

Table 5

Results of SNK post-hoc test of the significant terms in the ANOVA on the percentage of seed germination, the mean germination time (MGT) and the percentage of abnormal seedlings of *T. junceum* (T) and *G. flavum* (G) treated with bag leachates.

Total germination	*Species (S) x Exposure (E)*
	G < T: NE, BE, ME
	E x Pollution (Po):
	NE = BE < ME: L
	C = I = H < L: ME
MGT	*S x Plastic (P) x E x Po*
	T < G
	PE < MB: T (NE (I), ME (H)) - G (ME (I))
	MB < PE: G (NE, BE (I))
	NE = BE < ME: T (MB(H))
	NE = BE > ME: G (PE (I))
	NE > BE = ME: G (PE, MB (H))
	C = L = I < H: T (MB(ME))
	C > I = H > L: G (PE(NE))
	C > L = I = H: G (PE (BE, ME) -MB (BE))
	C = H > I = L: G (MB(NE))
Abnormalities	*Po*
	C = L =I < H

H: high pollution, I: intermediate pollution, L: low pollution, C: control or no pollution, NE: not-exposed bag, BE: beach-exposed bag, ME: marine-exposed (ME), MB: Mater-bi®, PE: high-density polyethylene (PE).

Table 6

Results of ANOVA analyses performed on radicle length, aboveground organ length and below- to aboveground ratio of *T. junceum* and *G. flavum* seedlings grown with leachates obtained from different amounts of not-exposed, beach- or marine-exposed MB or PE bags simulating different degrees of pollution. Significant results are in bold.

		Radicle length		Aboveground organ length		Below– to aboveground ratio	
Source	d.f.	F	p	F	p	F	p
Species (S)	1	138.10	**<0.001**	7.71	**0.005**	16.85	**<0.001**
Plastic (P)	1	12.23	**<0.001**	8.70	**0.003**	0.01	0.904
Exposure (E)	2	2.94	0.054	3.69	**0.026**	0.34	0.709
Pollution (Po)	3	1.35	0.259	5.77	**<0.001**	4.90	**0.002**
S x P	1	7.25	**0.007**	0.04	0.842	6.75	**0.009**
S x E	2	0.30	0.742	2.01	0.136	1.53	0.217
S x Po	3	4.81	**0.002**	0.72	0.540	2.60	0.053
P x E	2	1.92	0.148	2.34	0.098	1.44	0.238
P x Po	3	1.91	0.128	1.41	0.239	0.95	0.417
E x Po	6	1.00	0.428	1.55	0.162	1.30	0.257
S x P x E	2	0.41	0.664	5.73	**0.003**	5.64	**0.004**
S x P x Po	3	0.93	0.428	0.42	0.741	0.24	0.870
S x E x Po	6	1.20	0.306	2.41	**0.027**	2.49	**0.023**
P x E x Po	6	1.89	0.084	0.58	0.747	1.45	0.196
S x P x E x Po	6	4.29	**<0.001**	3.68	**0.001**	2.27	**0.037**
Residual	240						

Table 7

Results of SNK post-hoc test on the statistically significant term Plastic x Exposure x Pollution in the ANOVA on radicle length, aboveground organ length and below- to aboveground ratio of *T. junceum* (T) and *G. flavum* (G) seedlings grown with bag leachates.

Radicle length	**T < G**: PE (NE (L, I), BE (I, H), ME (L, I, H)) - MB (NE (H), BE, ME (L, H))
	MB < PE: T (NE (H)) - G (NE (L), BE (I, H), ME (H))
	PE < MB: G (BE (L))
	NE = ME > BE: G (PE (L))
	NE < BE = ME: G (MB (L))
	H < I = L = C: T (MB (NE)
	L < C = I = H: G (PE (BE)
Aboveground organ length	**T < G**: PE (NE (L), ME (I)) - MB (BE (L))
	G < T: PE (BE (L, I)) - MB (ME (L))
	MB < PE: T (BE (L)), G (NE, ME (L))
	PE < MB: G (BE (L))
	NE = ME < BE: T (PE (I))
	NE > ME > BE: G (PE (L))
	L > C = I = H: G (PE (NE))
Below- to aboveground ratio	**T < G**: PE (BE (L, I, H), ME (H)) - MB (NE (H), ME (L))
	MB < PE: G (BE (I))
	PE < MB: T (BE (H, L))
	NE = BE > ME: T (MB (L))
	I > H = L= C: G (PE (BE))
	H > I = L = C: G (PE (ME))
	H =I > L = C: G (MB(NE))

H: high pollution, I: intermediate pollution, L: low pollution, C: control or no pollution, NE: not-exposed bag, BE: beach-exposed bag, ME: marine-exposed (ME), MB: Mater-bi®, PE: high-density polyethylene (PE).

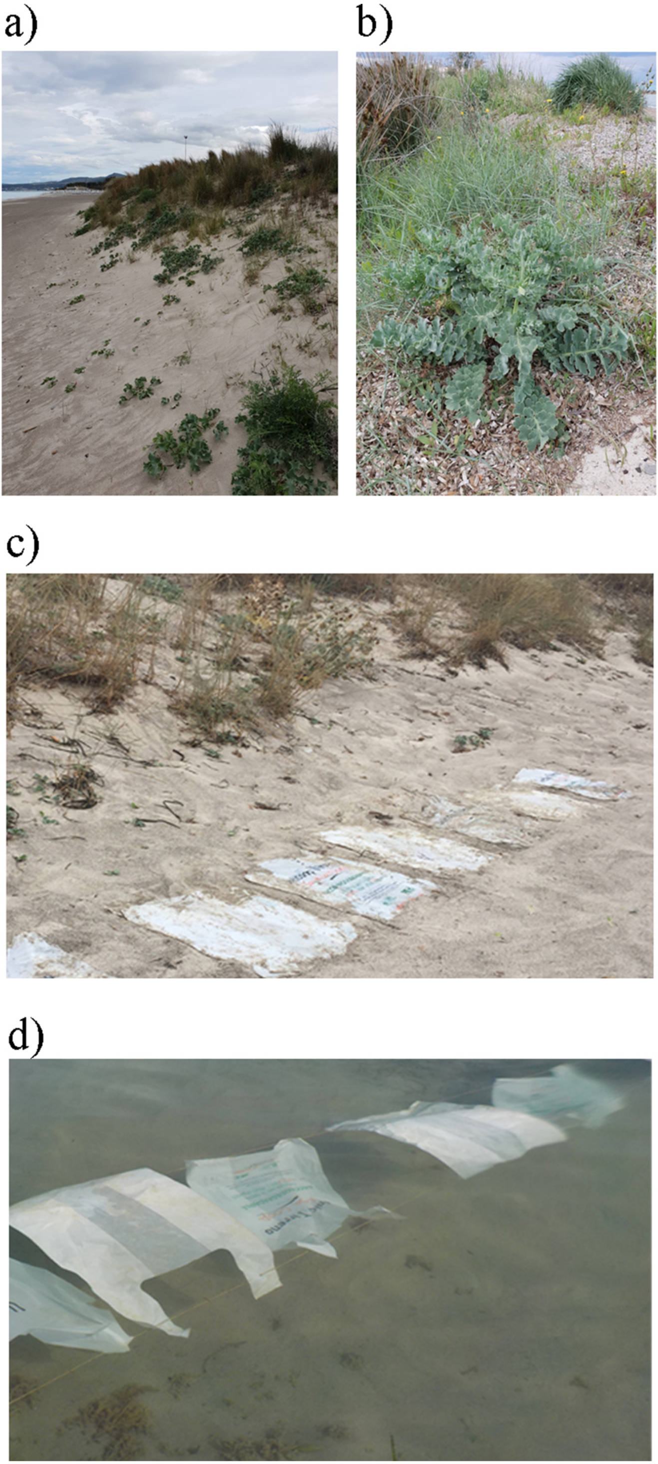

Figure 1

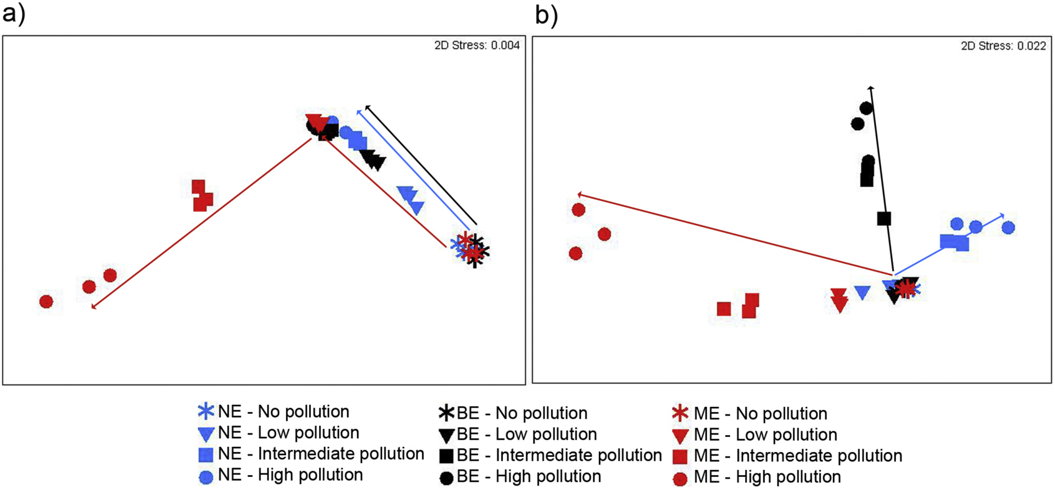
a)
2D Stress: 0.004
b)
2D Stress: 0.022
NE - No pollution
NE - Low pollution
NE - Intermediate pollution
NE - High pollution
BE - No pollution
BE - Low pollution
BE - Intermediate pollution
BE - High pollution
ME - No pollution
ME - Low pollution
ME - Intermediate pollution
ME - High pollution

Figure 2

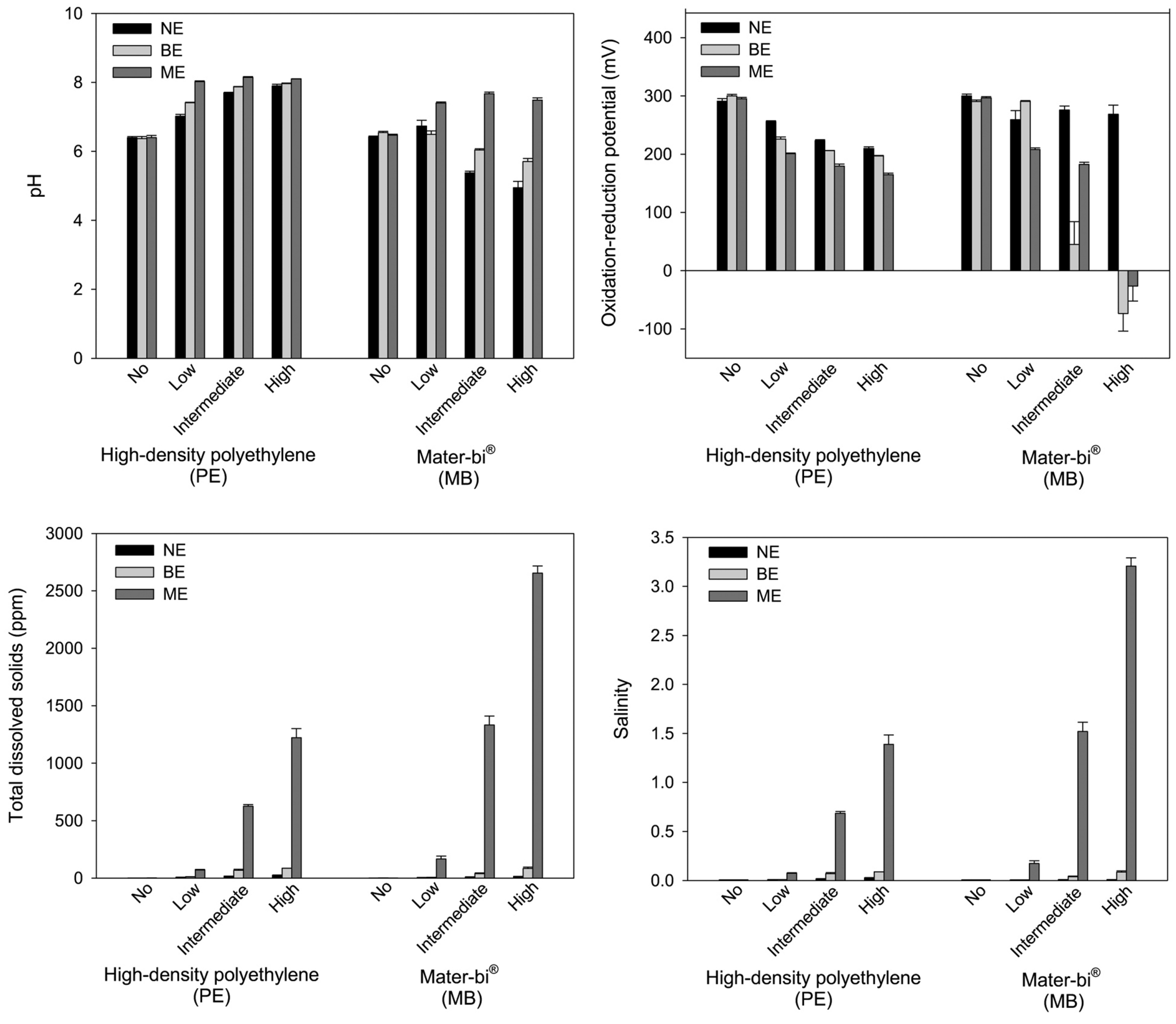
pH
10
8
6
4
2
0
NE
BE
ME
No
Low
Intermediate
High
No
Low
Intermediate
High
High-density polyethylene
(PE)
Mater-bi®
(MB)
Oxidation-reduction potential (mV)
400
300
200
100
0
-100
NE
BE
ME
No
Low
Intermediate
High
No
Low
Intermediate
High
High-density polyethylene
(PE)
Mater-bi®
(MB)
Total dissolved solids (ppm)
3000
2500
2000
1500
1000
500
0
NE
BE
ME
No
Low
Intermediate
High
No
Low
Intermediate
High
High-density polyethylene
(PE)
Mater-bi®
(MB)
Salinity
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
NE
BE
ME
No
Low
Intermediate
High
No
Low
Intermediate
High
High-density polyethylene
(PE)
Mater-bi®
(MB)

Figure 3

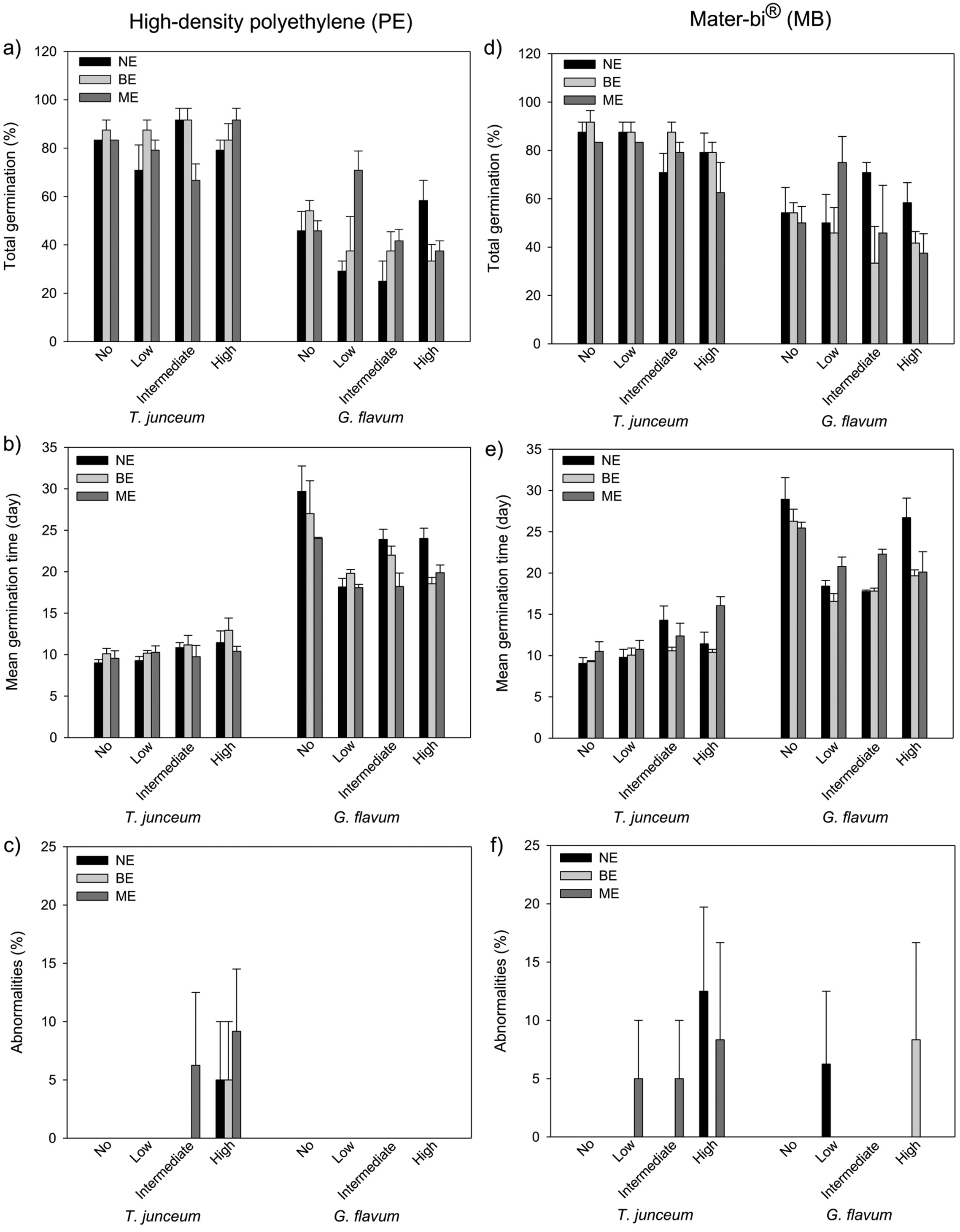
High-density polyethylene (PE)
Mater-bi® (MB)
a)
b)
c)
d)
e)
f)
NE
BE
ME
Total germination (%)
Mean germination time (day)
Abnormalities (%)
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
No
Low
Intermediate
High
T. junceum
G. flavum

Figure 4

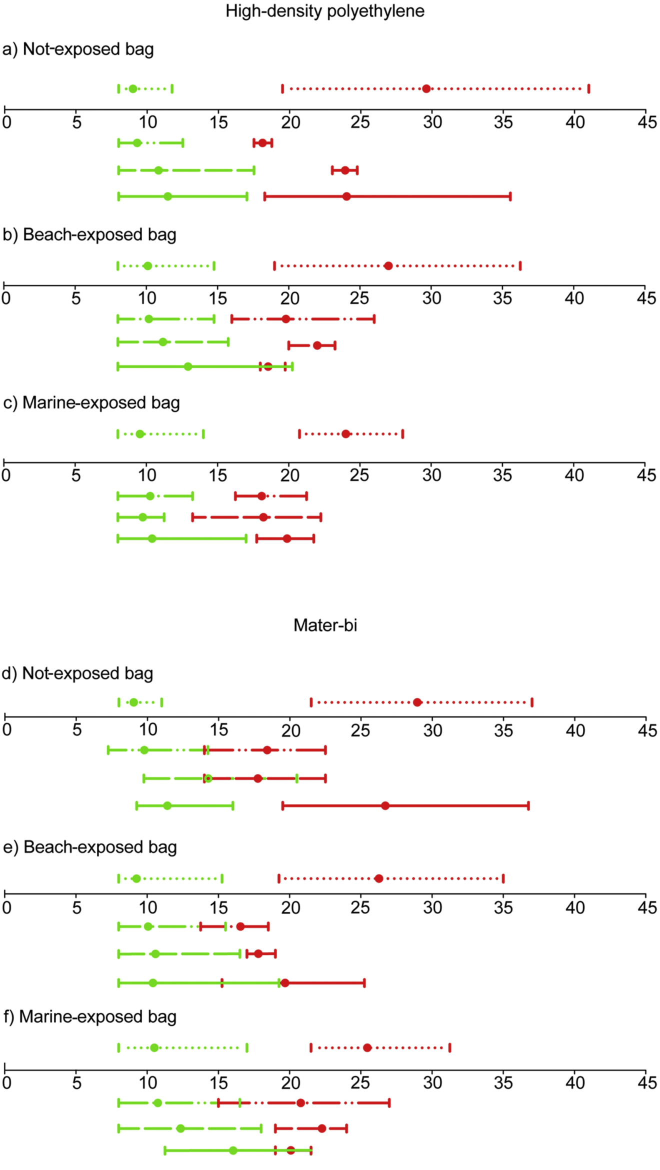
High-density polyethylene
a) Not-exposed bag
0 5 10 15 20 25 30 35 40 45
b) Beach-exposed bag
0 5 10 15 20 25 30 35 40 45
c) Marine-exposed bag
0 5 10 15 20 25 30 35 40 45
Mater-bi
d) Not-exposed bag
0 5 10 15 20 25 30 35 40 45
e) Beach-exposed bag
0 5 10 15 20 25 30 35 40 45
f) Marine-exposed bag
0 5 10 15 20 25 30 35 40 45

Figure 5

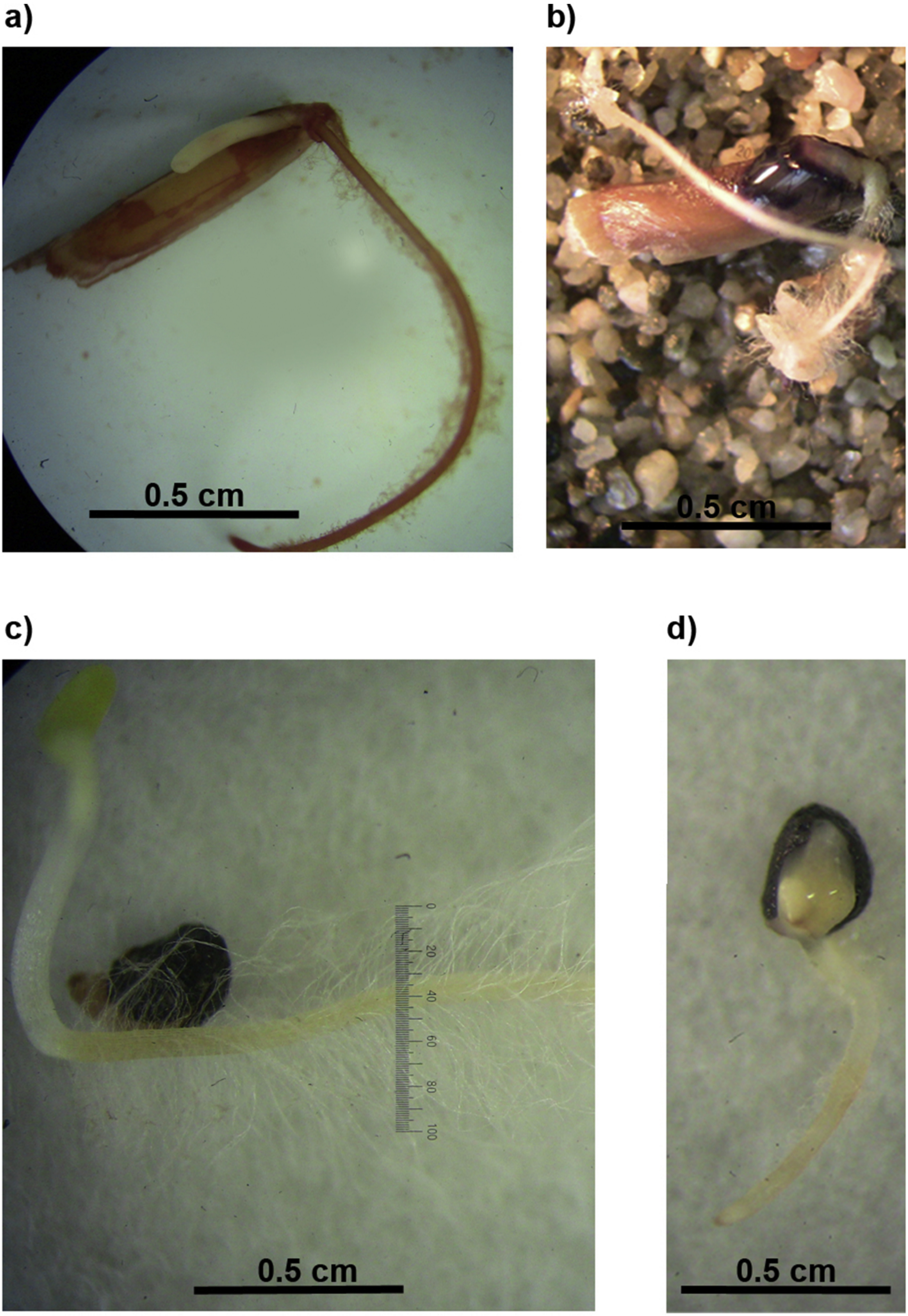

Figure 6

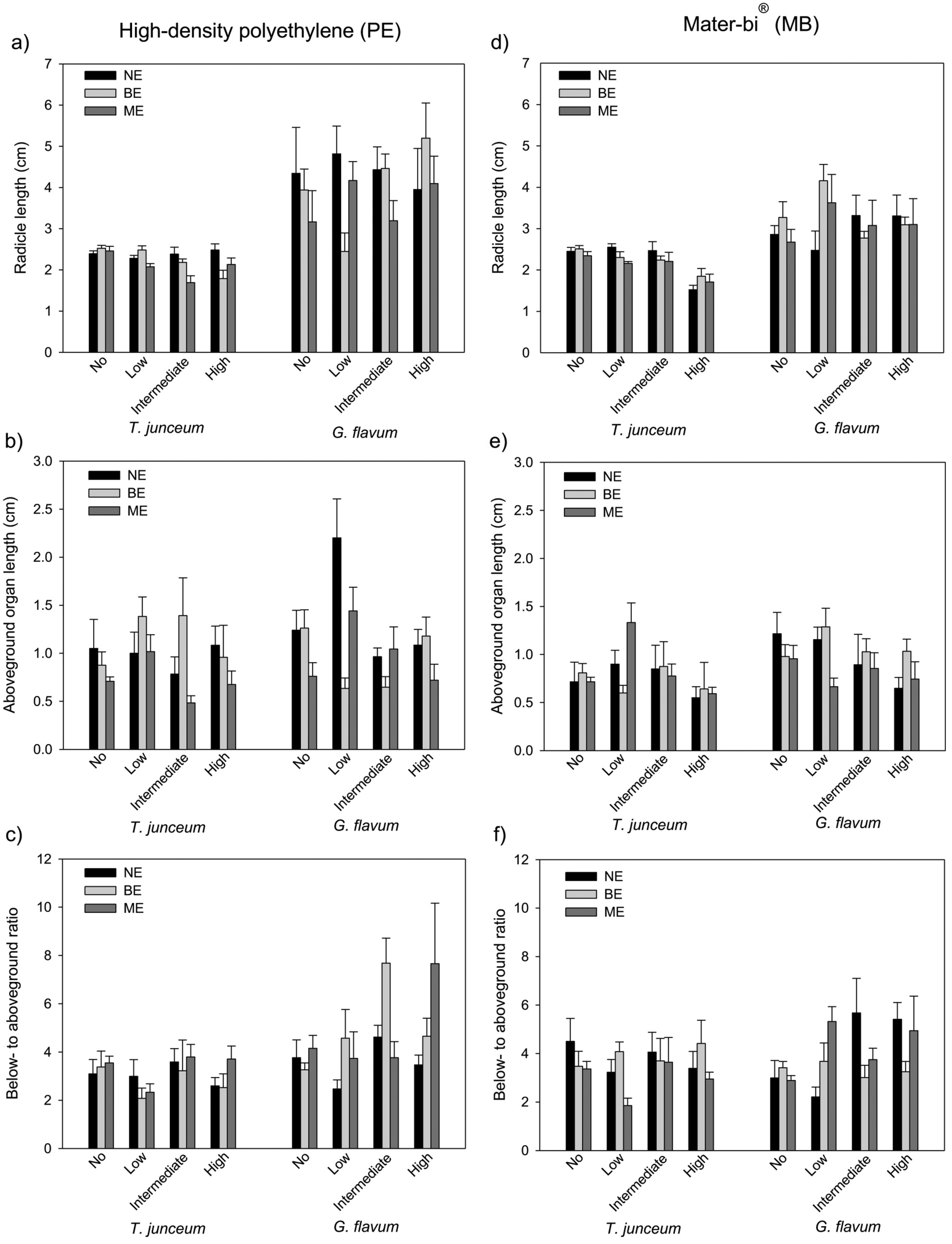
High-density polyethylene (PE)
Mater-bi® (MB)
a)
b)
c)
d)
e)
f)
NE
BE
ME
Radicle length (cm)
Aboveground organ length (cm)
Below- to aboveground ratio
0
1
2
3
4
5
6
7
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12
No
Low
Intermediate
High
T. junceum
G. flavum

Figure 7