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Abstract: The presence of plastic bags on coastal dunes worldwide is well documented. Plastic bags contain additives that during rainfall events can leach out from bags into sand dune and be absorbed by seeds and roots of plants. Dune plants play a fundamental role in dune system formation, yet the possible impact of bag leaching on their establishment and development has been neglected. We assessed in laboratory whether (i) not biodegradable bags (high-density polyethylene, PE) and new generation of compostable bags (Mater-bi®, MB) would influence via leaching water chemical/physical properties and (ii) leachates would affect germination and seedling growth using Thinopyrum junceum and Glaucium flavum as models. Leachates were obtained from different amounts of not-exposed and bags exposed to beach or marine conditions simulating various pollution degrees (none, low, intermediate and high pollution). All water variables were affected by leaching. The magnitude of these alterations depended on bag type and environmental exposure. Seeds of T. junceum treated with the high concentration of marine-exposed MB bag leachate germinated later than controls while those of G. flavum treated with the remaining leachates germinated earlier. For both species, leachates from the low concentration of PE and MB marine-exposed bags increased seed germinability. A short radicle was observed in T. junceum seedlings treated with not-exposed MB bag leachates. Glaucium flavum seedlings treated with beach- and marine-exposed PE bags and not-exposed MB bags leachates showed a greater below-aboveground length ratio and those grown with the low concentration of not-exposed PE bag leachate had a longer hypocotyl compared to controls. Leachates from the high concentration of PE and MB bag caused seedling anomalies in both species. These findings indicate that not biodegradable and compostable bags may interact with abiotic/biotic factors and affect via leaching germination phenology, seedling establishment and plant interactions with consequences on dune community structure.

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Opposed Reviewers:

Dear Editor,

My co-authors (Virginia Menicagli and Claudio Lardicci) and I are pleased to submit the enclosed manuscript “Exposure of coastal dune vegetation to leachates from plastic bags: a neglected effect of plastic litter” as Research Article in Science of the Total Environment.

The paper focuses on plastic pollution, and in particular on the potential effects of plastic bags on dune plants via leaching, i.e. the release of chemicals from plastics in natural environments. Many previous studies on plastic litter have investigated the effects of bags on a variety of organisms via ingestion or entanglement, but only few studies have dealt with plastic bag leaching and its impact on plants. Recently, a study has demonstrated that when in contact with water, chemicals can be released from plastic bags and migrate into the water phase, contaminating water available to plants. However, the potential effects of this phenomenon on dune vegetation that strictly depend on the availability of water and grow in a habitat highly impacted by plastics are completely ignored. Therefore, in this study the effects of the leaching of conventional not biodegradable bags and biodegradable/compostable shopper bags on the most vulnerable life history stages of dune plants, germination and early seedling growth, were examined. Conventional bags are a consistent fraction of plastic litter found in coastal environments. Biodegradable/compostable bags made with Mater-bi$^{®}$ (a starch-based polymer) have been marked in Europe to reduce the impact associated to plastic littering and disposal, but their presence in natural habitats has recently been observed. These bags are considered as an eco-friendly alternative to conventional ones, and they are specially designed to be disposed in industrial or home compost facilities at the end of their life. However, an increased number of studies indicate that once entered natural environments compostable bags degrade slowly and can affect marine organism and communities. Firstly, we performed a laboratory leaching experiment to examine whether (i) both types of bag 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 or sand burial would influence water variables. Then, we conducted a germination and seedling growth test to investigate whether bag leachates would affect seed germinability, germination phenology and seedling growth of *Thinopyrum junceum* (L.) Á. Löve and *Glaucium flavum* Crantz, two dune plant species belonging to different taxonomic groups.

The results of the leaching experiment demonstrate that conventional and compostable bags once deposited on sand dunes considerably altered via leaching chemical/physical characteristics of the water available to dune plants during rainfall events. Water quality depended on the type of bag material and the environmental conditions experienced by the bag. The results of the germination and early seedling growth test show that bag leachates interfered with factors controlling seed dormancy and seedling emergence. Both germination phenology and seedling growth of the two species are differentially affected by leachates, and the direction and the magnitude of these effects depended on type and amount of bag as well as on the environmental conditions experienced by the bag before being deposited close to seeds.

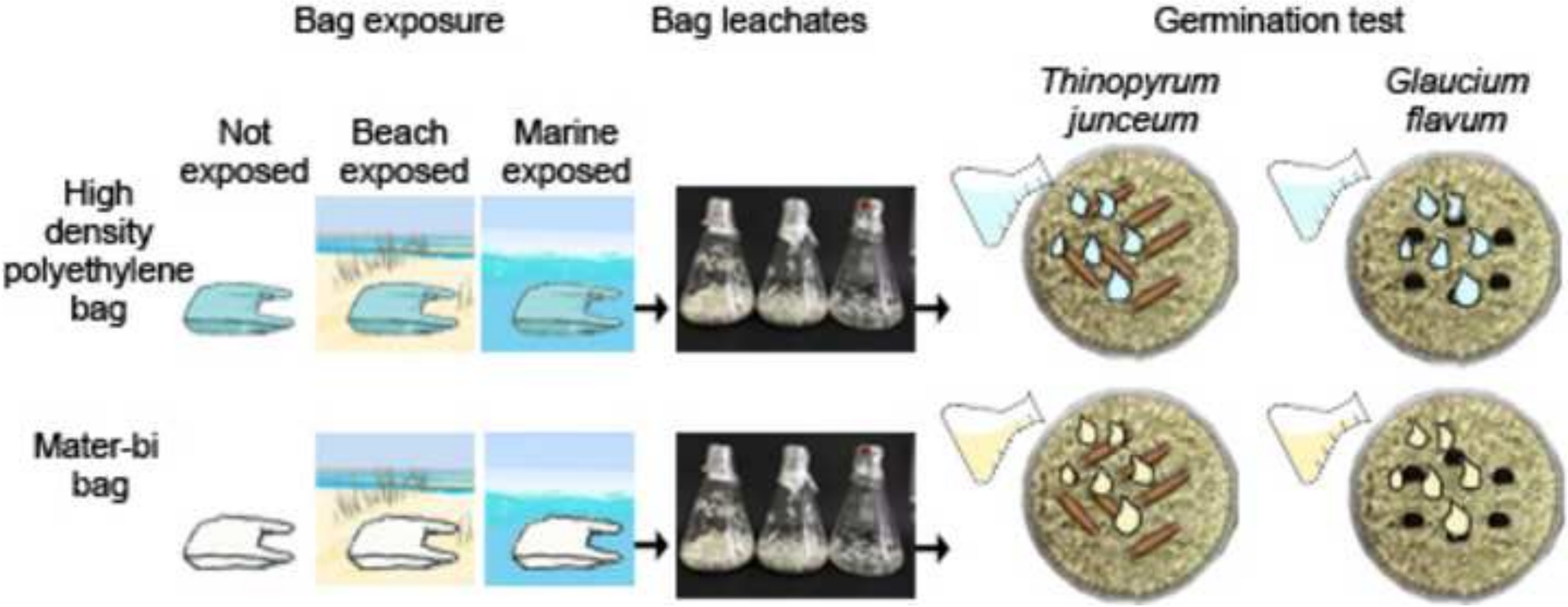
This manuscript fits the Aims and Scope of the journal, as it examines the impact of discarded plastics (anthroposphere) derived from land-based or marine-based sources (hydrosphere) via leaching by rainwater (atmosphere) on dune plants (biosphere) that play a pivotal role in maintaining and structuring coastal dune systems. Our results are based on experimental ecological data, and their discussion from an ecological management point of view could be useful to formulate new effective environmental policy to limit the impact of plastic pollution on natural habitats.

On behalf of all co-authors,

Yours sincerely,
Elena Balestri

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

*Graphical Abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

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.

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

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1. Introduction

Coastal dunes consist of a narrow 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; Provoost et al., 2011). 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; 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 intense 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 (Law, 2017; Moore, 2008; Rochman et al., 2015; Talsness et al., 2009; Wilcox et al., 2018). 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; Nakashima et al., 2012; 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, and the dicotyledon *Glaucium flavum* Crantz.

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), a group of bags (hereafter referred to not-exposed bags, NE) was left in laboratory at a temperature of 22 °C (± 1 SE) to test the effects of bag material itself on chemical/physical properties of the water. Other two groups of bags were exposed outdoor over a period of 10 days for weathering (Balestri et al., 2019). The first bag group (hereafter referred to as beach-exposed bags, BE) was placed in an upper beach area (average daily temperature of 7.1 ± 1.6 °C, total amount of precipitations of 60.8 mm and mean daylength of 11 hours) at Rosignano Solvay (43°23’N 10°26’ E, Italy). Bags were carefully laid out on the sandy substrate and fixed with pebbles to avoid dispersion. The second group of bags (hereafter referred to as marine-exposed bags, ME) was immersed in seawater in a shallow (0.5 m depth) coastal zone at Rosignano Solvay. Each bag was secured with cotton wires to a sample holder anchored to the bottom and left to move freely in the water column (average seawater temperature of 13.7 ± 0.1 °C). 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) similar to that of the rainwater of the coastal Mediterranean basin (Loye-Pilot et al., 1986) was used. 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* (yellow horned-poppy). Seeds were harvested from plants inhabiting Rosignano Solvay dune system in July 2017 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 overlapped, and they substantially differed from samples belonging to the plastic pollution treatments (Fig. 1). 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. 2 and Table 2). The impact of each type of bag on pH leachates increased with increasing pollution (Fig. 2 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. 2 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. 2 and Table 2). A significant greater amount of total dissolved solids (TDS) was measured in plastic leachates compared to controls (Fig. 2 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. 2 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. 2 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. 2 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. 3 and 4 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. 3 and 4 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. 3 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. 3 and 5 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. 6 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. 6 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. 6 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. 6 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. 6 and Table 7).

4. Discussion

Coastal dune vegetation establishment and development are strongly influenced by the availability and amount of rainwater (Maun, 2009). Results of our experiment simulating the leaching of conventional and compostable bags by rainwater on sand dunes demonstrate that both types of bag altered water chemical/physical characteristics, 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 nature, alterations of chemical/physical characteristics of soil water can have considerable effects on plant development. Indeed, the pH and the ORP values of water in sandy soils can influence the mobility and the availability of nutrients and carbonate, while soil salinity is the major selective force in seed germination and seedling growth of dune plants (Husson, 2013; Maun, 2009; Sykes and Wilson, 1989).

Results of our seed germination experiment indicate that both the types of bag affected via leaching seed germination processes and seedling growth. As observed for water chemical/physical variables, the magnitude and direction of bag 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 (Balestri et al., 2019) and to the variations of water chemical/physical characteristic that 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 release of high amounts of salts in water, as previous studies have shown an increase of mean germination timing of seeds this species 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. This stimulatory effect was possibly due to the release in water of low amount of salts deposited on the bags during seawater immersion. In fact, moderate salt concentrations have been shown to increase seed germination percentage of some dune species (Ungar, 1978; Woodell, 1983).

As 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 the 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 presence in the water phase of plastic additives and non-intentionally added compounds, such as for example bisphenol A (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).

In dune environments, shifts in germination phenology and alterations in biomass allocation to above-belowground organs can have a considerable impact on plant population dynamics and structure of communities. For example, an advance or a delay of seed germination timing of a species could lead to an overlap of its germination spread (i.e. the time elapsed between the first and the last seed germination event) with that of a neighbor species and hence increase the intensity of interspecific interactions (Maun, 2009). On the other hand, a reduction of the germination spread of a species could increase the intensity of intraspecific interactions among co-specific seedlings. 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).

On the basis of the findings of the present study, different plant population establishment scenarios could be hypothesized. For example, in sand dune areas not impacted by plastic bags, seeds of *T. junceum* are expected to germinate earlier than those of *G. flavum,* and the germination spreads of these species would never overlap. In areas polluted by PE bags, irrespectively of their exposure, seeds of *G. flavum* buried close to bags would germinate earlier and their germination spread would be reduced as compared to that of seeds located far away from bags (Fig. 4a,b,c). Under this scenario, the chance of interspecific competition among *G. flavum* seedlings near to the bags would increase, and emerging seedlings would morphologically differ from those grown in non-polluted areas. In areas polluted by MB bags, directly deposited or aged on beaches, the germination behavior *G. flavum* would be similar to that described in the above scenario, but the germination spread of this species would overlap to that of *T. junceum* (Fig. 4d,e). Under this scenario, *G. flavum* seedlings would greatly differ in developmental stage and the chance of both intra- and interspecific competition among seedlings would be enhanced. In addition, many *G. flavum* seedlings emerging close to the bags directly abandoned in dunes would differ in the pattern of allocation of biomass from those emerged in not polluted areas. Lastly, in dune areas highly polluted by MB bags transported by the sea, *T. junceum* seeds buried close to bags would germinate later than those located far away from bags (Fig. 4f), and this would lead to a seedling population more heterogeneous in size. Delayed germination timing would also cause an overlap of the seed germination spreads of *T. junceum* and *G. flavum* and increase the chance of interspecific competition among seedlings (Fig. 4f). Irrespectively of the type of scenario, in highly polluted dune areas a significant number of germinated seeds of both study species would not emerge and survive lacking of the above-ground organ.

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.

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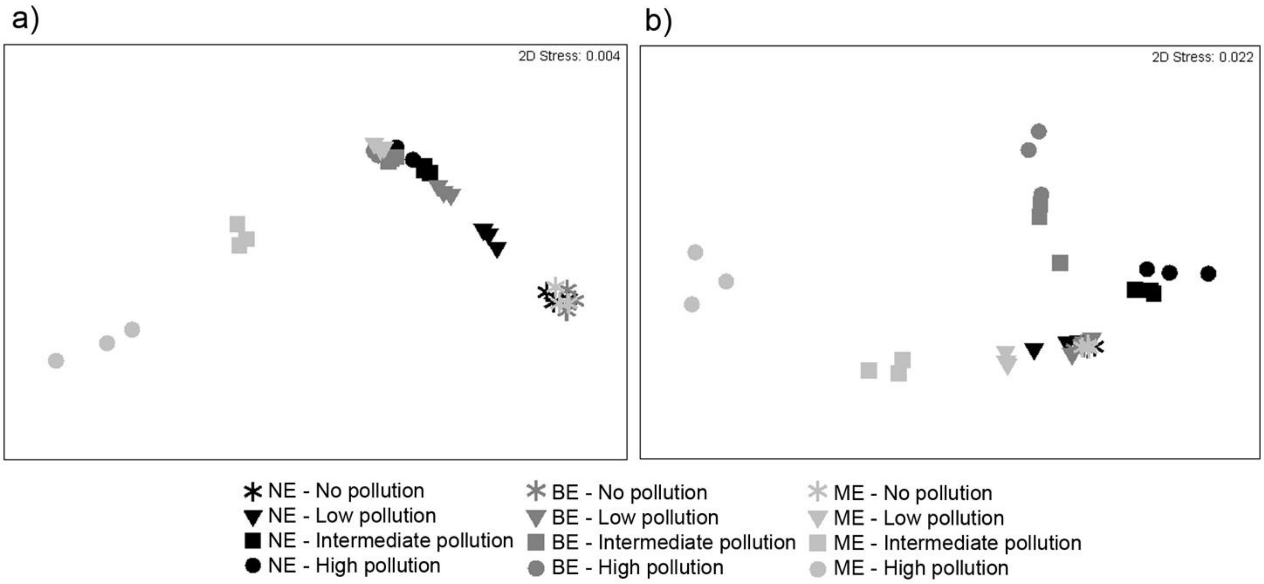

Fig. 1. Non-metric multidimensional scaling ordination (MDS) of chemical/physical characteristics of leachates from obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE (a) and MB (b) bags simulating different degrees of pollution.

2-column fitting image

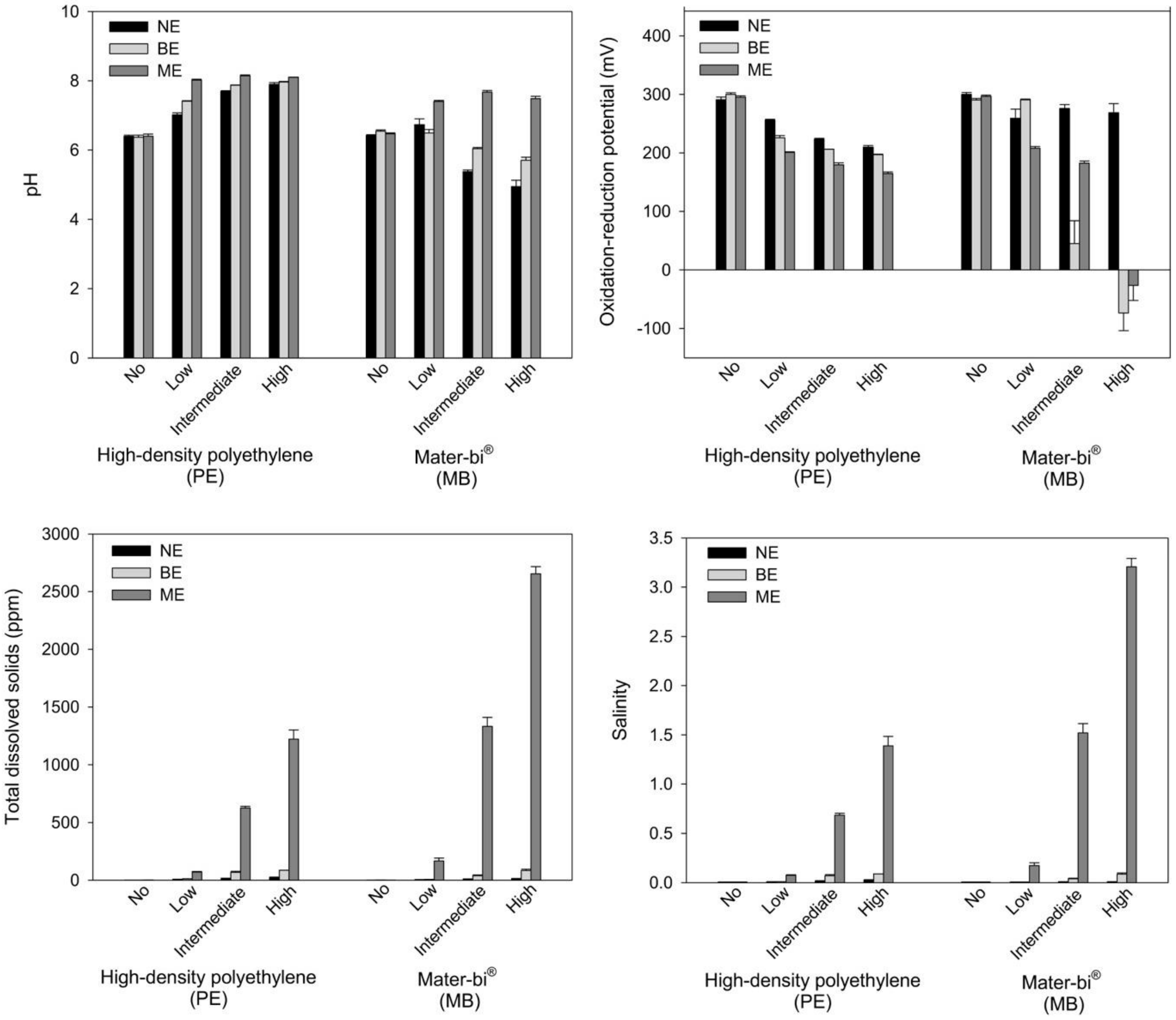

Fig. 2. Chemical/physical variables (pH, oxidation-reduction potential, total dissolved solids and salinity) of leachates obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE and MB bags simulating different degrees of pollution. Data are mean ± SE, n = 3.

2- column fitting image

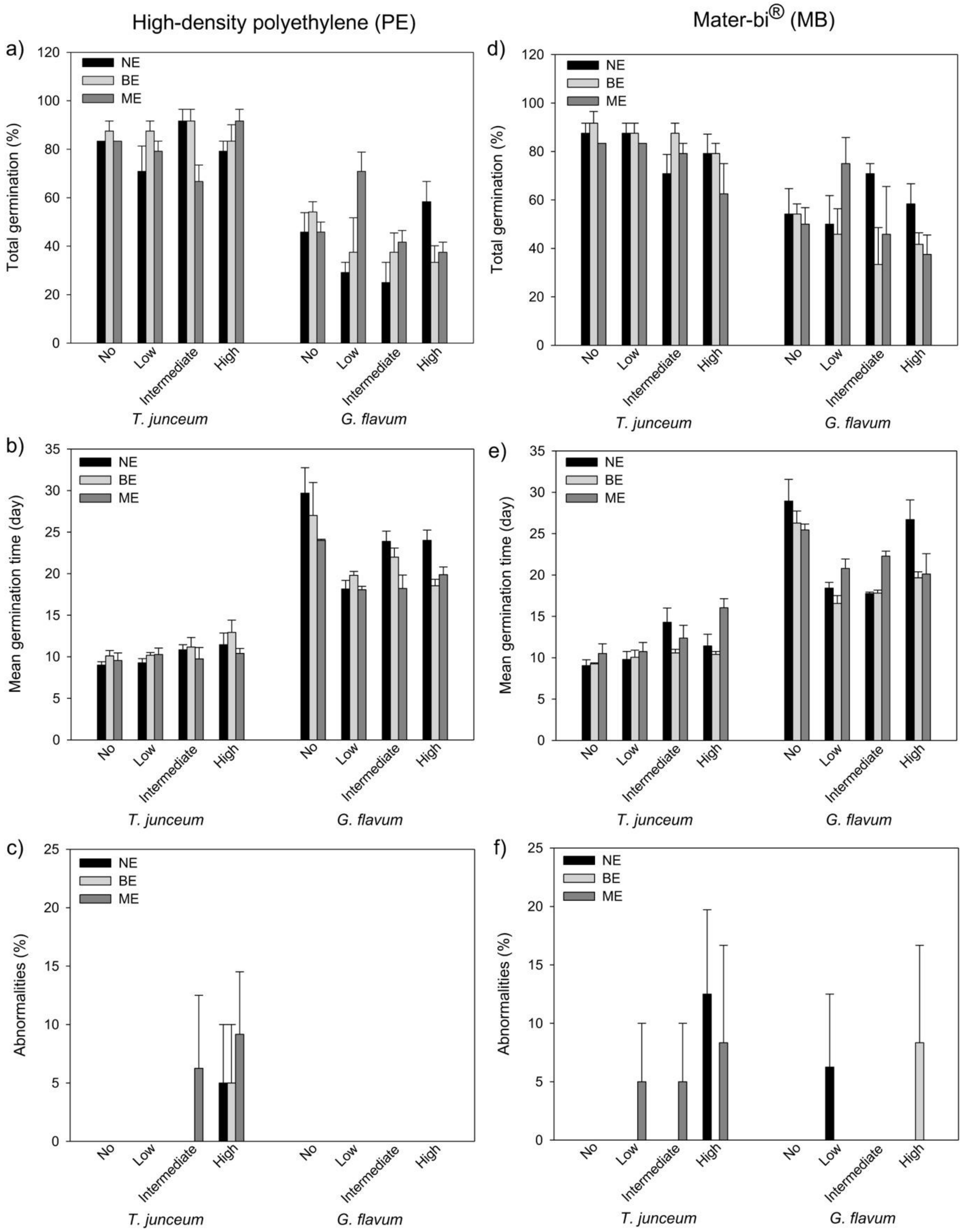

Fig. 3. Percentage of germination and mean germination time of seeds and percentage of abnormal seedlings of *T. junceum* and *G. flavum* grown with leachates obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE (a, b, c) and MB (d, e, f) bags simulating different degrees of pollution. Data are mean ± SE, n = 4.

2-column fitting image

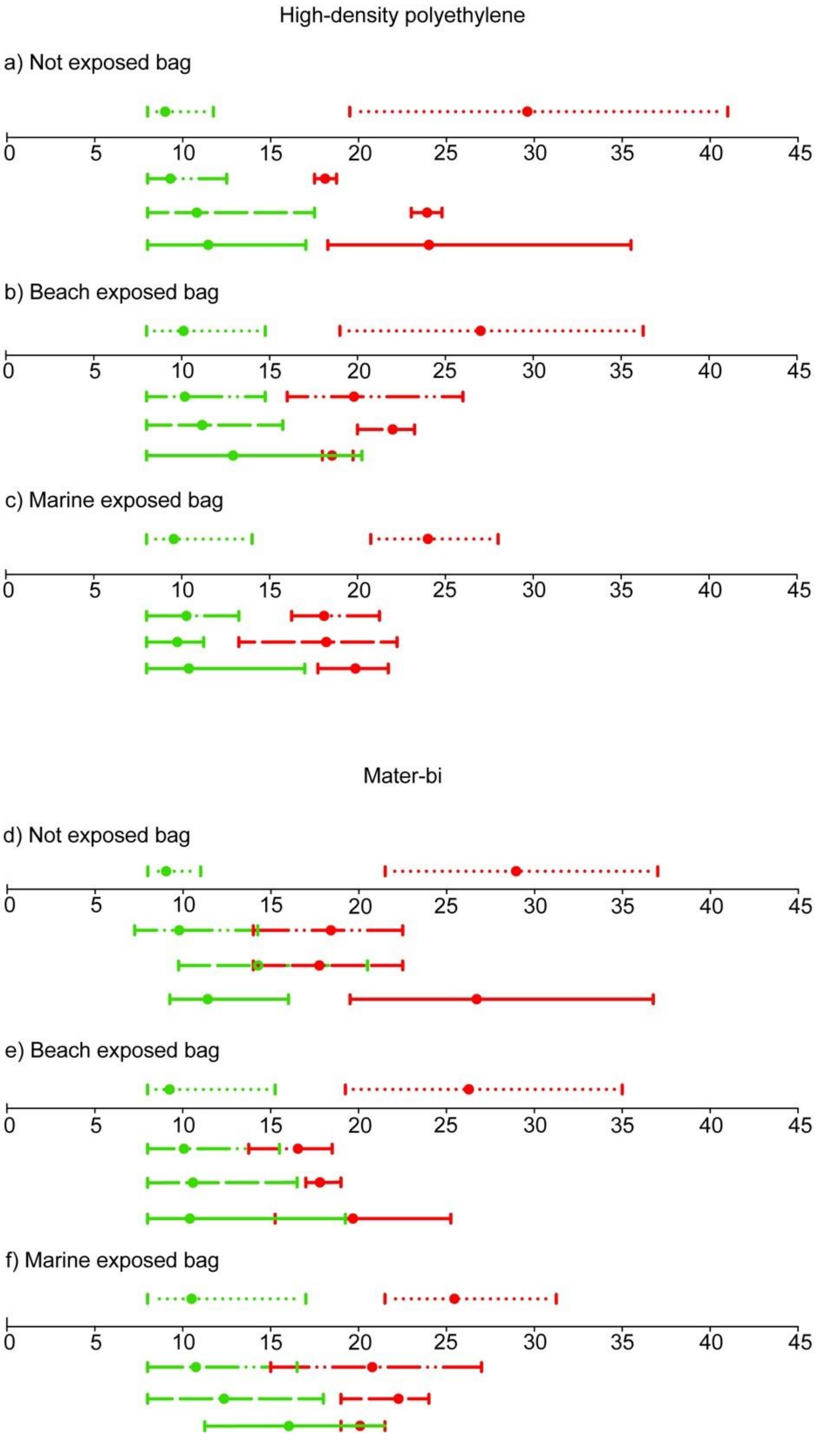

Fig. 4. The time spread of germination and the mean germination time of *T. junceum* (green) and *G. flavum* (red) seeds treated with bag leachates at various pollution degrees (——— high, — — intermediate, —··— low and ······· no pollution).

2-column fitting image

Color image in online version and grayscale image in printed version

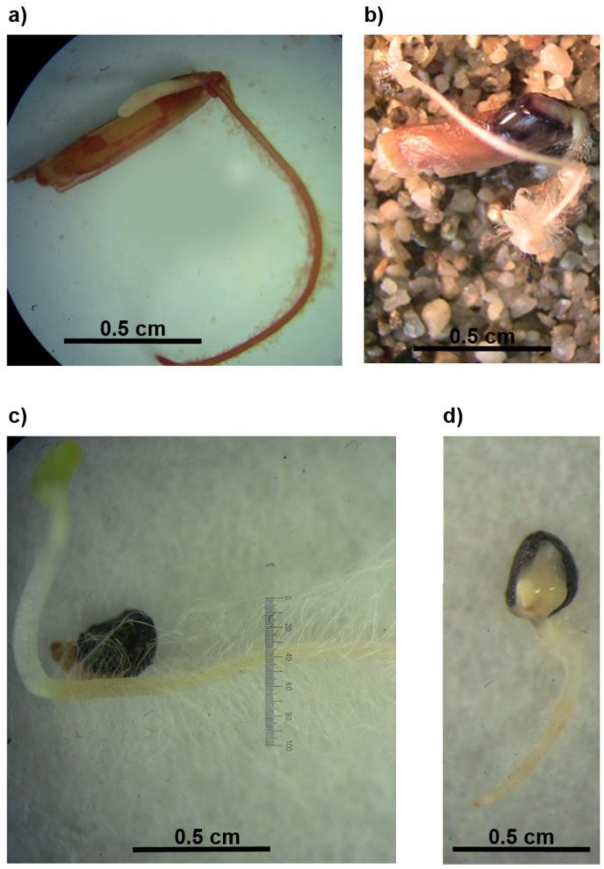

Fig. 5. Normal and abnormal seedlings lacking aboveground organs of *T. junceum* (a, b) and *G. flavum* (c, d) grown with bag leachates.

Single column-fitting image

Color image in online version and grayscale image in printed version

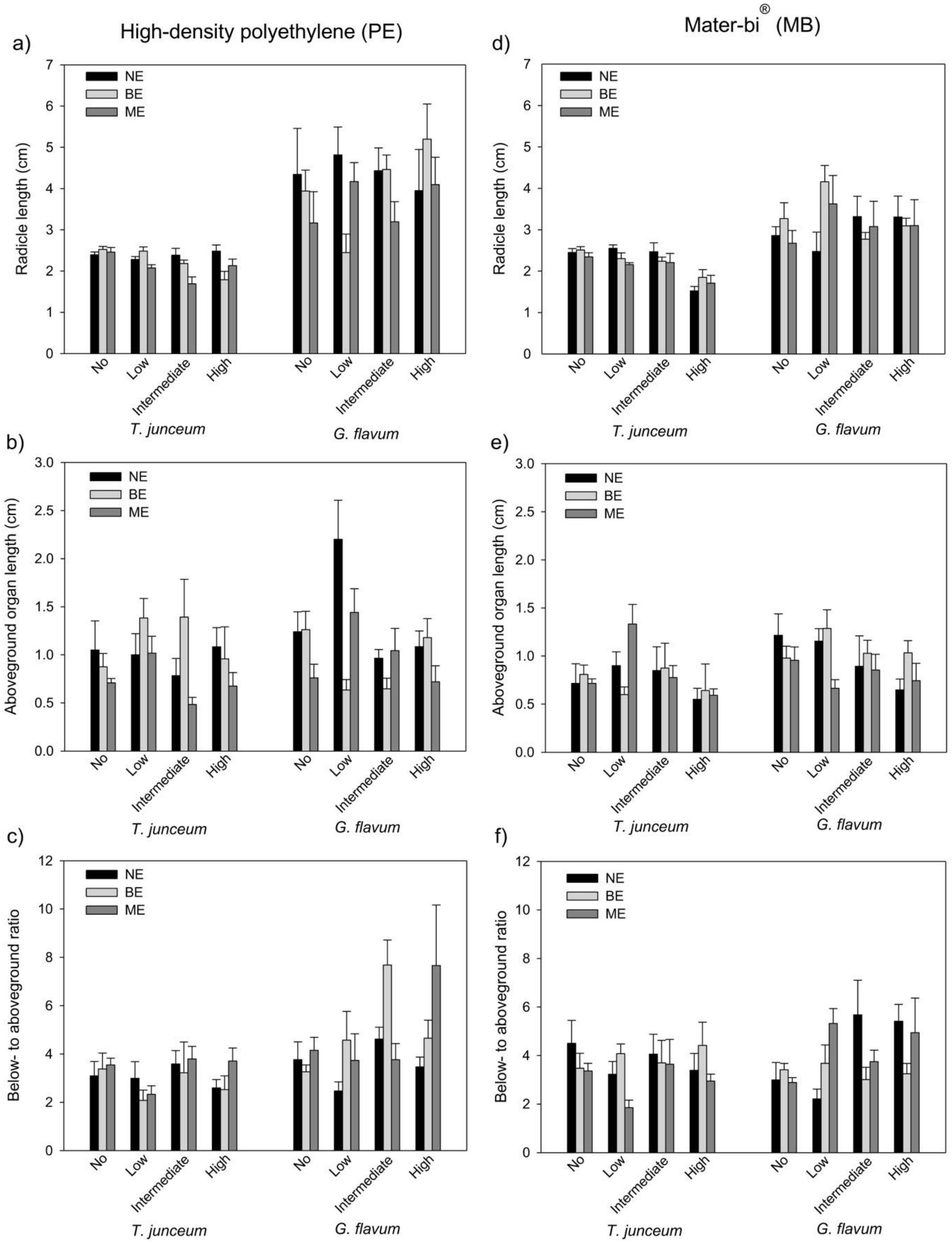

Fig. 6. Radicle length, aboveground organ length, and below- to aboveground ratio of seedlings grown with leachates obtained from different amounts of not-exposed (NE), beach-exposed (BE) and marine-exposed (ME) PE (a, b, c) and MB (d, e, f) bags simulating different degrees of pollution. Data are mean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