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Combined effect of plastic litter and increased atmospheric nitrogen deposition on vegetative propagules of dune plants: A further threat to coastal ecosystems☆

Virginia Menicagli [a], Elena Balestri [a, *], Flavia Vallerini [a], Alberto Castelli [a, c], Claudio Lardicci [b, c]

[a] Department of Biology, University of Pisa, Via Derna 1, 56126, Pisa, Italy
[b] Department of Earth Sciences, University of Pisa, Via S. Maria 53, 56126, Pisa, Italy
[c] Centre for Climate Change Impact, University of Pisa, Via Del Borghetto 80, Pisa, 56124, Italy

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ABSTRACT

Large amounts of non-biodegradable plastics are currently deposited on beach-dune systems, and biodegradable plastics could enter these already declining habitats in coming years. Yet, the impacts of plastics on vegetative recruitment, a plant strategy playing a key role in dune stabilization, are unknown. Whether these pollutants interact with increased atmospheric nitrogen (N) deposition, a major global driver of plant biodiversity loss, in affecting plant communities of such nutrient-poor habitats, and how plant-plant interactions mediate their effects need to be explored. In a one-year field experiments, we examined individual and combined effects of plastic (non-biodegradable, biodegradable), N deposition (ambient, elevated) and biotic condition (no interaction, interaction with a conspecific or with a hetero-specific) on the colonization success and growth of vegetative propagules of dune plants. *Thinopyrum junceum* and *Sporobolus pumilus* were chosen as models because they co-occur along Mediterranean dunes and differ in ecological role (dune- *vs.* non dune-building) and photosynthetic pathway (C3 *vs.* C4). For both species, survival probability was reduced by non-biodegradable plastic and elevated N by up to 100%. *Thinopyrum junceum* survival was also reduced by *S. pumilus* presence. Elevated N and biodegradable plastic reduced *T. junceum* shoot biomass when grown alone and with a conspecific, respectively; these factors in combination mitigated their negative individual effects on root biomass. Biodegradable plastic increased *S. pumilus* shoot and root biomass, and in combination with elevated N caused a greater biomass investment in belowground (root plus rhizome) than aboveground organs. Non-biodegradable plastic may be a further threat to dune habitats by reducing plant colonization. Biodegradable plastic and increased N deposition could favour the generalist *S. pumilus* and hinder the dune-building *T. junceum*. These findings highlight the urgency of implementing measures for preventing plastic deposition on beaches and reducing N input.

1. Introduction

Plastic litter is a major emerging global environmental problem (Barnes et al., 2009; Maity and Pramanick, 2020), and current projections suggest that coastal environments are likely to continue to receive plastic waste in the future (Jambeck et al., 2015). The impact of plastics on vegetated coastal habitats of conservation concern, such as seagrass beds and sand dunes (Directive 92/43/EEC, 1992), has only recently been addressed (Balestri et al., 2017; Menicagli et al., 2019a; NOAA, 2016). Yet, whether plastic litter may interact with other global change related stressors that are likely to affect these habitats needs to be explored. This issue is of high relevance, as the combined effects of multiple, co-occurring environmental stressors on plant traits and processes can often result in unexpected responses (Crain et al., 2008; Paine et al., 1998). In addition, how plant-plant interactions could mediate the individual and combined effects of plastic and of other co-occurring stressors on dune vegetation should investigated. These interactions play a

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* Corresponding author. University of Pisa, via Derna 1, 56126, Pisa, Italy.
E-mail address: elena.balestri@unipi.it (E. Balestri).

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fundamental role in determining plant community structure and dynamics, and they can modulate the effects of environmental drivers by promoting plant survival in extreme conditions (facilitation) or replacing less tolerant species to environmental changes (competition; Brooker, 2006; Arroyo et al., 2015).

Being located at the interface of land and sea, beaches and dunes have become "hotspots" of plastic litter accumulation (Andriolo et al., 2020; Ceccarini et al., 2018; De Francesco et al., 2019; Šilc et al., 2018). These systems are ecologically and economically important since they perform critical ecological functions and provide valuable goods and services (Barbier et al., 2011; Drius et al., 2019), but anthropogenic pressures and sea level rise are leading to a dramatic dune habitat decline worldwide (Feagin et al., 2005; Malavasi et al., 2016). Dune formation is driven by the interaction between sediment deposition and the establishment and spread of a few plant species (Maun, 2009) adapted to cope with stressful harsh conditions (i.e. low nutrient and water availability, García-Mora et al., 1999; Hesp, 1991). Plant-plant interactions can vary from competitive to neutral or facilitative, depending on the stress and disturbance levels (Franks, 2003; Martínez and García-Franco, 2008; Vaz et al., 2015). The accumulation of plastics made of conventional non-biodegradable polymers, in particular polyethylene and polypropylene, on beaches could pose a novel type of stress to dune plants (Menicagli et al., 2019a,b; Poeta et al., 2017; Šilc et al., 2018). Recent studies have shown that non-biodegradable plastic bags, which are currently a relevant component of the beach litter (Alshawafi et al., 2017; Andriolo et al., 2020; Šilc et al., 2018), can change sediment porewater physical and chemical parameters (e.g., pH, salinity and oxygen-reduction potential) and release chemical compounds (e.g., bisphenol A) into soil, inducing variations in the seedling emergence pattern of sensitive species (Balestri et al., 2019; Menicagli et al., 2019a,b). Changes in the emergence and growth pattern of seedlings exposed to biodegradable/compostable bags have also been observed, raising concerns about their harmlessness for natural environments (Menicagli et al., 2019a,b). These bags are considered as an eco-friendly alternative to non-biodegradable ones and are designed to be disposed at the end of their life cycle in proper industrial facilities. But, when released in natural habitats they can cause sediment and water acidification and release compounds well before being biodegraded (Balestri et al., 2019; Bandopadhyay et al., 2018; Narancic et al., 2018; Serrano-Ruíz et al., 2018, 2020).

In addition, dune plants have to face increased deposition of nitrogen (N), one of the major threats to terrestrial plant biodiversity (BassiriRad, 2015; Bobbink et al., 2003; Pakeman et al., 2016). During the past century, emissions of nitrogenous atmospheric pollutants has increased markedly, from an estimated N input of about 1–5 kg ha^{-1} year^{-1} to 20–60 kg ha^{-1} year^{-1} in some European ecosystems (Bobbink, 1998; Galloway et al., 2008). Impacts of elevated N deposition on plant communities are mainly mediated by N enrichment and changes in soil chemistry, such as acidification and nutrient imbalance (Bobbink et al., 2010; Stevens et al., 2018). Enhanced sensitivity to secondary environmental drivers such as extreme weather, herbivory and disease is also an important mechanism (Phoenix et al., 2012; Stevens et al., 2018). In addition, there is a quite variation in plant ability to respond to increased soil N availability. For example, plants with a C3 photosynthetic pathway are less efficient than those with a C4 pathway in using N (Ripley et al., 2010; Sage and Pearcy, 1987; Yuan et al., 2007), and the competitive balance between these plants can be influenced by soil N variations through changes in the relative importance of belowground and aboveground competition (Tilman, 1988). The impacts of increased N deposition are likely to be greatest in nitrogen-deficient systems, such as coastal dunes, because a higher N availability can change community composition by increasing the dominance of more nutrient-demanding species over those stress-tolerant adapted to low nutrient conditions (Ceulemans et al., 2017; McLendon and Redente, 1992 Wang et al., 2019), as already occurred along U.S. eastern and European coasts (Day et al., 2018; Remke et al., 2009). However, large variations in vegetation responses to N inputs have been reported among geographical areas and calcareous and acidic dunes (Aggenbach et al., 2017; Bird and Choi, 2017; Day et al., 2018; Pakeman et al., 2016; Plassmann et al., 2009). For European shifting dunes, the estimated critical N threshold, the lower exposure level at which significant detrimental effects begin to occur, ranges from 10 to 20 kg N ha^{-1}year^{-1} (Bobbink et al., 2010). Chronic atmospheric N deposition and plastic pollution have the potential to change some sediment chemical/physical parameters (Bobbink et al., 2010; Carson et al., 2011), and they will probably continue to affect coastal habitats in the coming decades (BassiriRad, 2015; Stevens et al., 2018; Windsor et al., 2019). Therefore, understanding whether and if so, how this novel combination of stressors may affect dune plant communities may help to provide a more holistic view of dune habitat vulnerability and identify priority conservation/mitigation actions.

In a one-year field experiment, we examined the individual and combined effects of macro-plastic, N deposition (current and predicted in the Mediterranean for 2050) and biotic condition (no interaction, interaction with a conspecific or with a hetero-specific neighbour) on the performance (in terms of colonization success and growth) of vegetative propagules of dune plants. A traditional non-biodegradable and a biodegradable/compostable plastic were chosen for testing. This latter was included as the global production of biodegradable plastics is predicted to increase in the next future (EU Parliament, 2018; European bioplastics, 2018) and reach the level of traditional ones. We focused on vegetative propagules because the stabilization of new dune areas strongly depends on their successful establishment and growth (Harris and Davy, 1986; Maun, 2009). As models, we selected two clonal grasses with different photosynthetic pathway (C3 *vs.* C4) and ecological role that can co-occur along Mediterranean dunes; *Thinopyrum junceum* (L.) Á. Love, a typical dune-building C3 species (van Puijenbroek et al., 2017; Vogel et al., 1986), and *Sporobolus pumilus* (Roth) P.M. Peterson & Saarela, a generalist C4 species not involved in the dune formation process (Brantley et al., 2014; Pyankov et al., 2010). We hypothesized that (i) non-biodegradable and biodegradable/compostable plastic would affect differentially the colonization success and plant growth based on their different behaviour in natural environments and potential influence on physical and chemical substrate properties observed in previous studies; (ii) elevated N deposition would influence differentially the growth of the two species based on their different water and N use efficiency; (iii) plastic and increased N deposition would influence interactively plant performance due to their ability to alter substrate properties, and (iv) the presence of a neighbour would modulate (alleviate or exacerbate) their effects, depending on the nature of plant-plant interactions (facilitative or competitive).

2. Material and methods

2.1. Study species

Thinopyrum junceum and *S. pumilus* are perennial rhizomatous grasses inhabiting European dune systems. *Thinopyrum junceum*, previously known as *Elymus farctus* (common name: sand couch), is a pioneer species responsible for the early dune formation (van

Puijenbroek et al., 2017). *Sporobolus pumilus*, previously known as *Spartina versicolor* (common name: cordgrass), grows in a range of habitats including disturbed dune depressions occasionally waterlogged by storm surge (Sarmati et al., 2019) and does not substantially contribute to dune formation (Brantley et al., 2014). This species was likely introduced in Europe from North Atlantic populations during the past century (Sarmati et al., 2019). In Mediterranean dune systems, the two species can co-exist forming the *Elymo farcti-Spartinetum Junceae* association (Sarmati et al., 2019).

2.2. Site description and experimental design

The experiment was conducted in a dune system of the Tuscany (Italy, 43°21′06.11″N 10°27′21.64″E) from 2018 to 2019. At this site, the average minimum and maximum monthly temperature ranged from 4.3 (in January) to 29.8 °C (in August), and the cumulative precipitation was approximately 670 mm during the experimental period (data from SIR Toscana-Servizio Idrologico della Regione Toscana, www.sir.toscana.it). Total N deposition rate along the Tuscan coast ranged from 4.7 to 5 kg N ha^{-1} year $^{-1}$ (Marchetto et al., 2014). Prior to the start of the experiment (April 2018), sand pH was 8.81 ± 0.01, and total N and P content was 0.084 ± 0.000 mg/g and 0.059 ± 0.001 mg/g, respectively (full details on sand sample collection and chemical-physical analyses are provided in the Supplementary Materials).

At the site, a relatively homogenous area (approximately 660 m^2) along embryonic shifting dunes was selected based on the presence of typic Mediterranean dune plants including both the study species. The area was fenced to avoid the intrusion of herbivores and humans. Before the start of the experiment, the area was ideally divided in a grid of 1 m^2 quadrats, and plots of 25 cm × 25 cm were attributed to quadrats according to a completely randomized design. The average distance between the nearest plots was approximately 1.25 m (minimum and maximum distances were 0.75 and 1.75 m respectively). The substrate within each plot was manually excavated and sieved (1 mm mesh) to remove seedlings, seeds and other materials. Plots were randomly assigned to a factorial combination of the following treatments; Plastic litter (P): without plastic (WP), non-biodegradable bag (NBP), biodegradable/compostable bag (BP); Nitrogen (N): ambient nitrogen (AN), elevated nitrogen (EN); Biotic condition (B): no interaction with a neighbour (NoI), interaction with a conspecific neighbour (CoI), interaction with a hetero-specific neighbour (HeI). The "control" was the condition WPxNoIxAN (Fig. 1). Each of the 18 treatment combinations (3 × 2 × 3) was replicated 12 times, in total 216 plots for each of the two species as the target. In plots assigned to the plastic treatment, a piece of bag (25 × 25 cm), either non-biodegradable or biodegradable, was placed on the bottom at a depth of 10 cm and covered with previously sieved sand. The bag size was in the range of that of plastic macro-fragments commonly found along sandy shores (Vlachogianni et al., 2020), and the burial depth was in the range of that of plastic fragments buried in sand dunes (Ceccarini et al., 2018). Plots without plastic bags were filled with sieved sand. All the bags were purchased from Italian retailers. Non-biodegradable bags were made of high-density polyethylene (HDPE), and biodegradable/compostable bags were made of starch and vinyl-alcohol copolymers (Mater-Bi®) and complied with the European standard EN13432:2000. Vegetative propagules (rhizomes or stolons naturally detached from established plants by wave action) were harvested from local populations and consisted of similar-sized rhizome fragments of *T. junceum* (34.6 ± 1.8 cm tall, mean ± SE, with 3.5 ± 0.3 culms and 4 nodes) and *S. pumilus* (40.8 ± 2.3 cm tall with 3.8 ± 0.3 culms and 4 nodes). The size of propagules, in terms of number of nodes, was in the range of that of naturally detached propagules found along sandy shores (Harris and Davy, 1986; Maun, 2009). To recreate different biotic interactions that the species may experience, one propagule of the target species, either *S. pumilus* or *T. junceum*, was planted alone in the plot (no interaction) or with another propagule of the same species (i.e., in interaction with a conspecific neighbour, *T. junceum* + *T. junceum* or *S. pumilus* + *S. pumilus*) or with a propagule of the other species (i.e., in interaction with a hetero-specific neighbour, *T. junceum* + *S. pumilus* for *T. junceum* as the target and *S. pumilus* + *T. junceum* for *S. pumilus* as the target). Hence, *T. junceum* and *S. pumilus* alternated in the role of the target and the hetero-specific neighbour. Propagules were planted in defined positions, and the distance between propagules was about 8 cm. In plots assigned to EN, a N amount of 5.2 kg N ha^{-1} over a background deposition was added to simulate a total N input (10.2 kg N $ha^{-1}year^{-1}$) in the range of the atmospheric N inputs predicted in the Mediterranean for 2050 (10–15 kg N ha^{-1} $year^{-1}$, Phoenix et al., 2006). Nitrogen was supplied as 1:1 nitrate to ammonium fertilizer (NH_4NO_3, Petrokemija, Croatia). Since atmospheric N deposition may occur as pulses adding a relatively small amount of N to natural systems with each pulse (Galloway et al., 2008; Wang et al., 2019), the amount of fertilizer was divided in four doses that were separately applied during the experimental period. Before each application, the fertilizer was dissolved in 50 mL of distilled water, and the resulting solution was evenly sprayed on the plot substrate after a moderate-heavy precipitation event (≥30 mm day^{-1}, Alpert et al., 2002; Caloiero et al., 2016) to maintain the natural N input frequency. Plots assigned to AN treatment received each time an equal amount of distilled water only.

2.3. Observed variables

One month after planting, each target propagule within a plot was examined *in situ* to check for the presence of at least one new shoot, considered as an indicator of successful plant regeneration (de la Peña et al., 2011), and to determine the probability of plant establishment (yes, no). Since in some plots the target propagule failed to establish, the development of established target plants was monitored on an equal number (six) of plots randomly selected among those with alive target plant per each treatment. Plants were monitored weekly for the whole duration of the experiment. At the end of the experiment (April 2019), the status (dead or alive) of each target plant within plots was evaluated to determine the probability of plant survival, and all plants and plastic materials still present within plots were harvested and transported to the laboratory. For each plastic type, a subsample (20) of the retrieved materials was inspected using a stereomicroscope (Leica WILD M3C, Germany) to assess the deterioration status. As all biodegradable plastics were broken in small pieces, their deterioration status was estimated in terms of number and size fragments. As all non-biodegradable plastics were still entire, the number of holes/perforations in each plastic was counted. Plants were washed with tap water to remove sand, and those still alive were separated into shoot, rhizome and root tissues and weighted after drying at 70 °C for at least 72 h to determine their biomass. These metrics were considered as they provide different useful indications about the effects of the investigated factors on the horizontal space colonization ability (for rhizome), and nutrient/water uptake and sand stabilization efficiency (for roots). The below (rhizome plus roots) - to aboveground (shoot) biomass ratio was calculated as it is considered as an important indicator of changes in relative resource allocation that reflects plant adaptive responses to surrounding

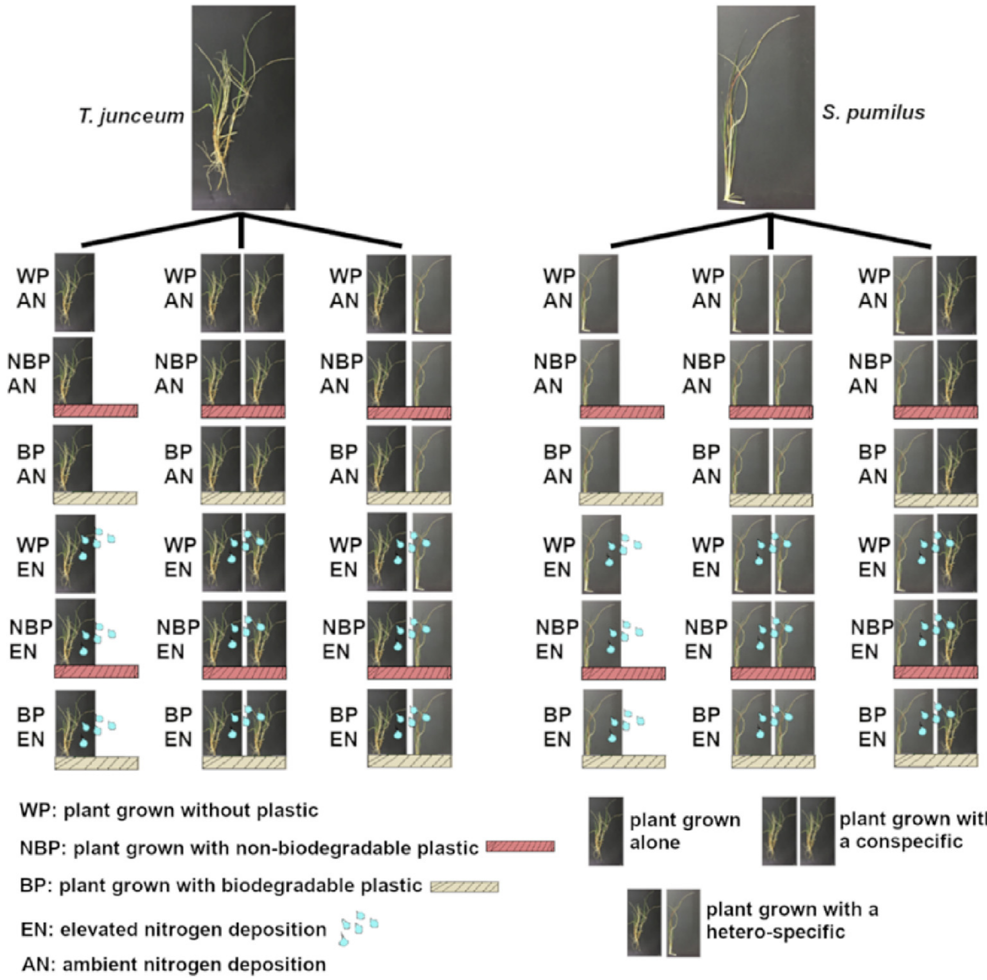

Fig. 1. Experimental design.

abiotic and biotic conditions (nutrient/water/space limitation and neighbour presence) (Qi et al., 2019). As only few *T. junceum* plants and none of *S. pumilus* ones flowered during the study period, reproductive effort was not evaluated.

2.4. Data analysis

The effects of plastic, N deposition and biotic condition on vegetative propagule recruitment and plant growth were analysed separately for each of the two species as the target. The probability of target propagule establishment and plant survival were analysed using generalized linear models (GLMs) with binomial error distributions and a logit link due to the binary nature of these variables (yes, no). For each variable, the statistical significance of fixed terms in the model was assessed with a Likelihood-ratio Test (LRT) of the change in deviance between a model with and a model without the term of interest (Zuur et al., 2009). The results of the models retaining those terms identified as being significant to the model according to LRT were reported in the Supplementary Materials. When significant effects were detected in the analyses, multiple post-hoc Tukey's HSD tests were used for pairwise comparisons of means.

To assess the effects the experimental factors on the growth of each species, separate univariate three-ways ANOVAs were performed on each variable. These analyses were carried out on three replicates, as in some treatments only three plants survived at the end of the experiment. In treatments with more alive plants, the three plants were selected at random. As few plants survived on buried non-biodegradable bags, the level non-biodegradable of the factor Plastic was excluded from the analyses for both the species. In addition, as few target *T. junceum* plants survived in the presence of biodegradable bag and hetero-specific neighbour, for this species as the target the level hetero-specific interaction of the factor Biotic condition was excluded from the analyses. Post-hoc Student-Newman-Keuls (SNK) tests were used to identify differences among levels of significant factors. Prior to ANOVA analyses, data were checked for normality and homogeneity of variances by using Shapiro-Wilk test and Cochran C test, respectively, and transformed to meet assumptions when necessary. For *T. junceum* rhizome biomass, no transformation was effective in removing the non-normal error distribution, and ANOVAs were performed on untransformed data as this analysis is robust to departures from the normality assumption (Underwood, 1997).

Generalized linear models were carried out in R software (v. 3.5.1; R Development Core Team, 2018) by using the glm function within the stats package and the lrtest function within lmtest package (Zeileis and Hothorn, 2002) while *post-hoc* Tukey's HSD tests were conducted by using the glht function within the multcomp package (Hothorn et al., 2008). ANOVA analyses were performed by using the lm function within the GAD package of R environment (Sandrini-Neto and Camargo, 2014).

3. Results

3.1. Plastic deterioration status

All non-biodegradable plastics retrieved from sand at the end of the experiment were still entire and showed various holes (28 ± 3, mean ± SE) with a diameter lower than 1 mm caused by root perforation. Instead, all biodegradable plastics were broken in smaller pieces (mean 24 ± 2, mean ± SE) of various size (from 0.6 cm to 9 cm), and most of them adhered to roots (Fig. S1).

3.2. Colonization success

One month after planting, the percentage of propagules of *T. junceum* and *S. pumilus* that had established a new plant ranged from 50 to 100% across all the treatments (Fig. 2a,b). For both the species, the probability of establishment was not significantly influenced by any factors or their interactions (Tables S1). However, many established plants died in summer (June–July, Table S3). For both the species as target, the probability of survival at the end of the experiment was negatively affected by the main factor Plastic. The reduction of survival was larger for plants established on NBP compared to those grown on BP and without plastic (Fig. 2c,d; Tables S1, S2). The probability of target survival was also influenced by the main effect Biotic condition for *T. junceum* and by N deposition for *S. pumilus*. For the former species, the likelihood of survival was lower when grown with a *S. pumilus* than with a conspecific neighbour regardless of plastic and N treatments (Fig. 2c; Table S2). For the latter species, the chance of survival was lower at the EN deposition than at the AN deposition (Fig. 2d; Table S2). This effect appeared to be influenced by NBP as the exclusion of this level of the factor Plastic from the survival analysis did not show any significant effect of N (Table S4).

3.3. Plant growth

The low number of plants survived in the presence of NBP did not allow us to compare their biomass variables with those of plants exposed to the other treatments. However, at a visual inspection the root system of these plants considerably differed from that of plants grown in WP and BP treatments, being it constituted by a tightly packed system of thin roots developed in the sand layer just above the bag fragment (Fig. S1).

ANOVA on shoot and root biomass of *T. junceum* detected a significant effect of the interaction between N deposition and Biotic condition. Specifically, plants grown alone had smaller biomasses at EN than at AN deposition, and those grown at AN had smaller biomasses in the presence of a conspecific than alone (Fig. 3a,c; Table 1). For shoot biomass, there was also a significant interaction between Plastic and Biotic condition. In the presence of BP, plants established with a conspecific were smaller than those grown alone (Fig. 3a; Table 1). Instead, for root biomass there was also a significant interaction between Plastic and N deposition. Without BP, root biomass was smaller at EN than at AN deposition, and in this latter condition it was smaller in the presence than in the absence of BP (Fig. 3c; Table 1). No significant differences for rhizome biomass and below-to aboveground biomass ratio were detected among treatments (Fig. 3b,d; Table 1).

For *S. pumilus*, shoot biomass was significantly affected by the main factor Plastic and Biotic condition (Table 1). Shoot biomass

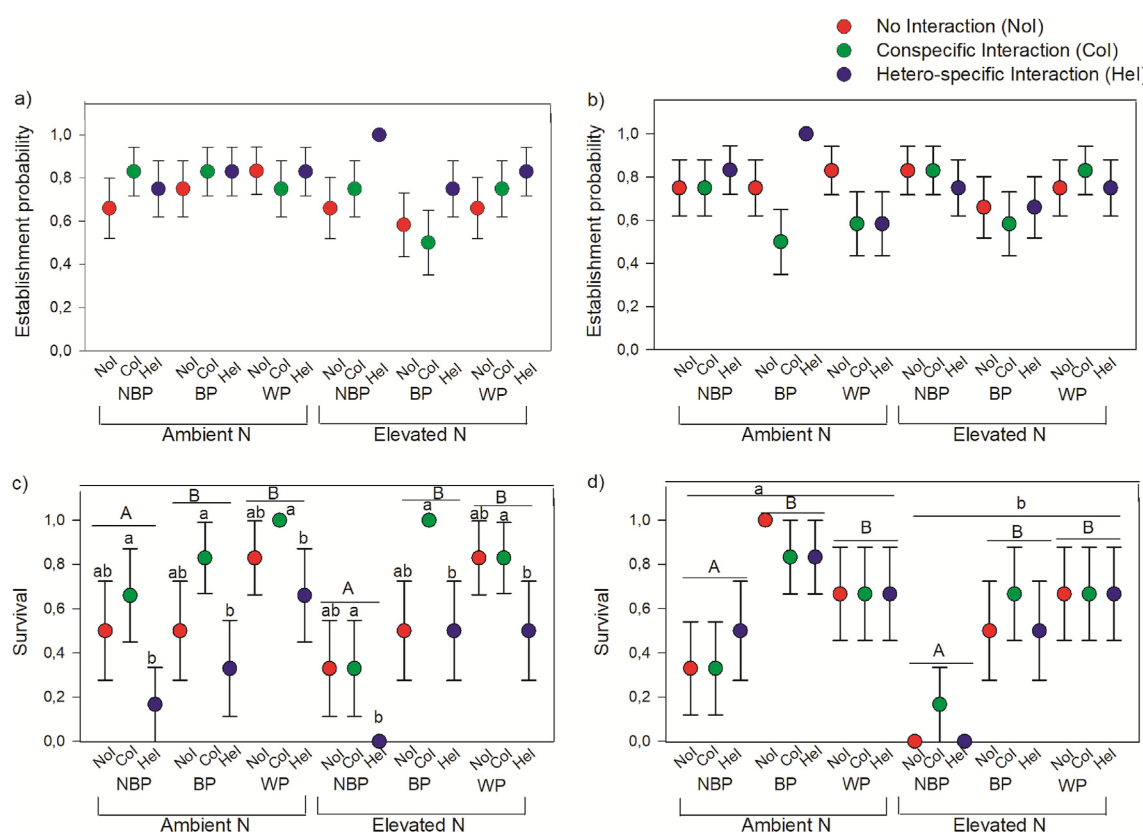

Fig. 2. Probability of propagule establishment (a, b) and plant survival (c, d) of *Thinopyrum junceum* (left panels) and *Sporobolus pumilus* (right panels) as the target grown without a neighbour (NoI) or with a conspecific (CoI) or a hetero-specific (HeI) neighbour in the presence of non-biodegradable plastic bag fragment (NBP), or biodegradable/compostable plastic bag macro-fragment (BP) or without plastic (WP) at ambient and elevated nitrogen (N) deposition. Mean ± se, n = 12 (a,b), n = 6 (c,d).

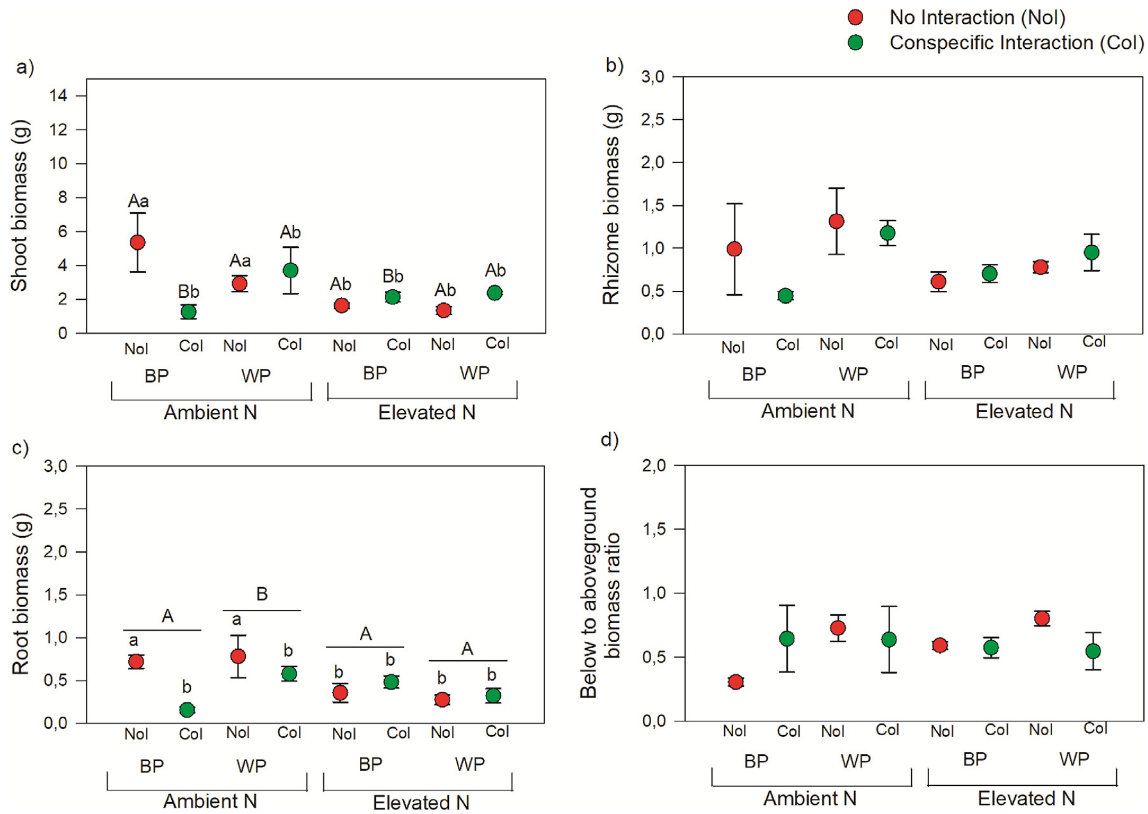

Fig. 3. Shoot biomass (a), rhizome biomass (b), root biomass (c) and below-to aboveground biomass ratio (d) of *Thinopyrum junceum* as the target grown without a neighbour (NoI) or with a conspecific (CoI) neighbour in the presence of biodegradable/compostable plastic bag macro-fragment (BP) or without plastic (WP) at ambient and elevated nitrogen (N) deposition. Mean ± se, n = 3.

was greater in plants grew with than without BP, and it was also greater in plants grew with a hetero-specific or alone than with a conspecific neighbour (Fig. 4a; Table 1). Root biomass was significantly affected by the main factor Plastic resulting greater with than without BP (Fig. 4c; Table 1). For rhizome biomass, there was a significant interaction among Plastic, N deposition and Biotic condition. At EN deposition, the rhizome biomass of plants grown with a neighbour, either conspecific or hetero-specific, was greater in the presence than in the absence of BP. In addition, with BP and a conspecific neighbour rhizome biomass was larger under EN than AN deposition (Fig. 4b; Table 1). At EN deposition, the rhizome biomass of plants grown in the presence of BP with a hetero-specific was also larger than that of plants grown alone while the opposite effect was found in plants grown without BP (Fig. 4b; Table 1). The interaction between Plastic and N deposition had a significant effect on the below-to aboveground biomass ratio. At EN, a higher ratio was observed in plants grown with than without BP, and when plants grew with BP the ratio was higher at EN than AN deposition (Fig. 4d; Table 1).

4. Discussion

Plastic litter is a global-scale problem, and the number of studies revealing the negative impact of this pollutant on coastal and marine animals has increased rapidly in the last decade (Bergmann et al., 2015). Yet, plants have received little attention, although the crucial role they play in building, stabilizing and maintaining coastal dune ecosystems. Our study is the first providing experimental evidence of the individual and combined effects of macroplastic (either non-biodegradable or biodegradable), increased N deposition and biotic interaction on dune habitat colonization by vegetative propagules of dune plants.

4.1. Colonization success

For both the two species as target, the establishment of propagule was not influenced by any of the investigated factors or their interactions. This could be due to the ability of vegetative propagules of dune plants to regenerate new plants using carbohydrates reserves stored in their rhizome (Maun, 2009). However, the survival of newly established plants was influenced by plastic. The survival probability of plants established on NBP resulted up to three times lower compared to that of plants grown without NBP, confirming our hypothesis about the importance of this pollutant for dune plants. These negative effects could be due to the well-known long durability and mechanical resistance of the polymer HDPE (Chamas et al., 2020) and the inability of plant roots to develop enough mechanical forces to fragment this material and acquire essential belowground resources for sustaining growth. Another possible explanation could be a reduction of water availability in soil caused by increased evaporation rate and more negative water potentials induced by the presence of buried plastic (De Jong, 1979). This is because most of plants died during the warmest months (June and July, Table S3). Other changes of sediment chemical-physical properties that could be due to the persistence of this plastic, for example soil pH, electrical conductivity, temperature and water permeability (Carson et al., 2011; Qi et al., 2020; Steinmetz et al., 2016; Zhang et al., 2020), cannot be excluded. The development of an extensive but shallow root system observed in the few plants survived in the presence of NBP

Table 1
Results of ANOVA analyses testing the effects of plastic litter (without plastic, biodegradable/compostable bag macro-fragment), nitrogen level (ambient, elevated) and biotic condition (no interaction, interaction with a conspecific or a hetero-specific neighbour) on plant variables for *Thinopyrum junceum* (a) and *Sporobolus pumilus* (b). For *T. junceum* the level interaction with a hetero-specific was excluded from the analysis due to the low number of survived plants. Bold values indicate significance at p < 0.05. Data transformations and results of SNK test are reported.

Source	df	Shoot biomass		Rhizome biomass		Root biomass		Below- to above ground biomass	
		F	p	F	p	F	p	F	p
a) *T. junceum*									
Plastic (P)	1	0.39	0.540	2.34	0.145	0.70	0.414	2.01	0.174
Nitrogen (N)	1	3.73	0.071	2.89	0.108	5.22	**0.036**	0.22	0.641
Biotic cond (B)	1	0.46	0.507	0.85	0.369	3.56	0.077	0.00	0.957
P x N	1	0.74	0.400	0.41	0.528	7.01	**0.017**	0.30	0.587
P x B	1	6.33	**0.022**	0.40	0.533	1.69	0.210	2.52	0.131
N x B	1	8.53	**0.010**	2.38	0.141	10.38	**0.005**	1.52	0.234
P x N x B	1	2.67	0.121	0.09	0.765	3.63	0.074	0.20	0.655
Residual	16								
Transformation		Log(x)		x^2		Sqrt(x)			
SNK test		CoI: BP < WP BP: CoI < NoI NoI: EN < AN AN: CoI < NoI				AN: BP < WP WP: EN < AN NoI: EN < AN AN: CoI < NoI			
b) *S. pumilus*									
Plastic (P)	1	4.57	**0.042**	7.91	**0.009**	9.27	**0.005**	3.72	0.065
Nitrogen (N)	1	0.18	0.674	2.35	0.138	0.11	0.740	1.60	0.217
Biotic cond (B)	2	4.44	**0.022**	1.91	0.168	1.26	0.299	0.33	0.720
P x N	1	1.21	0.281	0.93	0.342	0.74	0.395	6.85	**0.015**
P x B	2	2.78	0.081	2.89	0.074	0.33	0.715	0.85	0.438
N x B	2	0.57	0.567	1.70	0.202	2.96	0.070	1.20	0.316
P x N x B	2	1.43	0.258	3.88	**0.034**	0.88	0.426	2.25	0.126
Residual	24								
Transformation		Log(x)		Log(x)		Sqrt(x)		Log(x)	
SNK test		WP < BP CoI < NoI = HeI		BP-CoI: AN < EN CoI,HeI-EN: WP < BP BP-EN: NoI < HeI WP-EN: HeI < NoI		WP < BP		EN: WP < BP BP: AN < EN	

WP: without plastic; BP: biodegradable plastic; NoI: no neighbour or no interaction; CoI: plant grown with a conspecific; HeI: plant grown with a hetero-specific; AN: ambient level of nitrogen; EN: elevated level of nitrogen.

indicates that probably only the most vigorous plants might be able to circumnavigate the bag obstacle by reorienting root growth, an avoidance strategy adopted by some species (Santisree et al., 2012). However, these plants could be less efficient in trapping and consolidating sediment, and also more susceptible to physical disturbances, such as sand erosion and uprooting by wind, at least during their first year of growth, because of their shallow root system (Balestri and Lardicci, 2013). As the occurrence of macroplastics on beaches can be relevant, up to 1 fragment (from 2.5 to 50 cm in size) per linear meter (Vlachogianni et al., 2020), the negative effects of buried NBPs on plant survival could translate into the formation of numerous bare sand areas. This reduction in vegetation cover could affect dune mobility and promote under certain circumstances the formation of blowouts which increases the vulnerability of the dune systems to storms.

The absence of any substantial negative effects of BP on plant survival could be explained by the poor resistance of Mater-Bi® to perforation by roots and its fragmentation in small pieces unable to hinder root expansion. This finding is not in agreement with the negative effects caused by this type of bag on the emergence of seedlings of dune plants, including *T. junceum*, reported in a previous study conducted close to our experimental area (Menicagli et al., 2019b). However, these discrepancies could be ascribed to the greater size and vigour of vegetative propagules than seedlings and the position of the bag respect to plant material (below vs. above plastic).

The lower survival probability of *T. junceum* when grown with *S. pumilus* than when with a conspecific regardless of plastic and N deposition could be probably due to a greater competitive ability for local resources and/or larger size of this latter species (on average 10 g vs. 5 g dry weight of total biomass). Indeed, it has been shown that this C4 species under some circumstances may completely replace C3 species such as *Calamagrostis arenaria* (L.) Roth subsp. *arundinacea* (Husn.) Banfi, Galasso & Bartolucci (Fiorentin, 2007; Sarmati et al., 2019). As a result of the effects of the presence of *S. pumilus* and NBP, on average only 8.3% of the established *T. junceum* plants survived. Instead, the lower survival probability observed in *S. pumilus* when grown at EN than at AN condition could be mainly related to drought stress. Indeed, high N availability can increase the growth but also water consumption of plants (Liang et al., 2020), and in C4 species this could lead to metabolic limitations and a down-regulation of photosynthetic efficiency (Ripley et al., 2007, 2010). The negative effect of N on plant survival appeared to be exacerbated by the presence of NBP, as on average only 5.5% of the initially established *S. pumilus* plants survived as a result of the combination of EN deposition and NBP.

4.2. Plant growth

For most plant variables, the effects of plastic and N deposition were mediated by biotic conditions, and the direction of the effects on the two species was generally opposite, i.e., negative for *T. junceum* and positive for *S. pumilus*. At AN deposition rate, the shoot biomass of both *T. junceum* and *S. pumilus* plants were smaller when grown with a conspecific than as an isolated plant regardless of BP suggesting intraspecific competition for essential resources. The reduction of root biomass of *T. junceum* plants grown with BP compared to that of plants grown without plastic is in agreement

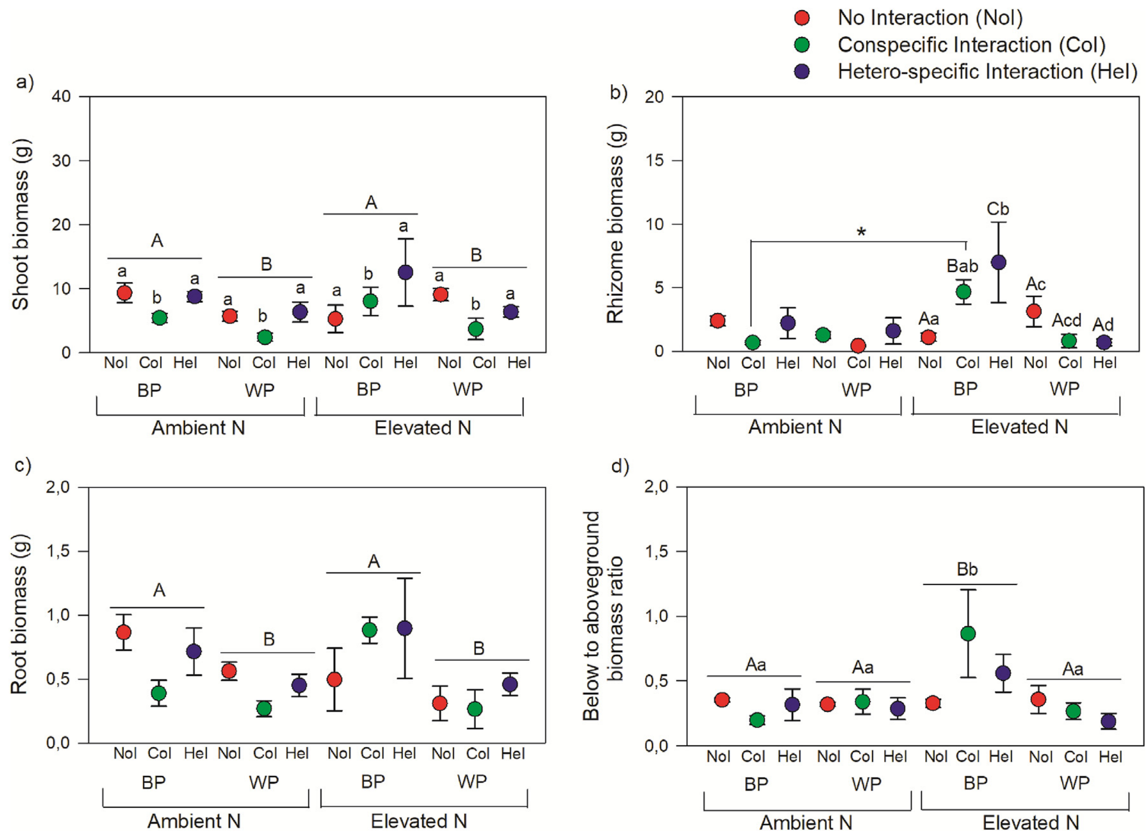

Fig. 4. Shoot biomass (a), rhizome biomass (b), root biomass (c) and below-to aboveground biomass ratio (d) of *Sporobolus pumilus* as the target grown without a neighbour (NoI) or with a conspecific (CoI) neighbour or with a hetero-specific (HeI) neighbour in the presence of biodegradable/compostable plastic bag macro-fragment (BP) or without plastic (WP) at ambient and elevated nitrogen (N) deposition. Mean ± se, n = 3.

with results of previous studies showing a reduced root growth in *T. junceum* seedlings (Menicagli et al., 2019b) and in crop species exposed to leachates obtained from Mater-Bi® plastics (Serrano-Ruíz et al., 2018). Negative effects on roots have also been observed in crop species grown in soils containing a biodegradable mulch made of starch-based plastic (Qi et al., 2018) like Mater-Bi®. As *T. junceum* generally requires alkaline sandy substrates to grow (approximately at pH 8; Greipsson, 2011; Maun, 2009), the negative effect on roots observed here could be related to a reduction of sand pH, a phenomenon observed following the contact of BP with soil and water (Menicagli et al., 2019a; Sintim et al., 2019), as well as to the release of degradation compounds from the bag into the sand layer below roots and to alterations of microbial activity. The reduction of shoot biomass observed in plants grown with a conspecific could be a result of a lower root biomass production combined with intraspecific competition for aboveground resources. In contrast, in *S. pumilus* BP increased biomass of roots and shoots. These positive effects indicate that BP might have alleviated plants from drought for example by increasing sand moisture availability, being Mater-Bi® a hydrophilic material (Milionis et al., 2014). Since *S. pumilus* grows better in acid-neutral soil (pH from 4.5 to 7.1; Lonard et al., 2010), this species might also have benefited from sand acidification following incorporation of BP in sand.

At the increased N deposition rate predicted in Mediterranean for the next decades, *T. junceum* plants grown alone did not differ from those grown with a conspecific in roots and shoots biomass while at AN plants grown alone had larger biomasses. This suggests that the supplied N might have negatively impacted the growth of isolated plants and that the presence of the conspecific might have mitigated these effects, probably by reducing the amount of N available per individual plant. The negative effects of EN observed here are in agreement with the decreased standing biomass and fine root production reported in other C3 species following N addition (White et al., 2012). They could be related to changes in soil properties linked to the higher N input for example decreased soil pH, accumulation of soil ammonium and lower efficiency in the uptake of N (especially nitrate) by *T. junceum* as observed in some grassland species (Britto and Kronzuncker, 2002; Brooker, 2006; Chen et al., 2015). Indeed, it has been shown that a lower soil pH can suppress the growth of some species and belowground microbial communities by increasing the concentrations of H^+ and Al^{3+} and decreasing those of base mineral cations (e.g., Ca^{2+}, Mg^{2+}, Na^+), and hence limiting nitrate uptake by plants (Chen et al., 2015; Stevens et al., 2010). In contrast, EN resulted in an increased rhizome biomass in *S. pumilus* but only when grown as an isolated plant. This suggests that this species might have benefited from the higher N supply and that interspecific competition for resources might have cancelled out positive N effects. The greater accumulation of rhizome biomass was partially in accordance with the results of previous studies showing that in nitrogen-limited environments increased N inputs can enhance belowground carbon allocation of C4 plants and alter biomass allocation by affecting above- and belowground productivity (Chen et al., 2015; Ripley et al., 2007; Sage and Pearcy, 1987; Wang et al., 2019; Zheng and Ma, 2018).

At the increased N deposition rate predicted in Mediterranean and in the presence of BP, the root biomass of *T. junceum* was reduced by approximately 40% compared to plants grown at ambient N deposition without BP. However, this reduction was less than that caused by the two factors when they acted in isolation, suggesting an antagonistic interaction of negative type according to the directional classification system proposed by Piggott et al. (2015). This could be due to the mitigation of the negative individual effects of the two factors on chemical/physical sand properties and a change in the balance between nitrogenous compounds. In contrast, the combined effect of BP and EN on below-to aboveground biomass ratio of *S. pumilus* was positive although the effect of each individual stressor was not statistically significant suggesting a synergistic interaction of positive type (Piggott et al., 2015) between these factors. This higher below-to aboveground biomass ratio was mainly due to an increased biomass allocation to belowground compartment. In addition, we found that the effects of BP and EN on the rhizome biomass of *S. pumilus* were mediated by biotic condition. The rhizome biomass of *S. pumilus* plant as the target grown with *T. junceum* at EN and BP was six-times greater than that of plants grown alone while it was four-times smaller than that of plants established alone without plastic. This could indicate that the presence of BP reversed the effect of EN on rhizome and possibly shifted interspecific interactions from competition to facilitation. Unfortunately, we could not examine the effect of *S. pumilus* on biomass accumulation and allocation pattern of *T. junceum* as the target, as there were not enough replicates for this treatment to perform statistical analyses. However, the overall low survival of *T. junceum* in the presence of *S. pumilus* suggests that this former species would not benefit from the latter one. Collectively, these results indicate that BP and EN could either exacerbate or mitigate their individual effect on the development of belowground organs of plants depending on the species and neighbour identity, favouring the spread of the generalist grass *S. pumilus* and hindering that of the dune-building *T. junceum*. This could have relevant consequences for dune system health leading to reduced dune accretion and sediment stability. Indeed, *S. pumilus* does not substantially contribute to dune formation by accumulating sand, as instead *T. junceum* does, and it also enhances the vulnerability of dunes to repeated disturbances like over-wash (Brantley et al., 2014). Although we have suggested several potential explanations, more research is needed to elucidate the specific physiological and/or environmental drivers behind the responses to BP and EN deposition observed here.

5. Conclusions

At a global scale, coastal dune ecosystems play a prominent role in coastal defence, wind and aerosol protection and biodiversity support. However, these highly valuable habitats are threatened by natural and anthropogenic factors including plastic pollution and N deposition. Our study reveals that non-biodegradable plastic litter could be a further threat to coastal dunes as it could dramatically reduce the colonization success by vegetative propagules of both dune-building and generalist species by up to 100% at EN deposition rate. It also demonstrates that BPs individually and in combination with EN and neighbour presence have the potential to affect differentially the growth of dune plant species and then their interactions. Importantly, our findings underline the relevance of better investigating the potential risks associated with the introduction of plastics in coastal dune environments and suggest that assessing the nature (additive and non-additive) and the direction (synergistic or antagonistic) of interactions among plastic litter, plant-plant interactions and global environmental stressors in future studies could improve our ability to predict the real impact of this pollutant and identify adequate interventions and restoration actions. Overall, lowering N deposition and setting up effective management actions aimed at reducing the entering of plastic waste in coastal environments and removing all plastics accumulated on beaches are urgently needed to mitigate the impact on coastal dune systems. Given the predicted global rise of the production of new generation of biodegradable/compostable plastics (EU Parliament, 2018; European bioplastics, 2018), also the implementation of more effective postconsumer management actions is fundamental to prevent their release into natural environments.

CRediT authorship contribution statement

Virginia Menicagli: Conceptualization, Formal analysis, Investigation, Visualization, Writing - original draft. **Elena Balestri:** Conceptualization, Formal analysis, Investigation, Visualization, Writing - original draft. **Flavia Vallerini:** Formal analysis, Investigation, Visualization. **Alberto Castelli:** Supervision, Writing - review & editing. **Claudio Lardicci:** Conceptualization, Investigation, Supervision, Writing - review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115281.

Credit authorship contribution statement

Virginia Menicagli: Conceptualization, Formal analysis, Investigation, Visualisation, Writing - original draft. **Elena Balestri**: Conceptualization, Formal analysis, Investigation, Visualisation, Writing - original draft. **Flavia Vallerini**: Formal analysis, Investigation, Visualisation. **Alberto Castelli**: Supervision, Writing - review & editing. **Claudio Lardicci**: Conceptualization, Investigation, Supervision, Writing - review & editing.

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