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Exposure of Bromus hordeaceus to fossil- and plant-based micro- and nanoplastics: Impacts and plant-plastic interactions vary depending on polymer type and growth phase

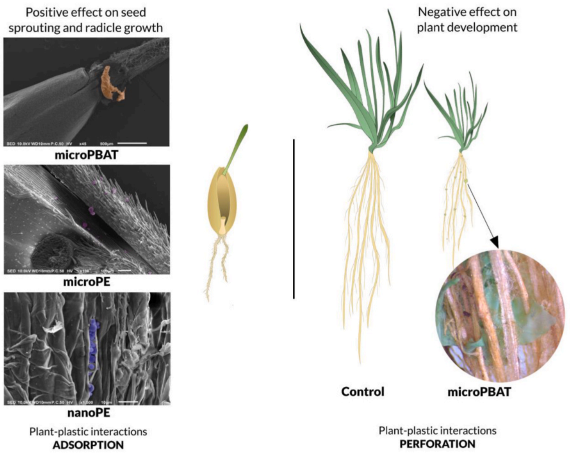
Inés María Alonso-Crespo [a,b,*], Alicia Mateos-Cárdenas [c,d]

[a] *Institute of Ecology, Leuphana University Lüneburg, Lüneburg, Germany*
[b] *Departamento de Ecoloxía, Grupo de Ecoloxía Animal (GEA), Universidade de Vigo, Vigo, Spain*
[c] *School of Biological, Earth and Environmental Sciences, University College Cork, North Mall, Cork, Ireland*
[d] *Environmental Research Institute, Lee Road, Cork, Ireland*

HIGHLIGHTS

- Roots penetrated microPBAT particles.
- Plastic particles were adsorbed on the seed coat and structures.
- Plastic particles stimulated radicle and sprout growth.
- MicroPBAT decreased plant growth.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic pollution, especially pollution by micro- and nanoplastics, is now considered a potential threat to all ecosystems, including terrestrial ecosystems such as grassland habitats. This study investigated the impacts of micro- and nano-sized plastics on *Bromus hordeaceus*, a common grass species in European grasslands. The micro and nanoparticles were fossil-based polyethylene (PE) or plant-based polybutylene adipate terephthalate (PBAT), and these two plastics were used at two different concentrations. Here, we report data on plant development and plastic-plant interactions from two different experiments, (1) an *in vitro* experiment to test seed germination and establishment and (2) a soil experiment to test plant development and plastic-plant interactions specifically investigated as a form of perforation. Results from the *in vitro* experiment indicate that while seed germination success was unaffected by plastic type, the presence of all plastic particle types acted as a stimulant, increasing

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* Corresponding author. Institute of Ecology, Leuphana University Lüneburg, Lüneburg, Germany.
E-mail address: inesalonsocrespo@gmail.com (I.M. Alonso-Crespo).

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the total length of radicles and sprouts of germinated seeds. Conversely, results from the soil experiment showed that the growth of *Bromus hordeaceus* was negatively affected by the presence of microPBAT in the soil during the pot assay.

Microscopic analysis confirmed that seed and plant structures interacted with all plastic particles via adsorption or perforation. This study demonstrates for the first time the ability of roots to penetrate plastics, especially microPBAT particles. Overall, our study concludes that both fossil-based and plant-based micro- and nano-plastics can influence plant growth, with effects varying based on plastic type, concentration, and plant growth phase. Further research is crucial to fully understand the intricate interactions between microplastics, soil properties, and plant development.

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1. Introduction

Plastic pollution is considered one of the significant environmental problems of our time ("UNEP," 2021). Traditional plastics (fossil-based) do not biodegrade in the way organic materials do. Instead, they undergo fragmentation, breaking down into particles known as microplastics (MPs) and even smaller nanoparticles (NPs). These microplastics can accumulate in ecosystems over long time. Microplastics are plastic particles ranging between 1 and 1000 μm in size made of a plastic polymer (Hartmann et al., 2019) and nanoplastics are plastic fragments smaller than 1 μm (Hartmann et al., 2016). They are categorized in two groups; (1) primary microplastics, industrially produced micro-scaled particles such as microbeads or pellets (Van Cauwenberghe et al., 2015) and (2) secondary microplastics, created by the degradation of larger plastics into smaller fragments through processes like photodegradation and weathering, followed by mechanical abrasion (Andrady et al., 2022), or alternatively through biological fragmentation (Laskar and Kumar, 2019; Mateos-Cárdenas et al., 2020). Secondary microplastics, such as fragments and fibers, are the most commonly microplastic type found in the environment (Rochman et al., 2019). With the rising concern of plastic pollution, some industries have switched their conventional fossil-based products to plant-based (bioplastic) alternatives with similar functional properties but largely unclear environmental fate and/or toxicity.

Initially, research on microplastics centered around marine ecosystems (Van Cauwenberghe et al., 2015), until Bank and Hansson (2019) introduced 'The Plastic Cycle'. This paper comprehensively illustrated how these pollutants are transported and where they end up, framing the problem of plastic pollution at the ecosystems level and as part of biogeochemical cycles. Moreover, this paper shifted the attention to terrestrial ecosystems as recipients of microplastics. Previous work indicated that terrestrial soils, and especially agriculture fields, could be major sinks of microplastics on the planet, potentially surpassing oceans as the primary plastic recipient (Nizzetto et al., 2016). Agricultural lands have been pointed out as one of the largest sinks of plastic in terrestrial ecosystems (Khalid et al., 2020; Rillig et al., 2017; Rochman, 2018; Weithmann et al., 2018; Yang et al., 2022). This has turned agro-ecosystems into the main point of focus for research in terrestrial plastics. Plastics can reach agricultural lands primarily through common management techniques such as mulching or sewage sludge applications (Nizzetto et al., 2016). To date, methodological inconsistencies are a major reason why the exact concentrations of nanoplastics and bio-microplastics in the environment remain uncertain, as reliable and standardized data is still lacking (Fojt et al., 2020; Xue et al., 2024). There is no single, agreed value to indicate the concentrations of plastic particles in the environment. Plastic concentrations vary per plastic and sample type, weathering and transport mechanisms in systems, among other variables (Moeck et al., 2023). However, the meta-analysis by Büks and Kaupenjohann (2020) analyzed microplastic concentrations in agricultural soils located in China and Europe, which have common agricultural practices such as mulching or application of sewage sludge. They found that agricultural soils can contain up to 13,000 particles per kilogram of dry soil, equivalent to 4.5 mg of plastic per kilogram of dry soil. There was a large variability within and between soil samples in this meta-analysis, driven primarily due to differences in sampling methods, polymer extraction, and identification techniques.

The subsequent spread of plastic from agro-ecosystems to surrounding ecosystems, such as grasslands is only a matter of time (Yang et al., 2022). This raises concerns over grassland biodiversity and food security (de Souza Machado et al., 2018; Khalid et al., 2020; Rochman et al., 2019). Although the study of the impacts of bioplastics on plants is still in its infancy, recent studies are starting to indicate that bioplastics can be hazardous (Brown et al., 2023; Liwarska-Bizukojc, 2023). However, little is known about how bioplastic pollution can impact native plants in terrestrial ecosystems. Grasslands, widespread worldwide, constitute extensively distributed ecosystems. Among them, European semi-natural grasslands stand out as one of Europe's most diverse but threatened ecosystems (Habel et al., 2013). Grassland vegetation is comprised of mixtures of grasses, legumes, and forbs (Carlier et al., 2009), with grasses usually being the most abundant plant functional group (Gibson, 2009). Grasslands provide a great variety of ecosystem functions and services (Petermann and Buzhdygan, 2021) with the plants present there playing key roles in nutrient cycles, carbon sequestration, and forming the foundation of food webs. Thus, there is an urgent need for studies investigating the effects of microplastics on grassland plant species, such as the one presented here.

In this study, we assess the impact of micro and nanoplastics on a grass species typically found in European grasslands, *Bromus hordeaceus*. Thus, *B. hordeaceus* serves as a phytometer. We exposed *B. hordeaceus* to fossil-based polyethylene (PE) micro- and nanoplastics as well as plant-based polybutylene adipate terephthalate (PBAT) microplastics. This study addressed the following questions:

1. Does the presence of fossil- or plant-based micro and nanoparticles affect the germination and establishment of *Bromus hordeaceus*?
2. Does the presence of fossil- or plant-based micro and nanoparticles affect the development and nitrogen uptake of *Bromus hordeaceus*?
3. Does *Bromus hordeaceus* interact with plastic particles in the soil?

2. Materials and methods

2.1. Plastic particles

Three different plastic particles made up of two different polymers were used in this study: (1) 0.2–9 μm white polyethylene nanobeads (Cospheric) –nanoPE-, (2) 40–48 μm white polyethylene microfragments (Sigma) –microPE- and (3) 500 μm polybutylene adipate terephthalate (PBAT) films –microPBAT-. The PBAT films were made in-house from Mater-Bi® BioBags (Novamont) using a Rommelsbacher EGK 200 coffee & spice grinder. Particles for this specific size were selected by sieving all fragments produced through a series of sieves to select the 500 μm size (See supplementary information for estimated concentrations particles per gram).

Two different experiments were carried out to test (1) the *in vitro* effects of the presence of plastic particles on the germination and establishment of *B. hordeaceus* and (2) the effects of the presence of plastic particles in soil on the development of *B. hordeaceus*.

For the *in vitro* experiment, all plastic particles (microPE and nanoPE and microPBAT) were applied independently in a solution with deionized (DI) water. The reasoning behind the selection of the concentrations for this experiment were to be able to test a low and a high concentration of particles under controlled conditions. The concentrations in the *in vitro* experiment are given in mg/mL because the particles were mixed with DI water and pipetted onto the filter papers in a petri dish. In the soil experiment, however, the concentrations are expressed in mg/g, as the particles were mixed with grams of soil. There are some estimates of microplastic concentrations in agricultural soils, e.g. 4.5 mg per kilogram of dry soil (Büks and Kaupenjohann, 2020). For our soil experiment, we established two concentration levels, and our selected low concentration was actually lower than the one estimated by Büks and Kaupenjohann (2020). Our low-concentration fossil-based (PE) microplastic treatment was set at 1 mg/kg of soil. A higher concentration (10 mg/kg of soil) was also used to investigate the potential effects of the plastics on plant growth. As for the concentrations of nanoplastics and microplastics of plant-based origin, there are no references for environmental concentrations, so the concentrations were adapted to way they were added to the experiment. Nanoplastics were pipetted from a solution made with DI water, microplastics were directly added in dry powder form. It should also be mentioned that the agricultural land from which the soil was sourced did not practice mulching or apply sewage sludge. The soil used in this study was not specifically tested for microplastic or nanoplastic presence, as this was beyond the scope of our research. We intentionally introduced defined particles to simulate contamination in our experimental setup. Additionally, the same soil was employed for control treatments, ensuring that any potential environmental contamination would affect all plants uniformly, thereby maintaining a consistent baseline for comparison.

2.2. *Plant species Bromus hordeaceus (soft brome) and soil type*

Bromus hordeaceus L. (Poaceae) is a grass species (monocotyledonous) commonly found in meadows, pastures and woodlands subjected to intensive forestry (FloraWeb-Bundesamt für Naturschutz (BfN), n.d.). It is a monocarpic species, typically annual, and is pollinated through either self-fertilization or wind-dispersed pollen (Kühn et al., 2004). This plant species was selected due to its ability to rapidly expand its roots throughout the space in the pot, making contact with all of the substrate in the area. This characteristic ensured that the roots quickly encountered the plastics present in the soil.

The selected soil for the soil experiment has characteristics from a typical of a dry acidic grassland: pH ($CaCl_2$) 4.9; organic matter content: 2.3%; total N: 0.07%; total C: 0.98%; C/N: 12.1.

2.3. *In Vitro experiment: Seed germination assay*

2.3.1. *Phase 1: Germination test of seeds spiked with plastics*

Four treatments were set up for the germination test: Control, microPBAT, microPE and nanoPE treatments. The experiment was performed in 90 mm size Petri dishes with five replicates (one replicate per petri dish) per treatment (Fig. 1). For the control treatment, 5 mL of DI water was pipetted on four stacked filter papers per replicate. In the case of the plastic treatments (micro and nano), three 5 mL solutions were made by mixing DI water with each micro- or nanoplastic, particles, respectively (Table 1). Next, 15 *B. hordeaceus* seeds were gently added on top of the filter papers in each Petri dish. All Petri dishes were sealed with polyfilm and placed in a tray at a 45° angle. The Petri dishes were then moved to a growth chamber under controlled conditions (average temperature = 21 °C/15 h day period – 9 h night period). The number of

Table 1
Polymer and particle types and concentrations applied in water in Petri dishes in each treatment.

Particle type	Polymer	Plastic amount per Petri dish (mg/ 5 mL)
Control	NA	0 mg
Nano	Polyethylene	12.5 mg
Micro	Polyethylene	12.5 mg
Micro	Polybutylene adipate terephthalate	2.5 mg

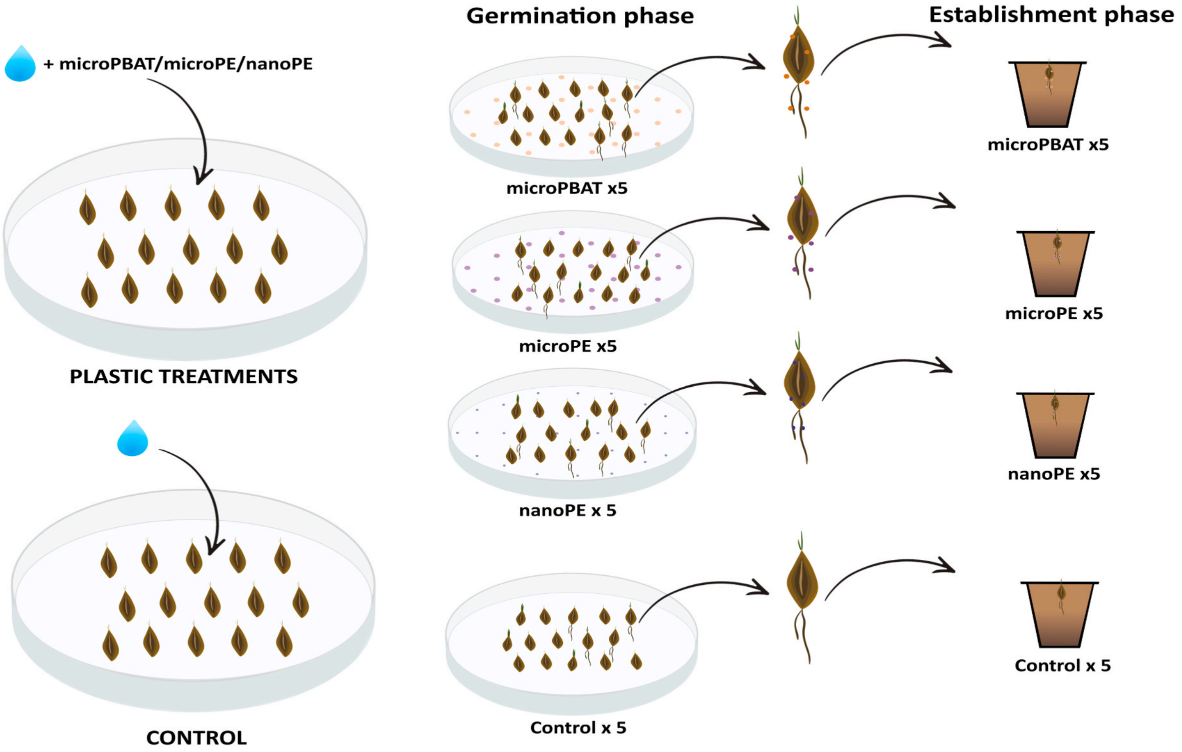

Fig. 1. Graphical representation of the different steps carried out in the *in Vitro* experiment: seed germination assay.

germinated seeds was counted at 96 h. Furthermore, at the end of this experiment (96 h), the seeds that had successfully germinated were imaged. ImageJ (ImageJ 1.53q) was used to gather radicle and sprout length data (cm).

2.3.2. *Phase 2: Establishment test of spiked germinated seeds*

After the germination phase, germinated seeds were identified, and one random seed per petri dish was transplanted into pots filled with cleaned soil (Fig. 1). Twenty pots (0.75 L in volume) were filled with 400 g of cleaned soil. Each pot received one seed from a specific treatment group, ensuring an equal representation of treatments (1 seed per petri dish, 5 seeds per treatment, and 20 seeds in total). Pots were equally watered with a total of 105 mL throughout the whole experiment. The emergence dates of the first, second and third leaf were recorded. The endpoint of this establishment test was reached when each plant had grown three full leaves. The experiment ended after 22 days.

2.4. *Soil experiment: Pot assay*

In order to test development of *B. hordeaceus* in the presence of plastic particles in the soil, four treatments were established (Control and microPBAT, microPE and nanoPE enriched soil) with two plastic particle concentrations (either high or low) except for the control treatment, which was free of plastic particles (Fig. 2, Table 2). Microplastic particles were added to the soil in dry powder form. In contrast, nanoplastics were added to the soil in a solution made by mixing the nanoplastics in powder form with DI water. Once the soil was spiked with these particles, the mixture was homogenised in a bucket by shaking it for 60 s to ensure even distribution of the particles. The homogenised mixture of soil and plastics was then placed in a pot. This process was repeated independently for each pot. A total of 35 pots, each with a volume of 2 L, were filled with 1500 g of soil mixed with the corresponding plastic particles for each treatment and replicate. *B. hordeaceus* seeds were pre-germinated in cleaned petri dishes during 72 h in a growth chamber (average temperature = 21 °C/15 h day period – 9 h night period). Two pre-germinated seeds per pot were initially selected to ensure the successful establishment of at least one plant. In those pots in which both seeds were successfully established, one was completely removed from the soil during the first week of the experiment to avoid confounding effects. Therefore, only one plant was grown per replicate. All pots were watered equally with a total of 1340 mL water per pot throughout the whole experiment and were kept at an average temperature = 21 °C/15 h day – 9 h night in a growth chamber.

Table 2
Polymer and particle types and concentrations applied in soil in each treatment.

Particle type	Polymer	Concentration	Plastic amount per pot (mg/1500 g)
Control	NA	NA	0
Nano	Polyethylene	Low	0.05 mg
Nano	Polyethylene	High	5 mg
Micro	Polyethylene	Low	1,5 g
Micro	Polyethylene	High	15 g
Micro	Polybutylene adipate terephthalate	Low	1,5 g
Micro	Polybutylene adipate terephthalate	High	15 g

2.4.1. *Nitrogen uptake*

At the beginning of the experiment, two 1 mL pipette tips were inserted in the soil to make a hole to allow the application of N-tracer at the end of the experiment. The tracer application was intended to be directly in the soil, avoiding contact with the leaves. Therefore, this step was conducted early in the experiment to prevent root damage that could result from inserting pipette tips. During the N-tracer application, the tips were removed, leaving a hole in the soil where the tracer was applied.

A total of 6 h before the harvest, the N_2 tracer was applied. The nitrogen solution consisted of 1.5 mM $K_{15}NO_3$, prepared by mixing 0.31272 g of $K_{15}NO_3$ (98%, Cortecnet) with 2 L of milliQ water. The pipette tips were removed from the soil and 3 mL of the solution were added per pot, 1.5 mL per hole, avoiding contact with the leaves. Harvest was done in the same order as the application of the tracer. Delta (δ) $_{15}N$ content in shoots was analyzed with an Elementar analyser as an indicator of N15 uptake.

2.4.2. *Plant growth*

After harvesting, the shoots were dried in an oven at 60 °C for 48 h and weighed to determine their dry mass (g). Roots were separated from the soil by rinsing with tap water and meticulously cleaned using

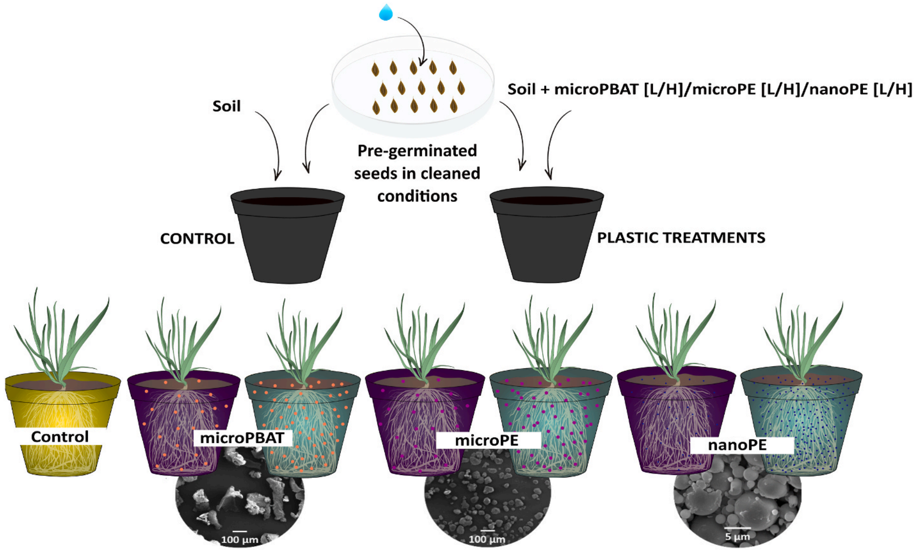

Fig. 2. Graphical representation of the different steps carried out in the soil experiment: pot assay. The control treatment is represented with a yellow pot. The low concentration treatment is represented with a purple pot, and the high concentration treatment is represented with a light blue pot. The plastic particles are color-coded as follows: orange for microPBAT, mauve for microPE, and dark blue for nanoPE. SEM images below show the plastic particles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tweezers and brushes to remove all visible plastic particles and organic matter (see Supplementary Figs. S1 and S2). Cleaned roots were then dried in an oven at 60 °C for 48 h and weighed to obtain the root dry weight (g).

2.5. Microscopy

Eight seeds per treatment were collected randomly from the *in Vitro* experiment: seed germination assay, and placed on filter papers for microscopic analysis. Two plants per treatment (roots and shoots) were selected from the soil experiment: pot assay, for microscopy analysis. A combination of Scanning Electron Microscopy (SEM, JEOL JSM-IT100) and light microscopy were used in this study. For SEM visualization of samples, they were gold-coated for 30 s using an Agar Scientific sputter coater with pumping system. SEM images were taken at working distance (WD) of 9–10 mm at high vacuum (H.V.) of HV = 50 and at x100, x220, x400, x700, x1500 and ×3700 magnification.

2.6. Statistical analysis

In Vitro experiment: A Generalized Linear Model (GLM) was employed to analyse discrete variables in the context of assessing the impact of different plastic types on the germination of *B. hordeaceus* seeds. The fixed factor in the analysis was the "Treatment", encompassing both particle size and plastic type, and represented as a categorical variable with four levels: Control, microPE, microPBAT, and nanoPE. The response variable in this case was the count of successfully germinated seeds (GerminationSuccess). Linear models (LM) were also employed to examine how *B. hordeaceus* germinated seeds exposure to different plastics during the germination phase influences production of radicles and sprouts, establishment success, and emergence speed. The fixed factor in the analysis was the Treatment, encompassing both particle size and plastic type, with four levels as previously described. Response variables in this case were radicle length development (cm) of germinated seeds (summation of the radicle length of all the radicles produced by one seed) (Radicle_Length), sprout length (cm) of germinated seeds (Sprout_Length) total number of established plants per treatment and day in which the first (First_leaf), second (Second_leaf) and third (Third_leaf) were first reported.

Soil experiment: LM were also utilised to investigate the effect of different plastic types spiked in soil in two concentrations on the growth and nutrient uptake of *B. hordeaceus* plants. Two fixed factors were included in the analysis. "Particle", encompassing both particle size and plastic type, and represented as a categorical variable with four levels: Control, microPE, microPBAT, and nanoPE, and "Concentration", which included two levels: high and low. As response variables were used SDW_g: shoot dry weight (g), RDW_g: root dry weight (g), PDW_g: plant dry weight (g), Average Delta (δ) of 15 N and Nitrogen accumulation (Naccum: (N_perc/100)*Shoot_biomass_g).

LMs were fitted using the 'lm' function from the "lme4″ R package (Bates et al., 2015). Post-hoc tests, when needed, were conducted using Tukey contrasts, and these tests were performed with the 'lsmeans' function from the "emmeans" R package (Lenth et al., 2018) or with 'glht' function from the "multcomp" R package (Hothorn et al., 2016). Data analysis was performed in R *ver.* 4.3.0 (<www.r-project.org>). The R package "tidyverse" (Wickham et al., 2019) was used to organise the data frames used in the analysis. Plots were created using "ggplot2" R package (Wickham, 2016), the color blind palette "viridis" (Garnier et al., 2018) and "ggpubr" (Kassambara, 2020).

3. Results

The results presented here refer to two different experiments. Firstly, an *in Vitro* experiment (section 3.1) where seedlings were grown *in vitro* in petri dishes in the presence of plastic particles and then transferred to a pot with clean soil (plastic free). In this test, the following parameters were measured: germination success, average sprout and radicle length and plant establishment. Secondly, a soil experiment: pot assay (section 3.2). In this test, seedlings were germinated under clean *in vitro* conditions and then transferred to contaminated soil that was spiked with plastic particles at two different concentrations (high or low) per polymer type. For this, shoot and root dry weight and nitrogen uptake were measured.

3.1. In Vitro experiment: Seed germination assay

3.1.1. Phase 1: Germination success

B. hordeaceus seeds were germinated in clean (control) or plastic spiked conditions to investigate any potential effects of the plastic particles at the germination stage. However, the presence of plastic particles did not significantly affect germination success (p-value = 0.7903) (Supplementary Fig. S4, Table S1).

3.1.2. Phase 1.1: Total sprout and radicle length (cm) of seeds

Sprout and radicle length in *B. hordeaceus* seeds were significantly affected by the plastic treatment during *in vitro* germination, compared to the control. Total radicle length was significantly higher in seeds grown in the presence of microPBAT, microPE and nanoPE particles compared with the Control (p-values <0.001). Total sprout length was also significantly higher for seeds grown in the presence of microPBAT (p-value<0.001) and nanoPE plastics (p-value = 0.00538), compared to control (Fig. 3, Supplementary Table S2).

3.1.3. Phase 2: Establishment of seeds germinated in the presence of plastic particles

No significant effects were observed in the establishment success of seeds that were germinated in the presence of plastic particles. However, on average, plants exposed to the microPBAT treatment exhibited a trend, with 50% lower establishment success compared to microPE and nanoPE treatments (Fig. 4). Also, establishment time was unaffected by any of the plastic treatments. The emergence dates of the first, second, and third leaves showed no significant variation in response to the presence of any of the polymers or plastic types.

3.2. Soil experiment: Pot assay

3.2.1. Plant development

Overall, the presence of microPBAT negatively affected *B. hordeaceus* shoot, root and plant dry weight, especially at high concentrations. Shoot and root dry weight were significantly lower when plants were exposed to the high concentrations of microPBAT particles compared to the control and the rest of the treatments. In addition, the presence of microPBAT in low concentrations also decreased shoot and root productivity compared to microPE and nanoPE treatments (Fig. 5, Supplementary Table S4 and Table S5, respectively). Likewise, when pulling all the productivity data together (SDW + RDW), plant dry weight was significantly less for all plants grown at the high concentration of microPBAT particles compared with the rest of the treatments. Plant dry weight was slightly higher for plants from the nanoPE treatment compared to the control treatment (Fig. 5c, Supplementary Table S6).

3.2.2. Nitrogen uptake

Average delta (δ) ˆ15 N values were higher for plants growing in soil spiked with microPBAT (Figure S5, Fig. 6a, Table S7). However, when comparing the nitrogen accumulation per gram of shoot biomass, a nitrogen dilution effect was detected, meaning that when growth rates decreased, the accumulation of nitrogen decreased as well. This was particularly the case for plants growing in soil spiked with microPBAT compared with high concentrations of microPE and nanoPE, with this effect more pronounced in soil with high concentrations of microPBAT (Fig. 6b, Table S8).

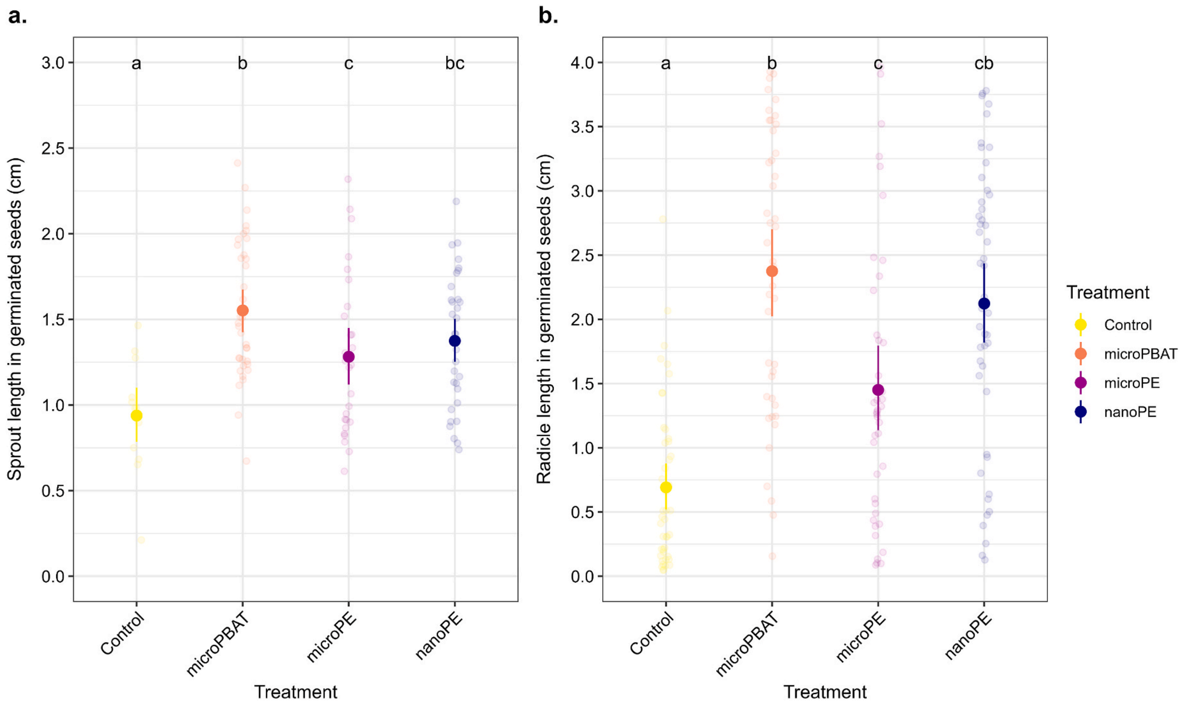

Fig. 3. **(a.)** Total radicle length and **(b.)** total sprout length of seeds germinated in the presence of different plastic types. Treatment refers to the plastic type to which the seeds were exposed. Control (yellow): No plastic present. MicroPBAT (orange): Microplastics of Polybutylene adipate terephthalate (bioplastic). MicroPE (purple): Microplastics of polyethylene (fossil-based). NanoPE (blue): Nanoplastics of polyethylene (fossil-based). Bigger dots represent the mean and lines represent the bootstrap confidence interval. Different letters indicate significant differences ($p < 0.05$) between treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

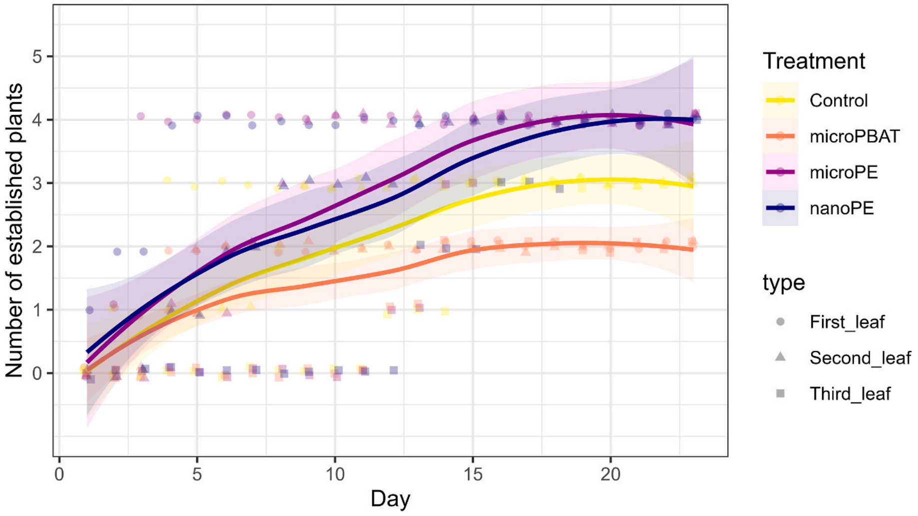

Fig. 4. Number of *B. hordeaceus* plants established over time from seeds germinated in the presence of plastics. Circles represent the appearance of the first leaf and triangles the appearance of the second one. The yellow line indicates seeds that were not exposed to plastics, showing their natural establishment success. Seeds exposed to microplastics of PBAT (bioplastic) are represented by the orange line, while the purple line represents seeds exposed to microplastics of polyethylene (fossil-based) and the blue line to nanoplastics of polyethylene (fossil-based). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. B. hordeaceus seeds interacted with all plastic particles and their roots perforated plant-based PBAT films

Plastic particles were detected adhering to the seed coat, radicles, and sprouts of seeds coming from the *in vitro* experiment (Fig. 7). A remarkable finding from the soil experiment is that roots were able to perforate plant-based PBAT films. This finding was first visually detected at the root cleaning stage of the plants and then imaged during microscopic analysis (Fig. 8 and Supplementary Fig. S3).

4 Discussion

4.1. In Vitro experiment: seed germination assay

Overall, no significant effects of plastics were observed on germination success; however, there was a notable variation in total radicle

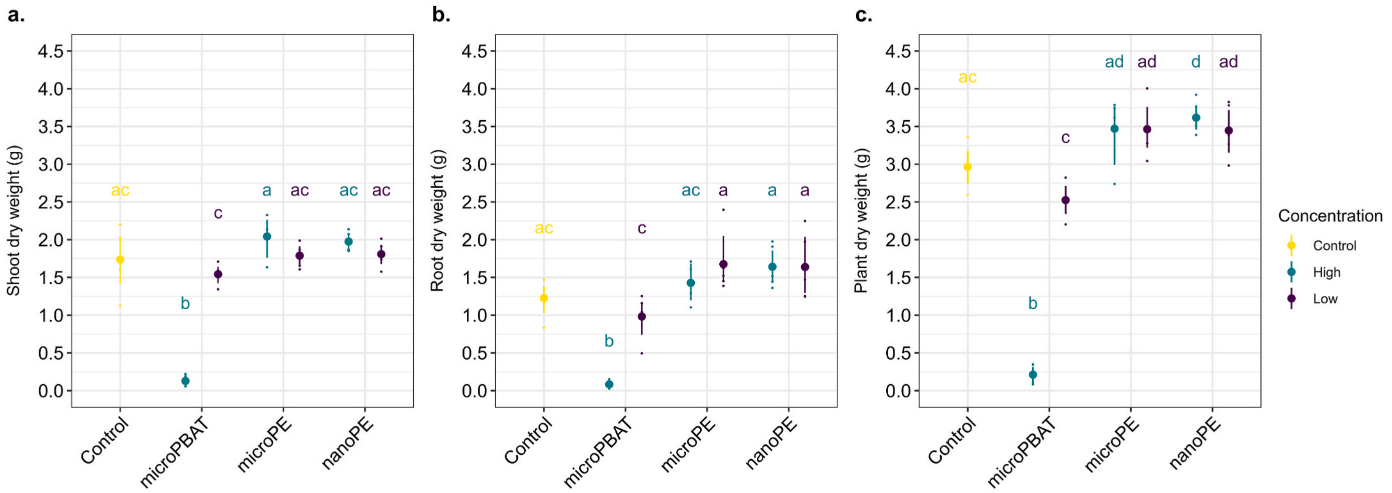

Fig. 5. **(a.)** Shoot dry weight (g), **(b.)** root dry weight (g) and **(c.)** plant dry weight (g) of *B. hordeaceus* plants growing in soil spiked with two concentrations of different plastic types and particles. The x-axis lists the different treatment to which the plants were exposed: control (plastic-free soil), microPBAT in soil (microplastics of polybutylene adipate terephthalate - bioplastic), microPE in soil (microplastics of polyethylene - fossil-based) and nanoPE (nanoplastics of polyethylene - fossil-based). Different colors represent the plastic particles concentration: yellow for the Control treatment with no plastic presence on the soil, blue for High concentration and purple for Low concentration. Bigger dots represent the mean and lines the bootstrap confidence interval. Different letters indicate significant differences ($p < 0.05$) between treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

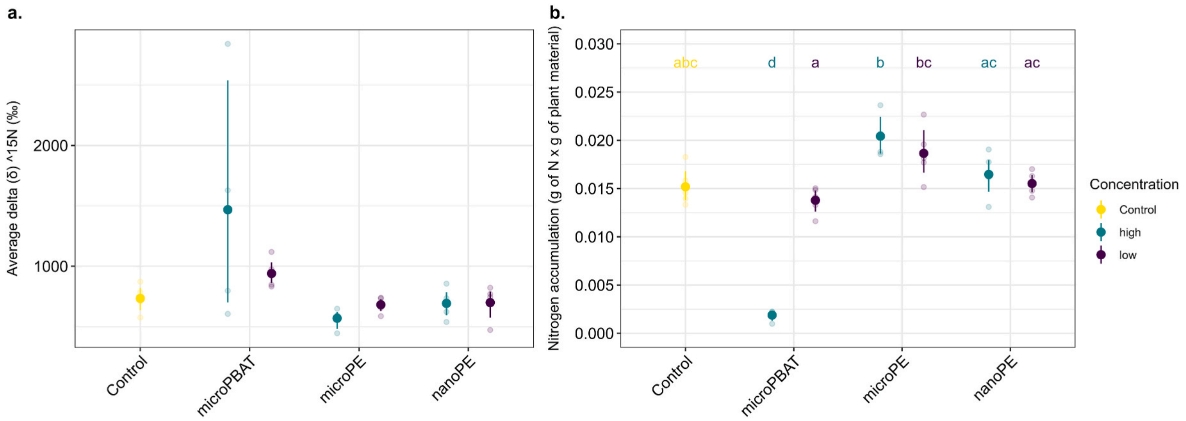

Fig. 6. **(a.)** Average delta (δ) ˆ15 N of *B. hordeaceus* plants growing in soil spiked with two concentrations of different plastic particles. **(b.)** Nitrogen concentration of *B. hordeaceus* plants growing in soil spiked with two concentrations of different plastic particles. Control (in yellow): no plastic present, microPBAT: presence of PBAT microplastics (Polybutylene adipate terephthalate, plant-based), microPE: presence of PE microplastics (polyethylene, fossil-based), nanoPE: presence of PE nanoplastics (fossil-based). High concentrations are represented in blue and low in purple. Bigger dots represent the mean and lines represent the bootstrap confidence interval. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and sprout length of seeds exposed to the different plastic. Seeds exhibited higher total radicle and sprout length across all plastic treatments compared to the control. Microscopy analysis visualized plastic particles adhered to the seed coat and radicles.

Previous studies showed a decrease in seed germination in different plant species exposed to different plastic types, concentrations and particle size (Bosker et al., 2019; Dong et al., 2022; Guo et al., 2022; Liao et al., 2019; Shi et al., 2022). Furthermore, in Dong et al. (2022) this negative effect was further reported in root and sprout length of seeds exposed to nanoparticles of polymethyl methacrylate (PMMA) with a decreased of both with an increased in concentration. These effects were related to the impediment of water and nutrient uptake due to the physical obstruction of pores by plastic particles. In contrast, in Lozano et al. (2022) despite observed differences in seed germination velocity, final seed germination percentage was unaffected by plastic presence in the soil, which was also observed for seeds exposed to bio-plastics (Liwarska-Bizukojc, 2023). Contrary to this, here we observed a stimulatory effect on the length of radicles and sprouts in seeds exposed to plastics. Potters et al. (2007) described various morphogenic responses in plants as acclimation strategies to cope with stressful environments, such as localised stimulation of cell division. This phenomenon may explain the results observed in our study. While the presence of microplastics did not affect germination success, germinated seeds exhibited increased radicle and sprout length, potentially as a strategy to cope with the presence of plastics. Upon transplanting these seeds, plastic exposure did not show significant differences in establishment success. However, a trend was noted: seeds exposed to microPBAT had a 50% lower establishment success rate compared to those exposed to microPE and nanoPE. We also observed an adsorption of the plastic particles on the seed coat and structures. Despite the lack of direct evidence that plastics adhering to plant structures are stressors or stimulants, it can be surmised that when plastic particles are present near plant structures, they could affect plant development through physical or chemical interactions with plant tissues (Mateos-Cárdenas et al., 2021; Roy et al., 2022). Although we did not investigate the uptake of plastic by plants in this experiment, previous studies have reported the uptake of plastic

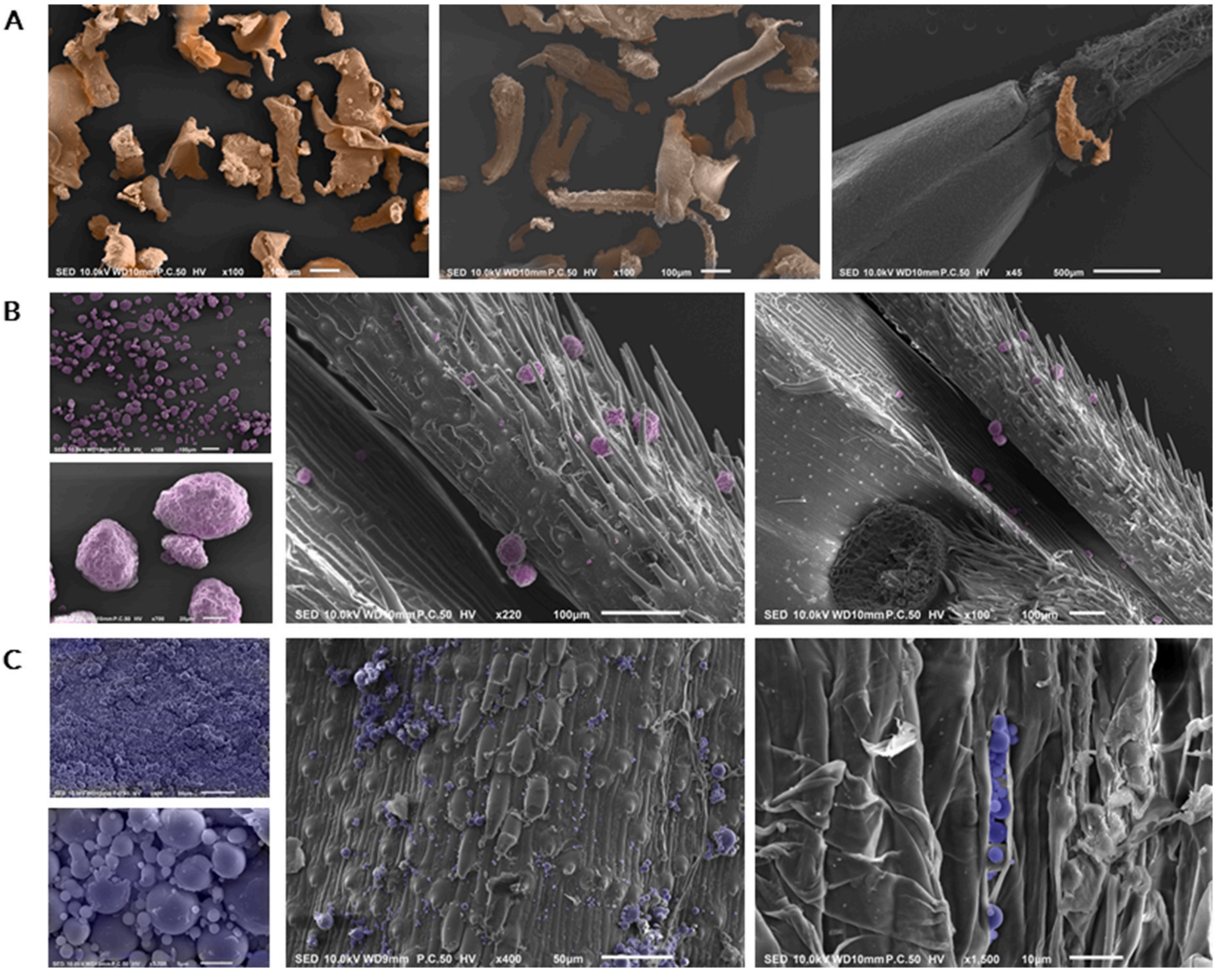

Fig. 7. SEM images of *B. hordeaceus* seeds from the seed germination assay under different treatments, **(A)** microPBAT particles adhering to seed coats, **(B)** microPE particles adhering to seed coats and **(C)** nanoPE particles adhering to seed coats. The color of the images (orange, purple and blue) has been artificially added to facilitate the visualization of plastic particles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

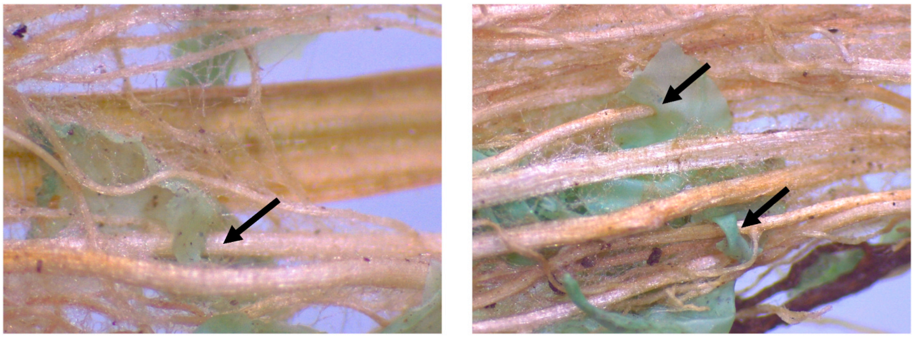

Fig. 8. MicroPBAT films perforated by *B. hordeaceus* roots, and observed at the end of the plant development assay. Arrows indicate microPBAT films in pale green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

nanoparticles by plants (Giorgetti et al., 2020; Wang et al., 2022) which could also trigger morphological strategies in plants depending on the plastic type but also on the growth phase of the plant.

4.2. Soil experiment: Pot assay

The presence of high concentrations of microPBAT particles in soil decreased *B. hordeaceus*'s productivity, while plants growing in the presence of high concentrations of nanoPE increased.

On the one hand, previous studies have shown adversely effects on plant development of traditional plastic (fossil-based) presence in grassland species (Gentili et al., 2022; Kleunen et al., 2020) and cultivars such as pumpkin, wheat or tomato (Colzi et al., 2022; Kleunen et al., 2020; Liao et al., 2019; Shi et al., 2022). Liwarska-Bizukojc (2023) also observed this negative effect for bio-plastics on root growth, and they also found a stimulation of shoots depending on the type of bio-plastic. Microplastics can affect root growth and elongation by adhering to roots surface (Boots et al., 2019; Wang et al., 2023) or clogging the pores hindering water and nutrient uptake (Gao et al., 2021) which affects the total development of the plant. On the other hand, microplastics have been reported as disruptors of soil physicochemical properties, and can diminish soil bulk density or increase soil water-holding capacity (de Souza Machado et al., 2018), modify soil aggregation (Liang et al., 2019; Lozano et al., 2021), soil pH (Zhao et al., 2021) or soil structure and, consequently, impact the soil water cycle (Wan et al., 2019).

Despite the fact that soil water content was not measured since it was not the objective of this study, it was observed that pots containing microPBAT particles in the soil were always wetter than the rest of the treatments, despite all pots having received the same amount of water during the whole experiment. Water retention studies, especially those that include bioplastics in their experimental design, are still scarce. It is known that fossil-based microplastics can affect the soil's evaporation dynamics and its ability to absorb and distribute moisture effectively (Cramer et al., 2023). For example, the presence of PE and PVC microparticles in sandy soil decreased pore size in the soil matrix and thus affected soil water storage (Jannesarahmadi et al., 2023). Furthermore, Shafea et al. (2023) showed the capacity of PET and PS microplastics to clog soil pores, hence affecting infiltration and water availability. It is reasonable to hypothesise that contrary to polyethylene, which is hydrophobic in nature (Bumbudsanpharoke et al., 2022), the ester groups in PBAT's molecular structure may induce a higher hydrophilicity. Maqbool et al. (2023) concluded that the degradation of biodegradable plastics can lead to an increase in soil water content at field capacity.

A key finding of this study is the fact that *B. hordeaceus's* roots have the capacity to penetrate microPBAT films. Root foraging behaviour has been extensively detailed (Lynch, 2022) and it is known that roots can alter their growth patterns to explore the surrounding environment and exploit available resources. In our study, the presence of microPBAT particles in the soil appears to disrupt this root exploration and increase the plant's energy expenditure, which may have contributed to the observed lower development of these plants. This finding implies that roots have no mechanism to sense and avoid such plastics. To the best of our knowledge, this is the first study to show the ability of roots to actually perforate plastic fragments, of any polymer origin. It can be speculated that when part of the root surface is in direct contact with plastics, this acts as a barrier, preventing the absorption of water and/or nutrients, thus slowing the growth of the plants. Previous studies have detected an effect of microplastics on the nutrient bioavailability in soil, which can have a cascading effect on plant growth (Zhao et al., 2021). Microplastic presence can influence key ecological functions such as nutrient cycling including mineralisation and denitrification (de Souza Machado et al., 2018; Seeley et al., 2020). Although the results for the average delta (δ) ˆ15 N content were significantly higher in plants exposed to microPBAT particles, the reduced biomass production in these plants caused a dilution effect. This dilution effect prevented definitive conclusions about the relationship between ˆ15 N uptake and plant development. However, when considering both the quantity of nitrogen and the amount of plant material produced, the nitrogen accumulation per plant was lower in plants exposed to microPBAT compared to those in other treatments and this effect was stronger at higher concentrations of this plastics.

Altogether, the adsorption of the plastic particles to the roots and the perforation of the microPBAT plastics by roots may have led to a decrease in the effective root surface area for water and nutrient uptake, potentially also aggravated by changes in soil water content soil due to the hydrophilicity of the microPBAT, resulting in reduced plant growth when exposed to microPBAT. On the contrary, the possibility that the nanoplastics may have been able to penetrate the plant tissue may have triggered a stimulatory reaction in the plant, as occurred in the *in vitro* experiment during the development of radicles and sprouts of germinated seeds. Hormesis (Kendig et al., 2010) has previously been proposed as a mechanism that could generate this type of response to low doses of plastics (Liwarska-Bizukojc, 2023). The absorption and adsorption of nanoplastics to roots could have incentivised a greater biomass in these plants as a morphological strategy of adaptation to the environment. However, it is still unknown how this effect could persist or change with longer study times. Plastic size, polymer type and growth phase influence the effects of the presence of plastic particle on plants, making it difficult to determine ecological risk assessments on the effects of their presence (Kumari et al., 2022). Further research on plant mechanisms to cope with microplastic stress, toxicity and plant-plastic interactions are needed to understand the risk of the plastic presence in natural systems.

5. Conclusions

This study provides important insights into the effects of micro- and nanoplastics on a grassland species, *Bromus hordeaceus,* across different developmental stages. Our findings reveal that the presence of polyethylene (PE) and polybutylene adipate terephthalate (PBAT) plastic particles may stimulate early seed development, particularly by enhancing radicle and sprout length. However, we observed distinct plastic-specific effects as plant development progressed. While nanoPE particles promoted plant growth, microPBAT particles negatively impacted plant establishment and overall growth performance. It is important to note that the particle concentrations used in this study were selected based on practical considerations and to facilitate testing under controlled conditions. These concentrations may not reflect environmental levels, as the precise concentrations of nanoplastics and bioplastics in soil have yet to be determined. Independently on concentration levels, a novel and significant discovery was that *B. hordeaceus* roots were able to perforate PBAT microplastics, demonstrating a previously unreported plant-plastic interaction. This finding highlights the complex and potentially disruptive nature of plant responses to microplastic exposure. These results emphasize the need for further research to explore the broader ecological implications of plant-plastic interactions, particularly in natural ecosystems where microplastics are increasingly prevalent. Understanding these dynamics will be essential for predicting how plastic pollution could influence terrestrial ecosystems, plant health, and ecosystem processes.

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CRediT authorship contribution statement

Inés María Alonso-Crespo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Dr. **Alicia Mateos-Cárdenas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.chemosphere.2024.143715.

Data availability

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References

Andrady, A.L., Barnes, P.W., Bornman, J.F., Gouin, T., Madronich, S., White, C.C., Zepp, R.G., Jansen, M.A.K., 2022. Oxidation and fragmentation of plastics in a changing environment; from UV-radiation to biological degradation. Sci. Total Environ. 851, 158022. https://doi.org/10.1016/j.scitotenv.2022.158022.

Bank, M.S., Hansson, S.V., 2019. The plastic cycle: a novel and holistic paradigm for the anthropocene. Environ. Sci. Technol. 53, 7177–7179. https://doi.org/10.1021/acs.est.9b02942.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Boots, B., Russell, C.W., Green, D.S., 2019. Effects of microplastics in soil ecosystems: above and below ground. Environ. Sci. Technol. 53, 11496–11506. https://doi.org/10.1021/acs.est.9b03304.

Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere 226, 774–781. https://doi.org/10.1016/j.chemosphere.2019.03.163.

Brown, R.W., Chadwick, D.R., Zang, H., Graf, M., Liu, X., Wang, K., Greenfield, L.M., Jones, D.L., 2023. Bioplastic (PHBV) addition to soil alters microbial community structure and negatively affects plant-microbial metabolic functioning in maize. J. Hazard Mater. 441, 129959. https://doi.org/10.1016/j.jhazmat.2022.129959.

Büks, F., Kaupenjohann, M., 2020. Global concentrations of microplastic in soils, a review. Soil Discussions 1–26. https://doi.org/10.5194/soil-2020-50.

Bumbudsanpharoke, N., Wongphan, P., Promhuad, K., Leelaphiwat, P., Harnkarnsujarit, N., 2022. Morphology and permeability of bio-based poly(butylene adipate-co-terephthalate) (PBAT), poly(butylene succinate) (PBS) and linear low-density polyethylene (LLDPE) blend films control shelf-life of packaged bread. Food Control 132, 108541. https://doi.org/10.1016/j.foodcont.2021.108541.

Carlier, L., Rotar, I., Vlahova, M., 2009. Importance and functions of grasslands. Not. Bot. Hort. Agrobot. Cluj-Napoca.

Colzi, I., Renna, L., Bianchi, E., Castellani, M.B., Coppi, A., Pignattelli, S., Loppi, S., Gonnelli, C., 2022. Impact of microplastics on growth, photosynthesis and essential elements in Cucurbita pepo L. J. Hazard Mater. 423, 127238. https://doi.org/10.1016/j.jhazmat.2021.127238.

Cramer, A., Benard, P., Zarebanadkouki, M., Kaestner, A., Carminati, A., 2023. Microplastic induces soil water repellency and limits capillary flow. Vadose Zone J. 22. https://doi.org/10.1002/vzj2.20215.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of microplastics on the soil biophysical environment. Environ. Sci. Technol. 52, 9656–9665. https://doi.org/10.1021/acs.est.8b02212.

Dong, R., Liu, R., Xu, Y., Liu, W., Wang, L., Liang, X., Huang, Q., Sun, Y., 2022. Single and joint toxicity of polymethyl methacrylate microplastics and as (V) on rapeseed (Brassia campestris L.). Chemosphere 291, 133066. https://doi.org/10.1016/j.chemosphere.2021.133066.

FloraWeb-Bundesamt für Naturschutz (BfN), n.d. FloraWeb [WWW Document]. FloraWeb. URL https://www.floraweb.de (accessed 4.14.23).

Fojt, J., David, J., Přikryl, R., Řezáčová, V., Kučerík, J., 2020. A critical review of the overlooked challenge of determining micro-bioplastics in soil. Sci. Total Environ. 745, 140975. https://doi.org/10.1016/j.scitotenv.2020.140975.

Gao, M., Xu, Y., Liu, Y., Wang, S., Wang, C., Dong, Y., Song, Z., 2021. Effect of polystyrene on di-butyl phthalate (DBP) bioavailability and DBP-induced phytotoxicity in lettuce. Environ. Pollut. 268, 115870. https://doi.org/10.1016/j.envpol.2020.115870.

Garnier, S., Ross, N., Rudis, B., Sciaini, M., Scherer, C., 2018. viridis: Default Color Maps from 'matplotlib'. R package version 0.5, 1 2018.

Gentili, R., Quaglini, L., Cardarelli, E., Caronni, S., Montagnani, C., Citterio, S., 2022. Toxic impact of soil microplastics (PVC) on two weeds: changes in growth, phenology and photosynthesis efficiency. Agronomy 12, 1219. https://doi.org/10.3390/agronomy12051219.

Gibson, D.J., 2009. Grasses and Grassland Ecology. OUP, Oxford.

Giorgetti, L., Spanò, C., Muccifora, S., Bottega, S., Barbieri, F., Bellani, L., Ruffini Castiglione, M., 2020. Exploring the interaction between polystyrene nanoplastics and Allium cepa during germination: internalization in root cells, induction of toxicity and oxidative stress. Plant Physiol. Biochem. 149, 170–177. https://doi.org/10.1016/j.plaphy.2020.02.014.

Guo, M., Zhao, F., Tian, L., Ni, K., Lu, Y., Borah, P., 2022. Effects of polystyrene microplastics on the seed germination of herbaceous ornamental plants. Sci. Total Environ. 809, 151100. https://doi.org/10.1016/j.scitotenv.2021.151100.

Habel, J.C., Dengler, J., Janišová, M., Török, P., Wellstein, C., Wiezik, M., 2013. European grassland ecosystems: threatened hotspots of biodiversity. Biodivers. Conserv. 22, 2131–2138. https://doi.org/10.1007/s10531-013-0537-x.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297.

Hartmann, N., Nolte, T., Sørensen, M., Jensen, P., Baun, A., 2016. Aquatic ecotoxicity testing of nanoplastics-lessons learned from nanoecotoxicology. Lessons Learned From Nanoecotoxicology, DTU Environment.

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., Hothorn, M.T., 2016. Package 'multcomp.' Simultaneous Inference in General Parametric Models. Project for Statistical Computing, Vienna, Austria.

Jannesarahmadi, S., Aminzadeh, M., Raga, R., Shokri, N., 2023. Effects of microplastics on evaporation dynamics in porous media. Chemosphere 311, 137023. https://doi.org/10.1016/j.chemosphere.2022.137023.

Kassambara, A., 2020. Ggpubr: "ggplot2" based publication ready plots. R package version 0.4.0 2.

Kendig, E.L., Le, H.H., Belcher, S.M., 2010. Defining hormesis: evaluation of a complex concentration response phenomenon. Int. J. Toxicol. 29, 235–246. https://doi.org/10.1177/1091581810363012.

Khalid, N., Aqeel, M., Noman, A., 2020. Microplastics could be a threat to plants in terrestrial systems directly or indirectly. Environ. Pollut. 267, 115653. https://doi.org/10.1016/j.envpol.2020.115653.

Kleunen, M., Brumer, A., Gutbrod, L., Zhang, Z., 2020. A microplastic used as infill material in artificial sport turfs reduces plant growth. Plants People Planet 2, 157–166. https://doi.org/10.1002/ppp3.10071.

Kühn, I., Durka, W., Klotz, S., 2004. BiolFlor: a new plant-trait database as a tool for plant invasion ecology. Divers. Distrib. 10, 363–365.

Kumari, A., Rajput, V.D., Mandzhieva, S.S., Rajput, S., Minkina, T., Kaur, R., Sushkova, S., Kumari, P., Ranjan, A., Kalinitchenko, V.P., Glinushkin, A.P., 2022. Microplastic pollution: an emerging threat to terrestrial plants and insights into its remediation strategies. Plants 11. https://doi.org/10.3390/plants11030340.

Laskar, N., Kumar, U., 2019. Plastics and microplastics: a threat to environment. Environmental Technology & Innovation 14, 100352. https://doi.org/10.1016/j.eti.2019.100352.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Emmeans: Estimated marginal means. AKA least-squares means 1 (7).

Liang, Y., Lehmann, A., Ballhausen, M.-B., Muller, L., Rillig, M.C., 2019. Increasing temperature and microplastic fibers jointly influence soil aggregation by saprobic fungi. Front. Microbiol. 10, 2018. https://doi.org/10.3389/fmicb.2019.02018.

Liao, Y.-C., Nazygul, J., Li, M., Wang, X.-L., Jiang, L.-J., 2019. Effects of microplastics on the growth, physiology, and biochemical characteristics of wheat (Triticum aestivum). Huanjing Kexue 40, 4661–4667. https://doi.org/10.13227/j.hjkx.201903113.

Liwarska-Bizukojc, E., 2023. Effect of innovative bio-based plastics on early growth of higher plants. Polymers 15. https://doi.org/10.3390/polym15020438.

Lozano, Y.M., Caesaria, P.U., Rillig, M.C., 2022. Microplastics of different shapes increase seed germination synchrony while only films and fibers affect seed germination velocity. Front. Environ. Sci. Eng. China 10. https://doi.org/10.3389/fenvs.2022.1017349.

Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A., Rillig, M.C., 2021. Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. Front. Plant Sci. 12, 616645. https://doi.org/10.3389/fpls.2021.616645.

Lynch, J.P., 2022. Harnessing root architecture to address global challenges. Plant J. 109, 415–431. https://doi.org/10.1111/tpj.15560.

Maqbool, A., Soriano, M.-A., Gómez, J.A., 2023. Macro- and micro-plastics change soil physical properties: a systematic review. Environ. Res. Lett. 18, 123002. https://doi.org/10.1088/1748-9326/ad0a1a.

Mateos-Cárdenas, A., Jansen, A.R., O'Halloran, J., van Pelt, Jansen, M.A., 2021. Impacts of microplastics in the Irish freshwater environment, 377. Environmental Protection Agency Ireland Research Report, p. 61.

Mateos-Cárdenas, A., O'Halloran, J., van Pelt, F., 2020. Rapid fragmentation of microplastics by the freshwater amphipod Gammarus duebeni (Lillj.). Sci. Rep. 10 (1), 12799.

Moeck, C., Davies, G., Krause, S., Schneidewind, U., 2023. Microplastics and nanoplastics in agriculture—A potential source of soil and groundwater contamination? Grundwasser 28 (1), 23–35.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50, 10777–10779. https://doi.org/10.1021/acs.est.6b04140.

Petermann, J.S., Buzhdygan, O.Y., 2021. Grassland biodiversity. Curr. Biol. 31, R1195–R1201. https://doi.org/10.1016/j.cub.2021.06.060.

Potters, G., Pasternak, T.P., Guisez, Y., Palme, K.J., Jansen, M.A.K., 2007. Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci. 12, 98–105. https://doi.org/10.1016/j.tplants.2007.01.004.

Rillig, M.C., Ingraffia, R., de Souza Machado, A.A., 2017. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 8, 1805. https://doi.org/10.3389/fpls.2017.01805.

Rochman, C.M., 2018. Microplastics research-from sink to source. Science 360, 28–29. https://doi.org/10.1126/science.aar7734.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4371.

Roy, T., Dey, T.K., Jamal, M., 2022. Microplastic/nanoplastic toxicity in plants: an imminent concern. Environ. Monit. Assess. 195, 27. https://doi.org/10.1007/s10661-022-10654-z.

Seeley, M.E., Song, B., Passie, R., Hale, R.C., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. Nature communications 11 (1), 2372.

Shafea, L., Felde, V.J.M.N.L., Woche, S.K., Bachmann, J., Peth, S., 2023. Microplastics effects on wettability, pore sizes and saturated hydraulic conductivity of a loess topsoil. Geoderma 437, 116566. https://doi.org/10.1016/j.geoderma.2023.116566.

Shi, R., Liu, W., Lian, Y., Wang, Q., Zeb, A., Tang, J., 2022. Phytotoxicity of polystyrene, polyethylene and polypropylene microplastics on tomato (Lycopersicon esculentum L.). J. Environ. Manage. 317, 115441. https://doi.org/10.1016/j.jenvman.2022.115441.

Unep [WWW Document]. https://www.unep.org/interactives/beat-plastic-pollution/?gad_source=1&gclid=CjwKCAiAxaCvBhBaEiwAvsLmWIrfI6c33y6gXyId8CYqcV4TVxbF-ESK5HN_GGDjcHpzt74DCM8LHhoCJLQQAvD_BwE. accessed 3.6.24.

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in sediments: a review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. https://doi.org/10.1016/j.marenvres.2015.06.007.

Wan, Y., Wu, C., Xue, Q., Hui, X., 2019. Effects of plastic contamination on water evaporation and desiccation cracking in soil. Sci. Total Environ. 654, 576–582. https://doi.org/10.1016/j.scitotenv.2018.11.123.

Wang, J., Li, J., Liu, W., Zeb, A., Wang, Q., Zheng, Z., Shi, R., Lian, Y., Liu, L., 2023. Three typical microplastics affect the germination and growth of amaranth (Amaranthus mangostanus L.) seedlings. Plant Physiol. Biochem. 194, 589–599. https://doi.org/10.1016/j.plaphy.2022.12.007.

Wang, W., Yuan, W., Xu, E.G., Li, L., Zhang, H., Yang, Y., 2022. Uptake, translocation, and biological impacts of micro(nano)plastics in terrestrial plants: progress and prospects. Environ. Res. 203, 111867. https://doi.org/10.1016/j.envres.2021.111867.

Weithmann, N., Möller, J.N., Löder, M.G.J., Piehl, S., Laforsch, C., Freitag, R., 2018. Organic fertilizer as a vehicle for the entry of microplastic into the environment. Sci. Adv. 4, eaap8060. https://doi.org/10.1126/sciadv.aap8060.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. https://doi.org/10.1007/978-0-387-78171-6.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. J. Open Source Softw. 4, 1686. https://doi.org/10.21105/joss.01686.

Xue, Y., Song, K., Wang, Z., Xia, Z., Li, R., Wang, Q., Li, L., 2024. Nanoplastics occurrence, detection methods, and impact on the nitrogen cycle: a review. Environ. Chem. Lett. 22, 2241–2255. https://doi.org/10.1007/s10311-024-01764-w.

Yang, Z., Lü, F., Zhang, H., Wang, W., Xu, X., Shao, L., Che, Z., Lu, B., Ye, J., He, P., 2022. A neglected transport of plastic debris to cities from farmland in remote arid regions. Sci. Total Environ. 807, 150982. https://doi.org/10.1016/j.scitotenv.2021.150982.

Zhao, T., Lozano, Y.M., Rillig, M.C., 2021. Microplastics increase soil pH and decrease microbial activities as a function of microplastic shape, polymer type, and exposure time. Front. Environ. Sci. Eng. China 9. https://doi.org/10.3389/fenvs.2021.675803.