RESEARCH ARTICLE

Plastic particles and their additives promote plant invasion through physicochemical mechanisms on seed germination

Yudi M. Lozano[1,2] | Lena Landt[1] | Matthias C. Rillig[1,2]

[1]Institute of Biology, Plant Ecology, Freie Universität Berlin, Berlin, Germany

[2]Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

Correspondence
Yudi M. Lozano
Email: yudyja@gmail.com

Abstract

1. Plant invasions pose a major threat to terrestrial biodiversity, and microplastic pollution in soil could exacerbate this problem. Seed germination, a crucial stage for plants, can be affected by microplastics through both physical interference of plastic particles and chemical leaching from plastic additives.

2. We conducted a greenhouse experiment using native and invasive plant species of European grasslands, and evaluated individual and combined effects of plastic particles and additives on germination parameters.

3. We found that plastic particles primarily affected seed germination as physical agents, while additives from these particles exerted a comparatively lesser impact.

4. Particles negatively affected seed germination of all native species. Germination velocity, synchrony and total germination decreased by ~30%, ~11% and ~11%, respectively, in soils containing plastic particles compared to those without. Certain species were negatively affected by plastic additives. For *Achillea millefolium* and *Dactylis glomerata*, germination velocity and total germination decreased by ~26% and ~7%, respectively, while germination synchrony of *Dactylis glomerata* decreased by ~21% in soils with additives than without them. Plastic particles may have blocked seed pores and inhibited hypocotyl and radicle growth, while toxic compounds from additives may have disrupted key germination processes.

5. By contrast, particles and additives generally did not affect invasive species, suggesting that the negative effects of plastic experienced by natives, whether physical or chemical, were counteracted by effects resulting from the novel conditions created by microplastics, which may include the amelioration of soil physical properties such as increased soil porosity and aeration, and potential positive plant–soil feedbacks. Invasive species may profit from windows of variable resource availability, with positive effects on seed germination.

6. *Synthesis*. Plastic particles and additives may promote plant invasion by negatively affecting seed germination of native species while having neutral effects on invasive species. The delayed germination of natives due to microplastics poses a potential threat, leading to competitive disadvantages, reduced reproductive

success and increased vulnerability to invasive species. Microplastic pollution appears to favour invasive species over natives during the early stages of seed germination, highlighting that the negative effect of microplastics on terrestrial ecosystems may be more severe than previously thought.

1 | INTRODUCTION

Terrestrial biodiversity is rapidly decreasing as a consequence of several factors of global change, including soil pollution (IPBES, 2019). Of particular concern is pollution with microplastics (plastic particles <5 mm), which is recognized as an important threat to ecosystems worldwide (Sigmund et al., 2023). Microplastics can contaminate terrestrial systems through soil amendments, plastic mulching, irrigation, flooding, atmospheric input and littering or street run-off (Rochman et al., 2019), with both positive and negative effects on plant–soil systems (Boots et al., 2019; Huang et al., 2019). For instance, in European grasslands, the biomass of the native species *Daucus carota* can increase in response to the presence of microplastic films in the soil (Huang et al., 2019; Lozano, Lehnert, et al., 2021). This effect is thought to occur because plastic particles could improve soil properties such as porosity and aeration (de Souza Machado et al., 2019; Lozano, Aguilar-Trigueros, et al., 2021). By contrast, the biomass of the native species *Lolium perenne* may decrease (Boots et al., 2019), presumably due to toxic effects from microplastic leachates. However, not only are native plants affected by microplastics, invasive plants may potentially benefit from microplastic pollution due to traits that enhance their ability to thrive in polluted environments (Lozano & Rillig, 2024). Research shows that microplastic fibres can enhance the growth of species of invasive character such as the range-expanding species *Calamagrostis epigejos* (Lozano & Rillig, 2020). Microplastic beads can increase fine root biomass and photosynthesis efficiency in invasive plants compared to native species under dry–wet water cycles (Zhang et al., 2024). Also, microplastic fragments may have negligible effects on the growth of the invasive plant *Solidago canadensis*, while having negative effects on its native counterpart *Solidago decurrens* (Li et al., 2024). This advantage can be expected, as invasive species could benefit more from the novel environmental conditions created by microplastics, such as changes in soil water content, aeration, microbial activity and aggregation (de Souza Machado et al., 2019; Lozano, Aguilar-Trigueros, et al., 2021). In addition, invasive species can possess advantageous traits that might help them to avoid or better tolerate microplastic pollution (Lozano & Rillig, 2024), enabling them to thrive in human-altered environments (Montesinos, 2021), which are most likely the ones exposed to higher levels of plastic pollution. Nonetheless,

microplastics can also inhibit the establishment of invasive plants like *Amaranthus palmeri* (Meng et al., 2023), and in some cases, they may have stronger negative effects on invasive plant biomass compared to native plants (Fu et al., 2024).

Multiple strategies such as allelopathy, herbivory and propagule pressure—closely linked with seed germination—enable invasive species to outperform native species (Bennett et al., 2011). While seed germination is critical for both native and invasive species, it plays a particular crucial role in the spread and establishment of invasive species (Daneshgar & Jose, 2009; Gioria et al., 2018). Invasive species often germinate faster and in greater proportions than native species (Guido et al., 2017; Lozano et al., 2019), with germination traits providing a key advantage in invasiveness (Moravcova et al., 2010; Palma et al., 2021). For example, species that germinate earlier can benefit from the early access to resources, space and reduced competition at initial stages of establishment (Gioria et al., 2018), which has been observed to be of greater benefit to several invasive plants in comparison to native species (Dickson et al., 2012). Additionally, synchronous germination plays a key role in the successful establishment of invasive species in novel environments (Gioria et al., 2018), as it influences not only the post-germination conditions that native or invasive specs experience, but also key processes like flowering and pollination time, which ultimately affect their growth and survival within the community, and thus their invasion potential (Gioria et al., 2018). The number of seeds that successfully germinate is another critical factor, as it directly influences plant population density and reproductive success, which is closely tied to the persistence of the seed bank in the soil—an important factor in the long-term success of invasive plant species (Gioria et al., 2021). Nonetheless, the relationship between seed germination and plant invasion is complex and can vary across different dimensions such as spread rate, local abundance or environmental range (Palma et al., 2021).

Plant invasion could be exacerbated by the current global change scenarios (Dai et al., 2022), in particular, by the presence of microplastics in the soil (Li et al., 2024). Studies of microplastic effects on seed germination either in native or invasive species are rather scarce and suggest mostly negative effects. For instance, polystyrene particles may decrease germination rate of species such as *Lepidium sativum* (Bosker et al., 2019; Pflugmacher et al., 2020), while having negligible effects on wheat seeds (Lian

et al., 2020). Similarly, microplastics of different shapes may decrease velocity and increase synchrony of seed germination of native species like *Daucus carota* (Lozano et al., 2022). Whether microplastics could promote the success of invasive plant species through their effects on seed germination parameters (e.g. promoting a faster and/or synchronous germination) remains a gap in our understanding.

Microplastics could potentially alter seed germination through physical and/or chemical mechanisms, highlighting different modes of action (Lozano et al., 2022). On the one hand, there could be a physical mechanism determined by the physical presence of microplastics (i.e. particles) in the soil, which may affect initial and later stages of seed germination through the blockage of seed pores (Bosker et al., 2019), hypocotyl and/or radicle growth. However, plastic particles could improve soil properties such as porosity and aeration (de Souza Machado et al., 2019; Lozano, Aguilar-Trigueros, et al., 2021) with potential positive consequences on seed germination. On the other hand, a chemical mechanism determined by the leaching of additives and chemical substances into the soil could also affect seed germination. Toxic compounds associated with microplastics (Lithner et al., 2011) can interfere with enzyme activity, such as amylase, which is essential for germination (Sethy & Gosh, 2013), ultimately resulting in negative effects. Both physical and chemical effects have been shown to affect the root length of the aquatic plant *Lemna minor* (Boots et al., 2023) and the growth of *Daucus carota* (Lozano, Perlenfein et al., 2024). However, we have not yet disentangled physical and chemical effects of microplastics on seed germination, nor has it been determined whether they differentially affect native and invasive plant species, potentially increasing the competitive ability of invasive species.

Thus, we evaluated the individual and combined effects of plastic particles and their additives on seed germination of native and invasive species in European grasslands. We aimed to (i) disentangle the physical (particles) and chemical (additives) effects of microplastics on seed germination and (ii) determine whether microplastics might enhance seed germination of invasive species over natives. Specifically, we hypothesized that the positive physical effects of plastic particles on soil properties would overcome the negative effects of seed pore blockage, thus promoting seed germination. By contrast, we hypothesized that the chemical additives from microplastics could have toxic effects on seeds, negatively affecting seed germination. Additionally, we hypothesized that invasive species, with their unique characteristics, might be better able to exploit the novel conditions created by microplastics compared to native species. To do so, we established a microcosm experiment where plastic particles (physical effect), additives (chemical effect) and their combined effect were evaluated on germination parameters of seven plant species (native and invasive) of grasslands in Germany. We evaluated seed germination parameters such as total germination, germination velocity and germination synchrony. We also determined the chemical additives released by plastics and their potential toxicity, by using different techniques, including untargeted LC-MS and ICP-OES analyses.

2 | MATERIALS AND METHODS

2.1 | Plant species selection

We selected a variety of native and invasive plant species to represent a typical grassland ecosystem in central Europe. Specifically, we selected four non-invasive grassland species native from Europe: *Achillea millefolium* L. (forb), *Dactylis glomerata* L. (grass), *Daucus carota* L. (forb) and *Trifolium repens* L. (forb) (Federal Agency for Nature Conservation, 2019). These native species are common, frequently co-occur in Central European grasslands and represent different functional groups (Lozano et al., 2020). Our native species may also exhibit an invasive character in America (Swearingen & Bargeron, 2016; USDA, 2024). We also selected three invasive species of European grasslands: *Bunias orientalis* L. (forb), *Tanacetum vulgare* L. (forb) and *Calamagrostis epigejos* L. *Roth* (grass) (Priede, 2022; Thevenot et al., 2022). *Bunias orientalis* and *Tanacetum vulgare* are invasive alien species in Central Europe that exhibit several traits characteristic of invasive species. They produce abundant seeds; form large, persistent seed banks; demonstrate phenotypic plasticity; and are often associated with anthropogenically disturbed habitats. Their rapid spread in recent decades is closely linked to human activities (Priede, 2022; Thevenot et al., 2022). Similarly, *Calamagrostis epigejos* is a range-expanding species that has invaded many semi-natural grasslands in Central Europe. As a perennial, rhizomatous plant, it utilizes a guerrilla clonal growth strategy to rapidly colonize new areas while also establishing a persistent seed bank (Dostál & Kovář, 2013; Těšitel et al., 2017). For our experiment, seeds of these species were obtained from commercial suppliers in the region (Rieger-Hofmann GmbH, Blaufelden, Germany).

2.2 | Microplastic selection and degradation

Plastic mulching is a major source of microplastics in agroecosystems (Khalid et al., 2023), exerting significant pressure on nearby natural grasslands. As plastic mulch from agricultural fields degrades, it breaks down into microplastics. These particles and their leachates can be transported by water run-off or atmospheric input into adjacent natural grasslands, potentially altering the plants and soil in these ecosystems. We selected low density polyethylene (PE) plastic films (silo film black, Folien-Bernhardt, thickness: 0.07 mm), widely used in the agriculture industry, which was cut and sieved through a 1-mm mesh to produce microplastic films ~1 mm^2 in size. Then, following Lozano et al. (2023), these films were exposed to UV-C degradation (254 nm irradiation) by using a photodegradation chamber with three 36 W UV-C lamps. Photodegradation is commonly performed using either UV-A or UV-C lamps. The former more closely simulates natural sunlight, but it takes several months to induce

changes in material properties. UV-C, while less reflective of natural ageing acts faster, with effects visible in a few weeks (Waldman & Rillig, 2020). Nonetheless, both UV-A and UV-C wavelengths induce similar degradation outcomes—such as polymer chain breaking, volatile organic compound production, reactive oxygen species formation and surface cracking—allowing for meaningful comparisons (De Freitas et al., 2022; Wu et al., 2023). In the UV-C chamber, the average incident energy was of 20.98 Wm^{-2} (photometer; item number HD 2302.0, DeltaOHM). Microplastic films were randomly distributed in the chamber and exposed to UV-C light for 2 weeks, with their positions shifted twice during this period. Previous assays indicated that a 2-week exposure effectively photodegraded the plastic, as evidenced by alterations in its physical and chemical properties. These changes included increased brittleness, surface microcracks, water absorption and the broadening of hydroxyl and carbonyl bands (Lozano et al., 2023). We used photodegraded plastics, which better represent the plastics entering soil environments, as even initially intact (pristine) plastics on soil surfaces, like those used in mulching and greenhouses, degrade with exposure.

2.3 | Additives and plastic particle treatments

Water extractable additives (additives from now on) were obtained modifying a protocol developed by Kim et al. (2020). We mixed 0.84 g of degraded microplastic films with 50 mL of distilled water in a 60-mL syringe and carefully removed the air pockets in the syringe. The syringes were closed with parafilm and vortexed for 5 s. We then incubated the syringes for 15 days at 40°C, a temperature achievable by Western European soils, under current climate conditions (García-García et al., 2023; Noyelle et al., 2023). Previous lab tests showed that 15 days of exposure suffices to extract additives using water. Syringes with only distilled water were also incubated at the same temperature to be used as control. After that, we filtered the extracts of each syringe with a sterile syringe filter (pore size 0.45 μm, Carl Roth, GmbH, Germany) to obtain the 'additives'. The plastic particles left in the syringe were washed three times with distilled water to obtain plastic from which water extractable additives have been removed. This yielded material for the two treatments: 'additives' and 'plastic particles'. The additives treatment corresponds to the quantity of particles used in the plastic particles treatment. As particles would have released additional additives during the experiment (Kim et al., 2020), we consider the effects of 'additives' in this study a potentially slight underestimate; at the same time, the pure effect of the plastic particles may eventually also have been mixed with some effects of additives for the same reason. We measured total organic carbon (TOC) by a combustion catalytic oxidation method (Shimadzu TOC-L, Shimadzu Corporation, Japan), and heavy metals such as Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), Zinc (Zn), Iron (Fe) and Manganese (Mn) by using the ICP-OES Avio 220 Max, PerkinElmer Inc., USA, in the water extracts from plastic (used to water the 'additives' and 'additives+particles' treatments) and in the distilled water (used to

water the 'particles' and 'control' treatments) (see below under experimental design). The TOC and heavy metal levels in the water extracts from plastics (additives) were clearly distinct from those in distilled water (see Section 3). We also conducted untargeted liquid chromatography–mass spectrometry (LC–MS) to identify chemical compounds in the water extracts. The detailed experimental procedures and data processing methods for the untargeted LC–MS analysis are outlined in Appendix S1.

2.4 | Soil preparation

We collected a sandy loam soil (Albic Luvisol; 0.07% N, 0.77% C, pH 6.66) from a dry grassland located in Dedelow, Brandenburg, Germany (53°37′ N, 13°77′ W), where our native species naturally grow and the selected invasive species have not yet been observed. Soil was air-dried, sieved through a 4-mm mesh size, homogenized and steamed in order to kill all possible seeds. A bottom layer of 160 g of dry soil was placed into each pot (9 cm diameter, 7.2 cm height, 500 mL) and watered with 35 mL of distilled water until saturation. Then, an additional layer of dry soil was mixed with degraded plastic particles at a concentration of 0.4% (w/w). That is, 0.76 g of plastic particles was mixed into 190 g of soil for each pot. This relatively high concentration of plastic particles in soil is intended to simulate potential scenarios in the future if plastic use is not reduced (Meizoso-Regueira et al., 2024). Plastic particles were mixed with the soil for 1 min in a large container, before placing it into each pot. Double size pots were used for *Bunias orientalis* because of the larger size of its seeds, but the same proportion of plastic particles and soil was kept. Soil preparation was done separately for each pot in order to provide an equal distribution of plastic particles throughout the soil.

2.5 | Experimental design

In May 2023, we established an experiment in a glasshouse with a temperature regime at 22/18°C day/night, a relative humidity of ~40% and a daylight period of 12 h. These conditions were previously tested and ensure adequate seed germination of these species. To disentangle the physical effects of plastic particles from the chemical effect of additives on seed germination, we established four treatments: control (C), additives (A), plastic particles (P) and a combination of additives and plastic particles (AP). To achieve this, soil without added plastic particles was watered with distilled water for the 'control' (C) treatment and with additives for the 'additives' (A) treatment. Additionally, soil mixed with plastic particles was watered with distilled water for the 'plastic particles' (P) and with additives for the 'additives + plastic particles' (AP) treatment. At the start of the experiment, pots were watered with 35 mL of either additives or distilled water. We thus had two microplastic treatments (particles and additives, each with 2 levels: absent, present)×10 replicates×7 plant species (4 native and 3 invasive) resulting in a total of 280 pots.

Seeds were surface-sterilized with 4% sodium hypochlorite for 5 min and 75% ethanol for 2 min, thoroughly rinsed with sterile water and kept at 4°C for ~5 days. Then, 50 seeds of each species were randomly placed into each pot, and germination, defined as seedling emergence, was recorded every 2 days for 3 weeks, when no new germination was observed for three consecutive counting days for all plant species, except *Bunias orientalis*, which reached total germination around the fifth week after sowing. Seedlings were removed after emergence. To mitigate any potential border effects, seeds were planted with a 1 cm distance from the edge of the pot and to a depth of twice the seed size. Based on previous assays with this soil, the pots were watered twice a week by gently spraying 20 mL of distilled water onto the soil surface to maintain water-holding capacity at ~70%. Pre-germination assay indicated germination of ~80%, except for *Calamagrostis epigejos* and *Tanacetum vulgare*, which had a germination rate of ~50% ($n = 3$). All pots were randomly distributed in the glasshouse and their position shifted twice during the experiment to homogenize environmental conditions.

2.6 | Germination indices calculation

To disentangle the effect of plastic particles and additives on seed germination of native and invasive species, we calculated the (i) total germination percentage, indicating the potential to complete germination (where the minimum is 0% and the maximum is 100%), (ii) the index of germination velocity, which measures how rapidly seeds germinate (with 0 representing the slowest and 100 the fastest germination rate) and (iii) the index of germination synchrony, which reflects the degree of overlap in germination. A value of 0 indicates minimal overlap (asynchrony), while a value of 1 represents maximum overlap (synchrony). This index focuses on the timing of germination (Aravind et al., 2021; Ranal & Santana, 2006). Germination parameters were calculated using the functions 'GermPercent', 'GermSpeed' and 'GermSynchrony' from the 'germinationmetrics' R package (Aravind et al., 2021). These functions consider both the quantity of germinated seeds and the counting interval (see calculations in Appendix S2).

2.7 | Phylogenetic relationships

In order to determine whether the phylogenetic signal between species was a significant predictor of germination parameter responses to microplastic treatments, we first applied the Blomberg's K test, which evaluates the tendency for related species to resemble each other more than they resemble species drawn at random from the phylogenetic tree (Blomberg et al., 2003). To do so, we constructed the phylogenetic tree by subsetting our plant species from the Daphne phylogeny (a phylogeny that encompasses all species in the trait databases BIOLFLOR, PLANTATT and Biobase 2003 and that includes a large European flora; Durka & Michalski, 2012) (Figure S1).

Then, we estimated for each germination parameter, the Blomberg's K with the function 'phylosig' from the R package 'phytools'. Values of $K < 1.0$ describe data with less phylogenetic signal than expected, while values of $K > 1.0$ describe data with greater phylogenetic signal than expected (Blomberg et al., 2003). Moreover, we calculated a phylogenetic distance matrix of the plant species by using the function 'cophenetic' from the R package 'stats'. We then subjected the distance matrix to a principal coordinate analysis by using the function 'cmdscale' from the same package. By doing so, we summarized the amount of variance explained by phylogenetic distance into a few independent principal axes (Diniz-Filho et al., 1998; Legendre & Legendre, 1998). The first two principal coordinate analysis axes, which represented ~80% of phylogenetic variation, were extracted and used as the phylogenetic covariate in further analysis (referred as 'phylogeny' from now on).

2.8 | Statistical analyses

2.8.1 | Redundancy analysis and linear models

We performed a redundancy analysis (RDA) to assess the response of the germination parameters to microplastic treatments. To account for phylogenetic relatedness, we included the first two principal coordinate analysis axes of the phylogenetic distance matrix as covariate (i.e. germination parameters were the standardized response variables, microplastic treatments and species identity were the explanatory variables, while phylogeny was the covariate) (see R script in Appendix S3). RDA and significance testing were performed using the function 'rda' and 'ANOVA.cca', respectively, both from the R package 'vegan' (Oksanen et al., 2021).

We also performed linear models to test the effect of additives and plastic particles on seed germination parameters of invasive and native species. Plastic particles (absent, present), additives (absent, present), provenance (native, invasive) and their interactions were established as fixed factors. Species was nested within provenance and together with phylogeny were included as a random factor in the model (see R script in Appendix S4). Afterwards, we performed linear models for each individual species. When the assumptions of normality and homogeneity of residuals were not met, we used both the 'bartlett.test' function and graphical inspection of residuals to identify the factors contributing to heteroscedasticity in each model. For these factors, we applied the 'varIdent' function from the 'nlme' R package (Pinheiro et al., 2021). For example, if the factor 'additives' caused heteroscedasticity, we specified it as 'varIdent (form = ~1 | additives)' and incorporated it as 'weights' within the model. Since after this procedure, some germination parameters were not suited for general linear models, we implemented generalized linear models with a quasibinomial distribution and a logit link function to avoid overdispersion. Additionally to comparing treatments using the Tukey test, we performed a Dunnett test to show differences between the control and the microplastic treatments. When the interaction between plastic particles and additives was significant, we did

not consider the main effects, that is, the individual effects of plastic particles and/or additives.

Additionally, effect sizes were estimated to show the variability in the response of the germination parameters, by comparing the combined effect of additives and particles (AP), which aims to mimic the microplastics usually found in the soil, with the control pots. We did so by using a bootstrap-coupled estimation 'dabestr' R package (Ho et al., 2019). Median values were used as measure of central tendency for the bootstrapped effect sizes, as they are less sensitive to outliers and skew. Positive effects indicate that the germination parameter values are greater with microplastics (AP) than in control soils. Negative effects indicate the opposite, while neutral effects indicate a similar response between AP and the control treatment.

After determining if microplastics as they occur in the soil (i.e. the combined action of particles and additives (AP)) could affect seed germination, which we demonstrated using effect sizes, the next step was to disentangle the effects of microplastic additives from those of plastic particles on germination parameters. To do so, we performed variance partitioning analyses to establish the importance of plastic particles and additives explaining the variation in each germination parameter. This was done by using the 'varpart' function from the 'vegan' R package (Oksanen et al., 2021) as follows: varpart (germinationparameter, ~ additives, ~ pieces). Partition was based on linear regression as the response variables were single vectors (Semchenko et al., 2018). The testable fractions: additives (present, absent) and plastic particles (present, absent) were analysed through a redundancy analyses being additives or particles used as 'conditional' of the other fraction (e.g. RDA$_{additives}$=totalgermination ~ additives + condition (particles)). Then, the 'anova' function was used to test the significance of each model (Borcard et al., 2018). The adjusted coefficients of determination in regressions could take negative values, which were interpreted as zeros (Legendre & Legendre, 2012). Statistical analyses were done in R 4.2.3 (R Core Team, 2023).

3 | RESULTS

We evaluated the effect of plastic particles and additives on total germination, germination velocity and synchrony of native and invasive species of grasslands. First, by using effects sizes, we found that microplastics in soil, that is the combination of particles and additives as it occurs in the soil (AP), affected the germination parameters (velocity, synchrony and total germination) of native species, whereas they did not affect those of invasive species. Then, through variation partitioning, we disentangled the effects of physical particles from chemical additives and found that, overall, germination of native species was better explained by particles and to some extent by additives, with such effects being mainly negative. By contrast, germination parameters of invasive species were generally not affected by plastic particles or additives (Figure 1). These patterns were consistent across both species and provenance levels (Figures 1–4; Tables S3 and S4). Specific results for each germination parameter are provided below.

3.1 | Water extractable additives

Total organic carbon and heavy metals were higher in additives extracted from plastics than in the control water (Figure S2; Table S1). TOC, iron (Fe) and manganese (Mn) contents followed such pattern. Cadmium (Cd), cobalt (Co), chromium (Cr) and nickel (Ni) showed a concentration below that of the blank (bi-distilled water) and were not included in the results.

Untargeted LC-MS analysis of the extractable additive solution from polyethylene films and the solvent blank was performed in both positive and negative ion modes (3 samples; 2 replicates of the microplastic and 1 control). Water extractable additives showed 13 and 10 features in positive and negative ion modes, respectively. Features such as bis[2-(4-aminoanilino)ethyl]-methyl-prop-2-enylazanium-methane was found in the positive mode; while features such as (1-methylcyclohexyl) 2-morpholin-4-ium-4-ylacetate was found in the negative ion mode (Supporting Information .xlsx file). No information on the function and use of these tentatively annotated compounds was available in PubChem, the largest open-access chemical database.

3.2 | Phylogenetic relationships

We found that phylogenetic signal was not a significant predictor of the germination responses to microplastic treatments, as we had K index values close to zero (weak signal). Specifically, for total germination, the phylogenetic signal (K) was 0.16, with a p-value of 0.94 (based on 1000 randomizations). For germination velocity, we observed K = 0.32 and a p-value of 0.61, while for synchrony, K was 0.49 with a p-value of 0.29. Furthermore, phylogeny had a minimal effect on the variation in seed germination parameters, accounting for only 0.45% of the total variation. By contrast, the combined effects of microplastic treatments and species identity explained 70.5% of the observed variation in germination parameters (Table S2).

3.3 | Total germination

We evaluated the effect of microplastics in soil (i.e. the combined effect of additives and particles; AP treatment) on total germination and found that microplastics (AP) negatively affected germination of native species, whereas they did not affect that of invasive species (Figures 1a and 2a; Tables S3 and S4). Next, we separated the physical effect of particles on total germination from the chemical effect of additives and found that particles mostly explained the germination of native species, followed by a key secondary role of additives. Specifically, germination of the natives *Daucus carota*, *Trifolium repens* and *Dactylis glomerata* were better explained by plastic particles than by additives (Figure 2b). Germination of these species decreased by 15%, 13% and 9%, respectively, in soils with plastic particles (P, AP) than in their counterpart soils without plastic particles (A, C) (Figure 2c; Figure S3). Nonetheless, for *Dactylis glomerata*, additives

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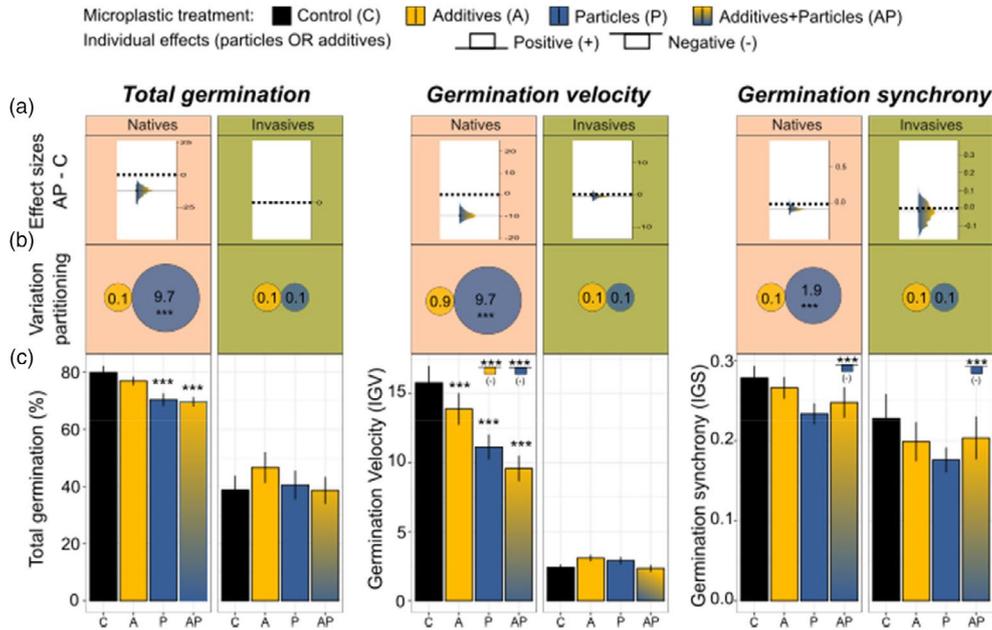
FIGURE 1 Summary of germination parameters for native and invasive species. Microplastic additives (A), particles (P) and their combined effect (AP) on total germination, germination velocity and synchrony of native and invasive species. In (a), effect sizes of the microplastic effect (i.e. Additives + Particles; AP), which mimics the plastic entering the soil, was compared to the Control (C; horizontal dotted line). Variance is displayed as means and 95% confidence intervals. The sampling error distribution is represented by a filled blue–yellow curve. Then, in (b), we disentangled the effects of the plastic particles and their additives/leachates on the response variable: Yellow circles represent additives and blue circles represent plastic particles, accounting for the variance in the germination parameter. Circle sizes are based on ranges and are an approximation of the values they represent. The variance explained is based on adjusted R^2 values enclosed within the circles. In (c), microplastic individual (main) effects of additives or particles—being positive ⊔ or negative ⊓ —indicate higher or lower germination when additives (yellow) or particles (blue) were present compared to their absence; in such cases, interactions among factors (particles, additives and provenance) were not significant (Tukey test). Significant interactions among factors were assessed using the Tukey test (letters above the bars). Differences between microplastic treatments and the control were assessed using the Dunnett test (asterisks above the bars). Significance was established at ***$p < 0.05$; *$p < 0.1$. $N = 10$.
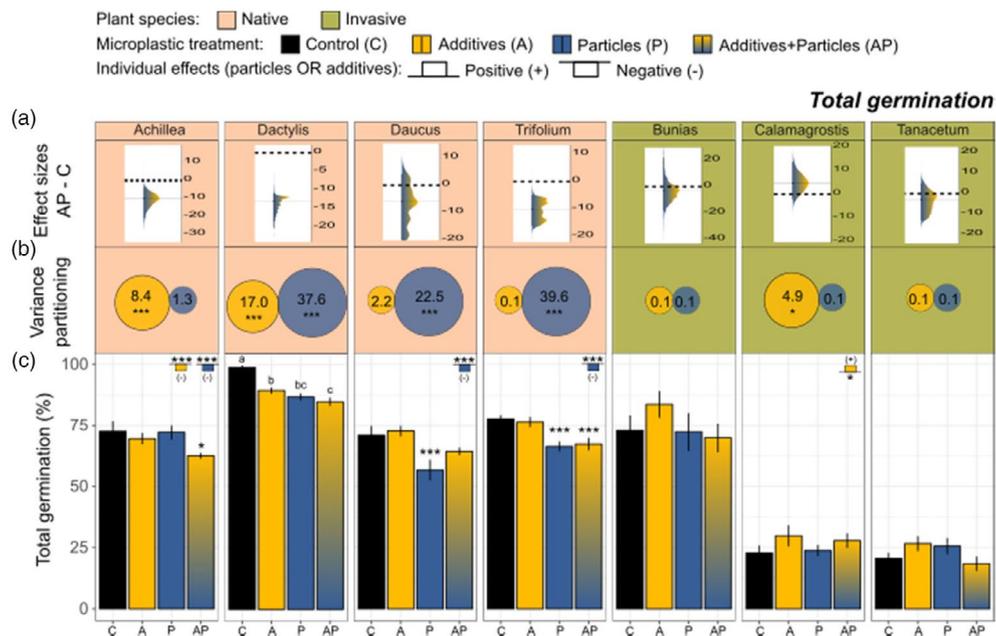
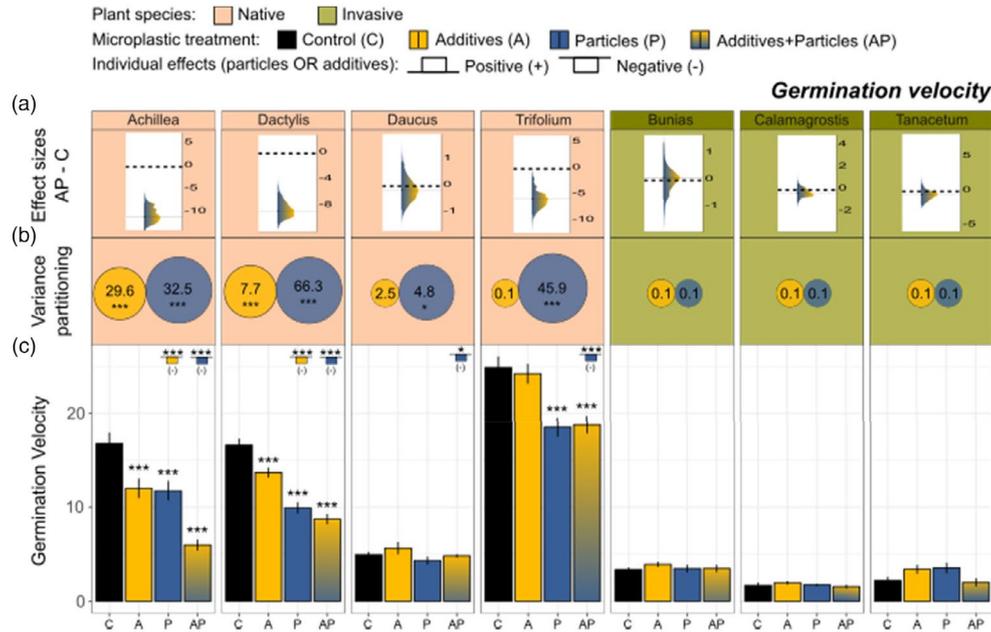**FIGURE 2** Total seed germination of native and invasive species. See details of the legend in Figure 1.

FIGURE 3 Seed germination velocity of native and invasive species. See details of the legend in Figure 1.
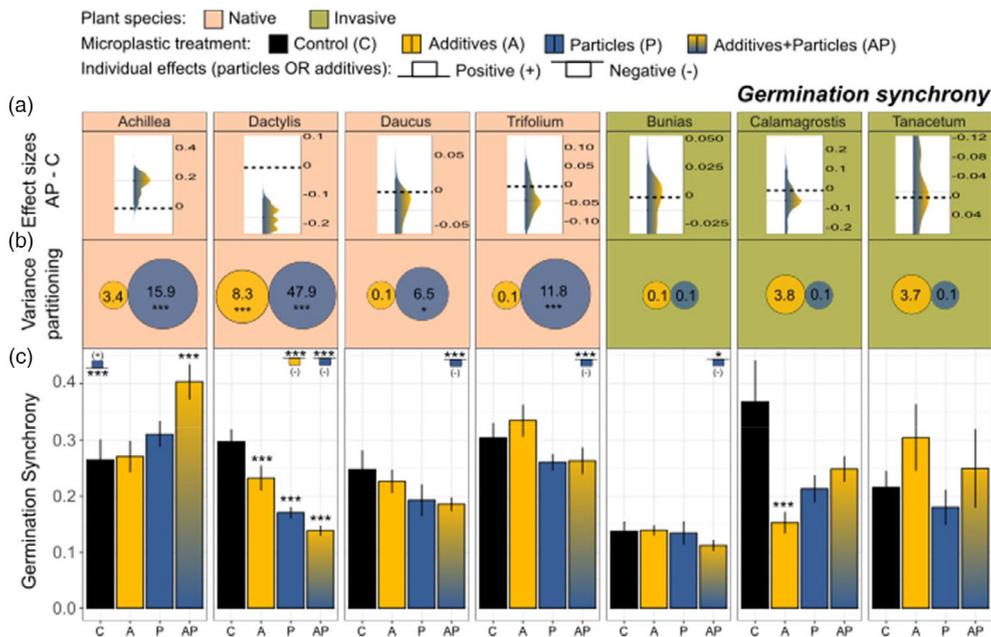

FIGURE 4 Seed germination synchrony of native and invasive species. See details of the legend in Figure 1.

also played a role as they decreased its germination by 9% in comparison with the control (Figure 2c). Although germination of the native *Achillea millefolium* decreased with plastic particles (P, AP), this was better explained by additives (A, AP), where germination decreased by 8% in comparison with soils without additives (P, C; Figure 2b,c). By contrast, plastic particles or additives effects on invasive species were practically negligible (Figure 2b,c); we only observed that additives marginally increased the total germination of *Calamagrostis epigejos* by ~23% (p=0.09), without any other microplastic treatment affecting other invasive species (Figure 2b,c; Table S4).

3.4 | Germination velocity

We evaluated the effect of microplastics in soil (i.e. the combined effect of additives and particles (AP)) on germination velocity. Our results showed that microplastics (AP) affected germination velocity of native species, whereas they did not affect that of invasive species (Figures 1a and 3a). Subsequently, we found that particles better explained the variation in germination velocity of native species, while additives played a key secondary role for some of them (Figure 3b). Specifically, germination velocity of *Achillea millefolium*,

Dactylis glomerata, *Daucus carota* and *Trifolium repens* decreased by 38%, 38%, 14% and 24%, respectively, in soils with plastic particles (P, AP) than in their counterpart soils without them (A, C; Figure 3c). *Achillea millefolium* and *Dactylis glomerata* germination velocity was also explained by additives, which decreased by 37% and 15%, respectively, in soils with additives (A, AP) than without them (P, C; Figure 3c). Overall, particles and additives decreased germination velocity of native species in comparison with the control (Figure 3c). By contrast, particles or additives did not affect germination velocity of any invasive species (Figure 3b,c).

3.5 | Germination synchrony

We also evaluated the effect of microplastics in soil (i.e. the combined effect of additives and particles (AP)) on germination synchrony (Figure 4a). Our results showed that microplastics affected germination synchrony of native species whereas they did not affect that of invasive species (Figure 4a). Then, we found that plastic particles better explained germination synchrony of all native species (Figures 1a and 4b). Specifically, *Dactylis glomerata*, *Daucus carota* and *Trifolium repens* decreased its germination synchrony by 42%, 20% and 18%, respectively, while *Achillea millefolium* increased it by 33% in soils with plastic particles (P, AP) than in their counterpart soils without them (A, C; Figure 4b,c). Nonetheless, additives also played a role for *Dactylis glomerata*; its germination synchrony decreased by 21% in soils with additives (A, AP) than without them (P, C; Figure 4b,c). By contrast, particles or additives practically did not affect germination synchrony of any invasive species (Figure 4b,c). We only observed that the invasive *Bunias orientalis* had slightly decreased germination synchrony in soils with plastic particles (P, AP) than without them (A, C), while the invasive *Calamagrostis epigejos* showed a similar pattern but in soils watered with additives (A) compared to the control (C) (Figure 4c).

4 | DISCUSSION

We disentangled different mechanisms by which microplastics affect seed germination of native and invasive species of European grasslands. Plastic particles as physical agents had a stronger effect on seed germination than chemical additives. We observed that particles and additives negatively affected germination of native species while they did not affect germination of invasive species.

4.1 | Particles and additives negatively affected germination of native species

Plastic particles, primarily influencing germination through physical effects, were the most important factor in explaining the germination parameters of native species. Specifically, particles negatively affected germination, which may be due to the fact that plastic

particles clog seed pores inhibiting water uptake, thus delaying germination (Bosker et al., 2019). Further visual inspection in additional pots revealed plastic particles adhering to the surfaces of seeds and radicles. We observed plastic particles lodged in the channels and appendages ('barbs') of *Daucus carota* seeds, as well as in hairs and palea of *Dactylis glomerata seeds (personal observation)*. Additives played a key secondary role, which similarly to particles, decreased seed germination parameters of native species. In that sense, total organic carbon (TOC), which shows the amount of organic compounds in the water, increased in water extracts from microplastics (additives) compared to control water, showing that plastic could have leached organic contaminants, among other substances into the soil (Figure S2). This is to be expected, as plastic films may contain hazardous monomers, and can carry many pollutants that can be leached into the water, such as plasticizers, colorants, heavy metals and other harmful agents. These substances may have contributed to the higher TOC and are known to be toxic for seed germination (Balestri et al., 2019; Lithner et al., 2011; Rochman et al., 2019; Wang et al., 2024). Indeed, leachates from polyethylene films have been shown to induce slight oxidative stress affecting germination of cotton seeds (He et al., 2023). Although the concentration of these chemical substances in our soil did not exceed the established critical levels for heavy metals in Germany (Schütze, 2003), it is likely that other compounds leached out undetected, or that some screened compounds would be toxic, though information on their toxicity remains unavailable. Overall, all these potentially toxic compounds could affect the activity of enzymes key for seed germination (Sethy & Gosh, 2013). For instance, manganese and iron whose concentrations were higher in water extracts (additives) from plastic films than in control water could potentially have negative effects on soil bacteria and thus decrease germination rate, helping to explain the negative effects of additives on seed germination.

4.2 | Neither particles nor additives affected the germination of invasive species

By contrast, overall the individual or combined effect of plastic particles and additives did not affect germination of invasive species. Particles could similarly block seed pores, hypocotyl and radicle growth, and additives could cause toxic effects on the seeds of all species irrespective of their invasive character. However, we showed that particles and additives barely affected invasive species, which does not mean that such negative effects did not occur, but rather that they were counteracted by other positive effects, as for example, those associated with the novel environmental conditions created by microplastics. These conditions include the amelioration of soil physical properties such as increased soil porosity, reduced bulk density, enhanced aeration (Lozano & Rillig, 2020; Wan et al., 2019) and potential positive plant–soil feedbacks (Lozano & Rillig, 2022). Microplastics change the very fabric of the soil by altering soil water regimes (de Souza Machado et al., 2019; Wan et al., 2019), which we hypothesize may open the window for the establishment of invasive

species according to the fluctuating resource availability postulate (Davis et al., 2000), which suggests that these species may take advantage of conditions of variable resource availability, especially at early stages of development (Wolkovich & Cleland, 2014). While native species may also benefit from these novel conditions, it has been suggested that invasive species possess better environmental tolerance, phenotypic plasticity and a set of competitive traits that could give them an advantage over native species, particularly in soils polluted with microplastics (Lozano & Rillig, 2024). Positive plant–soil feedbacks are known to facilitate plant invasion (Klironomos, 2002). For instance, invasive species may alter soil properties, including nutrient cycling, microbial communities and soil structure to promote their own growth (Ehrenfeld, 2003). Additionally, invasive species can accumulate beneficial microorganisms or reduce soil pathogens further reinforcing their dominance (Klironomos, 2002). While seeds may not have exerted large effects on soils here, soil microorganisms would have affected seed germination (Lozano et al., 2017, 2019; Perea et al., 2023). In that regard, our invasive species were not observed in the grassland where we collected the soil for this experiment. Thus, it is likely that invasive species might evade control by local soil pathogens and instead benefit from the soil microbes present, exemplifying a case of positive soil effects (Callaway et al., 2004) that may counteract the negative effects of plastic particles and additives. However, further testing is required to confirm these hypotheses.

4.3 | Environmental implications of particles and additives on seed germination

Plastic particles and to a lesser extent additives, delayed seed germination of native species, while for invasive species, plastic particles and additives had a neutral effect. Seed germination delay is a serious issue, especially for native species, as they typically possess smaller seed banks of shorter longevity in comparison with invasive species (Bakker et al., 2019). As microplastic effects may persist in the soil for a long time (Speißer, 2024), native seed banks and thus germination, can be comparatively more threatened by microplastics than seed banks of invasive species. Native seeds may lose their viability before the stress caused by plastic particles and/or additives is removed. Likewise, germination delay of native species may imply a competitive disadvantage, as early invasive species may be the first to capture available resources. That delay in germination may also affect their reproductive success as the abundance and diversity of pollinators change depending on the time of germination (Kehrberger & Holzschuh, 2019). This, in turn, would increase the competitive ability of invasive species, exacerbating the negative consequences of species invasion on terrestrial ecosystems functionality.

Unexpectedly, invasive species such as *Calamagrostis epigejos* or *Tanacetum vulgare* showed lower seed germination rates than native species. This could be attributed to seed dormancy as a mechanism to delay germination until conditions are optimal. Long-term seed dormancy has been observed to facilitate invasion of the tree

Ailanthus altissima (Redwood et al., 2019). *Calamagrostis epigejos* and *Tanacetum vulgare* might follow a similar pattern to avoid challenges such as microplastic toxicity. A follow-up experiment should be conducted to test this hypothesis.

Overall, plastic particles and additives decreased germination synchrony of native species. An asynchronous germination may increase their probability of germinating at a safe site (Gioria et al., 2018), a strategy from which invasive species seem to profit as well. For instance, *Calamagrostis epigejos* was more asynchronous in its germination with additives, which coupled with its higher total germination, suggest that, in soils polluted with microplastics, additives could potentially contribute to further expanding its distribution range. Interestingly, performance of this species has also been favoured by the presence of microplastic fibres in soil (Lozano & Rillig, 2020).

4.4 | Particles and additives threaten grasslands by indirectly promoting invasive species

Our study showed neutral effects of microplastics (additives or particles) on invasive species, while these effects were all negative for native species, which could indirectly enhance the success of invasive species. Grasslands cover close to one-third of the Earth's terrestrial surface are critical for providing important ecosystem services such as agricultural services, water supply and flow regulation, among others (Bengtsson et al., 2019). Thus, their decline due to the conversion to arable land for production of animal feed crops and, conversely, lack of management and abandonment (Queiroz et al., 2014) has had important consequences on global ecosystem functionality. These have left grasslands prone to plant species invasions (Vilà & Ibáñez, 2011), and such ongoing phenomena might be promoted by microplastics. Microplastics affect the functionality of terrestrial ecosystems (Lozano, Aguilar-Trigueros, et al., 2021) and potentially promote the spread of invasive plant species (Li et al., 2024; Lozano & Rillig, 2020, 2024). Research into the physical and chemical mechanisms by which microplastics affect the performance of several invasive and native species across their introduced and native ranges is essential. For example, studies should examine the performance traits of different invasive species including growth patterns and reproductive strategies. Specific traits such as seed production rate, viability and longevity, dispersal mechanisms and seedling growth can be studied in the introduced range, and compared with those observed in the native range. This research is particularly valuable under field conditions that reflect current global change scenarios. Further research should also consider that photodegradation of plastic particles is a time-dependent process, heavily influenced by specific climatic conditions including UV exposure, temperature and humidity. As photodegradation accelerates the release of chemical additives, the amount of additives leached into the soil under field conditions may be higher than believed. Consequently, their negative effects on seed germination may have been underestimated. It should also be noted that even after degradation, plastics leave a legacy in the soil that may enhance the competitive success of

invasive species (Lozano & Rillig, 2022). Therefore, long-term experiments are needed to evaluate the effect of microplastics on seed germination parameters to gain a deeper understanding of the persistence, viability and dynamics of seed banks of invasive and native species in microplastic polluted soils.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

We have no conflict of interest to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.w6m905qzr (Lozano, Landt, & Rillig, 2024).

ORCID

Yudi M. Lozano https://orcid.org/0000-0002-0967-8219
Matthias C. Rillig https://orcid.org/0000-0003-3541-7853

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: Phylogenetic tree of native and invasive plant species based on Daphne (Durka & Michalski, 2012).

Figure S2: Concentration of total organic carbon (TOC, $mg\,L^{-1}$) and heavy metals ($\mu g\,L^{-1}$) in water extractable additives from microplastic films of polyethylene.

Figure S3: Cumulative germination of seeds from native and invasive plant species typical from grassland environments.

Appendix S1: Untargeted LC-MS of the Water Extractable Additive Solution.

Appendix S2: Calculation of germination indices.

Appendix S3: R script used for the RDA analyses.

Appendix S4: R script used for linear models of the set of species.

Table S1: Total organic carbon (TOC) and heavy metals in water extractable additives from polyethylene films of 1 mm compared to control water.

Table S2: Variance proportion explained by the RDA model.

Table S3: Microplastic particles (P) additives (A) provenance (Pr) and their combined effect on total germination, germination velocity and germination synchrony of seeds from native and invasive plant species typical of grasslands.

Table S4: Microplastic additives (A), plastic particles (P) and their combined effect (AP) on total germination, germination velocity and germination synchrony of seeds from native and invasive species typical of grasslands ($n = 10$).

Table S5: Seed size (mm^2) of native and invasive species typical of grassland ecosystems (* range expanding species).