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Disentangling mechanisms by which microplastic films affect plant-soil systems: physical effects of particles can override toxic effects of additives

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Abstract

Background Microplastics, polymer-based particles < 5 mm, affect plant–soil systems positively or negatively, suggesting there are different modes of action. Microplastics, as particles, have physical effects but the leaching of additives likely contributes chemical mechanisms, both of which may be dependent on microplastic size. To disentangle such mechanisms, we established a controlled experiment involving polypropylene and polyethylene films of small, medium and large size, and we evaluated the individual and combined effect of plastic particles and additives (leachates from plastic particles) on soil properties and plant performance of the phytometer *Daucus carota* and on bare soils.

Results We find that additives better explained variation in soil properties (e.g., 44.6% vs 1.3%). Soil respiration and aggregation were negatively affected for additives, likely due to the presence of toxic substances. Overall, such effects increased as plastic size decreased. By contrast, plastic particles better explained plant biomass responses. The positive effect of particles on aeration which may promote root penetration and nutrient uptake, and microplastics itself as a source of carbon potentially promoting soil microbial activity, help explain the positive effect of particles on plant biomass. Plants mitigated the negative effects of additives on bare soils while enhancing the positive effects of particles. This improvement was likely linked to an increase in root activity and rhizodeposition, as plastic particles improved soil aeration. The combined effect of additives and particles, which mimics the microplastic found in the soil, mitigated their individual negative effects on plant–soil systems. As the negative effect of additives could have been masked by the positive effects of particles, simply reporting net positive effects would capture only part of the response.

Conclusions Additives and plastic particles differently affect soil properties and plant biomass. Additives primarily negatively affect soil properties due to toxic substances, while plastic particles enhance plant biomass likely by improving soil aeration. When examining microplastics effects on terrestrial systems (i.e., the combined effect of additives and particles), the negative effect of additives may be masked by the positive effects of plastic particles. Reporting only net positive effects risks overlooking these underlying negative effects. Plants can mitigate the negative impacts of additives and amplify the positive effects of plastic particles. Our study emphasizes the importance of investigating both the individual and combined effects of additives and particles to fully understand and address the impacts of microplastics on terrestrial ecosystems.

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Keywords Global change factors, Heavy metals, Microplastic size, Pollution, Total organic carbon, Toxicity, Water extractable additives

Introduction

Microplastics, plastic particles with their chemical additives, may enter the soil in various shapes, sizes and polymer types [1–3] through numerous pathways including soil amendments, plastic mulching, irrigation, atmospheric input, abrasion from car tires or road paint [3–5]. Their ubiquity and diverse physical and chemical properties make them a recognized threat to terrestrial ecosystems worldwide. Among them, films are particularly abundant, largely due to the use of agricultural plastics [6]. Plastic mulches, walk-in tunnels and low tunnel covers, silage, and temporary greenhouses, which are employed to increase crop yields [7, 8], contribute to soil pollution with microplastic films. Currently, about 3,500,000 metric tons of films are produced annually for the plasticulture market [9] posing a significant risk to terrestrial systems. These agricultural plastics are mostly made from polyethylene (e.g., low density polyethylene), though polypropylene and other polymers are also used depending on the application (e.g., silage, mulch) [7, 10]. Beyond agricultural lands, natural grasslands also face microplastic pollution. Grasslands are critical ecosystems that provide several services, including water supply, carbon storage, erosion control or climate mitigation [11, 12]. These services are essential and could be compromised by soil pollution with microplastics [13, 14].

Microplastic films may affect soil properties and plant performance positively or negatively. For instance, enzymatic activities such as urease or catalase can be enhanced in the presence of films [15], while by contrast soil aggregation may be decreased by them [16]. Additionally, plastic films can alter the soil water distribution, decreasing retention [17, 18]. These changes in soil properties can have multiple effects on plant performance, ranging from positive to negative. For instance, shoot and root mass can be increased by ~ 60% and ~ 59% with microplastic films [16], while seed germination rate and velocity can be negatively affected [19, 20].

These diverse effects of microplastics on plant–soil systems suggest that different mechanisms are involved, highlighting different modes of action. These include a physical mechanism driven by the presence of plastic particles in the soil, and a chemical mechanism, driven by the leaching of additives. Both mechanisms are strongly dependent on microplastic size. As microplastics enter the soil, they undergo fragmentation into smaller pieces due to degradation [21, 22], which implies an increase in the surface area:volume ratio, which may affect soil aeration and water flows, potentially exerting positive effects on soil aggregation, microbial activity, root development and plant growth [16, 23]. However, these positive effects of plastic particles can be counteracted by the negative effects of toxic chemicals, as the decrease in microplastic size also promotes the leaching of additives and impurities, which can have detrimental effects on soil biota and plant performance [24, 25].

Two of the most widely used microplastic films affecting terrestrial systems are polypropylene (PP) and polyethylene (PE). These films possess additives that can be released into the environment [26]. Specifically, these plastics typically contain process aids such as slip agents and lubricants, stabilizers like UV absorbers and antioxidants, and plasticizers including phthalates and citrates [26, 27]. Lubricants, surfactants, and unreacted hydrocarbons appear to contribute 18% and 17% of total additives, respectively [29]. The additives such as dipentyl phthalate and di-(2-ethylhexyl) adipate are commonly found in PE films, alongside stabilizers such as bisphenol A (BPA), TGIC, cadmium, and lead compounds [28, 29]. Many of these chemicals can cause irritation to the eyes, skin, or respiratory system, while others, including BPA and phthalates, are known endocrine disruptors [30]. Although polypropylene films contain fewer toxic additives than polyethylene, as reflected in their lower toxic effects on plant performance [31], the ingestion of these particles by soil organisms can still disrupt the food chain, posing risks to ecosystems and human health [32].

Plastic particles and the leaching of additives appear to be key mechanisms explaining the effects of microplastics on plant–soil systems. For example, pristine glitters, which may represent physical and chemical effects of such microplastics, along with their leachates, have been shown to reduce the root length of the aquatic plant *Lemna minor* [33]. However, the physical and chemical effects of microplastics on plant–soil systems can differ from their effects on bare soil without plants. For example, in soils without plants, the microplastics tended to decrease the number of newly formed aggregates and reduce overall aggregate stability [34, 35]. Conversely, when a plant is present, microplastics might have the opposite effect, increasing aggregate stability [14], suggesting that plants can mitigate the negative effects of microplastics on soil systems. Thus, here, we aimed to disentangle the mechanisms by which microplastic films affect plant–soil systems. Specifically, we sought to determine the roles of the physical presence of plastic particles and the chemical additives on soil properties and plant performance, and to assess the effects of microplastic size and polymer type on these mechanisms. Additionally, we investigated whether the presence of a plant in the system alters the magnitude and direction of microplastic physical and chemical effects on soils (e.g., mitigating or exacerbating such effects). To achieve this, we established a microcosm experiment using polyethylene and polypropylene films. We examined whether plastic particles, additives and their combined effect affect plant–soil systems as a function of plastic size (small, medium, and large) and polymer type (PE, PP). The experiment was carried out on bare soils and including the phytometer *Daucus carota subsp. carota.*

Materials and methods

Plant species and microplastic selection

We selected *Daucus carota* as a phytometer, which is a biennial herbaceous plant typical of grassland ecosystems [36] that shows clear responses to microplastics in soil [16, 37]. The seeds were obtained from commercial suppliers in the region (Rieger-Hofmann GmbH, Blaufelden, Germany). Also, we selected two widely used microplastic films. One is frequently used in agricultural systems as mulch, greenhouses, etc., (low density polyethylene, PE; black film, Folien-Bernhardt, thickness 0.07 mm), and the other widely used in several household items and in the packaging industry (polypropylene, PP; transparent folders of Cast Polypropylene STYLEX) [38].

Microplastic degradation

Plastics were exposed to UV-C degradation (254 nm irradiation) by using a photodegradation chamber with three 36 W UV-C lamps. The average incident energy of the chamber was 20.98 Wm^{-2} (photometer; item number HD 2302.0, DeltaOHM), which aimed to mimic the initial natural weathering of the plastic before entering the soil [37]. Polyethylene (PE) was degraded over a period of 2 weeks, while polypropylene (PP) was degraded during 2 days, the latter becoming too brittle after that time. Then, plastic was manually cut with scissors and separated using sieves of different mesh sizes. Three microplastic sizes were used: small (< 1 mm^2), medium ($1 < x < 2$ mm^2) and large ($2 < x < 4$ mm^2).

Photodegradation caused the emergence of brittleness, surface microcracks, water absorption and an increase in hydroxyl and carbonyl bands. Previous research has demonstrated that photodegradation of PP and PE films results in the formation or broadening of the hydroxyl band (3700–3250 cm^{-1}) and the appearance, increase, or broadening of the carbonyl band (1850–1550 cm^{-1}) [37]. Notably, the intensity of the hydroxyl and carbonyl peaks is more pronounced in PP than in PE suggesting that photodegradation affects the two polymers differently. The chemical structure of PP, which contains tertiary carbon atoms, makes it more prone to oxidation than PE, which primarily contains secondary carbon atoms. As a result, PE typically displays strong peaks for C–H bond stretching and bending but lacks significant hydroxyl or carbonyl signals [39]. Additionally, photodegraded PP films exhibit a 2484% increase in water absorption, while PE films showed a comparatively lower increase of 177% when compared to their pristine counterparts [37]. Based on this, we utilized different polymer types. Also, we used degraded plastics instead of pristine ones, as they more accurately represent the type of plastic that typically enters the soil environment.

Soil preparation and plastic particles treatment

We collected soil in Dedelow, Brandenburg, Germany (53 37’ N, 13 77’ W). The soil was a dry sandy loam (Albic Luvisol; 0.07% N, 0.77% C, pH: 6.66) from a dry grassland plant community. It was sieved (4 mm mesh size), homogenized, and mixed with microplastics at a concentration of 0.4% (w/w) for treatments requiring microplastic particles (see experimental design below). We used this relatively high concentration of plastic particles in soil, to simulate potential scenarios for the next 50–100 years if plastic used is not reduced [40]. Specifically, 0.76 g of microplastic was mixed into 190 g of dry soil for each pot (4 cm diameter, 21 cm height, 200 ml). The microplastics were manually mixed with the soil for 1 min in a large container to ensure even distribution before being placed into individual pots. Soil without added microplastic particles was handled similarly to provide similar disturbance levels.

Microplastic additives treatment

We used 0.76 g of plastic to prepare both the plastic particles and the water extractable additives (from now on additives) treatments. Here, we mixed 0.76 g of each microplastic into 45 ml of distilled water in a 50 ml syringe, ensuring air pockets were carefully removed without spilling water. The 45 ml of water corresponds to the amount needed to saturate 190 g of our soil, which allows the full interaction between the soil and the additives. The syringes were closed with parafilm and vortexed for 5 s. One syringe per replicate of each plastic size was used. We then incubated the syringes for 15 days at 40 °C in a lying position. Syringes with only distilled water were also incubated at the same temperature to be used as control. Using distilled water ensures the replicability of our research and allows for easier comparison with other studies. Subsequently, we filtered the extractions with a sterile syringe filter to minimize the risk of plastic particles contaminating the extraction (pore size 0.45 µm, Carl Roth, GmbH, Germany). Here we obtained the "microplastic additives" treatment. The microplastic particles left in the syringe were washed three times with distilled water to obtain plastic from which water extractable additives have been removed. Here we obtained the "plastic particles" treatment. During the experiment, plastic particles would have released additional additives over time [24]. As a result, the impact of these additives in our study may be slightly underestimated. Furthermore, the observed effects of the plastic particles themselves may also have been influenced by the toxic effects of these released additives.

Experimental design

In March 2022, we established the experiment in a glasshouse with a daylight period set at 12 h, 50 klx, and a temperature regime at 22/18C day/night with a relative humidity of 40%. To disentangle microplastic physical effects (plastic particles) from the chemical effects (additives) on plant–soil systems, we established four microplastic treatments. Soil without added films was watered with (i) distilled water (*control treatment*).or (ii) additives (*additives treatment*), while soil mixed with films from which additives were extracted was watered with (iii) distilled water (*plastic particles treatment*) or (iv) additives (*plastic + additives treatment*). This design was applied to each microplastic size and polymer type, and in two soil systems: bare soil and including a plant.

The experimental design consisted of two systems (soil, plant-soil) × 2 polymer types (PE, PP) × 3 microplastic sizes (small, medium, large) × 4 microplastic treatments × 6 replicates = 288 pots. Additionally, we included 12 control samples with plants and 15 control samples with bare soil to account for natural variability in the system and better distinguish true treatment effects from random fluctuations. *Daucus carota* seeds were surface-sterilized with 4% sodium hypochlorite for 5 min and 75% ethanol for 2 min, then thoroughly rinsed with distilled water. Three days after germination, seedlings of similar size were transplanted into individual pots, with one seedling per pot. At the start of the experiment, pots were watered with 35 ml of either the additive solution or distilled water. Two days later, an additional 10 ml of the same solution was applied to achieve saturation. Plants grew for 6 weeks and were watered every third day with 10 ml of distilled water during the first 2 weeks, and every second day with 20 ml thereafter, to maintain approximately 70% water holding capacity. All pots were randomly distributed in the glasshouse chamber, and their positions shifted twice during the experiment to homogenize environmental conditions. All plants survived until the end of the experiment. At harvest, plants were separated into above and belowground parts; soil was air-dried and stored at 25 °C for soil aggregation analyses, while fresh soil samples were used to measure soil respiration.

Measurements

We measured different chemical components of the water-extractable additives obtained from small, medium and large microplastics. Specifically we assessed: non-purgeable organic carbon (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. We also performed untargeted LC−MS of the water-extractable solutions whose experimental procedures and data processing are described in Appendix 1.

After harvest, we measured soil aggregation following a protocol by Kemper and Rosenau [41], modified by Lehmann et al. [42]. That is, we placed 4 g of dried soil (< 4 mm) on small sieves with a mesh size of 250 µm. Soil was rewetted with distilled water by capillarity and inserted into a sieving machine (Agrisearch Equipment, Eijkelkamp, Giesbeek, Netherlands) for 3 min where the agitation and re-wetting caused the treated aggregates to slake. Subsequently, we dried and weighed the water-stable fraction (dry matter) and we extracted the coarse matter, which was also dried at 60 °C for 24 h. Soil aggregation represented by water-stable aggregates was calculated as:

$$\text{WSA}\ (\%) = \left(\text{dry matter} - \text{coarse matter}\right)/(4.0\text{g} - \text{coarse matter}). \quad (1)$$

Additionally, we measured soil respiration via infrared gas analysis. We placed 25 g of fresh soil in 50 ml centrifuge tubes (Sarstedt AG & Co. KG, Nümbrecht Germany, item number 62.548.004) with modified lids to control gas exchange via a rubber septum (Supelco, Darmstadt, Germany, item number 27235 U). We measured CO_2 concentration (ppm) at two time points: First, we flushed the tubes with CO_2 free air for 5 min to measure CO_2 concentration at time zero. Then, soil samples were incubated at 20 °C for 24 h and we measured CO_2 concentration for the second time. At both times, we took 1-mL air sample and injected it to an infrared gas analyzer (LiCOR- 6400XT photosynthesis system, Li-Cor Biosciences, Lincoln, NE). The measurements were taken every ~ 2 s. The difference between the maximum and the minimum value (peak) was converted to ppm using the calibration equation (ppm = − 467 + 195.18 peak). Soil respiration was reported as the net CO_2 production (in ppm). At harvest, the roots were carefully removed from the soil and gently washed by hand. Subsequently, shoots and roots were dried at 60 °C for 72 h, after which their mass was determined.

Statistical analyses

To disentangle the physical effects of plastic particles from the chemical impact of additives on plant–soil systems, we performed variance partitioning analyses using the "vegan" R package. Likewise, to evaluate the effects of plastic size and polymer type on plant and soil variables, we applied linear models and multiple comparisons via the "nlme" and "multcomp" R packages. We also assessed whether plant presence altered the magnitude and direction of microplastic effects on soils, using the Relative Index of Interaction (RII). The chemical components of water-extractable additives, including total organic carbon (TOC) and heavy metals, were analyzed through linear models.

Variance partitioning modeling

The importance of plastic particles and additives in explaining the variation in soil properties and plant biomass was analyzed using variance partitioning "varpart" function from the "vegan" R package [43]. The partition was based on linear regression, as the response variables were single vectors (i.e., individual variables) [43, 44]. Each factor (additives, particles) was computed and tested using partial RDA, which helps to control for known linear effects or isolate the effect of a single explanatory variable [45]. The effect of additives was analyzed while accounting for the particles effect, and vice versa. We applied this approach to each response variable. For example, for soil respiration, we used the following code: rda.additives <−rda (soilrespiration ~ additives + Condition (particles)). The adjusted coefficients of determination in regressions could occasionally take negative values, which were interpreted as zeros [46]. Additives and plastic particles effects were analyzed with the "anova.cca" function [45].

Linear models and multiple comparisons

We performed general linear models to test the effect of microplastic treatments on our response variables. Since control samples were never exposed to any microplastic treatment, our design was not fully factorial. Instead, we analyzed the data using a single factor, 'treatment,' with 10 levels: 3 microplastic sizes (small, medium, large) × 3 microplastic mechanisms (additives, particles, additives + particles), plus a control (Appendix 2). We took into account the correlation between samples from the same plastic size by using the function "corCompSymm" from the "nlme" R package. The outliers, defined as data points lying more than 1.5 times the interquartile range above the upper quartile or below the lower quartile in a boxplot, were identified using "boxplot (variable) $out". We found n = 4 in respiration and n = 1 in aggregation measurements for soils exposed to PE films. Statistical analyses conducted with and without these outliers showed no significant differences. The residuals were checked to validate assumptions of normality and homogeneity. When heterogeneity of variances was present we implemented the function "varIdent" from the "nlme" R package to account for it in the treatment [47]. Then, for the selected model, we implemented the function "glht" from the "multcomp" R package [48, 49], to compare microplastic treatments within each plastic size (Tukey test) and each microplastic treatment versus the control (Dunnett test). The analyses were done independently for each soil system and polymer type.

Relative Index of Interaction (RII)

We calculated the Relative Index of Interaction (RII) to compare the direction and magnitude of microplastic physical and chemical effects on soil properties. By using a bootstrap procedure, we took a random replicate for the microplastic treatment (i.e., plastic particles, additives and the combination of them) and a second random replicate for the control (plant or soil, respectively). We then calculated the RII index following Armas et al. [50] as:

$$\text{RII soil property} = (Y_{\text{microplastic}} - Y_{\text{control}})/(Y_{\text{microplastic}} + Y_{\text{control}}). \quad (2)$$

where $Y_{\text{microplastic}}$, is the soil property value when the soil was affected by the microplastic treatment (i.e., plastic particles, additives or their combination); and Y_{control}, is the soil property value in control samples. For each soil property, we repeated the calculation of the RII index 999 times by bootstrap sampling with replacement [51]. It was calculated for each system (i.e., bare soil, including plant) and polymer type. This index ranged from − 1 to 1, with positive values indicating soil property values greater with microplastic treatments than in control samples and negative values indicating the opposite. Afterwards, we constructed 95% confidence intervals by using the function 'CI' from the "Rmisc" R package [52], and performed a Student's t-test to determine whether the mean value of RII index was different from zero. Statistical analyses were done in R 4.1.2 [53].

Results

Microplastic additives: total organic carbon and heavy metals increased in water extracts from microplastics of small size

The analyses of water extractable additives showed that overall, total organic carbon (TOC) and heavy metals were higher in additives extracted from microplastics of any size compared to control water. TOC increased by ~ 173%, ~ 116% and ~ 151% in additives extracted from small, medium and large PE films, respectively, and by around 175%, 141% and 117% in additives from PP films, compared to control water. Chromium content increased by ~ 591% and ~ 383% in additives from small and medium PE films compared to control water. Manganese, Fe and Ni contents were higher in water extracts from PE plastics of all sizes, with the highest increase (~ 1000%) observed in additives from small PE films. Copper levels increased by ~ 706% and ~ 772% in water extracts from medium and large PE plastics, respectively, and by ~ 265%, ~ 861% and ~ 156% in water extracts from small, medium and large PP films compared to control water. Cobalt increased by ~ 168% in water extracts from PP films of small size, while Cd and Pb content were not affected by microplastic size or polymer type (Fig. 1, Table S1).

Untargeted chemical screening of the extractable additive solution

Water-extractable additives from films of small, medium and large size, and the solvent blank (a total of 13 samples; 2 replicates for each microplastic type) were analyzed using LC–MS in both positive and negative ion modes. For polypropylene films, the small, medium, and large sizes revealed 8, 5, and 9 features, respectively, in the positive ion mode, while polyethylene films revealed 12, 15, and 14 features. The most common feature identified in the positive ion mode was 2-acetamido-3-hydroxyoctadecanoate. In the negative ion mode, polypropylene films revealed 7, 5, and 5 features, while polyethylene films revealed 6, 7, and 11 features, with the most common feature being (2S)-2-hydroxy-2-[[(Z)-octadec-9-enoyl]amino]acetate (supporting information; xlxs files). Information on the use or function of these tentatively annotated compounds was not available in PubChem.

Microplastic additives mostly explain variation in soil properties while plastic particles mostly explain the variation in plant performance

Microplastic additives played the most important role in determining soil properties while plastic particles was the key attribute explaining variation in plant biomass (Fig. 2). Specifically, the soil respiration was better explained by additives than by plastic particles of PE films in bare soils (6.4% vs 0.1%) or in a system with plant species (44.6% vs 1.3%) (Fig. 2a, b). No clear effects were found with PP films (Fig. 2c, d). Similarly, soil aggregation was better explained by additives, although plastic particles also played a role (Fig. 2e–h). That is, in bare soils, aggregation was better explained by additives than by plastic particles for both polymers (2.3% vs 0.1% with PE and 22.6% vs 0.1%% with PP). In soils with a plant, soil aggregation was also better explained by additives

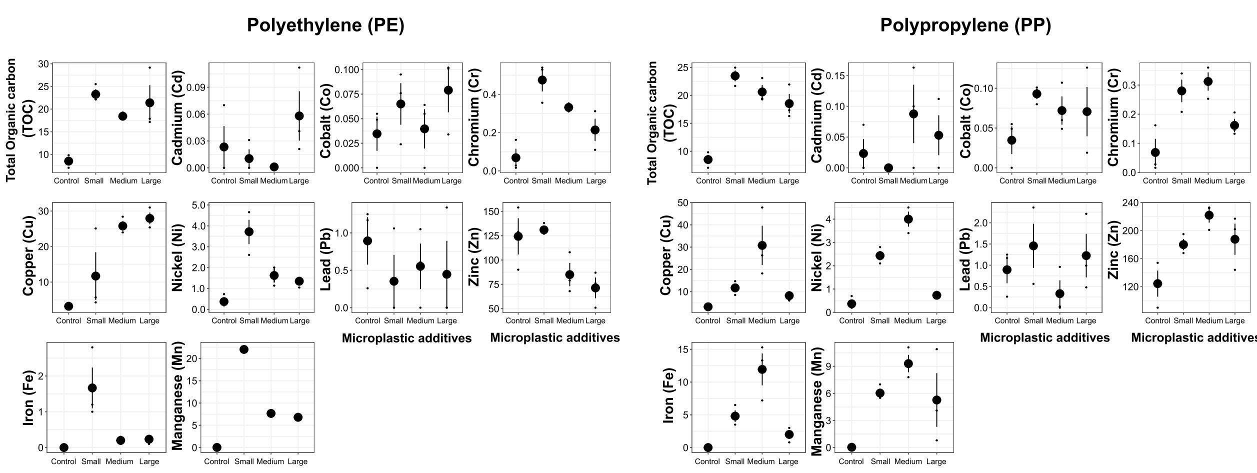

Fig. 1 Concentration of total organic carbon (TOC, mg L^{-1}) and heavy metals (μg L^{-1}) in water extractable additives from microplastics of small, medium and large size. Polyethylene (PE) and polypropylene (PP) were used as microplastics. Mean and standard error are represented. Data points are shown as circles. *N* = 3

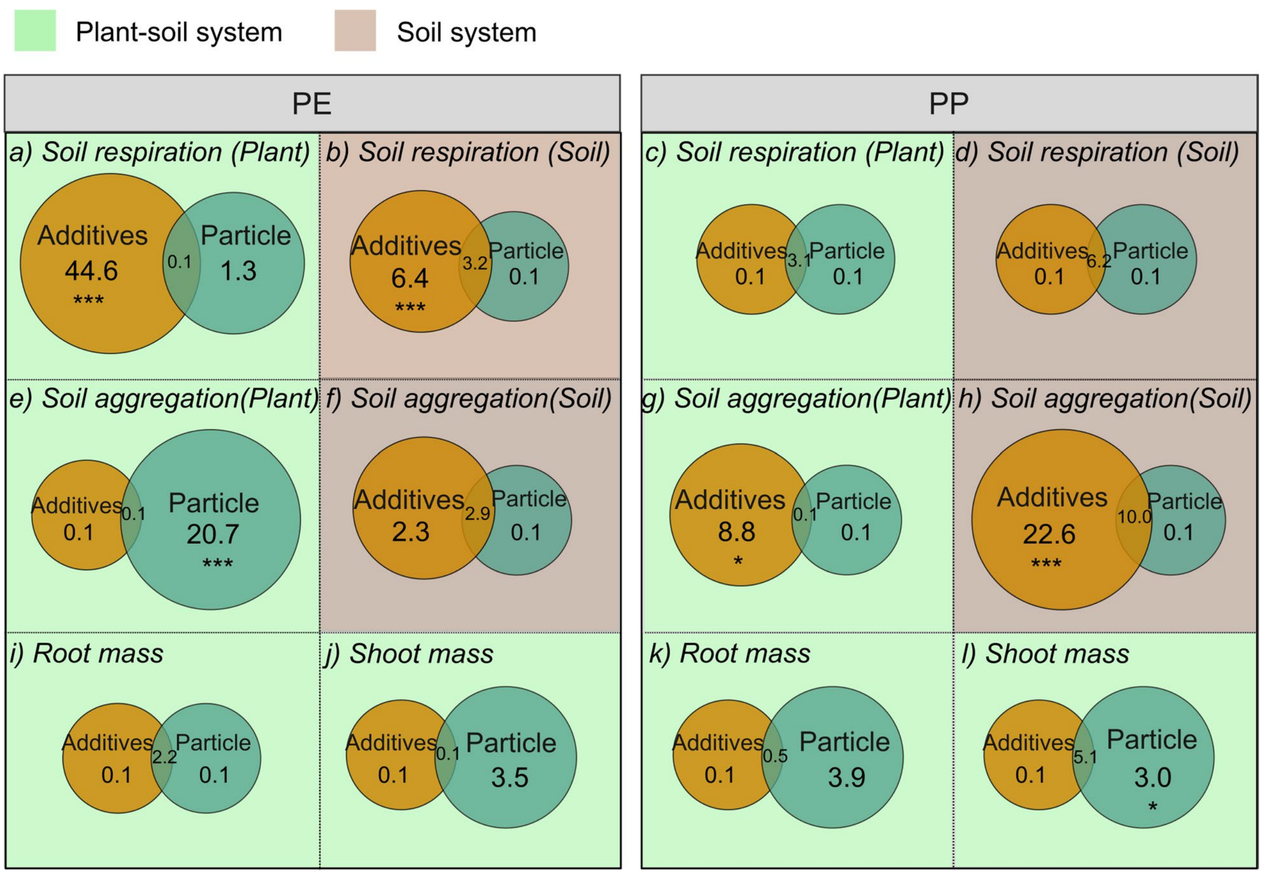

Fig. 2 Variance partitioning. Variation in soil respiration, soil aggregation, shoot and root mass in a system with bare soil or in one that includes a plant. Variance is explained by microplastic additives, plastic particle presence or their interaction. Films of polyethylene (PE) and polypropylene (PP) were used. Values close to zero are shown as 0.1. Variance explained is based on adjusted R^2 (*$p<0.1$, **$p<0.05$, ***$p<0.001$)

than by plastic particles of PP films (8.8% vs 0.1%), but the opposite pattern was found with PE films, as soil aggregation was better explained by plastic particles than by additives (20.7% vs 0.1%) (Fig. 2e–h). Lastly, root and shoot mass were better explained by plastic particles than by additives. Root mass was better explained by PP plastic particles (3.9%) than by additives (0.1%). Shoot mass was better explained by plastic particles (3.9% and 3.0%) than by additives (0.1%, 0.1%) irrespective of the polymer type (Fig. 2i–l).

Chemical and physical effects of microplastics on plant–soil systems depend on the plastic size: negative effects of additives from small plastic films

Overall, the additives were most important attribute explaining soil respiration while plastic particles played a secondary role (Fig. 2). These effects varied as a function of microplastic size (Fig. 3, Table S2-S4). In a system with a plant, the soil respiration decreased by ~ 22% with additives from small PE films and increased by ~ 23% with additives from larger PE films compared to control samples (Fig. 3a, Table S3). This reduced soil respiration with small films followed a similar pattern for both polymer types in bare soils (Fig. 3a, b, Table S4). Additionally, PE particles, particularly those of medium size, tended to negatively affect soil respiration in the presence of a plant.

Overall, the additives better explained soil aggregation, though particles also played a role (Fig. 2). For instance, with a plant, the soil aggregation increased by ~ 16% with additives from small PE films, while in bare soils it decreased by ~ 15% in comparison with their respective controls (Fig. 3c). Similarly, in soils with a plant, soil aggregation increased by ~ 17% with additives from medium PP films compared to control soils, but in bare soils, it decreased by ~ 21% with additives from large PP films (Fig. 3d). Lastly, with plants, soil aggregation increased by ~ 18% with large plastic particles of PE films compared to control samples (Fig. 3c). By contrast, root and shoot mass were better explained by plastic particles than by additives (Fig. 2). Specifically, root mass tended to increase with small and medium-sized PE particles,

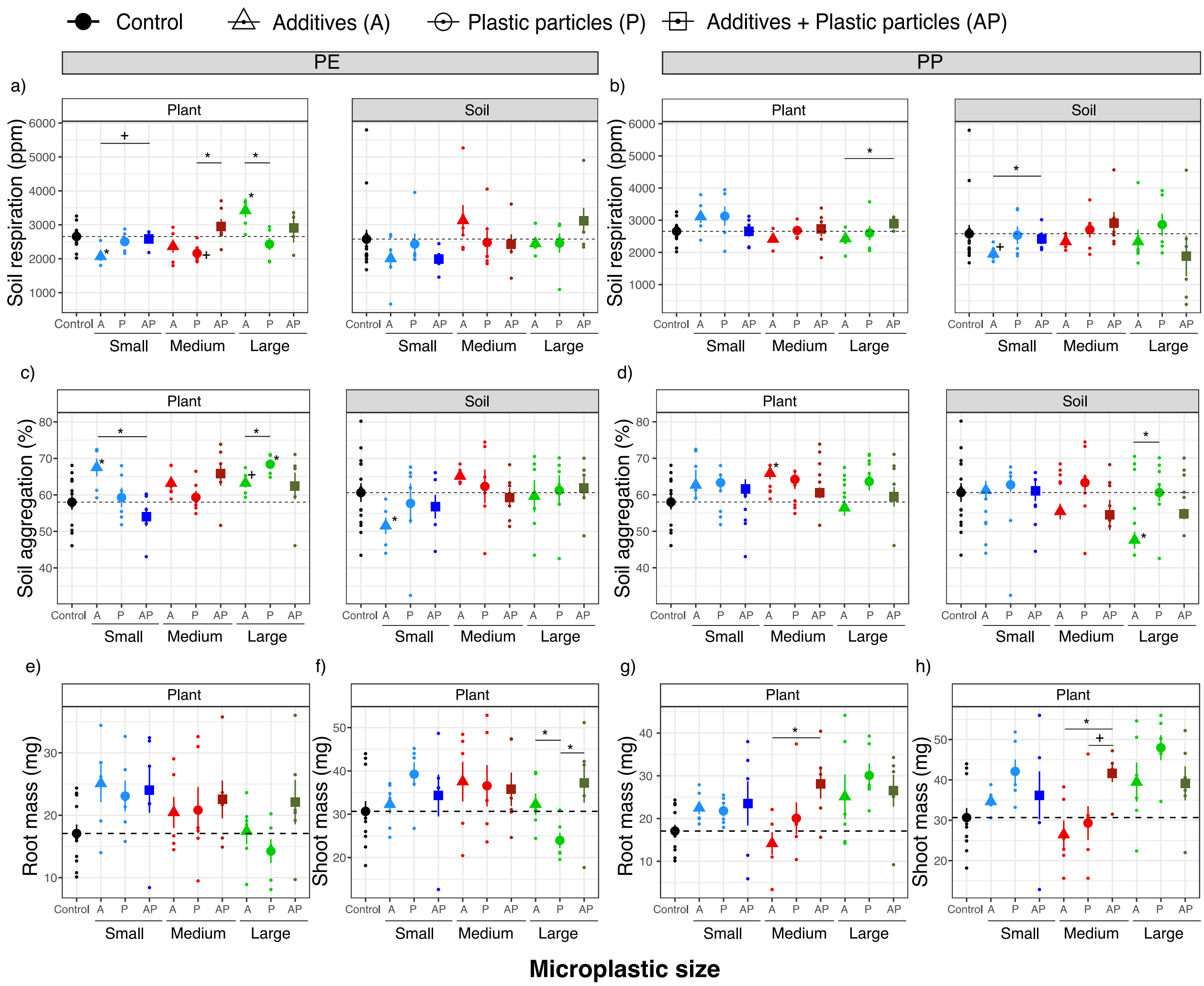

Fig. 3 Soil respiration, soil aggregation, root mass and shoot mass responses to additives (A), plastic particles (P) and the combination of additives and plastic particles (AP) in a system with plant or in bare soils. Polyethylene (PE) and Polypropylene (PP) films were used at small, medium and large sizes. Mean and standard error are represented. Data points are shown as circles. Soil aggregation was expressed as the percentage of water-stable aggregates. Significance was established at 0.05 (*) and 0.1 (+) for both Tukey and Dunnett test. Asterisks above the lines in the figure indicate differences between microplastic treatments (Tukey test), while asterisks next to error bars show differences between the soil with microplastics and the control samples (Dunnett test). $n=6$ for microplastics treatments, $n=12$ for control samples in a system with a plant, $n=15$ for control samples in bare soils

while tended to decrease with large PE particles compared to control samples (Fig. 3e). Similar pattern was observed for shoot mass (Fig. 3f).

Plant presence mitigates the negative effects of additives while enhancing the positive effects of plastic particles, as a function of plastic size

Soil respiration: single effects of additives and plastic particles

Overall, the magnitude of additives effect on soil respiration was greater than that of plastic particles, particularly for PE films (Fig. 4a, Table S5). Additives from small PE films negatively affected soil respiration in both bare soils and soils with a plant, while additives from large PE films positively affected soil respiration in bare soils. Such effect was further enhanced by the presence of the plant (Fig. 4a). The negative effect of additives from small PE films became more positive with additives from larger sizes. Similarly, the negative effect of additives from small PP films was mitigated by the presence of a plant, turning it into a positive effect (Fig. 4a). Plastic particles had a minimal impact on soil respiration. Polyethylene particles had a neutral

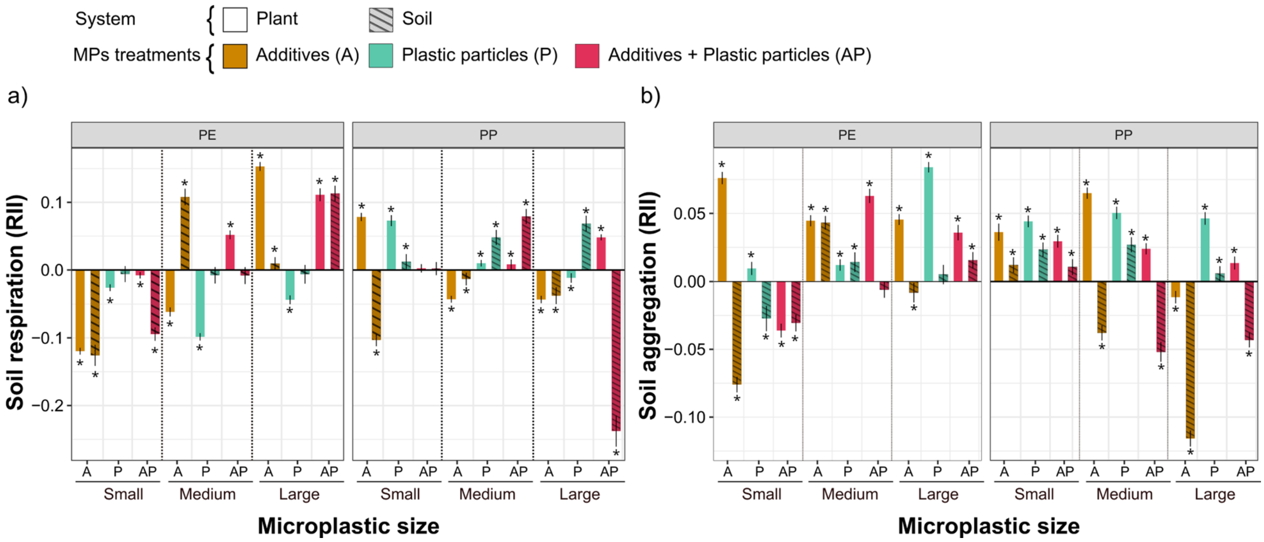

Fig. 4 Magnitude of microplastic chemical (additives) and physical (plastic particles) effects on soil respiration and aggregation, in a system with a plant or in bare soils. The Relative Index of Interaction (RII) compares each microplastic treatment against the control without microplastics. Microplastic treatments: additives (A), plastic particles (P) and their combined effect (AP) from microplastics of small, medium and large size. Polyethylene (PE) and polypropylene (PP) were used as plastic films. Positive values in the RII indicate higher trait value in the microplastics treatment than in control samples and negative values indicate the opposite. RII values different from zero are indicated by asterisk (*$p < 0.05$)

effect on respiration of bare soils, regardless of particle size, but caused a negative effect when a plant was present.

Soil aggregation: single effects of additives and plastic particles

Additives and plastic particles effects on soil aggregation tended to be positive (higher than control) when a plant was present, regardless of the polymer type (Fig. 4b). Additives played a key role in explaining soil aggregation (Fig. 2). Notably, the negative effect of additives from small and large PE films and from medium and large PP films on aggregation of bare soils was mitigated by the presence of a plant (Fig. 4b), while the positive effect of additives from small PP films on bare soils was enhanced due to the plant presence.

Plastic particles played a key role in explaining soil aggregation, especially PE films (Fig. 2), which always had a positive effect on soil aggregation when plants were present, regardless of the size (Fig. 4b). Indeed, the negative effect of small PE particles on bare soils was turned into a positive effect by the presence of a plant. Additionally, the neutral effect of large PE films on soil aggregation became positive with plants. Similarly, with PP films, the positive effects of plastic particles on soil aggregation in bare soils were always enhanced by the presence of the plant, regardless of the plastic size.

Combined effect of additives and plastic particles on soil properties

The combined effect of additives and plastic particles from small PE films was negative on soil respiration in bare soils, but this was mitigated by the presence of the plant. By contrast, large PE films had a positive effect on soil respiration, irrespective of the system. For PP films, the effect of small films on soil respiration was negligible, while the effects of medium and large films were mitigated by the presence of the plant (Fig. 4a). Regarding soil aggregation, the combined effect of additives and plastic particles from small PE films was negative regardless of the system. Conversely, the combined effect with large PE films was positive in bare soils, and enhanced by the presence of a plant. The neutral effect of medium PE films on soil aggregation became positive due to plant presence. Similarly, the combined effect with small PP films was positive for soil aggregation in bare soils and was enhanced by the plant presence. The negative effects of medium and large PP films on soil aggregation in bare soils were mitigated and turned positive by the presence of a plant (Fig. 4b).

Discussion

Microplastic additives mostly explain variation in soil properties

Additives played the most important role explaining the variation in soil properties, while plastic particles had a more secondary influence. Overall, this could be due to the additives directly impacting soil chemical and biological properties, which lead to rapid and noticeable effects on soil biota [54, 55]. In contrast, plastic particles primarily affect physical properties (e.g., soil porosity and aeration) [18, 56, 57], with their influence on soil biota being more indirect and comparatively slower to manifest. Consequently, the additives have a stronger, more immediate, and direct impact on soil properties such as respiration and aggregation.

In relation to these additives, total organic carbon, which measures the amount of organic compounds in the water [58], including organic pollutants, increased in water extracts from any microplastic. The heavy metals generally followed a trend of increasing concentration with smaller plastic sizes, as smaller particles have a higher surface area-to-volume ratio compared to medium and large particles. However, the copper levels were higher in the leachates from medium and large particles than from small ones. This may be due to the rougher surfaces and microcracks typically found in larger plastic pieces, which can accelerate copper release compared to smaller, more uniform pieces [59]. Such a phenomenon can be further amplified by photodegradation, as suggested by Feng et al. [60]. Nonetheless, the concentrations of heavy metals detected were below the toxicity threshold for agricultural soils [61]. This, as plastic films used in the packaging and agriculture industry are designed with substances that are difficult to extract with water, aiming to ensure human health [27, 30]. However, our results suggest that leached additives could affect soil properties, even at low concentrations. Indeed, as polypropylene and polyethylene films degraded, the formation of hydroxyl and carbonyl groups increases their water absorption capacity, facilitating the breakdown of the material [62]. This could promote the release of smaller chemical compounds including potentially toxic additives or byproducts, which are more likely to leach into the soil [21, 63]. Such leaching can have harmful effects on plant-soil systems, introducing contaminants into the soil food web and surrounding water systems, with potential impacts on a wide range of organisms.

The additives extracted from polyethylene and polypropylene had differing effects on soil properties. For instance, soil respiration was better explained by additives from PE films, while additives from PP films had no clear impact. Although PE and PP share some additives, they are designed for different purposes and thus contain distinct additives (e.g., color, with black PE film vs. transparent PP film). Additionally, since PP degrades faster, it may have released its additives before interacting with the soil [63]. Depending on the polymer type (PE or PP), plastics can also release degradation products such as NIAS (non-intentionally added substances) [64], which may further contribute to the differing effects of PE and PP on soil properties. We did not observe a clear effect of PP films on soil respiration, likely because many potentially hazardous additives were eliminated during degradation. However, we found that additives from PP films influenced soil aggregation, possibly due to chemical substances that remained or the presence of NIAS.

Our results showed that the additives from small films had higher concentrations of heavy metals and organic compounds compared to additives from larger sizes or control water. This can be attributed to a "leachable" layer at the plastic surface [38] which is more pronounced in small plastic particles due to their larger surface area. Polyethylene has been associated with metals such as Cr, Ni, Fe, and Mn, having negative effects on aquatic organisms [65–67], soil bacteria [68] and nematodes [24]. Therefore, the expected negative effects of additives on soil biota activity can explain the observed decrease in soil respiration and in soil aggregation. By contrast, the additives from small films positively affected soil aggregation in a system with plant. Polyvalent metal cations such as Fe, or Mn, another reactive metal co-occurring with Fe, which increased in additives from small plastic, may serve as cementing agents stabilizing organic matter [69] contributing to the formation of soil aggregates [70]; this, added to the plant presence whose roots help to entangle soil particles by producing mucilages and establishing mycorrhizal associations [70], promoted soil aggregation despite the negative effects of additives on this property.

Microplastic particles mostly explain variation in plant performance

In contrast, plastic particles, rather than additives, played the most important role in influencing plant biomass. This may be due to the soil substrate acting as a buffer, absorbing the additives leached from the microplastics and reducing the plant's exposure to contamination (or the compounds released were not phytotoxic). As a result, the positive effects of the plastic particles outweighed the potentially negative toxic impact of the additives. Plastic particles could enhance soil physical properties relevant for plant growth such as porosity, bulk density and aeration [18, 56, 57]. As suggested by previous research, these improvements may promote root penetration, nutrient uptake, rhizodeposition, microbial activity, and mycorrhizal associations [23, 56, 71, 72], resulting in increased root mass, and potentially greater shoot mass. The positive effects of plastic particles tend to be more evident with smaller plastics, becoming more negative as the size of the plastic (e.g., PE) increases. Large microplastic films can create water evaporation channels [18] reducing soil water content, and potentially limiting plant growth.

Chemical and physical effects of small microplastics on plant-soil systems are generally negative

Overall, the additives extracted from small microplastics had a more negative effect on soil respiration and aggregation than those from larger microplastics. Photodegradation fragments plastics into smaller pieces [21, 22], which leads to greater leaching of chemicals (additives and impurities) compared to larger plastics. The larger surface area of small plastics and specifically, the faster degradation of polypropylene may allow organic pollutants like pesticides and herbicides to adhere more readily [73], supporting the idea that smaller plastics are more toxic than larger ones [74].

Plants mitigate negative effects of additives while enhancing positive effects of plastic particles, as a function of plastic size

The positive effects of plant presence on soil respiration and aggregation can mitigate the toxicity of additives such as those from small polyethylene on soil aggregation. In fact, the negative effects of additives on bare soils became positive due to plant presence, explaining why previous research has found positive effects of microplastics on plant–soil systems (e.g., [16, 23]) despite their known toxicity [75].

Plant presence not only mitigated the negative effects of additives, but also enhanced the positive effects of plastic particles on soil aggregation. Polyethylene and polypropylene films of any size consistently exhibited a more positive effect on soil aggregation in the presence of a plant compared to bare soils. This enhancement can be attributed to roots and rhizodeposition promoting soil microbial activity and facilitating the entanglement of soil particles [70]. However, plastic particles of both polymers and any size (except small polypropylene particles), consistently exhibited a more negative effect on soil respiration in the presence of a plant. This output may be linked to nutrient competition between roots and soil microorganisms [76]. Although root–microorganism interactions are inherent in soil, plastic particles appear to exacerbate this competition, likely due to their hydrophobicity, electrostatic interaction, and other non-covalent forces [77], which enable plastic particles to absorb various organic and inorganic substances [78], potentially reducing nutrient availability in the soil. Indeed, research indicates that sediments with added microplastics exhibit higher concentrations of nutrients (C, N, P) than control sediments [79].

The combined effect of additives and plastic particles of small size mitigates their individual negative effects on soil properties and plant performance

The combined effect of additives and plastic particles largely mitigated the negative individual effects of small films on soil properties such as polyethylene negative effects on soil respiration. This likely occurred because the negative effects of additives, such as toxicity, were counterbalanced by the positive effects of plastic particles linked to the amelioration of soil physical properties such as soil porosity, bulk density, and aeration [18, 56, 57]. Similar patterns were observed in plant biomass with medium-sized polypropylene films. Although additives mostly affect plant–soil systems in a negative manner, with plastic particles having positive effects, opposite patterns could also be found depending on the polymer and plastic size. For instance, the additives can positively affect soil aggregation, as observed with small and large PE films or medium PP films, which can be potentially linked to the presence of organic substances with lower partition coefficients [21] that promote soil microbial activity [80]. These effects were potentially neutralized when plastic particles were added to the soil, as they can reduce soil water content, ultimately destabilizing soil particle aggregation.

Future directions and research opportunities

This study opens multiple opportunities for advancing our understanding of the effects of plastic particles and additives on terrestrial ecosystems. Since plastic particles have a primary effect on plant-soil systems, future research can include a broader spectrum of plastic shapes (e.g., fibers, foams, fragments) and polymer types (e.g., polystyrene, polyvinyl chloride). Exploring these effects across various plastic concentrations would allow the study of microplastic pollution gradients that better reflect real-world conditions. Likewise, studying the combined effects of multiple microplastic types acting together would provide insights into the potential for synergistic interactions, as these particles commonly coexist in natural environments.

We could have underestimated the positive effects of plastic particles, as toxic additives could have been released from the plastics during the experiment. To address this, future research might consider using plastic particles or similar inert materials that do not release additives, helping to better isolate the effects of the particles themselves from the effects of additives. While we conducted an untargeted screening of these chemical substances, detailed chemical analyses of the additives leached by each plastic type would be valuable, as the diverse chemical profiles may exert unique influences on plant–soil systems, and as these substances change during degradation and, due to their interaction with soil and water. In this regard, significant efforts should be made to broaden free access to chemical information on these substances.

Also, future research should investigate the effects of plastic particles and additives across a range of plant species, including those from different functional groups (e.g., grasses, forbs, legumes) and provenances (e.g., native, invasive). The studies could examine these effects both on individual plants and within plant communities across various soil types, which would provide a more comprehensive understanding of how microplastics influence plant–soil systems. Our findings highlight the crucial role of plants in mitigating the negative effects of microplastics on terrestrial ecosystems. However, this buffering effect can diminish if plastic pollution exceeds certain thresholds or if harmful additives are present at high concentrations. Therefore, the efforts to reduce plastic consumption are essential for protecting terrestrial ecosystems.

We should continue accounting for degraded plastic, which is the plastic that actually enters the soil. Degraded plastic has been shown to release more leachates (additives) than pristine plastic [22], significantly altering the effects of microplastics on plant-soil systems [37], our research represents an accelerated version of what happens in the field. However, under such conditions, plastic degradation could be affected by factors like precipitation, cloud cover, atmospheric moisture or the exposure to other environmental variables. Our results may still hold true in the field, but presumably at a slower rate. Thus, conducting similar studies under field conditions would also improve the ecological relevance of our findings, offering a clearer picture of how these environmental factors interact with microplastic particles and additives and their consequences on terrestrial ecosystems.

Conclusions

Microplastic additives negatively affect soil properties such as respiration and aggregation, due to their toxicity, with these effects increasing when additives are extracted from smaller plastic particles. By contrast, plastic particles appear to enhance plant biomass, as they primarily improve the physical environment for plant growth. We found that the positive effects of plastic particles can counterbalance the negative effects of additives. Then, when considering the effects of microplastics on terrestrial systems as a whole (i.e., the combined effect of additives and particles), the negative effects of additives might be masked by the positive effects of plastic particles. Our work here is important because simply reporting net positive effects of microplastics in plant–soil systems would overlook the negative effects that are also present. Additionally, our results indicate the crucial role of plants in mitigating the negative effects of microplastics on terrestrial systems.

Supplementary Information

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Supplementary Material 1.

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Author contributions
YML conceived the ideas and designed methodology with input from MCR. YML, CP, GB and YML established, maintained and harvested the experiment, and measured soil properties and plant performance parameters. YML analysed the data and wrote the manuscript. MCR commented on and edited the first draft. All authors contributed to the document and gave final approval for publication.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare no competing interests.

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