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Microplastics Can Change Soil Properties and Affect Plant Performance

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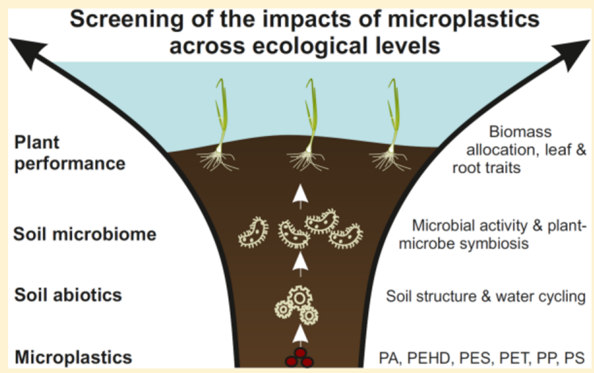
Supporting Information

ABSTRACT: Microplastics can affect biophysical properties of the soil. However, little is known about the cascade of events in fundamental levels of terrestrial ecosystems, i.e., starting with the changes in soil abiotic properties and propagating across the various components of soil−plant interactions, including soil microbial communities and plant traits. We investigated here the effects of six different microplastics (polyester fibers, polyamide beads, and four fragment types: polyethylene, polyester terephthalate, polypropylene, and polystyrene) on a broad suite of proxies for soil health and performance of spring onion (*Allium fistulosum*). Significant changes were observed in plant biomass, tissue elemental composition, root traits, and soil microbial activities. These plant and soil responses to microplastic exposure were used to propose a causal model for the mechanism of the effects. Impacts were dependent on particle type, i.e., microplastics with a shape similar to other natural soil particles elicited smaller differences from control. Changes in soil structure and water dynamics may explain the observed results in which polyester fibers and polyamide beads triggered the most pronounced impacts on plant traits and function. The findings reported here imply that the pervasive microplastic contamination in soil may have consequences for plant performance and thus for agroecosystems and terrestrial biodiversity.



INTRODUCTION

Microplastics are a diverse group of polymer-based particles (<5 mm) that have become iconic symbols of anthropogenic waste and environmental pollution.[1] Plastics are produced, used, and disposed of in terrestrial or continental systems where they interact with the biota.[2] Most of the plastic ever produced (4977 Mt) may be "environmentally available" in 2015 (i.e., in continental or aquatic systems), and this number could reach 12000 Mt by 2050,[3] with agricultural soils potentially storing more microplastic than oceanic basins.[4] A potentially important source of microplastics to soils is tire wear, but its abundance in relation to other particle types remains to be broadly determined. Notwithstanding, it is reported that microplastics in soils can reach >40000 particles kg^{-1}, with microplastic fibers as the predominant microplastic type (up to ∼92%), followed by fragments (4.1%).[5] Environmental microplastics such as fibers and fragments are secondary microplastics because they result from the disintegration or degradation of larger plastics. Their counterparts are beads and pellets (primary microplastics) manufactured for industrial and other applications. Eventually, primary microplastics might be accidentally released into the environment.[6]

There is a growing body of evidence suggesting that microplastics might cause environmental change in terrestrial systems.[2,7−10] Initial quantifications suggest that background concentrations might be as high as ∼0.002% of soil weight in Swiss natural reserves.[11] In roadside soils near industrial areas, levels ∼7% of soil weight are reported.[12] Other authors suggested that even higher contamination might occur in certain soils.[13] Such microplastic levels could affect soil chemistry, for instance, by altering the degradation of organic matter.[14] Moreover, microplastic-driven changes in soil

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properties are highly dependent on microplastic type.[15] Notwithstanding, there is a lack of empirical evidence on the potential effects of microplastic pollution on higher plants. Only one publication has reported some impacts of microplastic films on wheat growth at both vegetative and reproductive phases.[16]

In this study, we screen the potential effects of six different microplastics on a terrestrial plant−soil model. A suite of proxies of plant performance and functional traits in *Allium fistulosum* (spring onions) was analyzed. We explore changes in the soil environment caused by microplastics. Then, we analyze their effects on plant traits. We complement these analyses with an evaluation of potential functional changes on exposed plants. Finally, we propose a causal model for the observed impacts and discuss their potential implications.

■ MATERIALS AND METHODS

The test soil was a loamy sandy soil collected at the experimental facilities of Freie Universität Berlin (52°27′58" N, 13°18′10" E; Berlin, Germany) on April 4, 2017. This soil was immediately sieved to 5 mm to remove gravel and large roots and then stored at 4 °C until the beginning of the experiment. The physicochemical properties of this soil were extensively reported elsewhere (nitrogen content of ∼0.12%, carbon content of ∼1.87%, C−N ratio of ∼15.58, pH ∼ 7.1, and available phosphorus ∼ 69 mg kg^{-1}).[15,17,18]. We exposed the test soil to six microplastic types during ∼2 months and then inoculated seedlings of the spring onion *A. fistulosum* (see Supporting Information for details on the plant). The spring onions grew for an additional ∼1.5 months, after which a broad suite of proxies regarding soil and plant health were analyzed.

Microplastics. A primary microplastic and five secondary microplastics are studied here. The primary polyamide (PA) beads (Good Fellow- AM306010; Cambridge, U.K.) presented a nominal diameter of 15−20 μm.[15] Polyester (PES) fibers were obtained by manually cutting 100% polyester wool "Dolphin Baby" (product number 80313, Himalaya Co., Turkey). PES fibers had an average length of 5000 μm and an average diameter of 8 μm.[15] The other microplastics models were fabricated by cryo-milling pristine industrial pellets into microplastic fragments. For polyethylene high density (PEHD) and polypropylene (PP), the parental industrial pellets were 2−3 mm spheres. For polystyrene (PS) and polyethylene terephthalate (PET), parental material comprised 2−3 mm cylinders. The starting materials for these microplastics were obtained directly from production without significant additives or fillers. These industrial pellets were ground with a Retsch ZM 200 ultracentrifugal mill using a 2 mm ring sieve after embrittlement of pellets with liquid nitrogen. After drying, the ground materials were sieved (1 mm). PEHD fragments presented an average dimension of 643 μm, with most abundant sizes >800 μm as measured by laser diffraction. The most abundant sizes measured by laser diffraction for PET were 222−258 μm, with a median of 187 μm and 90th percentile of the largest dimension of ∼376 μm. For PP, most common sizes were between 647−754 μm, the median dimension was 624 μm and the 90th percentile was 816 μm. PS had most abundant sizes around 547−555 μm, a median of 492 μm, and a 90th percentile of 754 μm. Further images and particle size distributions as measured with microscopy for 60 of the PET, PP, and PS particles are presented in Figure S1. For practical reasons, we refer to the particle type by its polymer matrix. Disentangling the effects of the different polymers at various sizes and shapes is beyond the scope of this study.

Microplastic Addition to the Soil. Microplastics were microwaved (3 min) to minimize microbial contamination. As the plastics investigated here are mostly transparent to microwaves, their temperature did not approach melting points during microwaving.[15] A preliminary test revealed that Petri dishes with potato dextrose agar (Sigma-Aldrich, Germany) inoculated with microplastic particles after microwave did not display visible signs of microbial growth (∼2 months, ∼ 20 °C). Microplastics were then quickly added to the freshly collected soil. PES was added at 0.2% of soil fresh weight. All the other microplastics were added at 2.0% of soil fresh weight. Initial soil moisture content was ∼10.6 ± 0.3%. Our microplastic levels can be considered environmentally relevant for soils exposed to high human pressure. These levels were based on a previous experiment[15] in which noticeable changes on the soil biophysical environment were observed. Images of PET and PES particles added to the soils are presented in Figure S2A−C. The mixing of plastic and soil was performed in a glass beaker by stirring with a metal spoon ∼500 g of experimental soil during 15 min. A control treatment was included, with no plastic addition but equivalent stirring. The water holding capacity of the soils was then determined (see Supporting Information). Quantifications of plastics were not performed as there is no established methodology for extraction and measurement of microplastic concentrations in soils (of various compositions and shapes).

Exposure of Soil and Spring Onions to Microplastics. The experimental soils (200 g) were transferred to 200 mL glass beakers that were previously microwaved. These beakers with control (N = 24) and microplastic treated soil (N = 12 for each microplastic type) were covered with aluminum foil. We doubled the replicates in the control group to increase statistical accuracy and precision as all microplastic-treated samples would be compared to the controls. Beakers were then placed in the greenhouse of the Freie Universität Berlin at 21 ± 1 °C for ∼2 months (April 11−June 29, 2017). This first incubation allowed for interaction between the soil microbiome and the microplastic particles and potential leaching of plastics components. During the incubation, the experimental soils remained in the dark and water saturation was monitored ∼3 times a week to keep high moisture (i.e., brought to 90% of water holding capacity every time moisture was ∼30%). Watering was done by gently spraying distilled water on the soil surface.

At the end of the incubation period (June 29, 2017), nine seedlings originating from surface-sterilized seeds of the spring onions were introduced to half of the beakers. All beakers were kept in the greenhouse for an additional ∼1.5 months (until August 8 or 9, 2017) and watered every 2 days to 60% of water holding capacity. Thus, there were 12 replicates for control soil with plants and 12 replicates for control soil without plants (N = 12), and six replicates were available for each microplastic treatment with and without plants (N = 6).

Proxies of Soil and Plant Health. Evapotranspiration was assessed on July 25, 2017 by saturating the soils (100% of water holding capacity) with distilled water and following changes in weight over 72 h. The weight losses were converted into water loss (1 g ∼ 1 mL). Only the evapotranspiration for the third day is presented here because at that time, treatment differences were more pronounced. Although we refer to

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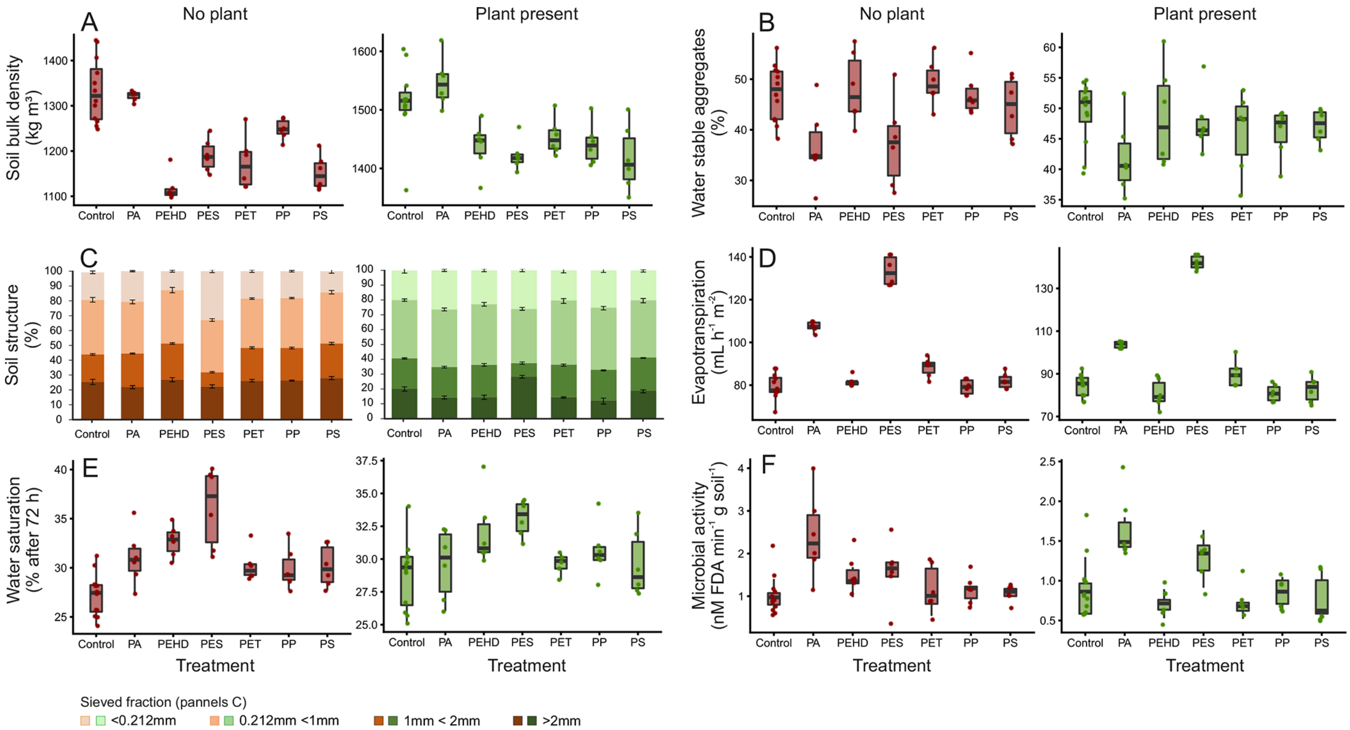

Figure 1. Effects of microplastics on soil environment and function. The presence of microplastics significantly affected soil bulk density (A), water stable aggregates (B), and soil structure (C) in bulk soils (brown) and rhizosphere (green). Such changes in soil structure significantly affected water evaporation (D), water availability (E), and soil microbial activity (F) either in the absence (brown) or presence (green) of plants. For panels A, B, and D−F, dots represent individual measured values, and boxplots display statistics (i.e., median, 25th and 75th percentile, and largest or smallest value extending from hinge up to 1.5-fold the interquartile range). For panel C, mean ± standard error (N = 6−12) is for percentage of mass of soil.

evapotranspiration throughout this manuscript, most water loss was due to evaporation. We could not disentangle evaporation from transpiration (see Supporting Information).

At harvest, the soil volume was measured to compute bulk density. Surface soil samples (0.5 g) were taken and microbial activity was assessed using hydrolysis of fluorescein diacetate (FDA)[19] with three analytical replicates and adaptations for a 96-well microplate reader (Tecan; Infinite M200, Mannedorf, Switzerland). Soil structure was assessed as reported in Machado et al.[15] Shortly, the whole soil was gently pushed through a set of stacked sieves (mesh openings of 4000, 2000, 1000, and 212 μm). After recording the weight of each sieved fraction, we reconstituted the sample and assessed water stable aggregates in a ∼ 4.0 g aliquot using a wet sieving apparatus (mesh opening of 250 μm, Eijkelkamp Co., Giesbeek, The Netherlands).

Above- and belowground plant organs were removed, and fresh weight was obtained for leaves (aerial and bulbs). Fresh roots were washed by hand with distilled water and then scanned (Epson Perfection V800 Photo scanner). The root traits of total length, average diameter, surface area, and volume were obtained with the WinRhizo TM scanner-based system (v. 2007; Regent Instruments Inc., Quebec, Canada). The dry weights of above- and belowground tissues were measured after 48 h of oven-drying at 60 °C. The difference between the fresh and dry weights of the leaf tissues was the water percentage, while the quotient between dry weight and root volume was the root tissue density. Specific root length was computed by dividing the root total length by the dry weight of the tissue. Root colonization by arbuscular mycorrhizal fungi (AMF) and non-AMF was assessed.[20] Finally, carbon and nitrogen contents in the photosynthetic leaves were analyzed with a Euro EA-CN 2 dual elemental analyzer (HEKA Tech, Wegberg, Germany). Other plant traits are derivations, e.g., root/leaf ratio.

Statistical Analyses and Causal Model. The statistics in the figures that follow (e.g., mean and quartiles in box plots) were computed with the default settings of ggplot2[21] for the software R.[22]

A Bayesian approach was adopted for statistical inference using the Stan language.[23] This is a probabilistic programming language used here to compute posterior probability functions for linear models through the Markov chain Monte Carlo methods. Stan was called through the rstan package.[23] The statistical inference was generally performed using the following 6-step pipeline. (I) For soil health proxies, the model structure was conceptually defined to test whether a particular end point could be modeled as a function of the interactive effects of microplastic treatment and plant presence. In the case of plant traits as a dependent variable, the only predictive variable was microplastic treatment. (II) A linear model was computed using the function stan_glm with normal prior and prior_intercept, QR= TRUE, and seed set to 12345. (III) The general diagnostic analyses of the linear model were assessed using the web app calling launch_shinystan. (IV) Comparisons of prior and posterior distributions with the 95% probability interval were performed with the function posterior_vs_prior. (V) The function loo was used to check whether any particular data point was too heavy on the posterior, and k_threshold was set to 0.7 whenever needed. (VI) The summary of the posterior probability distributions for the coefficients for each microplastic treatment was

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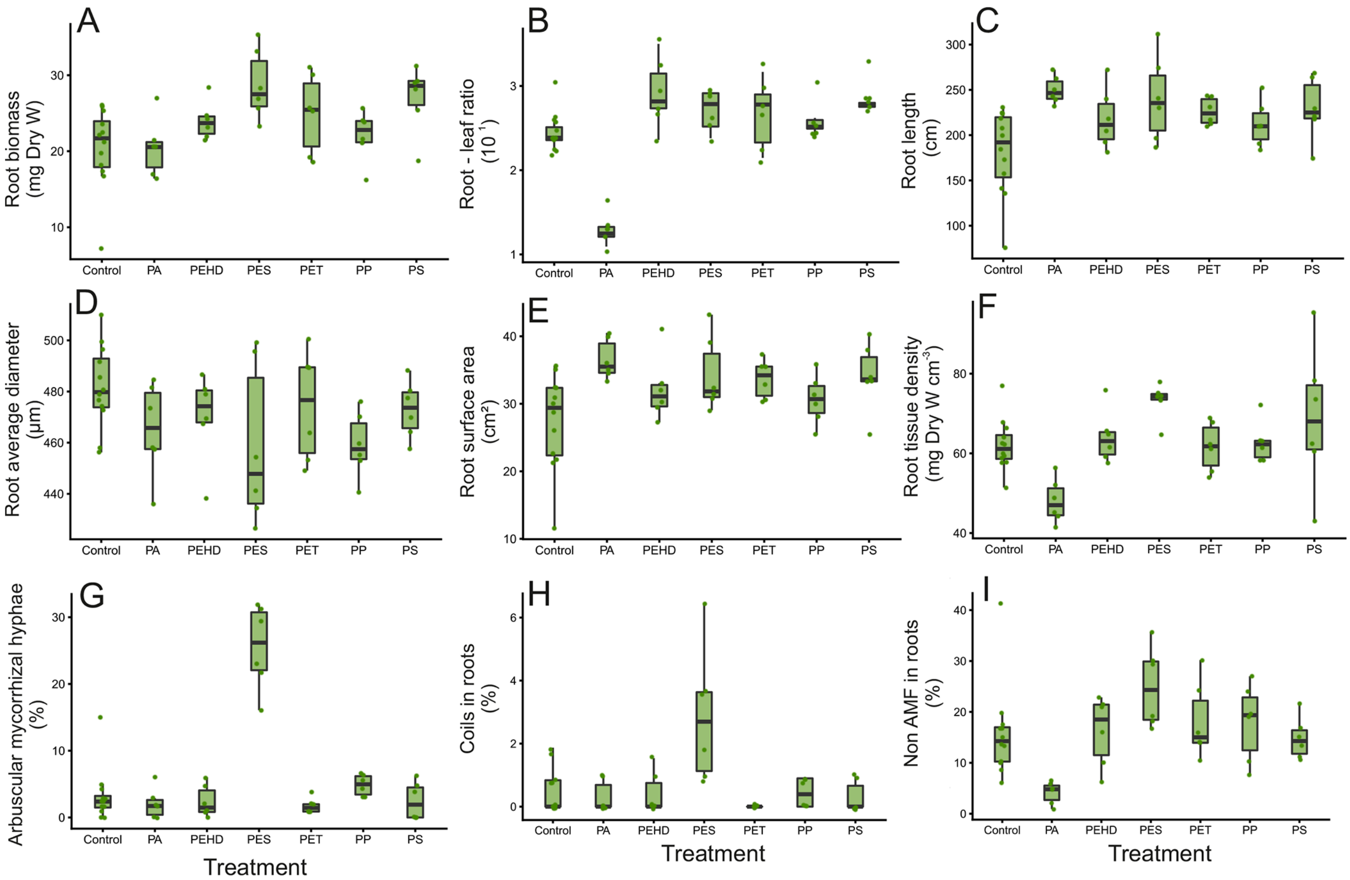

Figure 2. Effects of microplastics on root traits. The presence of microplastics significantly affected root biomass (A), root/leaf biomass ratio (B), root length (C), root diameter (D), root area (E), root tissue density (F), and root colonization by arbuscular mycorrhizal fungi (G), mycorrhizal fungal coils (H), and non arbuscular mycorrhizal fungal (non AMF) structures (I). Dots represent individual measured values, and boxplots display statistics (i.e., median, 25th and 75th percentile, and largest or smallest value extending from hinge up to 1.5-fold the interquartile range).

analyzed in the final model. We accepted as significantly affected the responses that presented posterior probability (hereafter "probability") with more than 75.0% of their density function at one side of the zero (i.e., no effect) value. In plain words, the current claims of significance are made when data support, with more than 75.0% of likelihood, that an effect (either positive or negative) exists. We also highlight probabilities higher than 97.5%, which imply that a 95.0% Bayesian confidence interval would not contain the observed mean value. For further details, please see the R scripts with all the Bayesian linear models, including model and MCMC diagnostic assessments, annotations for the reader, and inference comments that are provided as Supporting Information.

RESULTS AND DISCUSSION

Microplastics Can Change the Soil Environment. Microplastic addition resulted in altered physical soil parameters (Figure 1A−C) with consequences for water dynamics and microbial activity (Figure 1D−F). Soil bulk density was decreased by PEHD, PES, PET, PP, and PS (probability >97.5%, Figure 1A), while soil density was increased in the rhizosphere (probability >97.5%), and an interactive effect of microplastic treatments and plant presence was observed for all plastics (probability >75.0%) except PP. Concomitant decreases in water stable aggregates were significant for PA and PES (probability >97.5%) and for PS (probability >75.0%, Figure 1B). Rhizosphere presented higher water stable aggregates (probability >75.0%) and interacted with soils treated with PA, PES, PET, and PP (probability >75.0%). The soil structure was affected by all microplastic treatments with the intensity of effects depending on the microplastic type (Figure 1C), aggregate size fraction, and plant presence (probabilities >75.0%).

Evapotranspiration was increased by ∼35% by PA and ∼50% by PES (probability >97.5%), and smaller increases were associated with PEHD, PET, PS (probability >75.0%, Figure 1D). Spring onions increased the evapotranspiration (probability >97.5%, Figure 1D), and most plastics interacted with the plants to either increase (e.g., PES) or decrease (e.g., PA) evapotranspiration (probability >97.5%). Increases in evaporation were smaller than increases in water holding capacity. Therefore, the water availability was generally higher in soils treated with microplastics (probability >97.5%, Figure 1E), which was attenuated by plants (probability >97.5%). In turn, the general microbial metabolic activity was increased by PA, PEHD, and PES (probabilities >97.5%, Figure 1F) and decreased by interactive effects of plants and PA, PEHD, and PET (probabilities >75.0%).

Microplastics Can Alter Plant Root Traits. PES and PS triggered significant increases in root biomass (probability >97.5%), while a weaker effect was observed in plants exposed to PEHD, PET, and PP (probability >75.0%, Figure 2A). PA decreased the ratio between root and leaf dry biomass (Figure 2B) (probability >97.5%), while the exposure to PES, PET,

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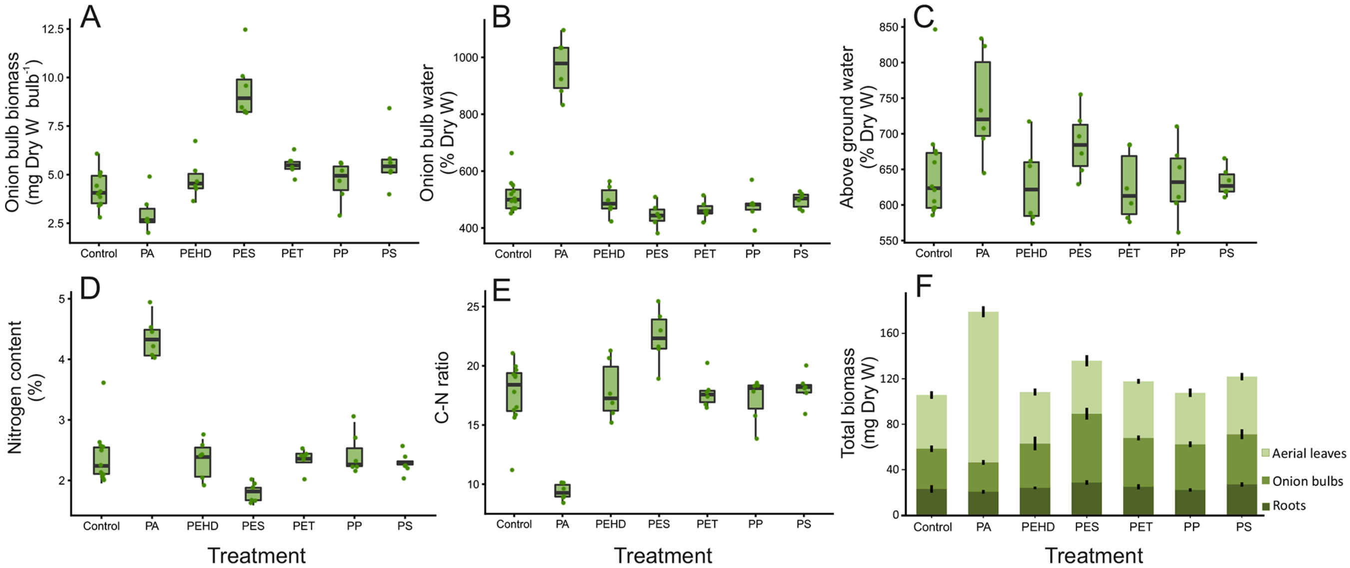

Figure 3. Effects of microplastics on proxies of general plant fitness. The presence of microplastics significantly affected biomass of onion bulb biomass (A), onion water content (B), above ground water (C), leaf composition (D, E), and total biomass allocation (F). For panels A−E, dots represent individual measured values, and boxplots display statistics (i.e., median, 25th and 75th percentile, and largest or smallest value extending from hinge up to 1.5-fold the interquartile range). Mean ± standard error (N = 6−12) are presented in panel F.

and PP significantly increased this ratio (probability >75.0%) as well as exposure to PEHD and PS (probability >97.5%). Moreover, all tested microplastics significantly increased total root length (probabilities >75.0%, Figure 2C) and decreased root average diameter (probabilities >75.0%, Figure 2D). With increased biomass of longer and finer roots, the total root area was increased by all microplastics (probabilities >75.0%, Figure 2E). PA caused a decrease on root tissue density (probability >97.5%); PES and PS triggered an increase of such response (probability >75.0%), and no significant effect was observed for PEHD, PET, and PP (Figure 2F).

Root symbioses were also affected by microplastic treatments. PES increased ∼8-fold the root colonization by AMF (probability >97.5%, Figure 2G), while PP caused an ∼1.4-fold increase (probability >75.0%), and PET caused a reduction of ∼50% in root colonization (probability >75.0%). In fact, PES triggered the strongest effect on the interaction of roots and the surrounding microbial communities as measured by the increase of mycorrhizal coils and non-AMF structures (probabilities >97.5%). With the exception of PP causing a small increase in colonization by coils (probability >75.0%) and PA decreasing non-AMF structures (probability >97.5%), other treatments had no detectable effects.

Microplastics Can Affect Plant Leaf Traits and Total Biomass. The dry biomass of onion bulbs was decreased in PA-treated plants (probability >97.5%, Figure 3A), while it nearly doubled after PES exposure (probability >97.5%, Figure 3A). In fact, all microplastic treatments were significantly different from control regarding the dry weight of onion bulbs. Likewise, the water content of onion bulbs increased 2-fold under PA exposure (probability >97.5%, Figure 3B) and decreased with PES, PET, and PP exposure (probability >75.0%). Water content of the aboveground tissue was less sensitive to microplastics, with significant increases observed only for PA and PES (probabilities >75.0%, Figure 3C). However, PA increased leaf nitrogen content, and PES significantly decreased it (probabilities >97.5%, Figure 3D). Thus, PA significantly decreased C−N ratio and PES increased it (probabilities >97.5%, Figure 3E). Total biomass was increased by PA and PES (probabilities >97.5%, Figure 3F). In the first case, the effect was driven by increases in aboveground leaf, while for the latter, there were increases in belowground bulb. It is worth mentioning that PA contains nitrogen in its composition which might be accountable for the observed effects (see the Section on the properties of plastic that affected soil and plant traits). Further increases in total biomass were observed for PET and PS (probabilities >75.0%). None of the microplastic treatments significantly decreased total biomass.

Properties of Plastic Particles That Affected Soil and Plant Traits. The PA were primary microplastic beads manufactured at relatively small sizes (15 μm) for industrial nylon production. Such virgin microplastics may contain high levels of compounds from the production process that are adsorbed to the particles or so loosely interacting with the polymer matrix that they could be easily released into soils. Particularly for PA, its effects are likely explained by the enrichment of soil nitrogen. This is supported by the nearly 2-fold increase in leaf N content (Figure 3D), an increase in total biomass (Figure 3F), and a relative decrease in the root-to-leaf ratio (Figure 2B). PA production involves polymerization of amines and carboxylic acids.[24] Thus, remaining monomers could leach into the soil causing effects analogous to fertilization. Future studies should quantify N content in soils contaminated with PA in order to provide additional evidence to this hypothesis. In this context, any further experiments including nitrogen analysis might need to consider that the PA-derived nitrogen could be released in some organic form that would be quickly metabolized by the microbiome directly on particle surfaces. Elemental or inorganic nitrogen analysis of the soils containing PA might not lead to direct information on nutrient bioavailability. Moreover, primary polymer-based pellets may contain additives (e.g., lubricants) on the surface and often organic phosphite antioxidant additives in the bulk, that are easily transformed to organic phosphates, that might break down to phosphate. The

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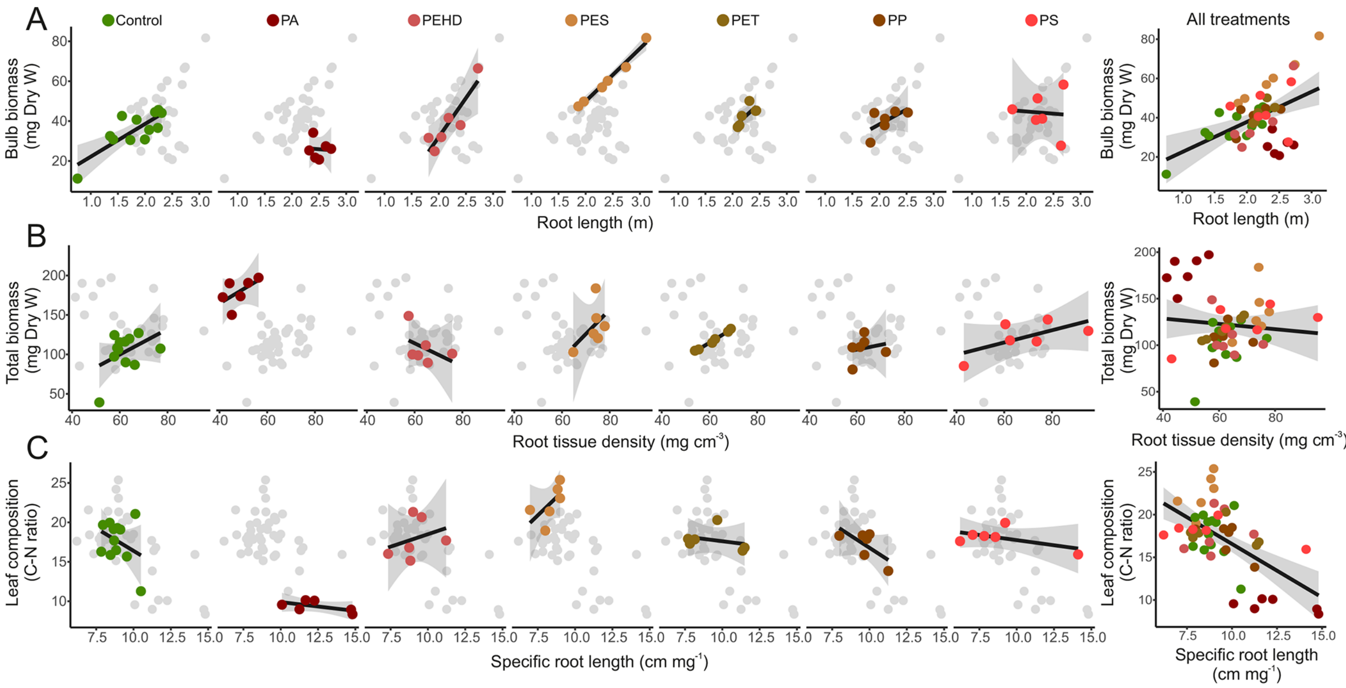

Figure 4. Microplastics' potential to alter plant functional traits. The selected allometric associations between root length and onion bulb biomass (row A), root tissue density and total biomass (row B), and specific root length and leaf composition (row C) display significant changes on slope or intercept of the relationships compared to controls. Dots represent individual measured values, black lines represent the linear regression, and gray area represents its 95% confidence interval. The slope of the positive relationship between root length and onion bulb biomass (probability >97.5%, row A-all treatments) is decreased by PA and PS (probabilities >75.0%, row A), while PEHD and PES significantly increase it (probabilities >75.0%). Likewise, the association between root tissue density and plant total biomass seemed to be affected by plastics (row B), where PA shifted the intercept (probability >75.0%), and PEHD and PP negatively influenced the slope of that interaction (probability >75.0%). Another example of a functional above-belowground link affected is in the row C. Also, with exception of PP, all the other microplastic treatments affected the relationship between specific root length and aboveground leaf composition (probabilities >75.0%). Additional relationships among other plant traits affected by the microplastic treatments are available in Figures S4 and S5.

situation may become even more complex in the case of aged polymer particles as can be expected in real world soils.

Nevertheless, many other plastic polymers contain elements that may be biogeochemically active. For instance, polyacrylonitrile and polyaramide also contain nitrogen, while polytetrafluoroethylene is rich in fluor. It is hypothesized that in the long run, even the carbon in plastic polymers might constitute a relevant pool of carbon in soils and a future selective pressure for soil microbes.[25] While the relevance of such a biogeochemical role of plastic-borne carbon is unknown, impacts on other elements were already assessed. For instance, Fuller and Gautam reported levels of polyvinyl chloride (PVC) in Australian soils that were around ∼7% of soil weight.[12] In that study, soil chlorinity correlated with plastic content near industrial areas where PVC was used.[12] Taking the environmental evidence together with our experiment, it is likely that certain microplastics could cause biogeochemical changes via leaching of components.

The PES microplastic fibers were the largest microplastics considered here. They were the plastic particles with the strongest effects on soil structure and interactions with water (Figure 1). The mechanisms of PES impacts on the soil biophysical environment in a common garden experiment without plants is discussed elsewhere.[15] The linear shape, size, and flexibility of such particles are very different from most natural components of soils and thus the likely drivers of the effects on such soil biophysical properties.[15] In turn, such effects could explain the observed changes in root structure (Figure 2) and biomass allocation (Figure 3F) of spring onions. In fact, soil bulk density, soil aggregation, and water dynamics, which were the end points affected most strongly by PES in the current experiment, are potential drivers of responses on plant traits. For instance, high soil bulk density increases penetration resistance, thus decreasing rootability. Indeed, spring onions exposed to PES had a ∼ 40% average increase in root biomass, together with an average decrease of ∼5% in root diameter.

Another interesting effect on PES-exposed plants was the change in leaf elemental composition, i.e., the altered N content (Figure 3D), C content (Figure S5), and their ratio (Figure 3E). Intraspecific changes in this particular trait are often associated with changes in plant physiological status or nutrient availability.[26] Alteration in plant physiology would be possible since PES-treated soils had substantially enhanced water holding capacity (Figure S3), kept water saturation higher for longer periods (Figure 1E), and increased water levels in aboveground plant tissues (Figure 3C). In fact, multiple physiological proxies of photosynthetic efficiency can respond to such alterations in water dynamics.[26,27] Moreover, such altered water cycling might also affect the availability of nutrients either by changing chemical speciation processes within soils or by impacting the activity of soil microbes. While we did not access chemical speciation, we observed evidence for altered microbial activity in multiple ways. Microbial activity was enhanced in PES-treated bulk soil and rhizosphere (Figure 1F), which is likely important for several biogeochemical transformations affecting nutrient fate such as mineralization or denitrification. PES treatment also significantly

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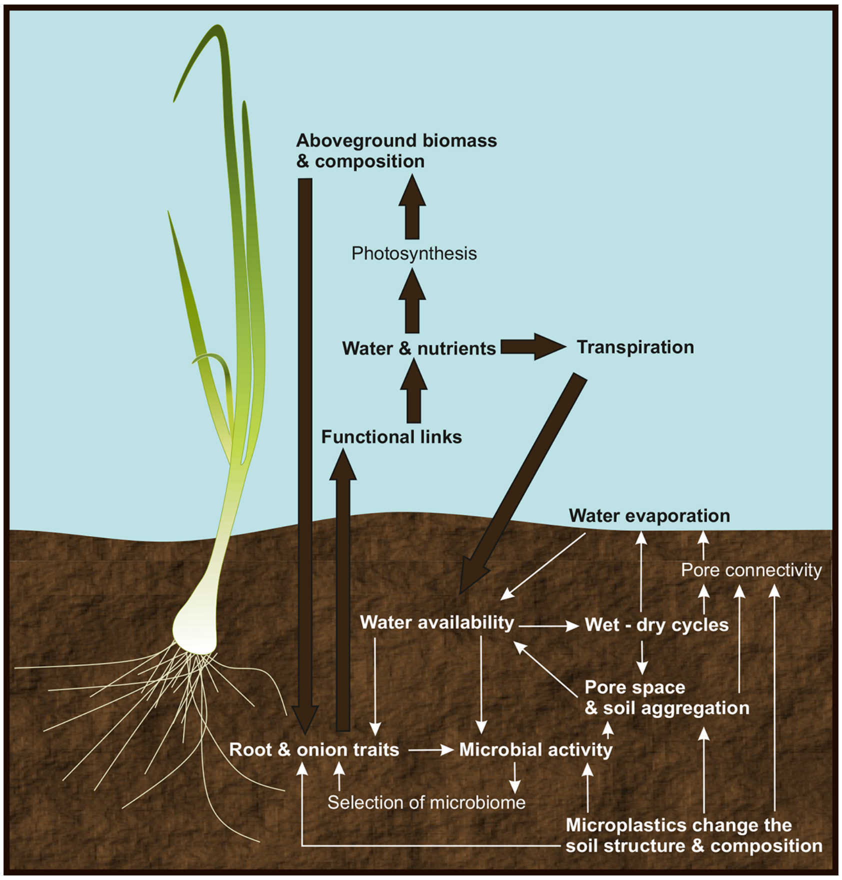

Figure 5. Causal diagram of the observed effects of microplastics on spring onions. Microplastics change the soil structure and composition triggering a cascade of shifts in the soil biophysical environment (white arrows). The microplastic particles that caused the most noticeable effects also differed most substantially in shape, size, or composition from the natural soil particles. The plants, in turn, adjust their traits to the new condition (brown arrows). Black and white fonts represent above- and belowground causal nodes, respectively. Processes and parameters in bold are supported by the collected empirical evidence in the current study. Empirical evidence remains to be investigated for microbiome selectivity, pore connectivity, and photosynthesis. A detailed explanation of this causal model is presented in the Supporting Information.

enhanced the colonization of spring onions roots by soil microbes. The abundance of AMF hyphae (Figure 2G) and their nutrient exchange structures (arbuscules Figure S5D, coils Figure 2H) inside the roots were substantially higher in PES-exposed plants. Mycorrhizal associations can increase nutrient availability and affect plant nutrient content. In fact, the mycorrhizal symbiosis often confers beneficial effects to plant growth, which might have contributed to the increase in biomass of PES-exposed plants.[28]

Compared to PES fibers, the PEHD fragments had less pronounced effects on soil structure (Figure 1C). This is likely due to the fact that PEHD particles were more similar in size and shape to the natural particles of the tested sand loamy soil (see[15]). However, PEHD still caused a substantial decrease in soil bulk density (Figure 1B) and changes in water dynamics (Figure 1D,E), which might have affected both soil microbes and spring onions. Moreover, the formula of polyethylene polymer is $(C_2H_4)_n$, implying that PEHD particles likely contained smaller amounts of elements capable of eliciting plant responses such as the nitrogen from PA beads. Therefore, a relatively limited impact on soil physics and the relative absence of nutrients likely both contributed to the less pronounced effects of PEHD fragments on soil parameters and plant traits. Some root traits responded to the decrease in bulk density (e.g., Figure 2B,C), while most of the plant traits remained unaffected.

The PET, PP, PS microplastics used here also presented size ranges and shapes more similar to the natural particles in a sandy loamy soil (Figure S1). Like PEHD, the other fragments also present a polymer matrix basically composed of carbon and hydrogen and therefore are likely less capable of triggering biogeochemical changes in the soil. As a consequence, these particles also instigated weaker effects in soil structure, water dynamics, and microbial activities. In a similar fashion as PEHD, the other fragments only substantially affected soil bulk density. Nevertheless, these changes were associated with multiple allometric responses of the above- and belowground traits of spring onions (Figure 4).

A Causal Model for the Effects of Microplastics and Its Implications for Terrestrial Systems. The current study captured the effects of six microplastic types in one particular plant–soil system. Therefore, generalizations need to be made with caution (see Supporting Information on the limitations of this assessment). Nevertheless, our results suggest multiple mechanisms of impacts of microplastics with relevant potential consequences for terrestrial systems.

The nature of the impacts discussed above can be combined with our current understanding of the fate and effects of microplastics in terrestrial systems[2,10] and with other recent empirical evidence of microplastic effects in soils[15,16,29] to propose a causal model (Figure 5). In this conceptual model, the effects of microplastics are mostly attributable to changes

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in the soil biophysical environment that affect growth and other responses of the spring onions. A detailed explanation of this causal model is presented in the Supporting Information.

The changes in the interaction of soil with water and their implications on water cycling (water holding capacity, evapotranspiration, and duration of water saturation period) are relevant for numerous processes spanning from microscale microbial activity to watershed-scale water management.[27,30,31] Similarly, the shifts in microbial activity as well as soil composition, as elicited by virgin PA beads, may have a range of biogeochemical consequences. Moreover, terrestrial plants provide the bulk of organic carbon for continental terrestrial and aquatic food webs. The fate of this organic matter in natural ecosystems depends on its quantity, quality, as well as spatial distribution.[26] In this sense, changes in plant biomass (Figure 3F), carbon/nitrogen ratios (Figure 3E), and biomass allocation (Figure 2B) are important for continental biogeochemistry and ecosystem functions.

PES is the most commonly reported type of environmental microplastic,[5,6] including its presence in sewage sludge used as an agricultural amendment. PES, as most of the other microplastics, significantly enhanced belowground biomass (onion bulbs and roots) with minor effects on aboveground biomass. Higher total biomass, which is often used as the ultimate proxy of plant fitness,[26,32] was observed as the result of many microplastic treatments. However, plastic particles with different properties (i.e., much larger, much smaller, or distinct constitution) might well trigger very different responses in soils and plants. Therefore, a generally positive effect of microplastics on plants cannot be postulated at present.

In conclusion, our findings imply that pervasive microplastic contamination may have consequences for agroecosystems and general terrestrial biodiversity. Further studies on the potential effects of microplastics with regard to other plant species, particle types, and environmental conditions are required to further unravel the full extent of terrestrial environmental change potentially triggered by this class of anthropogenic particles.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b01339.

Libraries, data importing, Bayesian statistical inference (all the Bayesian linear models, including model and MCMC diagnostic assessments, notations for the reader, and inference comments) (TXT)

Experimental results (TXT)

Figures and detailed discussion on the causal model (PDF)

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Notes

The authors declare no competing financial interest.

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