






RESEARCH ARTICLE

Effects of multiple microplastic types on growth of winter wheat and soil properties vary in different agricultural soils

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Societal Impact Statement

Winter wheat is one of the most important crops in the world. Microplastics, as an emerging pollutant, are widespread in agricultural soils due to various modern agricultural practices and can have adverse impacts on agricultural soils and plant growth. Herein, we investigated the effects of 10 types of microplastics on the properties of three agricultural soil types and the growth of winter wheat. This study contributes insights toward the conservation of agricultural soils and potential wheat yield responses to microplastic. Understanding the mechanisms that underpin the differences in responses to this pollutant class is of great importance for management recommendations.

Summary

- Microplastics (MPs) (size < 5 mm) are increasingly recognized as anthropogenic contaminants that severely affect terrestrial ecosystems. These particles are always detected as a mixture of various polymer types and shapes. However, we have limited knowledge of the effect of combined MPs on plant–soil systems.
- To address this, we selected 10 types of MP, applied to three soil types singly and in combination along an increasing gradient of 1, 2, 5, 8, and 10 MP types at a content of 0.4% (w/v). After 8 weeks of pre-incubation, winter wheat (TOBAK) was grown in each pot for another 8 weeks. Shoot and root biomass, soil aggregation, and carbon and nitrogen content were measured.
- The effects of the same MP on both soil and plant properties were drastically different (in size and effect direction) in the different soil types. However, no clear patterns were observed along an increasing number of microplastic types, suggesting that knowing the number of microplastic types in a sample, at equity of overall concentration, does not help predict effects.
- In contrast, our findings reveal the complex effects of multiple MPs on the soil–plant system and highlight that soil properties need to be taken into consideration when studying MP effects on terrestrial systems.

KEYWORDS

microplastic, multiple level, plant–soil system, soil properties, winter wheat

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1 | INTRODUCTION

Microplastics (MPs) (particle size < 5 mm) have garnered substantial interest as emerging anthropogenic contaminants owing to their ubiquitous presence across diverse environments and the potential threats they pose to all ecosystems (Rillig & Lehmann, 2020; Rillig, 2012; Huang et al., 2021). Substantial efforts have been undertaken to investigate their distribution and subsequent impacts on aquatic ecosystems, revealing that most of the aquatic ecosystems were under serious threat from the presence of MPs (Qiang & Cheng, 2021). However, growing evidence has identified that terrestrial ecosystems are the largest sink for MPs. Terrestrial ecosystems, particularly soils, are also vulnerable to MPs due to the low degradation and recycling rate of MPs in soil (Ren et al., 2022). About 79% of plastic waste is eventually deposited in terrestrial environments (Geyer et al., 2017). An estimated 359 million tons of plastic are annually transported into terrestrial ecosystems (Zhou et al., 2021). The annual accumulation of MPs in the soil is estimated to be 4–23 times larger than that in the oceans (Horton et al., 2017). Given the quantity of MPs deposited into terrestrial environments, the potential threats of MPs to soil ecosystems need to be urgently studied.

Agricultural ecosystems are a hotspot of MP accumulation in terrestrial environments due to various modern agricultural practices (Hofmann et al., 2023; Rillig & Lehmann, 2020). The main paths of macro- and micro-plastic entering agricultural soil include plastic mulch, plastic-coated fertilizers, biofertilizers (digested sewage sludge, organic waste composting, etc.), wastewater irrigation, atmospheric deposition, and others (Mahon et al., 2017). Among them, the usage of plastic mulch and organic fertilizers likely contributed most to MP pollution in agricultural soils. Agricultural soils might receive more MPs than the whole oceanic basins (Nizzetto et al., 2016). Thus, MP pollution in agroecosystems is getting increasingly severe with potential consequences for soil biophysical properties and plant performance (Fei et al., 2020; Shen et al., 2022). Existing studies in agroecosystems have yielded ambiguous and inconsistent results. MPs can influence plant growth, crop yield, nutrient utilization efficiency, and soil properties (Li et al., 2020). But size and direction of MP effects were related to MP type, shape, concentration, and size, as well as soil type, and plant species (Rillig, Lehmann, et al., 2019). Previous studies indicated that plastic mulch film and polyethylene (PE) both greatly influenced wheat growth (Liu et al., 2021); polystyrene (PS) could impact plant growth by altering the microbial metabolism and the correlation among microbes (Ren et al., 2021); polyamide (PA) increased the biomass of spring onions (Machado et al., 2019). The effect of MPs on soil properties was also highly variable. Machado et al. (2019) reported that PA fibers decreased soil aggregation, while polyester fibers had the opposite effect. Therefore, fundamental and in-depth studies on the effects of MPs in agroecosystems are necessary, especially studies taking into account the diversity of MP types and soil types.

MPs are a diverse contamination suite consisting of many types of polymers with different properties in terms of shape, size, additives, and degree of aging. Considering the various types and forms of polymers that are produced and applied, a single type of MP usually does

not occur just by itself in agricultural environments or anywhere else (Khalid et al., 2020). In soils, MPs always occur as a mixture of various polymer types and shapes (Tian et al., 2022; Wang et al., 2019). However, most studies have only focused on the effects of individual MPs on the soil or plants, in contrast to reality. Rillig, Ryo, et al. (2019) have revealed that the joint effects of a number of global change factors (up to 10 factors) could be predicted to a certain extent from knowing single-factor effects. Here, we pursue an analogous approach to that study, but with microplastic particle types to study the effect of combined MPs on the soil–plant system. Moreover, more research needs to investigate the effects of different kinds of MPs in different soil types. We thus target a significant research gap and expand knowledge about the effects of single and combined MPs on soil–plant systems in an agricultural context. Our study systematically explored the effect of ten different MP types applied individually in three different agricultural soils on soil biochemical properties and plant growth, as well as the effects of multiple combined MPs in the same soil–plant system. Our main hypotheses were that soil type matters for responses to MP, and that the diversity of MP (number of different MP particles, controlling for the overall amount of MP added) has a larger effect than single, individual MP additions.

2 | MATERIALS AND METHODS

2.1 | Soil preparation

In this experiment, we used three types of agricultural soil, which were collected from the topsoil (<30 cm) of three agricultural fields on different experimental stations across Germany. Following FAO classification, these three soil types were Albic Luvisol (soil A) (soil texture loamy sand; collected at 52° 47' N, 13° 29' E); Haplic Chernozem (soil B) (soil texture silty loam; collected at 51° 39' N, 11° 88' E); and Haplic Luvisol (soil C) (soil texture silty loam; collected at 50° 61' N, 7° 00' E). Further properties of these three soils according to Sümer et al. (2008), Altermann et al. (2005), and cka.uni-bonn.de are listed in Table S1.

All three soils were collected between November and December 2020. In each case, before use, the soil was collected in single sampling bags, sieved to 5 mm, and air-dried at 20 ± 2°C for ten days. During sieving, visible stones and organic matter residues were removed. Afterward, the soil was stored at room temperature until the beginning of the experiment. Each soil type was processed separately, and all the equipment in contact with the soil was previously sterilized with 70% ethanol to avoid cross-contamination between these three soils.

2.2 | Microplastics preparation

MP sources were chosen based on a literature search conducted in November 2020 focusing on studies reporting data on MP distribution and concentration from field surveys in agricultural environments

especially in European areas. We extracted information on the most abundant MP polymer types and shapes in various agroecosystems. Following this approach, we selected 10 types of MP encompassing three shapes and seven polymers as reported in Table S2. The three fiber MP sources were commercial primary MPs, while films and fragments were commercial materials that were manually cut to generate secondary MPs.

Different MPs were combined following the random sampling from a pool approach, as described in Rillig, Ryo, et al. (2019) and Brennan and Collins (2015). In these designs, “factors” (here are MP types) were randomly selected from a pool of “factors” to create replicates with a given number of MPs while de-emphasizing the composition and identity of the MPs. This allowed us to investigate the effects of an increasing number of MP sources in combination and to draw general conclusions about how changes in MP diversity would affect agroecosystem responses, regardless of MP identity. Then three soil types were contaminated with each of the 10 MP sources singularly or combined along an increasing gradient of 2, 5, 8, and 10 MP sources (Level 1, 2, 5, 8, and 10 MPs). All pots with MP received the same amount of MP (see below).

2.3 | Experimental design

The experiment was carried out in a climate-controlled greenhouse with a temperature of $20 \pm 2^\circ\text{C}$. We used 0.8 L (diameter = 4.8 cm; height = 44 cm) sterilized opaque pots and filled with 0.7 L of soil contaminated or not with MPs at the rate of 0.4% w/v. We approached soil contamination on a per-soil volume basis to simulate the actual condition happening in the agroecosystems where MP contamination happens on a surface basis. The three soil types used in this experiment differed in several physical–chemical properties, including bulk density, thus the contamination on a w/v basis led to a different amount of MP contamination on a weight basis among the three soil types. MPs were microwaved (2 min at 500 W) to minimize microbial contamination from the plastic material. Soil MP contamination was done separately for each experimental unit by stirring the two components (soil and MP) using the method proposed by Ingrafria et al. (2022). Briefly, the two components were mixed beforehand in a tray by hand, taking care to distribute the MP component homogeneously, and later added into a laboratory blender (Waring Blender LB20E). Soil and MPs were then gently mixed in the blender three times for 5–10 seconds. The same disturbance as the MP treatments was also applied to the control treatments.

We set up the control with 16 replicates ($n = 16$) per each soil type, 8 replicates for the treatments with a single MP contamination (10 MP identity; $n = 8$; 80 pots), and 10 replicates for the treatments with multiple MPs contamination (4 MP diversity, 2, 5, 8, and 10; $n = 10$; 40 pots); for a total of 136 pots per soil type [16 control + 80 (10 MP sources * 8 replicates) + 40 (4 MP combinations * 10 replicates)]. This design was applied for the three soil types; therefore, the whole experiment consisted of a total of 408 pots.

During the set-up of the experiment, pots were filled by replicate to avoid eventual bias due to time or other factors during operation (i.e. three replicates of each soil type for all the MP treatments were filled every day). Pots were then irrigated to field capacity water content and incubated for 8 weeks under natural light conditions (24 of March until the end of May 2021, $52^\circ 27' \text{ N}$, $13^\circ 18' \text{ E}$) in a climate-controlled greenhouse. We used this approach to allow for an interaction between MP and the surrounding soil environment. After these 8 weeks of pre-incubation, we sowed two seeds of winter wheat in each pot. One week later after all seeds were successfully germinated, we used tweezers to remove one seedling to keep one plant per pot. The tweezers were sterilized in alcohol in between pots.

During the entire experiment (incubation period and the plant growth period), irrigation was based on the water consumption of the control of each soil type, i.e., all treatments received the same amount of water as the control. Soil moisture was monitored twice a week by weighing, and the same amount of tap water, which brought the soil moisture of the control to 60% of field capacity, was added to all the experimental units of one soil type regardless of the microplastic treatments. The position of the pots was arranged in a completely randomized design, and re-randomization occurred every week. Each pot received 25 mg of N (equivalent to 138 kg/ha) as ammonium nitrate, applied in three equal events: 5 days after emergence (DAE), 10 DAE, and 15 DAE.

2.4 | Measurements

2.4.1 | Biomass

Whole plants were carefully removed from the pots. The shoot was directly cut and placed in a paper bag. For the root, after 8 weeks of incubation, the root system of winter wheat in most of the pots was so well developed that it was extremely difficult and time consuming to collect all the roots, including tiny roots scattered in the soil. So, we set a five-minute timer for each pot and collected as many roots as possible from the soil within five minutes. Even though this still caused some losses, we maintained the same processing for each pot to minimize the impact of this error. After collecting, the roots were gently washed by hand. Shoots and roots were then dried at 60°C for 72 hours, then biomass was weighed.

2.4.2 | C/N ratio

Carbon (C) and nitrogen (N) contents in the soil, root, and photosynthetic leaves were analyzed with a Euro EA-CN 2 dual elemental analyzer (HEKA Tech, Wegberg, Germany). All samples (soil, shoot, and root) were ground using a ball mill. Plant samples were collected from the biomass samples, and soil was dried under environmental temperature. Shoot and root parts were cut into small pieces before using the milling machine.

2.4.3 | Soil aggregation

Water-stable aggregate percentage was used to represent soil aggregation in this study, which was measured following a protocol by Kemper (Kemper et al., 1986). A total of 4.0 g dried sieved (2 mm) soil was placed into a 250 μm mesh sieve to be capillary rewetted with deionized water for 5 mins. Then we inserted the sieve in a sieving machine (Eijkelkamp, Netherlands) where the sieve was moved vertically for 3 mins. The fractions left in the sieve (dry matter) were dried in the oven at 60°C for 24 h and weighed. Afterward, coarse matter was extracted from dry matter and measured after drying the material in the same way. The calculation of percent water-stable aggregates (WSA) was $\% \text{ WSA} = (\text{water-stable aggregates} - \text{coarse matter}) / (4.0 \text{ g} - \text{coarse matter})$.

2.4.4 | Statistical analyses

All statistical analyses were done in R 4.3.1 (R Core Team, 2019). The effect of MP on shoot and root biomass, soil aggregation, and shoot, root, and soil carbon/nitrogen (C/N) ratio in different soil types were analyzed through linear models and multiple comparisons. First, the residuals of linear models were checked to validate assumptions of normality and homogeneity. When necessary, we implemented the function “varIdent” from the “vegan” R package to account for heterogeneity in variances. Then, we implemented the function “glht” and “Dunnett” test from the “multcomp” R package, to compare each microplastic treatment with the control (without MP). The plots were created with the graphic package “ggplot2” (Wickham, 2016).

3 | RESULTS

3.1 | Microplastic affects plant biomass

Generally, most of the treatments showed a negative influence on shoot biomass in soil A, except for treatment Level 8 and Level 10. The effect of MPs on shoot biomass tended to be neutral in soil B, while shoot biomass was promoted or unchanged (Figure 1, Table S3, Table S4, and Table S5). The influence of multiple MPs on shoot biomass in soil A showed a progressive increase with an increasing number of MPs (Figure 1C and Table S3), while no clear pattern was observed in soil B and Soil C. The effect of MPs on shoot biomass thus depended unexpectedly strongly on the soil type. In soil A and soil B, almost all polymer shapes decreased shoot biomass significantly. By contrast, all polymer shapes showed a positive influence in soil C (Figure 1A, Table S5).

Overall, all MP shapes and polymer types had a negative effect on root biomass in all three soil types (Figure 2, Table S3, Table S4, and Table S5). All single and multiple MP treatments decreased root biomass to varying degrees. The different multiple MPs did not follow any clear trend.

3.2 | Microplastic influence on plant nutrients

Among all MP shapes, only films showed a significant effect on shoot C/N in soil A (Table S3); all MP shapes decreased the shoot C/N to varying degrees in soil B, while in soil C film and fragment had a positive influence (Figure 3A). All treatments decreased the shoot C/N ratio to different degrees in soil B (Figure 3C and Table S4). No clear pattern was found in multiple MP treatments in all three soil types (Figure 3C). All MP shapes and polymer types tended to increase the root C/N in all soil types in this study, except for LDPE in soil A, and HDPE in soil B (Figure S1). No regular pattern was observed in the multiple MP treatments in any of the soil types.

3.3 | Microplastic affects soil aggregation

All the treatments except LDPE-Film decreased the WSA in soil A; all treatments had either a negative or no effect on WSA in soil B, which was exactly opposite to the performance in soil C (Figure 4, Table S3, Table S4, and Table S5). The performance of multiple MPs did not show any regularity with the increase of the MP levels in all soil types. All polymer shapes showed a negative influence both in soil A and soil B, while soil aggregation was increased by all MP shapes and types in soil C (Figure 4A).

4 | DISCUSSION

4.1 | Microplastic affects plant biomass

Numerous studies have indicated that the effect of MPs on plants can be highly variable (negative, neutral, or positive), depending on the MP size, shape, polymer type, aging time, and additives (Machado et al., 2019; Lozano & Rillig, 2020). The results of our study further strengthen the conclusion that different MP shapes and polymer types cause various influences on plant growth. However, there was no clear effect along different MP diversities (levels of different microplastic particle numbers) in this study. The impact of MPs on plant growth is generally attributed to two mechanisms: direct effects (including the toxicity of additives on MPs) or indirect effects via altering soil properties and microbial communities (Qi, Ossowicki, et al., 2020). The results of this study showed that the influence of MPs on shoot biomass showed a similar pattern to changes in soil aggregation, which can be taken as evidence of an indirect effect. In this study, the same MP shape performed inconsistently among different polymer types. This could be attributed to the different indirect effects caused by different polymer structures and different sizes. The same polymer with different shapes also showed opposite impacts. Similar results also showed that the performance of MP in soil was highly related to MP type and shape (Lehmann et al., 2020). Previous studies have shown that different MPs could influence plant growth in various ways. For instance,

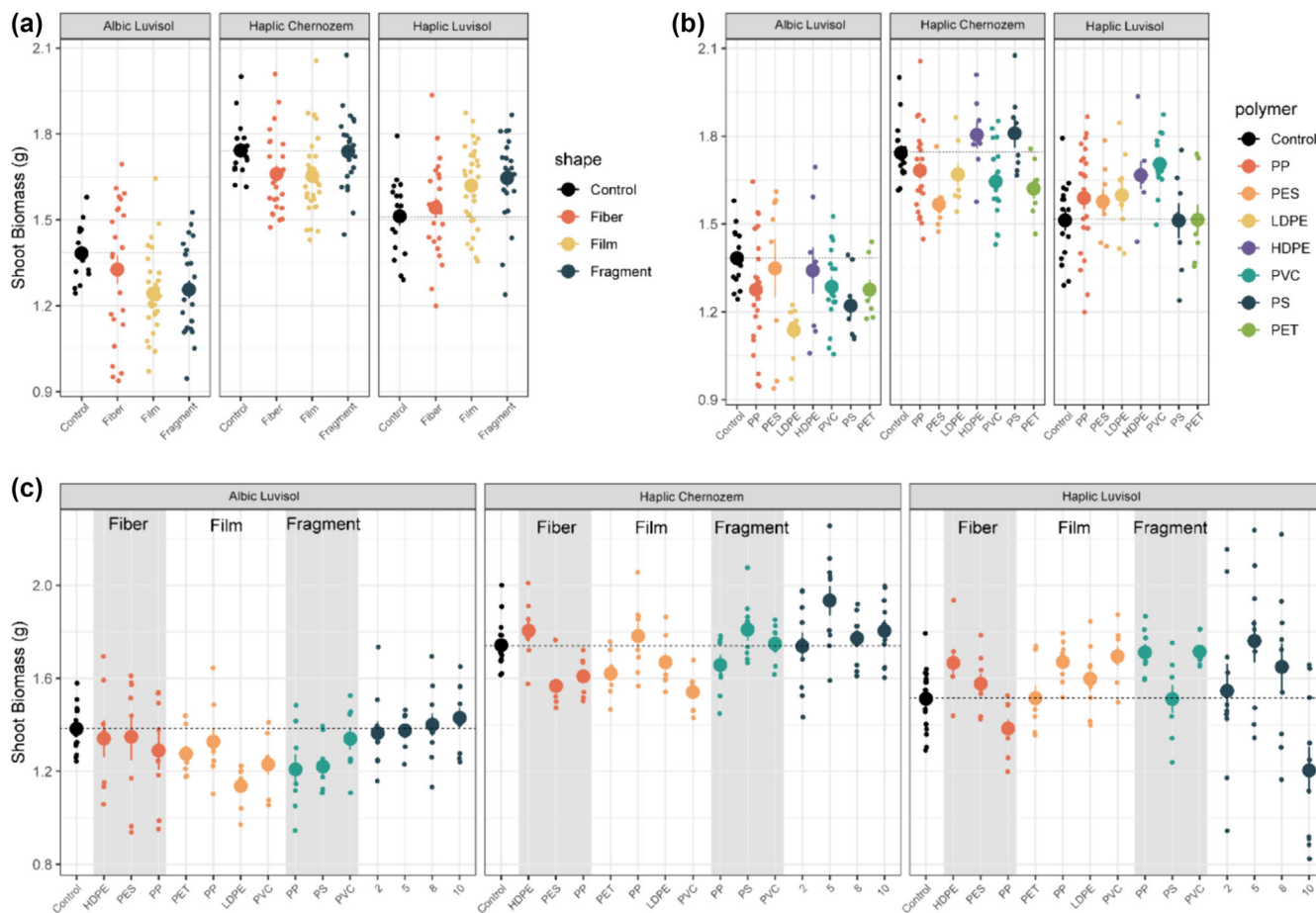


FIGURE 1 Shoot biomass response to microplastic (MP) shape (A), polymer type (B), and all individual treatments (C) in three soil types. Effect sizes and their variance are displayed as means and 95% confidence intervals. Horizontal dashed lines represent mean values of the control. Polymers: polypropylene (PP), polyester (PES), high-density polyethylene (HDPE), polyethylene terephthalate (PET), low-density polyethylene (LDPE), polyvinyl chloride (PVC), and polystyrene (PS). Levels 2, 5, 8, and 10 refer to mixtures of microplastics drawn from a pool of 10 different microplastics by following a ‘random draw from a pool’ approach (see Methods for details).

LDPE film could affect wheat biomass by altering rhizosphere bacterial community composition (Qi, Jones, et al., 2020); PVC was reported to inhibit plant growth because it could be adsorbed in the cell wall space of plant roots, affecting the absorption and transport of water and nutrients (An et al., 2021); MP fibers were reported to improve the water holding capacity and facilitate root penetration, which results in an improvement of soil water status, nutrient availability, and soil aeration so as to facilitate plant growth (Machado et al., 2018). PES fibers and PP fibers decreased shoot biomass in soil B, which was similar to the study from Ingrassia et al. (2022) who observed around a 30% decrease in maize biomass in their MP fiber treatment.

In this study, the negative effect of MPs on root biomass was obviously stronger than the effect on the aboveground part, as shown by the different decreased extent of root and shoot biomass. This may be due to the direct contact of the root with MPs, which is absent for the aboveground parts. Previous studies have also reported that MPs could cause mechanical damage to various crop roots, and reduce the root biomass of wheat, lettuce, soybean, and

corn (Gao et al., 2019; Li, Huang, et al., 2021; Li, Wang, et al., 2021). Since we did not pre-germinate the seeds in this study, the negative effect of MPs on roots could happen from the very beginning when we sowed the seeds. Exposure to MPs could cause a physical obstruction on seed pores by adhering to the seed surface, which prevents the uptake of water and nutrients, thus inhibiting seed germination - the most critical process for root development (Bosker et al., 2019; Pignattelli, Broccoli, Piccardo, Fellingine, et al., 2021; Pignattelli, Broccoli, Piccardo, Terlizzi, & Renzi, 2021). Afterward, MPs could also adhere to the root hairs of the germinated seed, which consistently influences root growth by hindering the transportation and uptake of water and nutrients and by influencing root respiration (Urbina et al., 2020). The size of MPs we used in this study was not yet at the nano level. However, MPs could have fragmented into even smaller debris after a total of 16 weeks of interaction with the soil environment. Thus, we cannot exclude the possibility of MPs being absorbed into the roots and the adverse effects that come with this. In addition, MPs could also affect root growth indirectly by altering the soil properties (Qi, Jones, et al., 2020; Qi, Ossowicki,

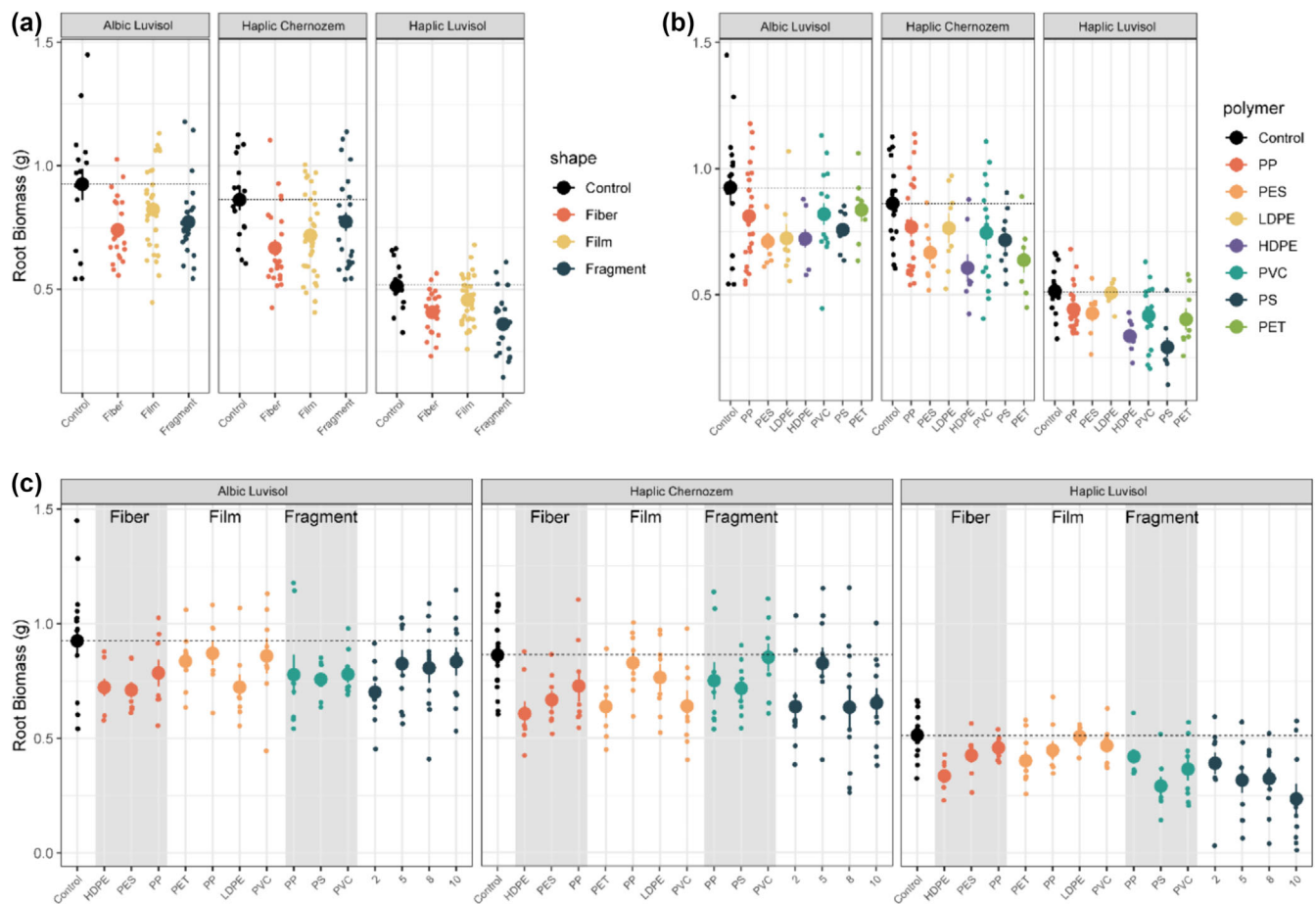


FIGURE 2 Root biomass response to microplastic (MP) shape (A), polymer type (B), and all treatments (C) in three soil types. Effect sizes and their variance are displayed as means and 95% confidence intervals. Horizontal dashed lines represent the mean values of the control. Polymers: polypropylene (PP), polyester (PES), high-density polyethylene (HDPE), polyethylene terephthalate (PET), low-density polyethylene (LDPE), polyvinyl chloride (PVC), and polystyrene (PS). Levels 2, 5, 8, and 10 refer to mixtures of microplastics drawn from a pool of 10 different microplastics by following a ‘random draw from a pool’ approach (see Methods for details).

et al., 2020). MPs could change soil aggregation, reduce the aeration and water permeability of the soil, and thereby affect plant growth (Lehmann et al., 2020). Changes in soil properties and water transport status may reduce the aeration permeability of the soil and block the effective absorption of water and nutrients by the root systems, thus affecting root growth. However, our result was also in contradiction with several previous findings, which showed an increase in the root biomass in MP-amended soil (Liu et al., 2022; Zang et al., 2020). Our study found effects on roots that differed from those of many other studies. This may be due to different reasons, for instance, soil types, plant species, germination, and different MPs. However, one key difference in our experiment was the pre-incubation period, during which we allowed the MP particles to interact with the soil prior to planting the seeds. It is likely that this was a key effect in our experiment. Given our current understanding, we cannot explain the striking dissimilarity in plant performance in the same MP treatments. Further studies are urgently required to explore the underlying mechanisms in greater depth.

4.2 | Microplastic influences plant nutrients

Nutrient cycling in soil–plant systems can be affected by MPs in profound ways, and different MPs can even have contrasting results on components of nutrient cycling (Fei et al., 2020; Liu et al., 2023). A change in plant nitrogen content can be also related to the root damage caused by MPs, thus negatively influencing N uptake and transport in plants. In this study, the effect of MPs on nutrient cycling differed among soil types. Previous studies also reported that soil type is a critical factor that needs to be taken into consideration when studying and predicting MP influence on nutrient cycling (Yan et al., 2021). The variations observed in soil physico-chemical, microbial populations, and biological parameters could potentially impact the relationship between MPs and soil components, resulting in diverse consequences (Li et al., 2023; Riveros et al., 2022). The distinctive composition of MPs has been considered a significant carbon source for soil (Rillig, 2018). Although the degradation of MPs is typically very slow, it is possible that

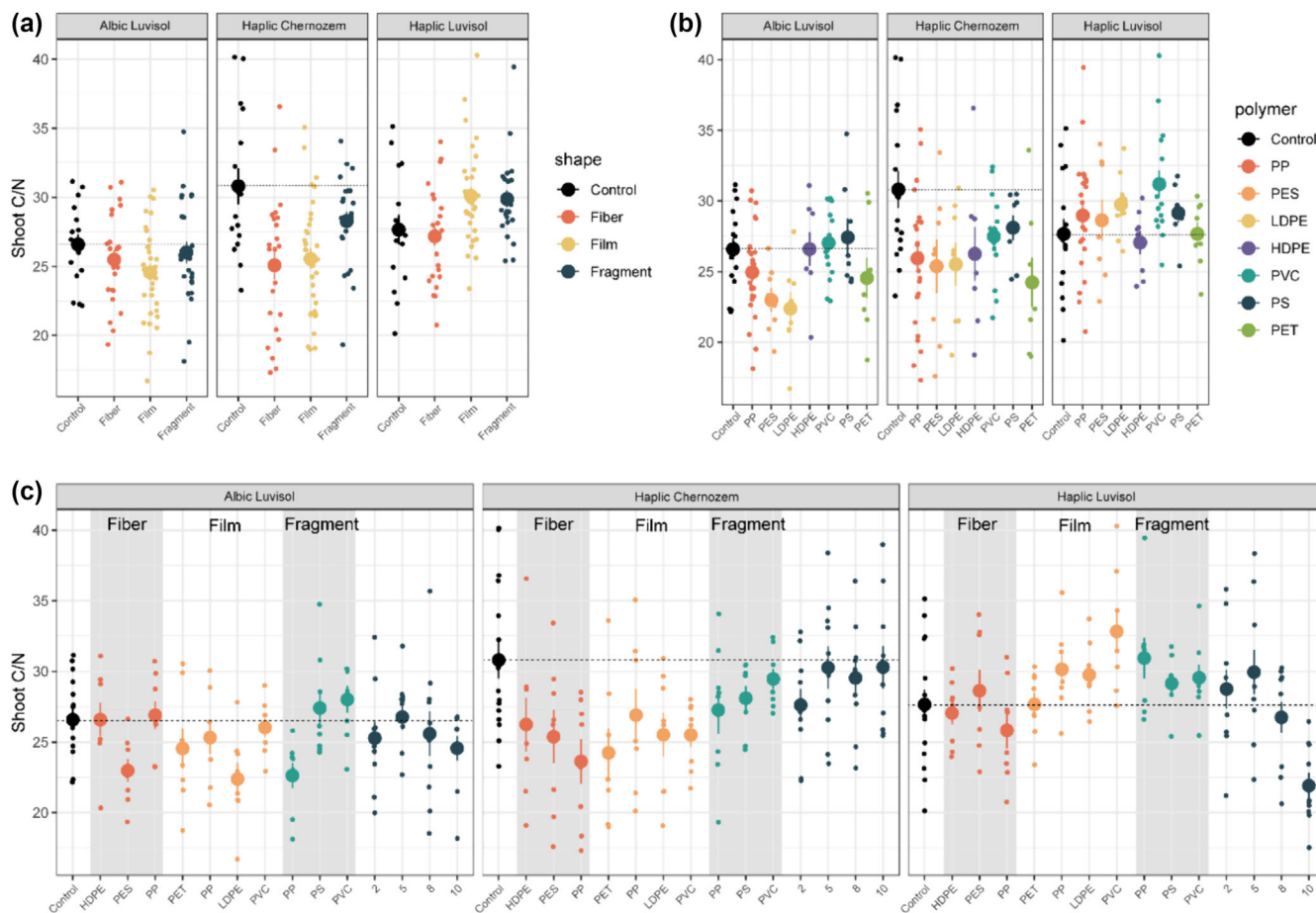


FIGURE 3 Shoot C/N ratio response to microplastic (MP) shape (A), polymer type (B), and all treatments (C) in three soil types. Effect sizes and their variance are displayed as means and 95% confidence intervals. Horizontal dashed lines represent the mean values of the control. Polymers: polypropylene (PP), polyester (PES), high-density polyethylene (HDPE), polyethylene terephthalate (PET), low-density polyethylene (LDPE), polyvinyl chloride (PVC), and polystyrene (PS). Levels 2, 5, 8, and 10 refer to mixtures of microplastics drawn from a pool of 10 different microplastics by following a ‘random draw from a pool’ approach (see Methods for details).

leachates served as a carbon source for microbes in this study, thus potentially causing microbial immobilization and thus shifts in N uptake by plants.

4.3 | Microplastic affects soil aggregation

The influence of MPs on soil aggregates in this study was highly variable, depending mainly on soil type, MP shape, and polymer type. Previous studies suggested that MPs are more likely to have a negative impact on soil aggregation (Machado et al., 2019). Once MPs are introduced into the soil, they could interfere with the formation of aggregates and decrease aggregate stability by damaging aggregates (Wan et al., 2019; Zhang et al., 2020). Soil aggregation is also strongly influenced by soil biota (Lehmann et al., 2017). A previous study demonstrated that MP could influence soil aggregates by changing the diversity of the soil bacterial community. For instance, the abundance and richness of Actinobacteria, which is one of the bacterial groups contributing the most to soil aggregation, decreased

with the presence of MP film (Zhao et al., 2021). We also observed a positive effect of MPs on soil aggregation in this study. This may be attributed to the different soil properties. Previous work also reported that MPs may increase the stability of soil aggregates by providing additional binding sites (Lozano et al., 2021). Introducing a plant could also greatly change soil aggregation responses, as root growth very likely affects the formation of soil aggregates. This is important to consider as many previous studies were conducted with soil in the absence of plants, lacking the feedback they could provide.

In conclusion, in this study, the effect of MPs on soil–plant systems was strongly dependent on the soil type, and MP type. This intriguing difference in the response of different soils to MPs is a challenge for future studies. Contrary to our expectation, we did not observe any clear patterns along an increasing number of MP types, when keeping the overall amount of MP constant. This means that, while knowing the type of MP contaminating a site is still important, we cannot draw strong conclusions from just knowing the diversity of particles found at a site to predict effects.

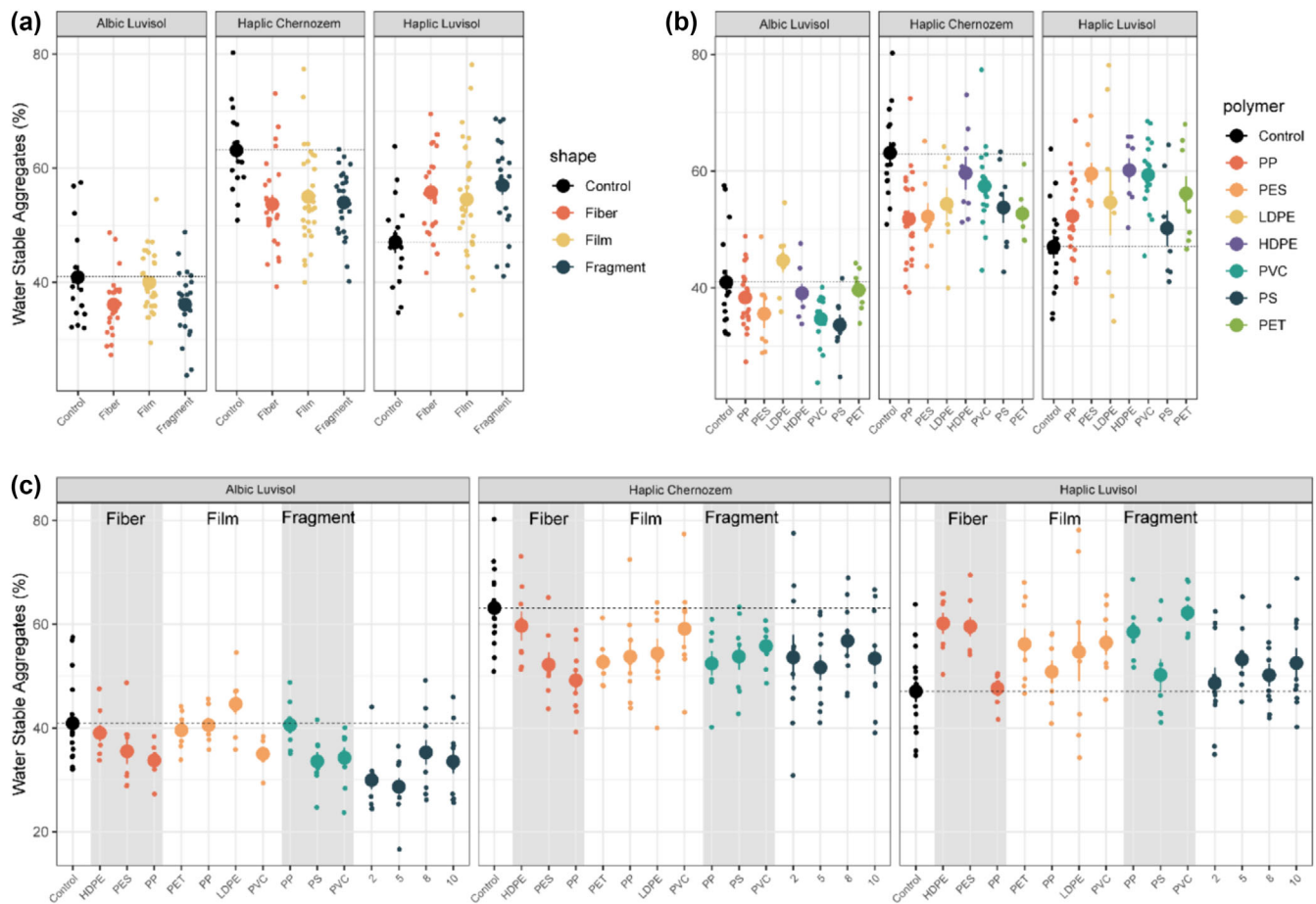


FIGURE 4 Water stable aggregation response to microplastic (MP) shape (A), polymer type (B), and all treatments (C) in three soil types. Effect sizes and their variance are displayed as means and 95% confidence intervals. Horizontal dashed lines represent the mean values of the control. Polymers: polypropylene (PP), polyester (PES), high-density polyethylene (HDPE), polyethylene terephthalate (PET), low-density polyethylene (LDPE), polyvinyl chloride (PVC), and polystyrene (PS). Levels 2, 5, 8, and 10 refer to mixtures of microplastics drawn from a pool of 10 different microplastics by following a ‘random draw from a pool’ approach (see methods for details).

AUTHOR CONTRIBUTIONS

Matthias Rillig and Rosolino Ingrassia conceived the ideas and designed experiment, with input from Nicolas Brüggemann and Michael Schloter; Hongyu Chen collected data, analyzed the data, and led the writing of the manuscript in close collaboration with Matthias Rillig. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in “figshare” at <https://doi.org/10.6084/m9.figshare.24794091.v1>. (Chen et al., 2024).

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REFERENCES

- Altermann, M., Rinklebe, J., Merbach, I., Körschens, M., Langer, U., & Hofmann, B. (2005). Chernozem-soil of the year 2005. *Journal of Plant Nutrition and Soil Science*, 168, 725–740.
- An, J., Liu, H. Y., & Zheng, Y. (2021). Effects of soil microplastics residue on soybean seedlings growth and the physiological and biochemical characteristics. *Journal of Sichuan Agricultural University*, 39(1), 41–46, 113.

- Bosker, T., Bouwman, L. J., Brun, N. R., Behrens, P., & Vijver, M. G. (2019). Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. *Chemosphere*, 226, 774–781. <https://doi.org/10.1016/j.chemosphere.2019.03.163>
- Brennan, G., & Collins, S. (2015). Growth responses of a green alga to multiple environmental drivers. *Nature Climate Change*, 5, 892–897. <https://doi.org/10.1038/nclimate2682>
- Chen, Hongyu; Ingrassia, Rosolino; Schloter, Michael; Brüggemann, Nicolas; C. Rillig Matthias (2024). Multiple microplastics soil-plant experiment. Figshare. Dataset. <https://doi.org/10.6084/m9.figshare.24794091.v1>
- Fei, Y., Huang, S., Zhang, H., Tong, Y., Wen, D., Xia, X., Wang, H., Luo, Y., & Barceló, D. (2020). Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. *Science of the Total Environment*, 707, 135634. <https://doi.org/10.1016/j.scitotenv.2019.135634>
- Gao, M., Liu, Y., & Song, Z. (2019). Effects of polyethylene microplastic on the phytotoxicity of di-n-butyl phthalate in lettuce (*Lactuca sativa* L. Var. ramosa Hort). *Chemosphere*, 237, 124482.
- Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, 3, e1700782. <https://doi.org/10.1126/sciadv.1700782>
- Hofmann, T., Ghoshal, S., & Tufenkji, N. (2023). Plastics can be used more sustainably in agriculture. *Communications Earth & Environment*, 4, 332. <https://doi.org/10.1038/s43247-023-00982-4>
- Horton, A. A., Walton, A., Spurgeon, D. J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment*, 586, 127–141. <https://doi.org/10.1016/j.scitotenv.2017.01.190>
- Huang, D., Tao, J., Cheng, M., Deng, R., Chen, S., & Yin, L. (2021). Microplastics and nanoplastics in the environment: Macroscopic transport and effects on creatures. *Journal of Hazardous Materials*, 407, 124399. <https://doi.org/10.1016/j.jhazmat.2020.124399>
- Ingrassia, R., Amato, G., Iovino, M., Rillig, M., Giambalvo, D., & Frenda, A. S. (2022). Polyester microplastic fibers in soil increase nitrogen loss via leaching and decrease plant biomass production and N uptake. *Environmental Research Letters*, 17, 054012. <https://doi.org/10.1088/1748-9326/ac652d>
- Kemper, W. D., & Rosenau, R. C. (1986). Aggregate stability and size distribution. In *Methods of Soil Analysis, Part I: Physical and Mineralogical Methods*--A. Klute (Ed.). Wiley: NJ, United States. 425–442. <https://doi.org/10.1016/s5583bookser5.1.2ed.c17>
- Khalid, N., Aqeel, M., & Noman, A. (2020). Microplastics could be a threat to plants in terrestrial systems directly or indirectly. *Environmental Pollution*, 267, 115653. <https://doi.org/10.1016/j.envpol.2020.115653>
- Lehmann, A.; Leifheit, E.F.; Gerdawischke, M.; Rillig, M.C. (2020). Microplastics have shape- and polymer-dependent effects on soil processes. bioRxiv.
- Lehmann, A., Zheng, W., & Rillig, M. C. (2017). Soil biota contributions to soil aggregation. *Nature Ecology and Evolution*, 1, 1828–1835. <https://doi.org/10.1038/s41559-017-0344-y>
- Li, B., Huang, S., Wang, H., Liu, M., Xue, S., Tang, D., Cheng, W., Fan, T., & Yang, X. (2021). Effects of plastic particles on germination and growth of soybean (*Glycine max*): A pot experiment under field condition. *Environmental Pollution*, 272, 116418. <https://doi.org/10.1016/j.envpol.2020.116418>
- Li, J., Song, Y., & Cai, Y. (2020). Focus topics on microplastics in soil: Analytical methods, occurrence, transport, and ecological risks. *Environmental Pollution*, 257, 113570. <https://doi.org/10.1016/j.envpol.2019.113570>
- Li, J., Yu, Y., & Zhang, Z. (2023). The positive effects of polypropylene and polyvinyl chloride microplastics on agricultural soil quality. *Journal of Soils and Sediments*, 23, 1304–1314. <https://doi.org/10.1007/s11368-022-03387-6>
- Li, S., Wang, T., Guo, J., Dong, Y., Wang, Z., Gong, L., & Li, X. (2021). Polystyrene microplastics disturb the redox homeostasis, carbohydrate metabolism and phytohormone regulatory network in barley. *Journal of Hazardous Materials*, 415, 125614. <https://doi.org/10.1016/j.jhazmat.2021.125614>
- Liu, S., Wang, J., Zhu, J., Wang, J., Wang, H., & Zhan, X. (2021). The joint toxicity of polyethylene microplastic and phenanthrene to wheat seedlings. *Chemosphere*, 282, 130967. <https://doi.org/10.1016/j.chemosphere.2021.130967>
- Liu, Y., Xiao, M., Shahbaz, M., Hu, Z., Zhu, Z., Lu, S., Yu, Y., Yao, H., Chen, J., & Ge, T. (2022). Microplastics in soil can increase nutrient uptake by wheat. *Journal of Hazardous Materials*, 438, 129547. <https://doi.org/10.1016/j.jhazmat.2022.129547>
- Liu, Y., Xu, F., Ding, L., Zhang, G., Bai, B., Han, Y., & Li, G. (2023). Microplastics reduce nitrogen uptake in peanut plants by damaging root cells and impairing soil nitrogen cycling. *Journal of Hazardous Materials*, 443, 130384. <https://doi.org/10.1016/j.jhazmat.2022.130384>
- Lozano, Y. M., Lehnert, T., Linck, L. T., Lehmann, A., & Rillig, M. C. (2021). Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. *Frontiers in Plant Science*, 12, 616645. <https://doi.org/10.3389/fpls.2021.616645>
- Lozano, Y. M., & Rillig, M. C. (2020). Effects of microplastic fibers and drought on plant communities. *Environmental Science & Technology*, 54, 6166–6173. <https://doi.org/10.1021/acs.est.0c01051>
- Machado, A. A. D., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rillig, M. C. (2018). Impacts of microplastics on the soil biophysical environment. *Environmental Science & Technology*, 52, 9656–9665. <https://doi.org/10.1021/acs.est.8b02212>
- Machado, A. A. D. S., Lau, C. W., Kloas, W., Bergmann, J., Bacheher, J. B., Faltin, E., Becker, R., Goerlich, A. S., & Rillig, M. C. (2019). Microplastics can change soil properties and affect plant performance. *Environmental Science & Technology*, 53, 6044–6052. <https://doi.org/10.1021/acs.est.9b01339>
- Mahon, A. M., O'Connell, B., Healy, M. G., O'Connor, I., Officer, R., Nash, R., & Morrison, L. (2017). Microplastics in sewage sludge. *Environmental Science & Technology*, 51, 810–818. <https://doi.org/10.1021/acs.est.6b04048>
- Nizzetto, L., Futter, M., & Langaas, S. (2016). Are agricultural soils dumps for microplastics of urban origin? *Environmental Science & Technology*, 50, 10777–10779. <https://doi.org/10.1021/acs.est.6b04140>
- Pignattelli, S., Broccoli, A., Piccardo, M., Felling, S., Terlizzi, A., & Renzi, M. (2021). Short-term physiological and biometrical responses of *Lepidium sativum* seedlings exposed to PET-made microplastics and acid rain. *Ecotoxicology and Environmental Safety*, 208, 111718. <https://doi.org/10.1016/j.ecoenv.2020.111718>
- Pignattelli, S., Broccoli, A., Piccardo, M., Terlizzi, A., & Renzi, M. (2021). Effects of polyethylene terephthalate (PET) microplastics and acid rain on physiology and growth of *Lepidium sativum*. *Environmental Pollution*, 282, 116997. <https://doi.org/10.1016/j.envpol.2021.116997>
- Qi, R., Jones, D. L., Li, Z., Liu, Q., & Yan, C. (2020). Behavior of microplastics and plastic film residues in the soil environment: A critical review. *Science of the Total Environment*, 703, 134722. <https://doi.org/10.1016/j.scitotenv.2019.134722>
- Qi, Y., Ossowicki, A., Yang, X., Huerta, L. E., Dini-Andreote, F., & Geissen, V. (2020). Effects of plastic mulch film residues on wheat rhizosphere and soil properties. *Journal of Hazardous Materials*, 387, 121711. <https://doi.org/10.1016/j.jhazmat.2019.121711>
- Qiang, L., & Cheng, J. (2021). Exposure to polystyrene microplastics impairs gonads of zebrafish (*Danio rerio*). *Chemosphere*, 263, 128161. <https://doi.org/10.1016/j.chemosphere.2020.128161>
- R Core Team. (2019). R: A Language and Environment for Statistical Computing. Available online at: <http://www.r-project.org>.
- Ren, X., Tang, J., Wang, L., & Liu, Q. (2021). Microplastics in soil-plant system: Effects of nano/microplastics on plant photosynthesis,

- rhizosphere microbes and soil properties in soil with different residues. *Plant and Soil*, 462, 561–576. <https://doi.org/10.1007/s11104-021-04869-1>
- Ren, X., Yin, S., Wang, L., & Tang, J. (2022). Microplastics in plant-microbes-soil system: A review on recent studies. *Science of the Total Environment*, 816, 151523. <https://doi.org/10.1016/j.scitotenv.2021.151523>
- Rillig, M. C. (2012). Microplastic in terrestrial ecosystems and the soil? *Environmental Science & Technology*, 46, 6453–6454. <https://doi.org/10.1021/es302011r>
- Rillig, M. C. (2018). Microplastic disguising as soil carbon storage. *Environmental Science & Technology*, 52(11), 6079–6080. <https://doi.org/10.1021/acs.est.8b02338>
- Rillig, M. C., & Lehmann, A. (2020). Microplastic in terrestrial ecosystems. *Science*, 368, 1430–1431. <https://doi.org/10.1126/science.abb5979>
- Rillig, M. C., Lehmann, A., Machado, A. A. D., & Yang, G. (2019). Microplastic effects on plants. *New Phytologist*, 223, 1066–1070. <https://doi.org/10.1111/nph.15794>
- Rillig, M. C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C. A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., & Yang, G. (2019). The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science*, 366, 886–890. <https://doi.org/10.1126/science.aay2832>
- Riveros, G., Urrutia, H., & Araya, J. (2022). Microplastic pollution on the soil and its consequences on the nitrogen cycle: A review. *Environmental Science and Pollution Research*, 29, 7997–8011. <https://doi.org/10.1007/s11356-021-17681-2>
- Shen, M., Song, B., Zhou, C., Almatrafi, E., Hu, T., Zeng, G., & Zhang, Y. (2022). Recent advances in impacts of microplastics on nitrogen cycling in the environment: A review. *Science of the Total Environment*, 815, 152740.
- Sümer, M. R., Zeitz, J., Ellmer, F., & Verch, G. (2008). Effects of different long-term soil management systems on some physical and chemical properties and crop production in soils in Berlin-Dahlem and Dedelow- Zalf Müncheberg (Germany). *International Meeting on Soil Fertility Land Management and Agroclimatology*, Turrkey: Anan Menderes Universitesi. 135–140.
- Tian, L., Jin, C., Ji, R., Ma, Y., & Yu, X. (2022). Microplastics in agricultural soils: Sources, effects, and their fate. *Current Opinion in Environmental Science & Health*, 25, 100311.
- Urbina, M. A., Correa, F., Aburto, F., & Ferrio, J. P. (2020). Adsorption of polyethylene microbeads and physiological effects on hydroponic maize. *Science of the Total Environment*, 741, 140216. <https://doi.org/10.1016/j.scitotenv.2020.140216>
- Wan, Y., Wu, C., Xue, Q., & Hui, X. (2019). Effects of plastic contamination on water evaporation and desiccation cracking in soil. *Science of the Total Environment*, 654, 576–582. <https://doi.org/10.1016/j.scitotenv.2018.11.123>
- Wang, T., Xie, T. L., & Xu, C. (2019). Microextractors applied in nuclear spent fuel reprocessing: Micro/mini plants and radiochemical analysis. *Critical Reviews in Environmental Science and Technology*, 49, 1–31. <https://doi.org/10.1080/10643389.2018.1530051>
- Wickham, C. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. <https://doi.org/10.1007/978-3-319-24277-4>
- Yan, Y., Chen, Z., & Zhu, F. (2021). Effect of polyvinyl chloride microplastics on bacterial community and nutrient status in two agricultural soils. *Bulletin of Environmental Contamination and Toxicology*, 107, 602–609. <https://doi.org/10.1007/s00128-020-02900-2>
- Zang, H., Zhou, J., Marshall, M. R., Chadwick, D. R., Wen, Y., & Jones, D. L. (2020). Microplastics in the agroecosystem: Are they an emerging threat to the plant-soil system? *Soil Biology and Biochemistry*, 148, 107926. <https://doi.org/10.1016/j.soilbio.2020.107926>
- Zhang, B., Yang, X., Chen, L., Chao, J., Teng, J., & Wang, Q. (2020). Microplastics in soils: A review of possible sources, analytical methods and ecological impacts. *Journal of Chemical Technology & Biotechnology*, 95, 2052–2068. <https://doi.org/10.1002/jctb.6334>
- Zhao, Z. Y., Wang, P. Y., Wang, Y. B., Zhou, R., Koskei, K., Munyasya, A. N., Liu, S. T., Wang, W., Su, Y. Z., & Xiong, Y. C. (2021). Fate of plastic film residues in agro-ecosystem and its effects on aggregate-associated soil carbon and nitrogen stocks. *Journal of Hazardous Materials*, 416, 125954. <https://doi.org/10.1016/j.jhazmat.2021.125954>
- Zhou, J., Wen, Y., Marshall, M. R., Zhao, J., Gui, H., Yang, Y., Zeng, Z., Jones, D. L., & Zang, H. (2021). Microplastics as an emerging threat to plant and soil health in agroecosystems. *Science of the Total Environment*, 787, 147444. <https://doi.org/10.1016/j.scitotenv.2021.147444>

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