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Nitrogen fertilization and arbuscular mycorrhizal fungi do not mitigate the adverse effects of soil contamination with polypropylene microfibers on maize growth☆

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ABSTRACT

Soil contamination with microplastics may adversely affect soil properties and functions and consequently crop productivity. In this study, we wanted to verify whether the adverse effects of microplastics in the soil on maize plants (*Zea mays* L.) are due to a reduction in nitrogen (N) availability and a reduced capacity to establish symbiotic relationships with arbuscular mycorrhizal (AM) fungi. To do this, we performed a pot experiment in which a clayey soil was exposed to two environmentally relevant concentrations of polypropylene (PP; one of the most used plastic materials) microfibers (0.4% and 0.8% w/w) with or without the addition of N fertilizer and with or without inoculation with AM fungi. The experiment began after the soil had been incubated at 23 °C for 5 months. Soil contamination with PP considerably reduced maize root and shoot biomass, leaf area, N uptake, and N content in tissue. The adverse effects increased with the concentration of PP in the soil. Adding N to the soil did not alleviate the detrimental effects of PP on plant growth, which suggests that other factors besides N availability played a major role. Similarly, although the presence of PP did not inhibit root colonization by AM fungi (no differences were observed for this trait between the uncontaminated and PP-contaminated soils), the addition of the fungal inoculum to the soil failed to mitigate the negative impact of PP on maize growth. Quite the opposite: mycorrhization further reduced maize root biomass accumulation. Undoubtedly, much research remains to be done to shed light on the mechanisms involved in determining plant behavior in microplastic-contaminated soils, which are most likely complex. This research is a priority given the magnitude of this contamination and its potential implications for human and environmental health.

1. Introduction

There is a growing recognition that pollution with microplastics (plastic particles smaller than 5 mm; Hartmann et al., 2019) poses a serious threat to aquatic and terrestrial ecosystems as well as human health (Karbalaei et al., 2018). It is likely that, given the continuous release of microplastic into the environment and the long time it takes for it to degrade, the problem will become more and more serious in the near future. Although public attention is mainly focused on marine environments, land areas are more contaminated with microplastics than oceans (Nizzetto et al., 2016). Microplastics are incorporated into the soil in different ways: through atmospheric deposition or the fragmentation of plastic products used in agriculture, irrigation, biosolid supply, fertilization, and so on (Crossman et al., 2020; Ren et al., 2021).

Microplastic contamination can have marked effects on the chemical-physical, structural, and hydrological characteristics of the soil (Ingraffia et al., 2022a; Qiu et al., 2022; Wang et al., 2022a), in turn altering the composition, structure, and activity of the soil microbiome and microfauna (Xu et al., 2020). However, the results of research in this field do not always agree, varying according to the characteristics of the soil, the polymer studied (type, shape, and size), the agronomic techniques applied, the duration of the experiment, and so on. With some exceptions, most studies have shown that microplastic pollution compromises plant growth directly and indirectly (Li et al., 2022; Wang et al., 2022b) by altering the characteristics of the soil and affecting soil microbes and animals. So, soil microplastic pollution affects soil fertility,

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also acting on the soil nutrient cycle and particularly on the soil nitrogen cycle (Lima et al., 2023; Seeley et al., 2020; Sun et al., 2022). This influence extends to various processes, including leaching, nitrification, denitrification, volatilization, and the alteration of enzymatic activities (Huang et al., 2023; Ingraffia et al., 2022b; Iqbal et al., 2020; Shen et al., 2022). Some authors have highlighted that changes in nitrogen availability for plants, induced by the presence of microplastics in the soil, can lead to negative impacts on plant growth (Ingraffia et al., 2022b; Liu et al., 2023a; Zhang et al., 2023).

The presence of microplastics in the soil, which affects microbial communities and activity, may affect the plant's ability to establish associative or symbiotic relationships, in particular with arbuscular mycorrhizal (AM) fungi (Leifheit et al., 2021). AM fungi play an extremely important role in terrestrial ecosystems by entangling soil aggregates and improving soil structure (Rillig and Mummey, 2006), supplying nutrients to their symbiotic host partner (Ingraffia et al., 2020), and helping plants overcome biotic and abiotic stresses (Puccio et al., 2023; Veresoglou and Rillig, 2011). Currently little is known about how microplastics in the soil affect populations of AM fungi. Recent studies have highlighted that microplastics in soil can influence the structure and diversity of the AM fungi community, exhibiting varying effects depending on polymer type and dosage (Liu et al., 2023b; Yang et al., 2021)). Moreover, de Souza Machado et al. (2019) observed varying effects of different microplastics on root colonization by AM fungi in spring onion. Polyester led to an 8-fold increase, polypropylene (PP) resulted in a 1.4-fold increase, and polyethylene terephthalate caused a 0.5-fold decrease. Similarly, in a study on winter wheat by Zang et al. (2020), it was found that polyvinyl chloride stimulated putative AM fungi, while polyethylene had no effect. The change in soil characteristics, in particular bulk density, whose reduction favors better ventilation of the soil, could favor root colonization by AM fungi (Lehmann et al., 2022). However, the presence of pollutants in the soil can reduce the number of arbuscles and spores (Wang et al., 2018; Wang, 2017; Desalme et al., 2012). The presence of microplastics could also affect AM symbiosis indirectly by changing the structure of the microbial community: penalizing or favoring the mycorrhizal helper bacteria can affect root colonization by AM fungi and their functionality (Leifheit et al., 2021). If AM fungi are affected by the presence of microplastics (either directly or indirectly), their ability to defend the plant from pathogens and help it overcome environmental stresses (salinity, drought, etc.) and nutritional stresses could be impaired. On the other hand, it is also possible that soil inoculation with AM fungi could be an effective strategy to mitigate the negative impacts of microplastics by improving nutrient availability and plant uptake, as observed Moreno-Jiménez et al. (2022). Nevertheless, this hypothesis requires further testing through additional studies.

We believe that filling in the gaps in knowledge on this topic is particularly important given current global climate change and the need to develop more sustainable and resilient agriculture and healthier food. Therefore, we designed a pot experiment to study the effects of soil contamination with different amounts of polypropylene (PP) microfibers on shoot and root growth in maize (*Zea mays* L.) plants, N uptake, and water use efficiency. We made the following hypotheses: 1) PP contamination adversely affects growth and resource use efficiency, and these adverse effects differ according to N availability; 2) the presence of PP in the soil reduces mycorrhizal colonization of roots; and 3) mycorrhization is nevertheless able to mitigate the adverse effects of PP on plant growth.

2. Materials and methods

2.1. Experimental design

The experiment was carried out at the Pietranera farm (Lima Mancuso Foundation; Santo Stefano Quisquina, AG, Italy; 37° 32′ 39.54″ N, 13° 31' 01.32" E; 162 m a.s.l.) in a wire house under a transparent plastic roof with open sides. A complete randomized factorial design with six replicates was used to study the following treatments:

1) three levels of microfiber contamination: uncontaminated control (Ctr), soil contaminated with 0.4% microfibers per dry soil (w/w; $PP_{[0.4]}$), soil contaminated with 0.8% microfibers per dry soil (w/w; $PP_{[0.8]}$);
2) AM fungal inoculation: plants grown in the presence (+Myc) or absence (–Myc) of AM fungi;
3) N fertilization: the addition of 0 or 80 mg N per pot (–N and +N, respectively).

2.2. Experimental setting and management

We used an agricultural soil (layer 0–30 cm) classified as a Typic Haploxerert (Vertisol); this soil type is widespread in Mediterranean environments with a flat or slightly sloping morphology. It is well structured, with a clay texture, good water and nutrient accessibility, a subalkaline reaction, and a fair or large presence of organic matter and other elements of fertility (phosphorous, potassium, N, etc.). Smectite (montmorillonite) is the dominant clay mineral. This soil is characterized by large, deep cracks along the profile during the dry season. It has a medium-high production potential. The soil used in this experiment had the following properties: clay 415 g kg^{-1}, silt 357 g kg^{-1}, and sand 228 g kg^{-1}; pH 7.74; total organic carbon 15.78 g kg^{-1} (Walkley–Black procedure); total N 1.54 g kg^{-1} (Kjeldhal); saturated electrical conductivity at 25 °C 1.89 dS m^{-1}; cation exchange capacity 30.0 cmol kg^{-1}.

After sampling in October 2020, the soil was air-dried, sieved at 600 μm, sterilized (three successive cycles of humidification, 24 h at room temperature, and 24 h in an oven at 130 °C), and stored at 4 °C until the start of the experiment. At sampling time, we checked to ensure that the soil was not contaminated with meso- and/or macroplastic particles; however, we did not carry out analytical procedures to assay contamination with smaller plastic particles, and therefore we cannot exclude the possibility that the control treatments might have contained detectable amounts of micro- and/or nanoplastic particles.

As regards contamination with microplastics, primary PP microfiber was used. We characterized the fibers by scanning at least 200 fibers 10 times on polyvinyl chloride trays (Epson Perfection Scan V800, 8 bit grayscale, 800 dpi). Scans were analyzed with ImageJ (ver. 1.53a; National Institutes of Health, Bethesda, MD, USA). The mean length of the fiber was 3 ± 0.4 mm, and the diameter was 21 ± 2 μm. We chose PP fiber as the contaminant because fibers are the main form of microplastic pollution in soil (Liu et al., 2018), and PP is the world's second most widely produced synthetic plastic (Geyer, 2020).

In December 2020 the microplastic was incorporated into the soil at two different concentrations (0.4% and 0.8% w/w) calculated based on the dry weight of the soil. The contamination levels were chosen based on the knowledge that microplastic concentration in soil can reach up to 7% (w: w; (Fuller and Gautam, 2016). Moreover, the contamination concentration we used is commonly applied in soil-plant studies, in which microplastic contamination created substantial variations in soil properties and plant response (Ingraffia et al., 2022b, 2022a; Lehmann et al., 2022; Lozano et al., 2021; de Souza Machado et al., 2019, 2018). We homogeneously incorporated the PP microfibers into the soil using the method proposed by Ingraffia et al. (2022b, 2022a). Briefly, we incorporated the microfibers into the soil by mixing the soil and microfibers in a laboratory blender (Waring® WSG30; Waring Commercial, Torrington, CT, USA). The microfibers were incorporated separately in each individual experimental unit. The soil and microfibers were mixed five times for 5 s each. Exactly the same disturbance was applied to the soil in the control treatment.

The experiment was carried out in polyvinyl chloride pots (72 in total) with a diameter of 5 cm and a height of 60 cm. Each pot was filled with 950 g soil not contaminated or contaminated with PP microfibers, irrigated by capillarity with distilled water until field capacity was

reached, and placed in a growth chamber in the dark at 23 ± 2 °C and 60 ± 5% relative humidity for about 5 months (incubation). During the incubation period, all pots were irrigated, again for capillarity, once a week.

At the end of April 2021, maize (cv. Iason F.1) was sown at 3 seeds per pot; pots had been previously sterilized in a solution with 3% sodium hypochlorite for 5 min. Immediately afterward all pots were irrigated to bring the substrate back to field capacity.

At sowing time, the natural microbial community of the soil, excluding AM fungi, was reintroduced in each pot. For this purpose, 4 kg unsterilized soil was diluted in distilled water (1:3 w/v); the suspension was stirred for 20 min at 140 rpm. After decanting, the suspension was filtered (through a triple 2.5 μm filter paper) to remove the natural AM fungal community. Then 100 mL filtrate was added to each pot. To the pots in the +Myc treatment was added 1 g of a commercial inoculum (Aegis Irriga, Italpollina, Rivoli Veronese, Italy) consisting of a mix of two species of AM fungi (Rhizophagus irregularis and Funneliformis mosseae), both at a density of 700 spores per gram. This commercial inoculum also contained 1 × 107 bacteria of the rhizosphere per gram of inoculum. To isolate the effects of the AM fungi, we extracted the bacterial community of the inoculum (and distributed it only in the pots of the –Myc treatment) using the same protocol used for the natural soil microbial community. We carried out the inoculation at sowing time by distributing the formulation in the top centimeters of the soil.

To each pot in the +N treatment was added a total of 86 mg ammonium sulfate with a 10% enrichment of ^{15}N isotope split equally over three dates (10, 20, and 30 days after plant emergence).

Ten days after emergence a thinning was carried out, leaving only one plant per pot. During the experiment, the plants were kept optimally watered, irrigated when the available water reached 70% of the maximum. We calculated the amount of water needed to bring the substrate back to field capacity by monitoring the change in pot weight (two measurements each week).

2.3. Measurements

At 50 days after emergence (when the maize plants had reached the stage of the sixth to seventh leaf), the shoot biomass of each pot was collected, separated into botanical fractions (leaves, culms, dry and senescent tissue), and weighed. The leaf surface was determined with a leaf area meter (LI-3100C; LiCOR, Lincoln, NE, USA). Each fraction was then dried in an oven at 40 °C (to constant weight) to determine the dry matter. The root biomass was carefully extracted by sieving and washing and then oven-dried at 40 °C to a constant weight.

For all plants, we assessed mycorrhizal status by visualizing mycorrhizal colonization. Root samples were taken from each plant; cleaned with successive treatments with KOH 10%, H_2O_2 10 vol, and HCl 10%; and stained with acid fuchsin (0.01%) in lactophenol, according to the technique of Phillips and Hayman (1970), partially modified as reported in Miceli et al. (2016). Excess dye was removed from the roots by immersion in clear lactophenol (25 mL distilled water, 25 mL glycerin, 25 mL lactic acid, 25 g phenol crystals) for 24 h. Mycorrhizal colonization (the percentage of stained tissue, with respect to the hyaline portion, on the unit length of root) was determined under a stereoscopic microscope (30×; Zeiss, Oberkochen, Germany) for 10 root fragments per plant, then averaged, referring to a total root length of about 30 cm (Vierheilig et al., 2005; Rajapakse and Miller, 1992; Kormanik and McGraw, 1982). Infected roots were dissected manually and root sections were mounted with a drop of lactophenol. AM fungi structures were observed under a light microscope (Axioskop; Zeiss) coupled to an AxioCam MRc5 (Zeiss) digital camera. Images were captured with AxioVision 4.6 (Zeiss).

Both shoot and root biomass fractions were ground to a fine powder (using a Qiagen TissueLyser II), gathered into a single sample (mixing 30% of the total shoot weight and 30% of the total root weight), and analyzed for the concentration of total N with the Dumas method (DuMaster D-480; Büchi Labortechnik, Flawil, Switzerland). Samples from the +N treatments were also analyzed for ^{15}N content with an elemental analyzer (NA1500; Carlo Erba, Milan, Italy) paired with a mass spectrophotometer (Isoprime, Cheadle, UK).

We obtained the total N uptake by multiplying the N content of the biomass by the amount of biomass in each pot. The ^{15}N concentration was used to determine the amount (^{15}Nrec) and percentage (%^{15}Nrec) of N recovered from the fertilizer with Equations (1) and (2), respectively:

$$^{15}Nrec = N_t \times \frac{atom\%\ ^{15}Nfp\ excess}{atom\%\ ^{15}Nfert\ excess} \quad (1)$$

$$\%^{15}Nrec = \frac{^{15}Nrec}{f} \times 100 \quad (2)$$

where N_t is N content (g pot^{-1}) in the biomass, atom% ^{15}Nfp excess is the ^{15}N isotopic excess (atom% ^{15}N−0.3663) in the fertilized plant, atom% ^{15}Nfert is the ^{15}N isotopic excess in the fertilizer, and f is the amount of fertilizer (g pot^{-1}).

Total biomass production (shoots and roots) and total water consumption ($water_{cons}$) were used to calculate water use efficiency (WUE) as follows:

$$WUE = \frac{biomass}{water_{cons}} \quad (3)$$

The $water_{cons}$ was calculated as the sum of all water applied during the experiment.

2.4. Statistical analysis

The data were analyzed in R (R Core Team, 2022) according to the experimental design. A three-way ANOVA was used to examine the effects of the applied treatments, and of their interaction. Model residuals were checked for heteroscedasticity and a normal distribution.

We compared all response variables between the groups ($PP_{[0.4]}$ or $PP_{[0.8]}$ vs. Ctr; +Myc vs. –Myc; +N vs. –N) using the "dabestr" package (Ho et al., 2019) to calculate effect sizes as unpaired mean differences and generate bias-corrected and accelerated bootstrapped 95% confidence intervals. The same procedure was used to estimate the effect of mycorrhizal inoculation and N fertilization within each microplastic treatment. We used this combined approach given the increasing recognition of the limitations of using only p values and avoiding dichotomous cutoffs (Ho et al., 2019; Wasserstein and Lazar, 2020).

We generated graphical representations of data using the "dabestr" and "tidyverse" (Wickham et al., 2019) packages.

3. Results

Table S1 shows the results of the ANOVA for the effects of the applied treatments and their interactions on maize growth and N parameters.

3.1. AM root colonization

Characteristic structures of AM fungi were observed in the roots of the inoculated (+Myc) plants (Fig. 1). The root colonization of AM fungi in the uninoculated treatments (–Myc) was negligible (on average, 1.2% of the root length was colonized). The percentage of colonization in the inoculated treatments was, on average, 13.4% without appreciable differences due to treatment (microplastic contamination or N fertilization; Fig. S1).

3.2. Effects of soil contamination with PP microfibers

On average, contamination with PP microfibers, even at the lower concentration (0.4%), induced marked decreases in leaf area (−18% compared to the uncontaminated control; Fig. 2A), shoot biomass (−11%; Fig. 3A), and root biomass (−20%; Fig. 4A). However, the

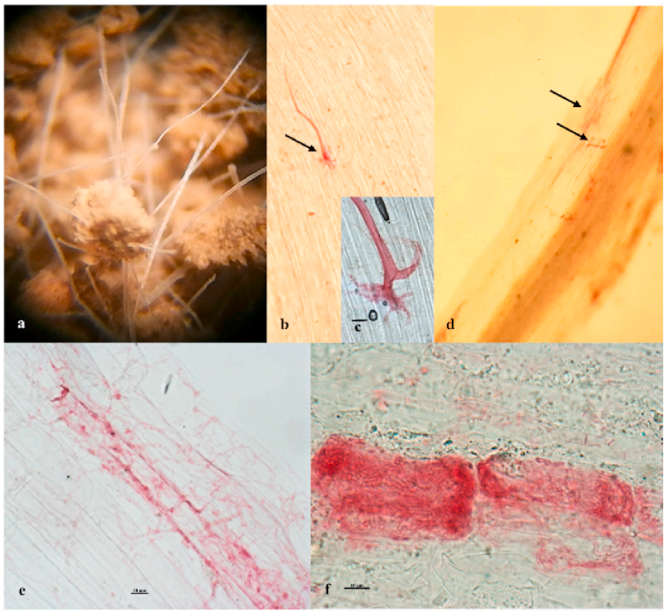

Fig. 1. (a) Polypropylene microfibers in soil aggregates. The development of an arbuscular mycorrhizal (AM) mycelium in maize roots (in fuchsia): (b) beginning of colonization by an extramatrical hypha (arrow, 30 ×) and (c) detail of the infection point (bar = 50 μm), (d) early stages of AM infection in the maize root cortex (arrows, 20 ×), (e) intercellular and intracellular AM fungal growth, (f) intracellular arbuscles in different stages of growth.

presence of microfibers resulted in an increase in the shoot/root ratio (+11%; Fig. 5A). Furthermore, PP contamination at the lower concentration (0.4%) resulted in reductions, compared to the control, in N content (−8%; Fig. 6A), N uptake (−22%; Fig. 7A), and fertilizer ^{15}N recovery (−21%; Fig. 8A). Water consumption (Table S2) was proportional to the accumulation of shoot biomass, and therefore no effect on water use efficiency was observed (Fig. 9A). Increasing the concentration of microplastic in the soil to 0.8% generally resulted in further small decreases in shoot biomass, N content, and uptake compared to the lower concentration of contamination. In contrast, root biomass and ^{15}N fertilizer recovery increased slightly as the concentration of microplastic increased from 0.4% to 0.8% (Figs. 4A and 8A).

3.3. Effects of soil inoculation with AM fungi

Mycorrhizal inoculation, on average, adversely affected shoot biomass (−7%), N content (1.33% in –Myc and 1.21% in +Myc), and N uptake (−14%; Figs. 2B, 6B and 7B). These adverse effects were more pronounced in soils contaminated with microplastics (Figs. 2D, 6D and 7D). Mycorrhization markedly reduced shoot and root biomass in the treatment contaminated with PP at the 0.4% concentration (−12% and −11%, respectively) but had much milder effects in the uncontaminated control (Figs. 3D–4D).

In the absence of mycorrhizal inoculum, ^{15}N fertilizer recovery did not differ significantly by PP contamination, but in the presence of AM fungi, lower values were observed at the lower PP concentration (Fig. 8C).

3.4. Effects of N fertilization

N fertilization, on average, had positive effects on leaf area (+10% compared to the unfertilized treatment), shoot biomass (+13%), and the shoot/root ratio (+16%; Figs. 2C, 3C and 5C). N fertilization had no effect on root biomass, N content, N uptake, and water use efficiency (Figs. 4C, 6C and 7C, and 9C).

Increased N availability due to N fertilization had only a slight effect, mitigating the adverse effect of the PP microfibers on the maize plants. Indeed, the increases in shoot biomass and the shoot/root ratio due to N fertilization were more consistent in soils contaminated with PP compared to the control (Figs. 3E–5E), whereas the opposite was observed for N uptake (Fig. 6E).

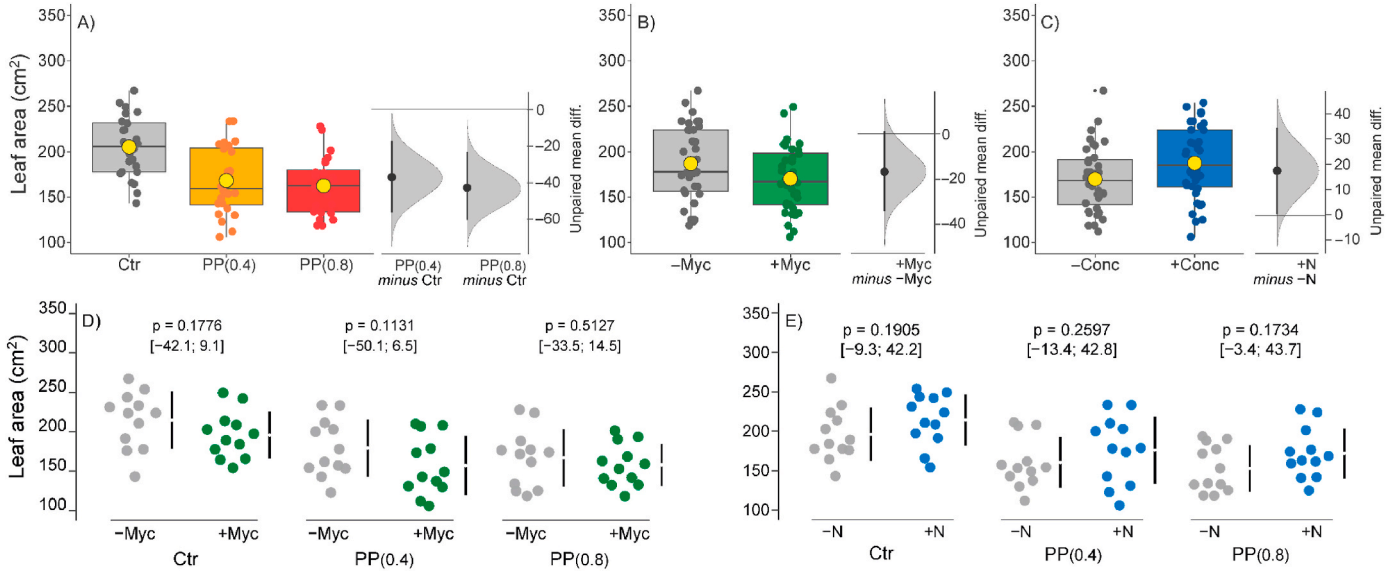

Fig. 2. Leaf area (cm^2). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A), mycorrhizal inoculation (B), nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
Ctr, uncontaminated control; PP(0.4) and PP(0.8), PP contamination at 0.4% and 0.8% (w/w), respectively; –Myc, uninoculated control; +Myc, inoculation with arbuscular mycorrhizal fungi; –N, unfertilized treatment; +N, N fertilization.

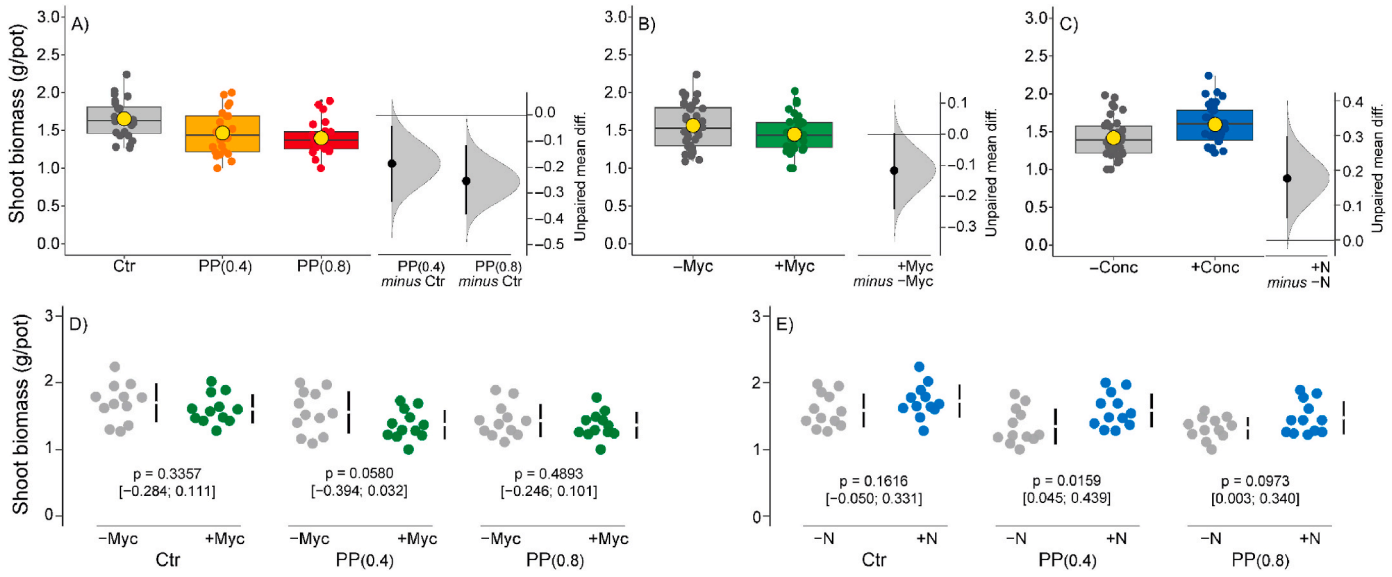

Fig. 3. Shoot biomass (g/pot). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B); nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
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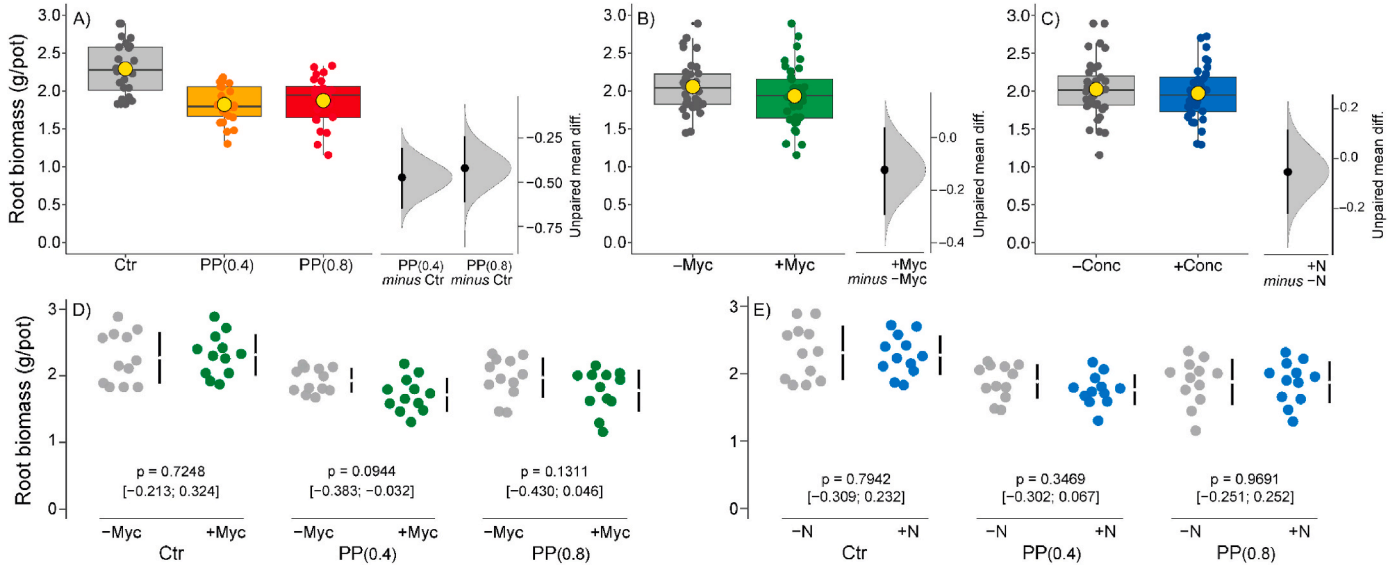

Fig. 4. Root biomass (g/pot). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B); nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
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4. Discussion

Data from this study clearly show that soil contamination with PP fiber drastically reduces the shoot and root growth of maize, its leaf area, the N content in tissue, and N uptake capacity. In general, adverse effects increased as the level of PP contamination increased. The effects of microplastic in soil on higher plants may vary widely, as reviewed by Li et al. (2022) and Wang et al. (2022b). This is not surprising, because the presence of microplastic in the soil can lead to different changes in the chemical-physical characteristics and hydrological parameters of soil depending on the initial characteristics of the substrate (Ingraffia et al., 2022a). Some authors, working on onion and carrot, have observed in sandy soils (sandy loam or loamy sand) significant increases in phytomass due to contamination with microplastic fibers of different polymers

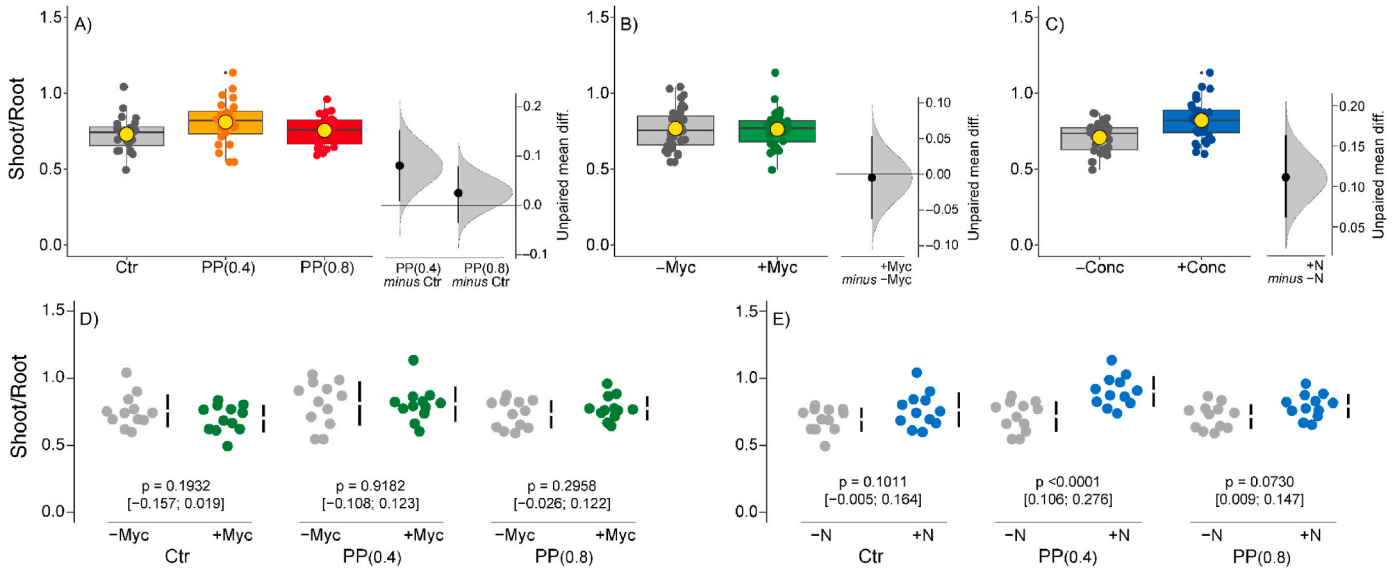

Fig. 5. Shoot/Root ratio. The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B); nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
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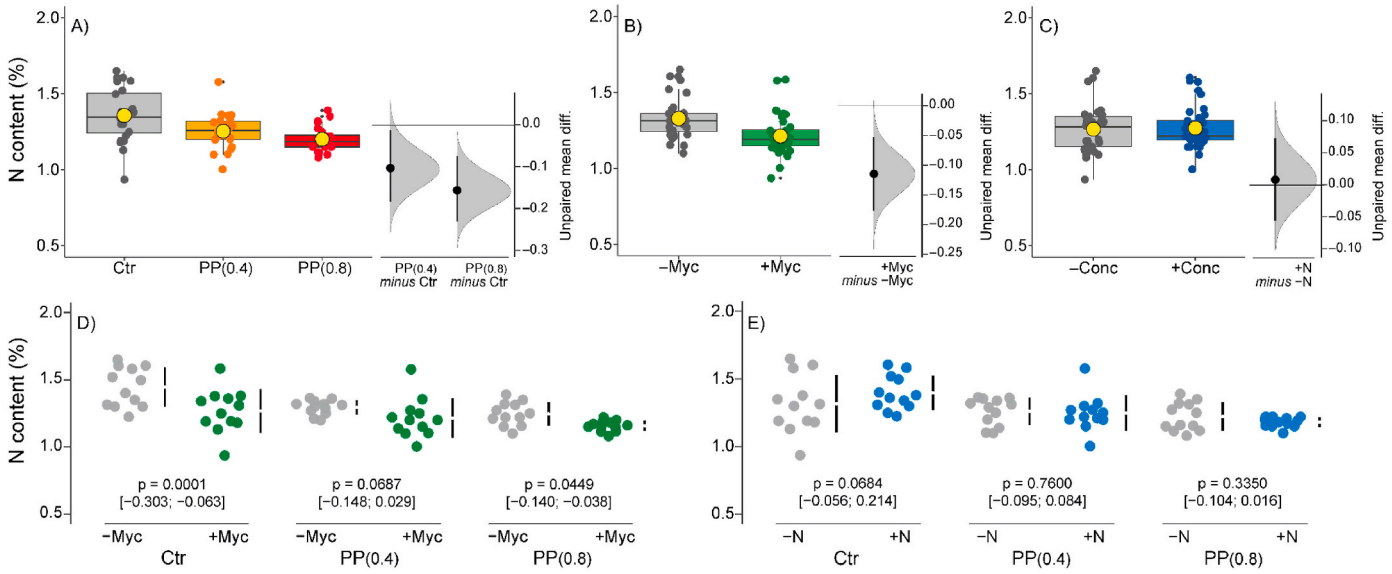

Fig. 6. Nitrogen content (%). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B); nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
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(polyester, polyethylene, PP, etc.) and have attributed them to a reduction in soil bulk density and a concomitant increase in water retention capacity (Lehmann et al., 2022; Lozano et al., 2021; de Souza Machado et al., 2018). These factors can facilitate root growth and improve water and nutrient supply to the plant. In the present study, the effects of microplastic contamination on soil physical and hydrological parameters were not evaluated. However, in previous research by Ingraffia et al. (2022a), which used the same soil as the present experiment (clay soil), only minor effects of microplastic contamination were observed on the apparent density and amount of water potentially available to plants. Therefore, we believe that the decreases in biomass observed in the contaminated treatments in this experiment are not attributable to the effects of microplastic on physical, structural, or hydrological characteristics of the soil.

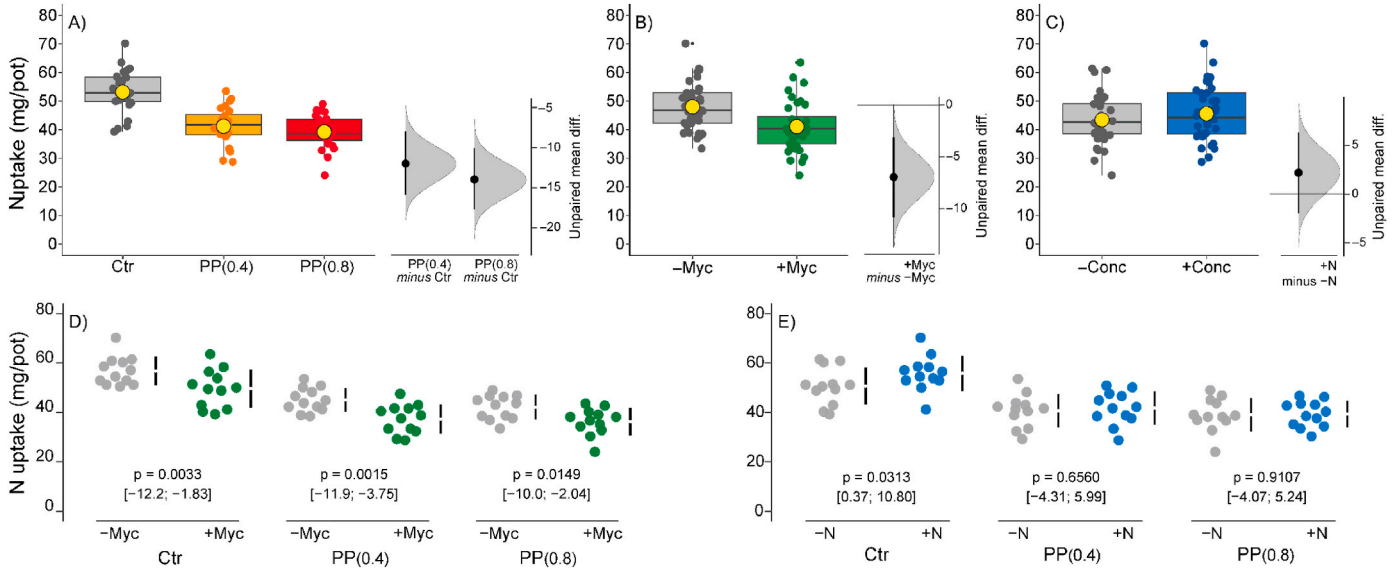

Fig. 7. Nitrogen uptake (mg/pot). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B); nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
Ctr, uncontaminated control; PP(0.4) and PP(0.8), PP contamination at 0.4% and 0.8% (w/w), respectively; –Myc, uninoculated control; +Myc, inoculation with arbuscular mycorrhizal fungi; –N, unfertilized treatment; +N, N fertilization.

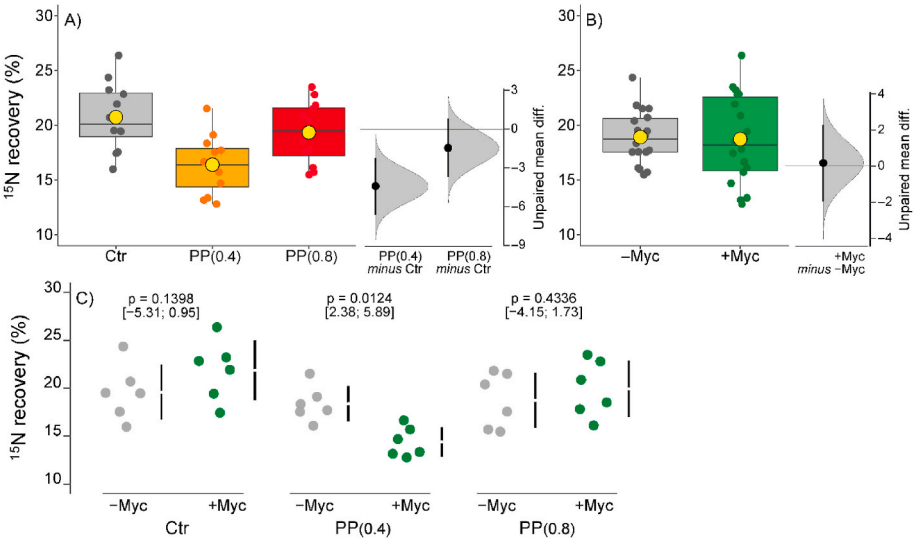

Fig. 8. ^{15}N fertilizer recovery (%). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (C). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
Ctr, uncontaminated control; PP(0.4) and PP(0.8), PP contamination at 0.4% and 0.8% (w/w), respectively; –Myc, uninoculated control; +Myc, inoculation with arbuscular mycorrhizal fungi.

Of course, the plant species could also be key to the discordant results reported in the literature on the response of higher plants to microplastics in the soil. In fact, as reported by Lozano and Rillig (2020), the presence of microplastics in the soil can influence different plant species in different ways, also changing the structure of plant communities. Several studies using different culture methods (soil culture and hydroponics) and different types and shapes of microplastic have highlighted a significant decrease in maize growth (Ingraffia et al., 2022b; Lian et al., 2021; Urbina et al., 2020; Wang et al., 2020). This, together with what was observed in this study, indicates that maize is particularly sensitive to exposure to microplastic in the soil.

Some authors have hypothesized that the adverse effects on plant growth of microplastics in the soil are attributable to a reduction in N availability induced by an alteration of the activity of key enzymes in N metabolism (e.g., acting on the processes of nitrification, denitrification, volatilization, or leaching; Ingraffia et al., 2022b; Iqbal et al., 2020; Sun et al., 2020). Moreover, we cannot exclude the possibility that the presence of microplastic, acting in some way as a physical barrier, can limit the ability of the plant to intercept N by limiting the expansion of the root system. This seems to be in line with the results of this experiment, in which, compared to the uncontaminated control, contamination with microplastic reduced the uptake of N by plants, with increasing effects as the concentration of microplastic in the soil increased. However, the fact that we also observed adverse effects of microplastic when we increased the availability of N for plants with fertilization leads us to believe that the availability of N was not the key factor determining the observed differences in the growth of plants.

Therefore, other direct and indirect mechanisms must have

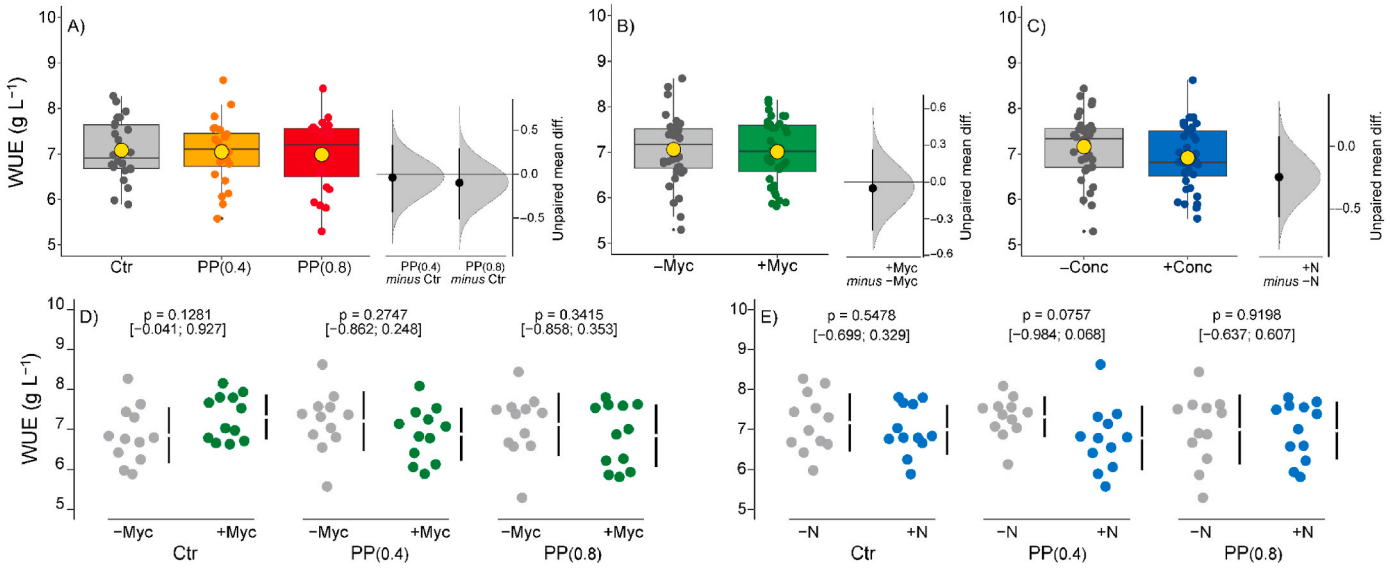

Fig. 9. Water use efficiency (WUE, g L^{-1}). The upper graphs show the mean effects of the applied treatments: polypropylene (PP) contamination (A); mycorrhizal inoculation (B); nitrogen (N) fertilization (C). Raw data and box plots are displayed. The filled curve indicates the resampled distribution of unpaired mean differences, given the observed data. The unpaired mean difference for each contrast is indicated by the black circle, and 95% confidence intervals are illustrated by black vertical lines. The lower graphs show raw data for the interactions between PP contamination and mycorrhizal inoculation (D) and between PP contamination and N fertilization (E). The p values for pairwise comparisons and estimated 95% confidence intervals (in brackets) are reported.
Ctr, uncontaminated control; PP(0.4) and PP(0.8), PP contamination at 0.4% and 0.8% (w/w), respectively; –Myc, uninoculated control; +Myc, inoculation with arbuscular mycorrhizal fungi; –N, unfertilized treatment; +N, N fertilization.

contributed significantly to the observed effects on plant growth. Some research has shown how microplastic and the compounds resulting from its degradation can exert toxic effects (genotoxicity, cytotoxicity, and phytotoxicity) on soil-grown plants by damaging plant roots (Chang et al., 2022; Giorgetti et al., 2020; Maity et al., 2020). In this experiment, degradation of the microplastic during the incubation period (which lasted about 5 months) may have led to the release of nanoparticles that may have been absorbed by the plant root system, causing damage to plant tissues. Moreover, it cannot be ruled out that the microplastic used in this experiment contained contaminants (plasticizer, flame retardants, thermal stabilizers, etc.) that, once released during the processes of fragmentation and degradation, could have exerted toxic effects on plant growth, as highlighted by Hahladakis et al. (2018) and Rozman et al. (2021). Finally, it is possible that the observed impact on the growth of the maize plants can be traced back to general degradation of the soil environment due to, for example, changes in the entity, structure, or activity of the microbial community as well as soil fauna; changes in the chemical-physical characteristics of the soil; the release of toxic compounds; or interference in the fertility cycle (Qi et al., 2020; Rillig et al., 2019; Gu et al., 2017). None of these factors alone is likely to explain the observed effects; it is more likely that they act jointly with different weights in different environmental conditions.

On average, mycorrhizal symbiosis had negative effects on the growth of maize plants in this study. In the inoculated treatments compared to the control, a significant reduction was observed in leaf area (−10%), aboveground biomass (−11%), and belowground biomass (−9%). In general, mycorrhizal symbiosis positively influences the growth and productivity of plants by favoring the removal of nutrients, in particular less mobile ones such as phosphorus, and resistance to biotic and abiotic stresses (Fileccia et al., 2017; Thirkell et al., 2016; Grümberg et al., 2015; Saia et al., 2014; Pozo et al., 2010). However, these advantages are not always realized. Often, in very fertile soils, the plant receives no benefit from symbiosis, and its growth can even be impaired by the considerable demand for carbon of fungi. Likewise, in poor soils AM fungi can compete with plants for nutrients, which often negatively affects plant growth and yield (Ingraffia et al., 2020; Püschel et al., 2016). Therefore, the impacts of mycorrhization appear largely variable depending on the plant's environment (climate, soil type, etc.) and the agronomic practices used (fertilization strategy, soil tillage, rotation, etc.; van der Heijden and Horton, 2009).

In this experiment, the percentage of mycorrhization of maize roots was not affected by the presence of microplastics. Indeed, we expected a reduction in mycorrhization, as the presence of pollutants in soil has been shown to reduce AM root colonization and infectivity (Wang, 2017; Ferrol et al., 2016; Desalme et al., 2012). Data on this topic are lacking, and existing findings are not always unambiguous (Lehmann et al., 2022; de Souza Machado et al., 2019). The environment (soil type, water availability, etc.) and the type of microplastic (main constituent and additives, shape, and size) may have considerable repercussions for plants' ability to establish symbiotic/associative relationships with microorganisms in the soil.

Finally, contrary to what we hypothesized, mycorrhizal symbiosis did not appear to be able to mitigate the negative effects of the presence of microplastics in the soil. On the contrary, as regards root growth, symbiosis somewhat exacerbated the negative effects of soil contamination with microplastic; this topic certainly deserves further study to qualify and quantify both the direct impact of microplastic on AM fungi and the indirect impacts via plants, soil properties, and/or the soil biome.

Further research is needed to identify the mechanisms underlying negative responses of plants in soil contaminated by microplastics. Researchers should be aware, however, that the different factors involved do not act individually but jointly change the properties, functions, and processes of the soil and the response of plants. Moreover, it is appropriate to verify any results in various agronomic contexts and with different types, forms, and concentrations of microplastics; this is certainly a priority, as it can lead to a more comprehensive understanding of this emerging anthropogenic problem.

5. Conclusions

In conclusion, the data from this study clearly show how, under the conditions we have described, soil contamination with PP microfibers drastically reduces the growth of maize and its ability to intercept N,

both native and supplied. The latter finding can have negative environmental implications because any N not intercepted by crops is a source of air and water pollution.

N fertilization did not mitigate the adverse effects of PP on plant growth, which shows that differences in N availability are not the key factor determining the observed adverse effects. Moreover, the presence of microplastics in the soil did not affect the ability of maize plants to activate symbiotic relationships with mycorrhizal fungi. However, unlike what we hypothesized, symbiosis did not appear to mitigate the adverse effects due to the presence of microplastics in the soil. Indeed, it further compromised the root growth of the maize plants. Given the current knowledge gap, more research is needed to elucidate the mechanisms driving the plant response in microplastic-contaminated soils.

Author statement

All the authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122146.

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