Journal of Hazardous Materials 480 (2024) 135781

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Polypropylene microfibers negatively affect soybean growth and nitrogen fixation regardless of soil type and mycorrhizae presence

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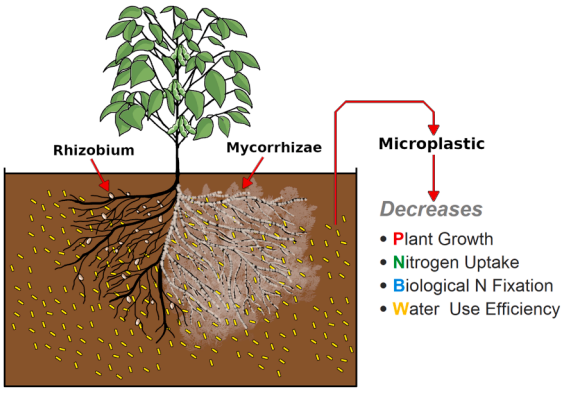
HIGHLIGHTS

- Polypropylene (PP) fibers inhibited soybean growth in two different soil types.
- 0.4 % PP in soil reduced biological nitrogen fixation (BNF) and water use efficiency.
- Increasing PP contamination to 0.8 % did not significantly change the effects.
- Mycorrhiza did not help plants overcome PP stress but mitigated the impact on BNF.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microplastics
Mycorrhizal symbiosis
Water use efficiency
Environmental pollution

ABSTRACT

Recent studies have indicated that soil contamination with microplastics (MPs) can negatively affect agricultural productivity, although these effects vary greatly depending on the context. Furthermore, the mechanisms behind these effects remain largely unknown. In this study, we examined the impact of two concentrations of polypropylene (PP) fibers in the soil (0.4 % and 0.8 % w/w) on soybean growth, nitrogen uptake, biological nitrogen fixation (BNF), and water use efficiency by growing plants in two soil types, with and without arbuscular mycorrhizal fungi (AMF). PP contamination consistently reduced vegetative growth (–12 %, on average compared to the control), with the severity of this effect varying significantly by soil type (more pronounced in Alfisol than in Vertisol). The extent of BNF progressively reduced with the increase in PP contamination level in both soils (on average, –17.1 % in PP0.4 and –27.5 % in PP0.8 compared to the control), which poses clear agro–environmental concerns. Water use efficiency was also reduced due to PP contamination but only in the Alfisol (–9 %, on average). Mycorrhizal symbiosis did not seem to help plants manage the stress caused by PP contamination, although it did lessen the negative impact on BNF. These findings are the first to demonstrate the

Abbreviations: AMF, arbuscular mycorrhizal fungi; BNF, biological nitrogen fixation; CEC, Cation Exchange Capacity; EC, Electrical Conductivity; MPs, microplastics; Ndfa, nitrogen derived from atmosphere; PP, polypropylene; TN, Total Nitrogen; TOC, Total Organic Carbon.

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https://doi.org/10.1016/j.jhazmat.2024.135781
Received 6 June 2024; Received in revised form 4 September 2024; Accepted 6 September 2024

effect of PP on BNF in soybean plants, underscoring the need to develop strategies to reduce PP pollution in the soil and to mitigate the impact of PP on the functionality and sustainability of agroecosystems.

1. Introduction

A significant portion of global plastic production ends up in the environment without proper treatment, leading to pollution in both aquatic and terrestrial ecosystems [1,2]. This pollution poses serious risks to human health [3], especially when plastics break down into particles smaller than 5 mm, known as microplastics (MPs) and nanoplastics. Soil is increasingly becoming a repository for MPs, which enter through various pathways such as atmospheric deposition, fragmentation of agricultural plastic products, irrigation with treated wastewater, fertilization, and more [4,5].

Several studies have shown that MP contamination affects the chemical, physical, structural, and hydrological properties of soil [6–8], potentially altering the soil microbiome and microfauna structure and activity [9]. Consequently, MP pollution affects soil fertility, altering nutrient cycles, particularly the nitrogen (N) cycle [10–12], with influences on processes like leaching, nitrification, denitrification, volatilization, and enzymatic activities [13–16]. Numerous studies have shown that MP pollution can negatively affect plant growth both directly and indirectly by impairing the plant's ability to absorb water and nutrients, increasing reactive oxygen species production, altering the mineral nutrient and trace element composition in roots and leaves, interfering with hormonal regulation, reducing chlorophyll concentration, and diminishing photosynthetic efficiency [17–19].

Additionally, MPs can impact the plant's ability to establish associative or symbiotic relationships, particularly with arbuscular mycorrhizal fungi (AMF) [20] and N-fixing bacteria [21]. Recent studies indicate that soils contaminated with MPs show increased markers of N fixation, such as the nifH gene [22], possibly due to increased populations of N-fixing genera like *Burkholderiaceae* [23] or *Bradyrhizobium* [24]. This suggests that MPs might enhance biological N fixation (BNF). However, the effects of MPs are influenced by various factors, including soil characteristics, polymer type, shape, size, agronomic management, and experimental duration. More research is needed to draw definitive conclusions.

Little is known about how MPs in soil affect AM fungal populations. These fungi are crucial for terrestrial ecosystems as they promote aggregate formation and enhance soil structure [25], supply nutrients to symbiotic host plants [26], and help plants overcome biotic and abiotic stresses [27–29]. Recent studies have shown that MPs influence the structure and diversity of AM fungal communities differently, depending on the type and concentration of polymer [30,31]. De Souza Machado et al. [32] observed an 8-fold increase in AMF root colonization in soil contaminated with polyester and a 1.4-fold increase with polypropylene, while polyethylene terephthalate resulted in halved root colonization. Similarly, Khan et al. [33] found that polystyrene and High-Density Polyethylene stimulated AMF root colonization in soybean. According to Lehmann et al. [34], MPs could alter soil characteristics such as bulk density, improving soil aeration and creating a favorable environment for AM fungi. However, other studies have reported no differences in root colonization due to the presence of MPs [11,31]. MPs in soil might also indirectly affect mycorrhizal helper bacteria, influencing AMF root colonization and functionality, and potentially impacting mycorrhizal symbiosis [20]. If MPs negatively affect AMF, their ability to protect plants from pathogens and environmental stresses (e.g., salinity, drought) and to support nutrient uptake could be compromised. On the other hand, it is also possible that inoculating soil with AMF might mitigate the negative impacts of MPs by enhancing nutrient availability and plant uptake, as suggested by Moreno-Jiménez et al. [35] However, this hypothesis requires further experimental validation.

Therefore, we conducted a pot experiment to investigate the impact of soil contamination with varying concentrations of polypropylene (PP) microfibers on soybean (*Glycine max* (L.) Merr.) shoot and root growth, N uptake, BNF, and water use efficiency. We hypothesized the following: 1) PP contamination negatively impacts plant growth, resource utilization efficiency, and BNF, with varying effects based on soil type; 2) the presence of PP in the soil reduces mycorrhizal colonization of roots; and 3) despite this reduction, mycorrhization can mitigate the negative effects of PP on plant growth.

2. Materials and methods

2.1. Experimental design

The experiment was carried out in southern Italy at Pietranera farm (Lima Mancuso Foundation in Santo Stefano Quisquina, AG, Italy; 37° 32′ 39.54″ N, 13° 31′ 01.32″ E; 162 m a.s.l.). It was conducted in two phases: the initial incubation phase involved PP microfibers interacting with the soil environment without plants for approximately 4 months in a growth chamber in the dark at 23 ± 2 °C and 60 ± 5 % relative humidity. In the second step, the experimental units were moved to a wire house covered with a transparent plastic roof with open sides and sown with soybean. The wire house shielded the experimental units from natural rain while permitting natural temperature variations. The soybean growing period spanned approximately 2 months commencing in April 2021 and concluding in June 2021 (temperature data collected from a weather station located within 200 m of the experimental site are reported in Supplementary material; Fig. S1).

We investigated the effect of PP microfiber at two diverse levels 0.4 % and 0.8 % weight on soil dry weight (w/w) and the presence of AMF on soybean performances in two soil types. Each treatment was replicated 12 times for a total of 144 experimental units [2 soil types (Vertisol and Alfisol); 3 MP treatments (0 PP addition, Ctr; 0.4 % w/w, PP0.4; 0.8 % w/w, PP0.8); 2 AM treatments (with, +myc; or without, –myc; AM inoculum); 12 replicates]. Experimental units were arranged in a completely randomized design.

2.2. Experimental setting and management

Both the soil types were collected from the first 30 cm of agricultural fields in October 2020. We chose the following two soils which are widely spread in the Mediterranean area:

- Typic Haploxerert (Vertisol). The soil is well structured, with a clay texture; 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.
- Typic Rhodoxeralfs (Alfisol). It is a typical soil widespread on the carbonate platforms of many Mediterranean environments. The color tends to be red due to the considerable presence of iron oxides linked to the leached clays. Kaolinite is the dominant clay mineral. This soil is characterized by strong pedological aridity, due to its calcareous nature, and low amounts of organic matter and fertility elements.

After sampling, the soil was air dried, sieved at 600 μm, sterilized through three successive cycles of humidification, 24 h at room temperature, and 24 h in an oven at 130 °C, and stored at 4 °C in sterilized plastic bags until the start of the experiment (1 month later). At sampling time, we checked and ensured that the soils were 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.

Both the soils were characterized as follows: particle size distribution and soil texture classification according to the United States Department of Agriculture [36]; total N (TN; Kjeldhal), total organic carbon (TOC; Walkley–Black procedure), pH, saturated electrical conductivity at 25 °C (EC), and cation exchange capacity (CEC). Soil properties are listed in Table 1.

For MP contamination, we used primary PP microfiber (STW, Schwarzwälder Textil–Werke Heinrich Kautzmann GmbH Aue 3 • D–77773 Schenkenzell). We chose PP microfiber because it is one of the main MP contaminants in soil [37,38]. 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.

MP was incorporated into the soil at two concentrations, 0.4 % (PP0.4) and 0.8 % (PP0.8) w/w, which corresponds to about 3.5×10^6 and 7.0×10^6 items kg^{-1} of soil dry weight respectively (items kg^{-1} were estimated based on the average of particle sizes and the density of PP being 0.91 g cm^{-3}). Although these concentrations of contamination exceed the typical amounts found in agroecosystems, they can hold potential environmental relevance in the near future. Indeed, concentrations of 4.1×10^5 items kg^{-1} and 1.6×10^5 items kg^{-1} have been found in woodland and vegetable land in China [39], while Crossman et al. [4] in Canada estimated that an agricultural field fertilized with biosolid can receive an annual MP addition up to 1.10×10^8 particles ha^{-1}. Moreover, the concentrations we applied are commonly used in studies focusing on the effect of MP soil–plant systems [6,11,14,34,35].

PP microfibers were homogeneously incorporated into the soil using the method proposed by Ingraffia et al. [6,14]. Briefly, the MP fibers were incorporated into the soil using a laboratory blender (Waring® WSG30; Waring Commercial, Torrington, CT, USA). The PP microfibers were incorporated separately for each individual experimental unit. The soil and MP fibers were mixed five times for 5 s each. The same disturbance was applied to the soil in the control treatment.

The experiment was carried out in 1.2 l pots (d= 5 cm; h= 60 cm) filled with soil (according to the bulk density of each soil type which was 0.76 g cm^{-3} and 0.82 g cm^{-3} for Vertisol and Alfisol respectively) contaminated or not with PP microfibers. Subsequently, all the pots were irrigated by capillarity to the field capacity and placed in a growth chamber for about 4 months as reported in the section "Experimental design". During the incubation period, pots were irrigated by capillarity to the field capacity once a week.

At the sowing time, the soil native microbiome, excluding AM fungi, was reintroduced in each pot. For this purpose, unsterilized soil was diluted in distilled water (1:3 w/v) and stirred for 20 min at 140 rpm. After decanting, the suspension was filtered through an 11 μm mesh to remove the natural AM fungal community. A total of 100 mL of filtrate was added to each pot.

Pots of +myc treatment were also added with 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 g^{-1}. The commercial inoculum contained also 1×10^7 of rhizosphere's bacteria per gram of inoculum. To isolate the effects of the AM fungi, the bacterial community of the inoculum was extracted (using the protocol described above for the native microbiome) and added to the –myc treatment pots.

On the 20 of April 2021, 3 seeds of soybean (cv. Galina) inoculated with *Bradyrhizobium japonicum* (Nitrosem Soja, Nitrosem srl, Genola, CN, Italy) were sown in each pot. All pots and seeds were previously sterilized in a solution of 3 % sodium hypochlorite for 5 min. After sowing, all pots were irrigated to field capacity. One week after emergence, plants germinated were thinned to have only one plant per pot.

Eventually, 6.9 mg ammonium sulfate were added to each pot with a 10 % enrichment of ^{15}N isotope split equally over three dates (10, 20, and 30 days after plant emergence). Maize was used as the reference crop for assessing N_2 fixation of the soybean using the ^{15}N isotope dilution technique [40]. The non–fixing reference plants were grown in the same conditions as the legume.

For the entire growing period, soil moisture was monitored twice a week through the gravimetric method to keep the soil moisture between 70 % and 95 % of water holding capacity. Water consumption of the different experimental treatments is reported in Fig. S2.

2.3. Measurements

Plants were harvested when soybeans reached the stage of V5–V6 (five-six unfolded trifoliolate leaves); this stage was reached 55 days after the emergence. Shoot biomass was harvested and separated into botanical fractions (leaves and stems) and weighed. Leaves were used to determine the leaf area using a leaf area meter (LI–3100 C; LiCOR, Lincoln, NE, USA). The root biomass was carefully extracted by sieving and washing. Shoot and root biomasses were later oven–dried at 40 °C to constant weight to determine the dry matter.

A portion of root biomass was used to assess mycorrhizal colonization. Roots were first cleaned with successive treatments with KOH 10 %, H_2O_2 10 vol, and HCl 10 %; and then stained with acid fuchsin (0.01 %) in lactophenol using the method proposed by Phillips and Hayman [41] modified as proposed by Miceli et al. [42]. 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 [43–45]. 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 Axio–Vision 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 total N and ^{15}N enrichment 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.

Data on the ^{15}N enrichment of biomass were used to calculate the percentage of soybean N derived from symbiotic N_2 fixation (%Ndfa):

Table 1
Physical and chemical properties of the two soils used in the experiment.

Soil	Clay	Silt	Sand	TN	TOC	pH	EC	CEC
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})		(dS m^{-1})	(cmol kg^{-1})
Vertisol	415	357	228	1.54	15.78	7.74	1.89	30.0
Alfisol	152	431	417	0.77	11.20	7.58	2.01	13.8

Clay, Silt, and Sand were classified according to USDA (Clay < 2 μm, Silt 2–50 μm, and Sand 50–2000 μm); TN, Total Nitrogen; TOC, Total Organic Carbon; EC, Electrical Conductivity; CEC, Cation Exchange Capacity.

$$\%Ndfa = \left(\frac{1 - atom\%^{15}N_{soy}}{^{15}N_{maize}}\right) \times 100$$

where atom% $^{15}N_{soy}$ represents the atom% ^{15}N excess of soybean tissue and atom%$^{15}N_{maize}$ represents the atom% ^{15}N excess of maize tissue. The ^{15}N–natural abundance of the atmosphere (0.3663 % ^{15}N) was used to calculate the atom% ^{15}N excess of both crops.

The amount of N fixed by the soybean was estimated as follows:

$$Nfixed = \left(\frac{N_{soy} \times \%Ndfa}{100}\right)$$

where N_{soy} represents the total N in the soybean aboveground biomass.

Total biomass production (shoots and roots; TB) and total water consumption (W_{appl}) were used to calculate water use efficiency (WUE) as follows:

$$WUE = \frac{TB}{W_{appl}}$$

The W_{appl} was calculated as the sum of all water applied during the experiment.

2.4. Statistical analysis

The data were analyzed in R software [46] 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 (PP0.4 or PP0.8 vs. Ctr) within the same treatment (soil-mycorrhiza combination) using the "dabestr" package [47] to calculate effect sizes as unpaired mean differences and the *P-values* for pairwise comparisons. Graphical data representations were generated using the "tidyverse" R package [48].

3. Results

3.1. AMF root colonization

The root colonization of the uninoculated treatments (–myc) was always null in all the treatments. In the inoculated plants (+myc), the characteristic structures of AM fungi were observed (Fig. 1). The percent inoculation varied between soil types (11.7 % in the Alfisol and 4.4 % in the Vertisol), but no variation was observed between the absence and presence of PP (both levels) in the soil (Table 2).

3.2. Biomass and leaf area

Overall, the growth of soybean plants was almost double in Alfisol compared to Vertisol; the differences were more marked for the roots than for the shoots (Table 2; Fig. 2).

On average, mycorrhizal colonization adversely affected shoot and root biomass. The effects of PP contamination varied in relation to soil type. In the Alfisol, contamination with PP resulted in a reduction of 12.9 % of shoot biomass and 21.2 % of root biomass (Fig. 2); this effect did not vary significantly either due to the level of contamination (0.4 vs 0.8 %) nor due to the presence or absence of mycorrhizae. In the Vertisol, contamination with PP had no effects on the growth of shoots and roots in the absence of mycorrhizae, while in the presence of mycorrhizae, contamination with PP resulted in a reduction of shoot biomass (–16.4 %) and particularly of root biomass (–24.2 %); also, in this case no difference was observed between the two levels of PP contamination.

The presence of PP also determined a significant reduction in leaf area in both Alfisol (on average, –9.6 %) and Vertisol (on average, –10.4 %); in the latter the negative effect was more marked in the +myc treatments (–15.4 %) compared to –myc treatments (–6.4 %; Fig. 3).

3.3. Nitrogen uptake and fixation, and water consumption

N content in the shoot tissues varied due to PP contamination differently with soil type (Fig. 4). In the Alfisol, contamination with both levels of PP determined a light increase of N content (on average +5.6 %); on the contrary, in the Vertisol, a reduction of N content was observed, moreover of increasing magnitude as the level of contamination increased (–7.9 % in PP0.4 and –8.6 % in PP0.8).

Soil contamination with PP determined an overall reduction in the amount of N accumulated in the shoot (Fig. 4); this effect was more marked in Vertisol (–19.1 %) than in Alfisol (–7.7 %). Mycorrhizal inoculation reduced the amount of N accumulated in the shoot (on average –11.8 %) regardless of PP and soil treatments.

The percentage of N derived from the atmosphere (%Ndfa) was decidedly higher in Alfisol than Vertisol (on average, 41.5 % and 16.0 %, respectively; Fig. 5). In uninoculated plants, %Ndfa progressively reduced with the increase in PP contamination level in both soils (on average, 31.2 % and 20.5 % in Ctr and PP0.8, respectively).

The mycorrhizal symbiosis appeared capable of mitigating this effect so much that in the inoculated plants the %Ndfa was, on average, 33.8 % in the control and 30.3 % in the higher contamination rate (PP0.8). Similar trends were observed for the amount of the biological fixed N (Fig. 5).

Water consumption (Fig. S2) decreased in both soils as the level of PP pollution increased; it was greater in non–mycorrhized plants compared to mycorrhized ones. Furthermore, the reduction in water consumption was associated with a lower water use efficiency (Fig. 6), particularly in Alfisol.

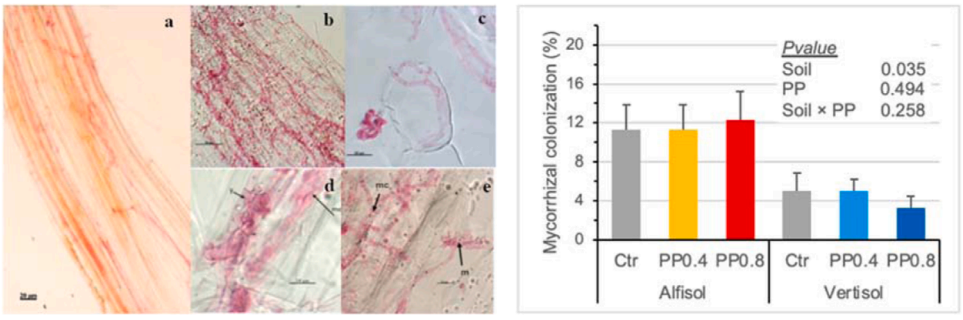

Fig. 1. Arbuscular mycorrhizal mycelium in a stained soybean root (a); particular of root cortex highly colonized (b); intracellular growth of mycelial structures (c); details of mycelial colonization (arrow identified by "mc" indicating mycelium) and arbuscules structures (arrow identified by "y" and "m" indicating young and mature arbuscules respectively) (d) and (e). On the right-hand side, the percentage of mycorrhizal colonization in the two soils (Alfisol and Vertisol) at different levels of polypropylene microfibers contamination (Ctr, no PP added; PP0.4, 0.4 % w/w added; PP0.8, 0.8 % w/w added) in presence (+myc) of AM fungal inoculum.

Table 2
Analysis of variance: P–values for the effects of the applied treatments (Soil: Alfisol and Vertisol; PP: different levels of polypropylene microfibers contamination; Myc: absence or presence of AM fungal inoculum) on the trait measured on soybean plants.

	Soil	*PP*	*Myc*	*Soil*PP*	*Soil*Myc*	*PP*Myc*	*Soil*PP*Myc*
Shoot biomass	< 0.001	< 0.001	< 0.001	0.089	0.021	0.818	0.172
Root biomass	< 0.001	< 0.001	0.003	0.002	0.972	0.694	0.561
Leaf area	< 0.001	< 0.001	< 0.001	0.267	0.013	0.855	0.788
N content	0.253	< 0.001	0.001	< 0.001	< 0.001	0.578	0.034
N uptake	< 0.001	< 0.001	< 0.001	0.636	0.376	0.806	0.571
%Ndfa	< 0.001	0.109	0.021	0.769	0.440	0.555	0.809
Fixed N	< 0.001	0.005	0.765	0.629	0.389	0.482	0.456
Water consumption	< 0.001	< 0.001	< 0.001	0.260	0.072	0.724	0.326
WUE	< 0.001	0.008	0.727	< 0.001	0.216	0.054	0.343

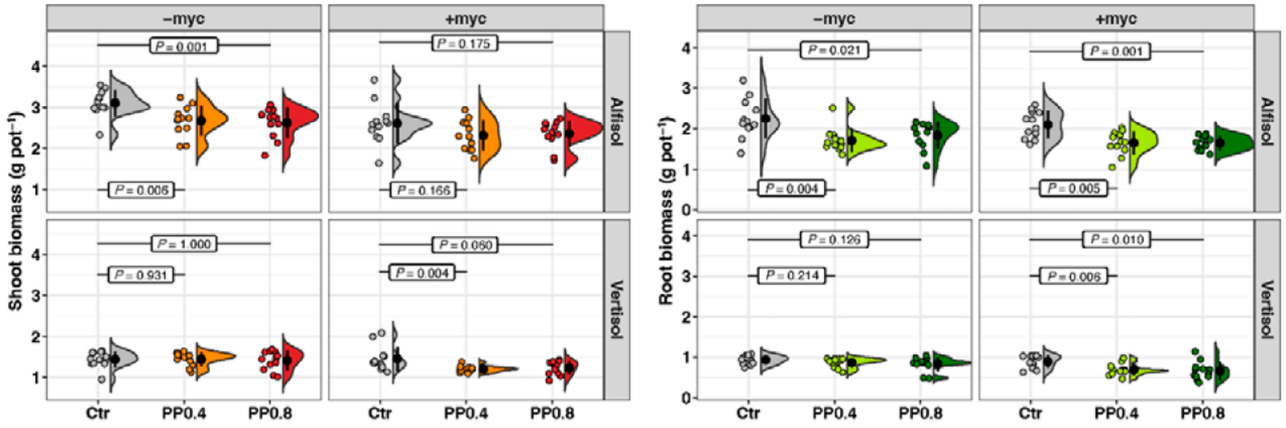

Fig. 2. Raw data (dots) and half-violin plots of shoot biomass (left) and root biomass (right) measured in the two soils (Alfisol and Vertisol) at different levels of polypropylene microfibers contamination (Ctr, no PP added; PP0.4, 0.4 % w/w added; PP0.8, 0.8 % w/w added), and in the absence (−myc) or presence (+myc) of AM fungal inoculum. Numeric data distributions are represented by half-violin plots, with the width of the plot showing the density distribution of the values. Circles inside plots represent means, with whiskers representing ± SE (n = 12). On the plots are reported the P values for pairwise comparisons between Ctr and PP0.4 or between Ctr and PP0.8 within the same treatment.

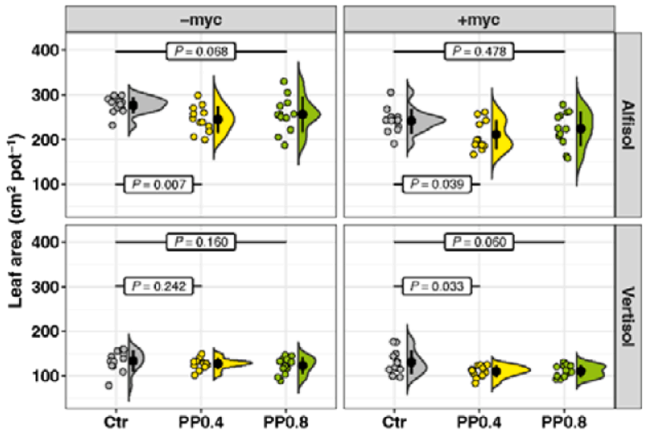

Fig. 3. Raw data (dots) and half-violin plots of leaf area measured in the two soils (Alfisol and Vertisol) at different levels of polypropylene microfibers contamination (Ctr, no PP added; PP0.4, 0.4 % w/w added; PP0.8, 0.8 % w/w added), and in the absence (−myc) or presence (+myc) of AM fungal inoculum. Numeric data distributions are represented by half-violin plots, with the width of the plot showing the density distribution of the values. Circles inside plots represent means, with whiskers representing ± SE (n = 12). On the plots are reported the P values for pairwise comparisons between Ctr and PP0.4 or between Ctr and PP0.8 within the same treatment.

4. Discussion

This research has shown that soil contaminated with PP microfibers negatively impacts soybean plants, depressing both root and shoot growth, reducing the photosynthesizing area, and decreasing the accumulation of N in the tissues, the share of N derived from the atmosphere, and water use efficiency. Several studies have highlighted that different shapes (fibers and fragments) of PP MPs in the soil might harm plant growth [11,49,50] through mechanisms such as changes in soil physicochemical characteristics and hydrological parameters (e.g., bulk density, state of aggregation and stability, water retention capacity [51, 52]), alterations in nutrient cycles affecting element availability for plants [53], and impacts on microbial structure and enzyme activity in nutrient cycles [54]. Liu et al. [55] found that PP soil pollution inhibits the growth and N uptake of peanut plants by damaging the plasma membrane of root cells, causing oxidative stress and disturbing soil N cycling. MPs and their degradation products can also damage plant roots, causing genotoxicity, cytotoxicity, and phytotoxicity [56,57]. In this experiment, the degradation of PP during the incubation period (which lasted about 5 months) likely led to the release of nanoparticles that may have been absorbed by the plant root system, causing tissue damage. Additionally, PP may have contained substances like plasticizers, flame retardants, thermal stabilizers, and others, which, once released during the fragmentation and degradation process, could have exerted toxic effects on plant growth, as reported in other studies [58–60]. These effects can vary widely with agronomic and pedo–climatic factors [61], so it is not surprising that other studies have reported null or positive effects on plant growth [62,63]. In this research, the depressive effects induced by PP contamination appeared more evident in Alfisol than in Vertisol. The experimental method, such as sieving soil

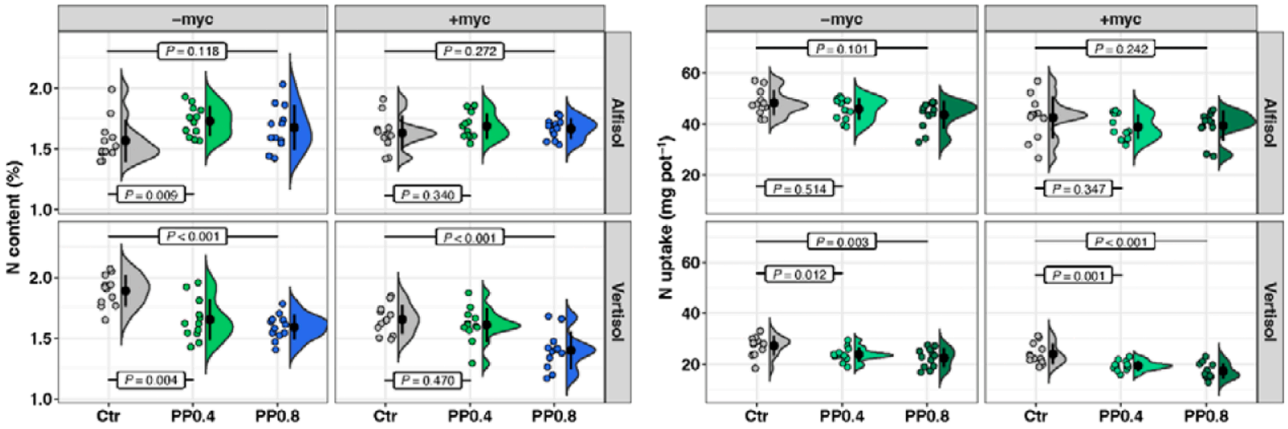

Fig. 4. Raw data (dots) and half-violin plots of nitrogen content (left) and nitrogen uptake (right) measured in the two soils (Alfisol and Vertisol) at different levels of polypropylene microfibers contamination (Ctr, no PP added; PP0.4, 0.4 % w/w added; PP0.8, 0.8 % w/w added), and in the absence (−myc) or presence (+myc) of AM fungal inoculum. Numeric data distributions are represented by half-violin plots, with the width of the plot showing the density distribution of the values. Circles inside plots represent means, with whiskers representing ± SE (n = 12). On the plots are reported the P values for pairwise comparisons between Ctr and PP0.4 or between Ctr and PP0.8 within the same treatment.

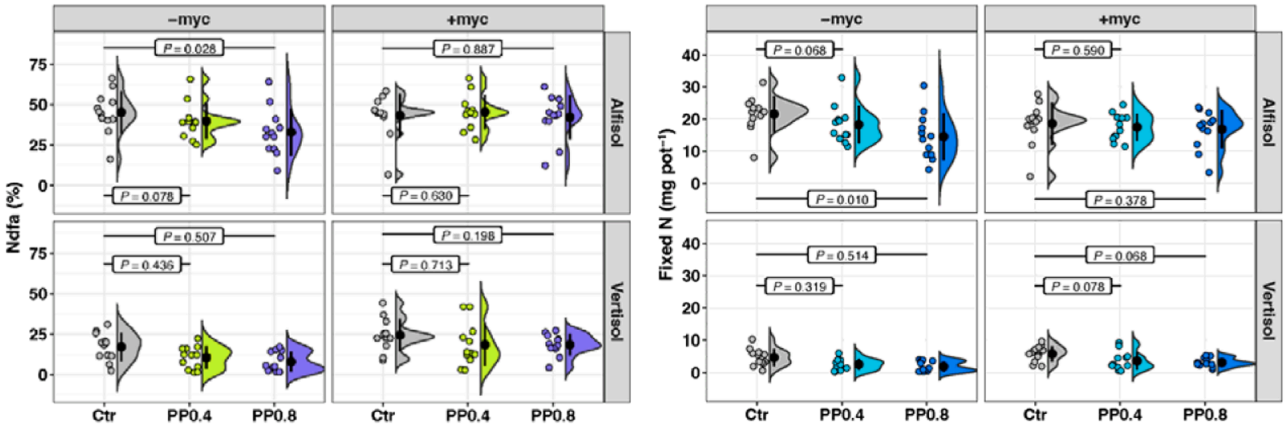

Fig. 5. Raw data (dots) and half-violin plots of nitrogen derived from the atmosphere (left) and fixed nitrogen (right) measured in the two soils (Alfisol and Vertisol) at different levels of polypropylene microfibers contamination (Ctr, no PP added; PP0.4, 0.4 % w/w added; PP0.8, 0.8 % w/w added), and in the absence (−myc) or presence (+myc) of AM fungal inoculum. Numeric data distributions are represented by half-violin plots, with the width of the plot showing the density distribution of the values. Circles inside plots represent means, with whiskers representing ± SE (n = 12). On the plots are reported the P values for pairwise comparisons between Ctr and PP0.4 or between Ctr and PP0.8 within the same treatment.

at 600 μm and using a homogenizer to incorporate PP fibers, might have contributed to excessive compaction and root asphyxia, especially in the Vertisol which was characterized by a higher clay content than the Alfisol. The presence of PP probably attenuated these effects by improving soil permeability and water movements, partially counterbalancing the negative impact of PP on soybean growth.

In this study, we observed a strong depressive effect of PP pollution on N biological fixation in soybeans. To our knowledge, this is the first study to evaluate the effect of MP pollution on symbiotic N fixation in soybeans. Various types of MPs have been shown to positively influence the relative abundance of N–fixing bacteria, particularly *Bradyrhizobium* genera [23,24], leading to the hypothesis that MP contamination could positively affect N fixation. Kim et al. [64] achieved similar results with sub-micron MPs at environmentally relevant concentrations.

The availability of N in the soil has a marked influence on the BNF process. However, the results of this research suggest that the adverse effects of PP on N fixation are not directly linked to variations in soil N availability. A reduction in N availability should increase the legume's dependence on symbiotic N fixation, as observed in many studies [40,65]. An increase in N availability in the substrate, which could explain the reduction of N derived from biological fixation, is unlikely given the drastic reduction in N uptake in plants grown in PP-polluted soil. The reduction in N fixation observed in this research can be attributed to other causes, such as MPs acting as a physical barrier limiting plant-rhizobia interaction [11,66], MP-induced oxidative stress damaging root cell plasma membranes and reducing root development and nodulation [55], and the general depressive effect of PP on plant growth reducing the plant's N needs and dependence on N fixation [67,68]. Additionally, the PP used in this experiment might have contained contaminants that exerted toxic effects on both plant growth and the rhizobial community. Soil contamination with PP reduced water consumption, which was expected given the reduced plant growth and, consequently, the transpiration needs. However, the presence of PP also reduced water use efficiency, confirming previous reports that PP contamination negatively interferes with important physiological functions of plants [69]. Water use efficiency is a key indicator of ecosystem function and performance [70], and this result is concerning, especially in light of predicted climate change impacts on water availability [71]. Improving water use efficiency is a priority for future agricultural sustainability.

In this experiment, mycorrhizal symbiosis reduced soybean growth, decreasing root and shoot growth by 11.9 % and 9.4 % respectively, and

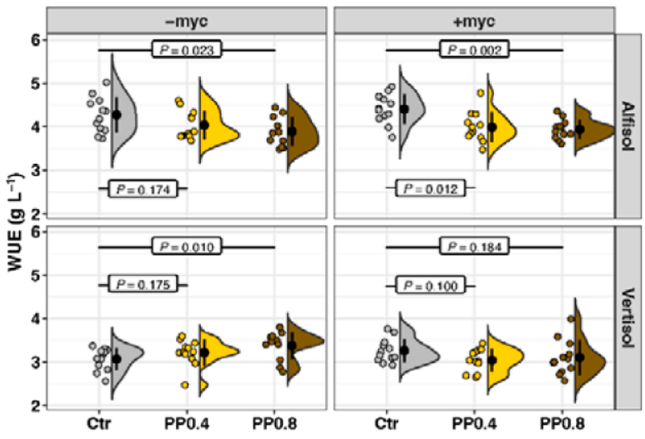

Fig. 6. Raw data (dots) and half-violin plots of water use efficiency (WUE) measured in the two soils (Alfisol and Vertisol) at different levels of polypropylene microfibers contamination (Ctr, no PP added; PP0.4, 0.4 % w/w added; PP0.8, 0.8 % w/w added), and in the absence (−myc) or presence (+myc) of AM fungal inoculum. Numeric data distributions are represented by half-violin plots, with the width of the plot showing the density distribution of the values. Circles inside plots represent means, with whiskers representing ± SE (n = 12). On the plots are reported the P values for pairwise comparisons between Ctr and PP0.4 or between Ctr and PP0.8 within the same treatment.

reducing leaf area by 11.4 % in uncontaminated controls. While mycorrhizal symbiosis generally supports plant growth by aiding nutrient uptake and stress resistance [72], its benefits are not always realized. In favorable environments, plants may not benefit from mycorrhizal symbiosis if their needs are already met, and their growth can be impaired by the carbon demand of fungi. In poor soils, mycorrhizal fungi can compete with plants for water and nutrients, penalizing plant growth. The impact of mycorrhization varies based on environmental factors and crop management [73]. In this experiment, PP presence did not affect the percentage of mycorrhization in soybean roots, consistent with previous observations in maize [11]. Information on this subject is limited and sometimes unclear [32,34]. Environmental conditions and MP characteristics can significantly impact plants' capacity to form symbiotic or associative connections with soil microorganisms. Contrary to our initial hypothesis, mycorrhizal symbiosis did not alleviate the adverse impacts of PP presence in the soil and even exacerbated the negative effects of PP contamination, particularly on root growth. It should be noted that the mycorrhization rate in this study was low, which may explain why mycorrhizal inoculation produced no positive effects. However, we observed that mycorrhizal symbiosis limited the impact of PP contamination on the BNF process. Other studies have shown that AM symbiosis can promote BNF efficiency by supporting plant growth, helping plants overcome stress conditions [74], and through functional synergy between root symbionts [75]. Further research is needed to better understand MPs' direct and indirect impacts on AM fungi and the soil biome.

5. Conclusions

This study highlights how soil contamination with PP significantly reduces both root and shoot growth of soybean plants. The response to PP contamination is strongly influenced by soil type, emphasizing the need to understand soil processes and mechanisms underlying different responses. PP pollution significantly affects N absorption and BNF, marking the first quantification of MP contamination effects on BNF. The negative environmental consequences are concerning as BNF is crucial for sustainable agriculture. Mycorrhizal symbiosis did not sufficiently assist plants in overcoming PP soil pollution stress, although it somewhat alleviated the adverse effects on BNF. However, the low mycorrhization rate observed may explain the lack of positive effects associated with mycorrhizal inoculation. This study underscores the significant threat that PP soil pollution poses to agricultural productivity and sustainability, highlighting the urgent need for strategies to reduce PP accumulation and mitigate its impact on agricultural systems.

Environmental implication

Plastic pollution in terrestrial ecosystems poses significant risks to agricultural stability and human health. Given the continuous release and slow degradation of plastics, the problem is likely to worsen. Soil contamination with polypropylene (PP) microplastics significantly reduces soybean growth and adversely affects nitrogen absorption and biological nitrogen fixation (BNF), with impacts differing by soil type. Mycorrhizal symbiosis offers limited relief from PP-induced stress, slightly mitigating its negative impact on BNF. Increased PP contamination level reduces BNF and water use efficiency, crucial for sustainable agriculture. These findings underscore the urgent need to develop strategies to reduce PP accumulation and preserve agroecosystem functionality and sustainability.

CRediT authorship contribution statement

Livio Torta: Writing – review & editing, Investigation. **Alfonso S. Frenda:** Writing – review & editing, Visualization, Funding acquisition, Formal analysis, Conceptualization. **Antonella Lo Porto:** Writing – review & editing, Visualization, Investigation. **Gaetano Amato:** Writing – review & editing, Visualization, Conceptualization. **Rosolino Ingraffia:** Writing – review & editing, Conceptualization. **Giacomo Gargano:** Writing – review & editing, Visualization, Investigation. **Dario Giambalvo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization.

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.

Acknowledgements

This research was partly funded by the University of Palermo, Italy. Open Access Funding was provided by the University of Palermo within the CRUI–CARE Agreement.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135781.

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