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Polyester microplastic fibers in soil increase nitrogen loss via leaching and decrease plant biomass production and N uptake

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Abstract

Microplastic contamination, like other global change factors, can induce effects on ecosystem functions and processes, affecting various soil biophysical properties. However, effects of such contaminants on nutrient cycles in agroecosystems are still poorly understood. We here performed two pot experiments to investigate the effect of polyester microplastic fibers (PMFs) on soil physical properties, nitrogen cycle, and plant performance in a maize-based agroecosystem. Moreover, we followed the N loss via leaching in soil contaminated or not with PMFs by simulating heavy rainfall events that mimic a future scenario of climate change. Our results show that soil contaminated with PMFs (at a concentration of 0.5% w/w) can jeopardize agroecosystem sustainability by affecting soil physical properties and in particular soil macro- and microporosity, the nitrogen cycle, and plant performance. In particular, we found that soil PMF contamination limited crop growth and N uptake by circa 30%, and consequently increased N loss via leaching. Overall, our findings show that soil contamination with PMFs may pose problems to future agricultural challenges like food security and environmental protection.

1. Introduction

Microplastic (i.e. plastic particles size smaller than 5 mm [1]) pollution is becoming widely recognized as a major environmental concern to both aquatic and terrestrial ecosystems [2, 3], with contamination observed also in the remote world regions [4, 5]. Moreover, given its ubiquity and expected long residence time [6, 7] it is likely that the problem will grow increasingly severe.

Microplastics can arrive and be incorporated into soil through several ways [8–12], and, just like other global change factors, can induce effects on ecosystem functions and processes [3]. Indeed, although the exploration of microplastics in terrestrial environments is a relatively recent topic, in the last years several authors have shown that microplastic can affect various soil properties (e.g. bulk density, water holding capacity, porosity etc.), microbial activity, and plant performance [13–20]. Moreover, microplastic particles may influence the nitrogen cycle in soil by affecting the activity of key enzymes and processes (as hypothesized by Iqbal *et al* [21]). The influence of microplastic on the soil environment, in particular on macroporosity, can further affect soil nitrogen fate by creating preferential paths for leaching. Nitrogen (N), especially in the nitrate form, is mobile, and thus soil microplastic contamination could affect nitrogen loss via leaching from agroecosystems. Moreover, the contamination may negatively affect the ability of plants to take up N, decreasing the use efficiency of N both native and applied with fertilizers and, consequently, increasing the risk of N loss. N loss from agroecosystems is a main agricultural issue worldwide having effects on: (a) production, as N is an essential crop nutrient and a decrease in the amount of N available to the crop can lead to a drastic reduction in crop yield, particularly in cereals; (b) environmental sustainability, as N leaching is a main pollutant of freshwater and directly related to water eutrophication [22]; and (c) economic sustainability, as higher N losses increase the required amount of fertilizer to support similar crop production [23].

Ongoing climate change has already increased the amount of heavy rainfall, and even more such changes are expected in the future [24, 25], which can *per se* increase the amount of N leaching from agroecosystems [26]. To our knowledge, no information is available on how the increasing soil microplastic contamination can affect this phenomenon. Different types of microplastics in different environments (i.e. water, sediment, soil) can affect different abiotic and biotic ecosystem compartments [27, 28], and within one environment (i.e. soil) different shapes and polymers can exert different effects on properties and processes [29]. Among the various microplastic types, polyester microplastic fibers (PMFs) is one of the most frequently detected types in agricultural environments [30, 31]. Here we performed two pot experiments to understand mechanistic interactions and processes due to soil PMF contamination in a microscale environment as a model system. In particular, our goals were to investigate the effects of this type of microplastic on: (a) soil physical properties; (b) maize growth and N uptake; and (c) N leaching from the agroecosystem. In Exp I, we investigated the effect of PMF on soil physical properties, namely bulk density and soil porosity, while in Exp II we examined how PMF contamination affects plants performance and the environmental sustainability of a maize based agroecosystem by monitoring N loss via leaching determined by heavy rain events during crop growth.

2. Materials and methods

2.1. Soil, polyester microplastic fibers, and incubation conditions

For both the experiments, we used an agricultural soil classified as Typic Xerorthent (Entisol), collected in the last week of October 2019 from the upper 30 cm of an agricultural field located in a typical Mediterranean environment in Southern Italy, Sicily (37.561368 °N latitude and 13.512904 °E longitude). After sampling, the soil was air dried, sieved (600 μm), and stored at 4 °C until the beginning of the experiment (end of December 2019) to minimize changes in the natural microbial community. We characterized the sieved soil, measuring particles size distribution [32]; total nitrogen (Kjeldhal); total organic carbon (Walkley–Black procedure; Nelson and Sommers 1996); pH; saturated electrical conductivity (25 °C); and cation exchange capacity (table 1).

For PMF contamination, we manually cut a 100% polyester white rope (Marlow, Marlowbraid classic rope) to generate secondary microplastic fibers (mean length: 2.87 mm, 1 sd = 0.31 mm; mean diameter: 87 μm, 1 sd = 3 μm). The fibers were characterized by scanning ten times at least 200 fibers on PVC trays (Epson Perfection Scan V800, 8 bit grayscale, 800 dpi). Scans were analyzed with WinRhizo (WinRHIZO Pro v. 2007d, Regent Instrument Inc., Quebec, Canada).

The generated microplastic fibers were incorporated into the air-dried soil at a concentration of 0.5% w/w. This PMFs level was similar to that used in previous studies, which reported noticeable changes on the soil biophysical environment and plant response [13, 16, 33, 34]. We are aware that this level of contamination is far beyond the current level observed in agricultural soils [31]. However, considering the slow degradation time and the amount of microplastics that continuously reaches the soil environment [35], in some areas, the contamination of soils is steadily increasing; therefore investigating the effects of high levels of contamination is of key importance as it may represent future scenarios.

We homogeneously incorporated the fibers into the soil by stirring with a laboratory blender (Waring® WSG30, Waring Commercial, Torrington, Connecticut). The fibers incorporation into the soil was done separately for each individual experimental unit. The fibers were added into the blender as a band sandwiched between two layers of soil. The mixture of soil and fibers was mixed five times for 5 s each. Exactly the same disturbance was also applied to the soil of the control treatment. In any case, the soil was poured into the pot and leveled with minimum compaction to obtain an initial soil dry bulk density of approximately 0.82 g cm^{-3}. The bottom part of the pots was closed using a cotton mesh to ensure that the above soil did not fall out, while allowing drainage.

For both experiments, the two treatments, soil contaminated with PMF (MP add) and the control (Ctr), were watered with distilled water to field capacity by capillary action and incubated in a growth chamber in the dark at 23 ± 2 °C and 60 ± 5% relative humidity for circa 5 months (from 13 December 2019 to 29 May 2020 for Exp I and from 30 December 2019 to 5 June 2020 for Exp II). During the incubation period, pots were watered twice a week with distilled water to field capacity by capillary action. Pots were re-distributed randomly at each irrigation event. We could not follow this protocol for 35 d (from 7 April to 15 May 2020) because of the national lockdown due to the coronavirus pandemic; however, after this period, we checked the pot status and no visual damage was observed.

Table 1. Properties of the soil used in the experiment.

Clay g kg^{-1}	Silt g kg^{-1}	Sand g kg^{-1}	TN g kg^{-1}	TOC g kg^{-1}	pH	EC dS m^{-1}	CEC cmol kg^{-1}
209	461	330	1.50	9.25	7.84	1.88	18.4

TN = total nitrogen; TOC = total organic carbon; EC = electrical conductivity; CEC = cation exchange capacity.

2.2. Exp I: polyester microplastic fibers effect on soil physical properties

The experiment was performed in 0.1 l pots (height = 5 cm; diameter = 5 cm) filled with 80 g of soil contaminated or not with PMF as reported above. The Ctr treatment was set up in eight replicates while the MP add treatment consisted of four replicates.

At the end of the incubation period, the soil samples were placed on the porous plate of a glass funnel and saturated from the bottom by progressively raising the water level in a graduated burette which can be adjusted in height [36]. Saturation was achieved in three steps of 24 h each followed by submersion for 2 h.

From saturation, soil samples were desorbed by imposing a sequence of eight pressure head values, h (m), down to −1.00 m. At each h level, we recorded the volume of drained water into the burette. The volumetric water content, θ (cm cm^{-3}), corresponding to $h = -0.1$ m, was calculated by adding the drained volumes to the value determined at $h = -1.00$ m after oven-drying.

Dry soil from each pot was then crushed and compacted in small samples (height = 5 cm; diameter = 5 cm) at the same bulk density measured on the larger samples for determination of volumetric water content corresponding to $h = -3.3, -10, -33$ and −150 m by a pressure plate apparatus [36]. All analyses were conducted in a laboratory under controlled conditions of temperature (mean $T = 22$ °C).

The dry bulk density, ρ_b (g cm^{-3}) was calculated by measuring the volume at $h = -1.00$ m and the oven-drying weight of soil samples.

Total soil porosity, ϕ, was determined as:

$$\phi = 1 - \frac{\rho_b}{\rho_s}$$

where ρ_s (g cm^{-3}) is the soil particle density that was assumed 2.60 g cm^{-3} as common for mineral soils.

Macroporosity was obtained as the difference between total porosity and θ at $h = -0.10$ m; microporosity was obtained as the difference between total porosity and macroporosity. The plant available water content (PAWC; cm^3 cm^{-3}) was calculated as the difference between the volumetric water content corresponding to $h = -150$ m and $h = -1.00$ m.

2.3. Exp II: polyester microplastic fibers effect on plant performance and soil processes

To investigate the effect of PMF contamination on plants performance and environmental sustainability, we used 0.6 l pots (height = 30 cm; diameter = 5 cm) purposely modified to collect the soil leachate (figure 1) filled with 500 g of soil. In this experiment both treatments were set up in six replicates each.

After the incubation period, one maize plant (*Zea mais* L. var. *saccharata*, cv Iason F.1) per pot was grown for 34 d. The crop was fertilized with 49 mg of N per pot as ammonium nitrate (YaraBela SULFAN, N 24%) distributed in five equal events along the crop growth (7, 12, 14, 19, 24 d after sowing; DAS) (figure 2).

Plants were kept in optimal conditions in terms of water supply throughout the entire experiment. Pots were weighed at least three times a week and variation in weight was attributed to evapotranspiration. Watering was pot specific; so at each irrigation event a specific volume of distilled water was added from above to each pot to reach field capacity. Field capacity was previously determined through a gravimetric method. Briefly, ten perforated crucibles were filled with 100 g substrate and placed in a basin with water up to half of the height of the crucibles. The crucibles were allowed to absorb water by capillarity until each was saturated. Excess water was allowed to drain, and the crucibles were weighed and then oven-dried at 105 °C to a constant weight. The difference in weight between the crucibles before and after the drying process represented the soil water content at field capacity.

We simulated three heavy rain events during crop growth: the first at 18 DAS, the second at 27 DAS, and the third at 32 DAS just before the harvest (figure 2); thus the first event occurred after the 3rd fertilizer supply and the other two when the entire dose of N had been supplied. At these dates, the amount of water needed to bring the substrate back to field capacity was added of 30 ml (equivalent to a rainfall of 15 mm, so simulating a heavy rain event not unusual for the Mediterranean environment during spring). The day after each heavy rain event the leachate was collected (figures 1 and 2) and stored at −20 °C for further analysis.

The experiment was performed indoors at Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37.545064 °N latitude, 13.517530 °E longitude; 160 m a.s.l.) with a temperature regime of 23 ± 2 °C and natural daylight period.

Pots were re-randomized at every leachate collection time. At the end of the experiment (34 DAS; when corn plants reached the phase of seven leaves unfolded), above- (shoot) and belowground (root)

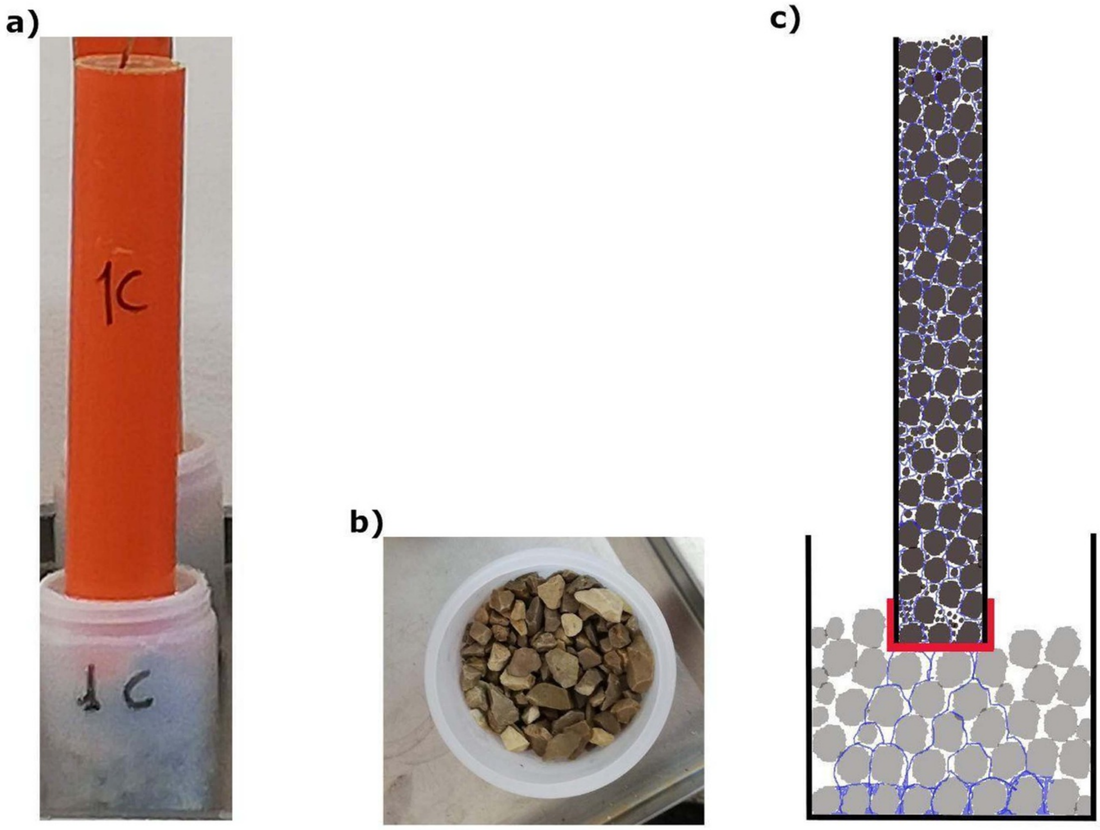

Figure 1. (a) Photo of pot used in the experiment; (b) leachate collection pot; (c) design schematic of the pot used in the experiment: in brown soil particles; in red cotton mesh; in grey gravel; and in blue soil solution.

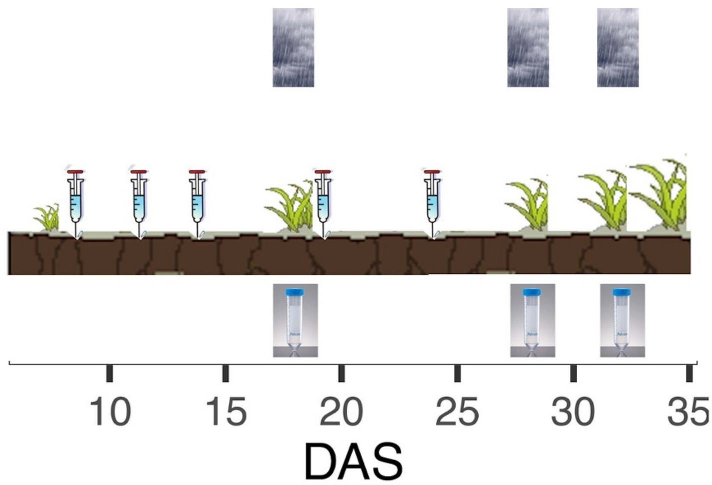

Figure 2. Scheme of experimental management during crop growth (DAS: days after sowing). Syringes indicate fertilization events; rain clouds indicate the heavy rain events; falcon tubes indicate the leachate collection time points.

biomasses were harvested and dry weights recorded. A root sub-sample was extracted and scanned using the same method described previously for the fibers characterization. Scan data and dried root mass were used to calculate total root length and specific root length (SRL).

Both biomass fractions were separately ground to a fine powder and used to assay the N concentration with the Dumas method (flash combustion with an automatic N analyzer; DuMaster D-480, Büchi Labortechnik AG, Flawil, Switzerland).

From each pot, a soil sample was collected and the soil ammonium and nitrate concentration was quantified using a colorimetric method. Briefly, 10 g soil was extracted in 100 ml of a 2 M potassium chloride-extractable solution and shaken for 1 h at 140 rpm. The solution was filtered through filter paper (Whatman 42) and used for assaying the ammonium and

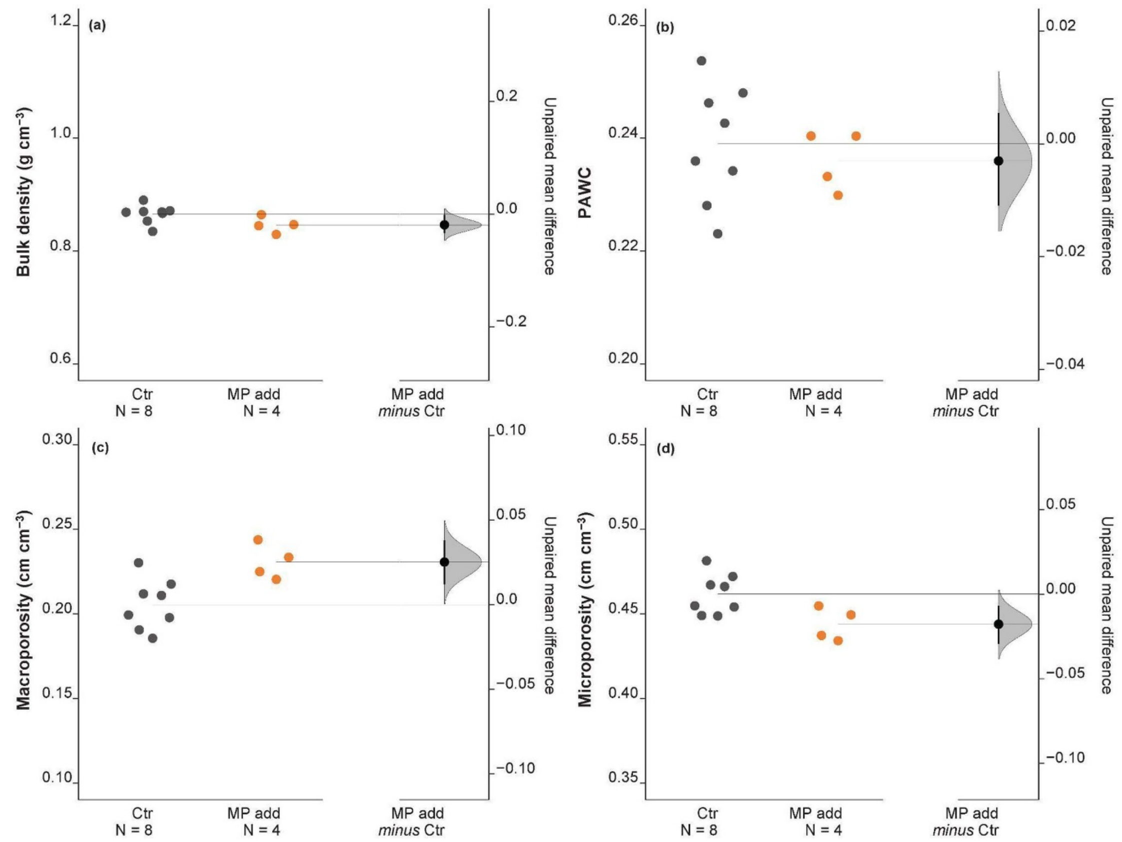

Figure 3. (a) Bulk density; (b) field capacity water content; (c) macroporosity; (d) microporosity. Raw data of control (Ctr, grey dots) and PMFs addition treatment (MP add, brown dots) are shown in the plot. The filled curve indicates the resampled distribution of unpaired mean difference, given the observed data. Horizontally aligned with the mean of the test group, unpaired mean difference is indicated by the black circle. The 95% CIs are illustrated by the black vertical lines.

nitrate concentration using the AA100 AutoAnalyzer (SEAL Analytical GmbH, Norderstedt, Germany).

The three collected leachates were filtered through filter paper (Whatman 42) and the ammonium and nitrate concentration was assayed using the same colorimetric method.

The total biomass production (tot biom; shoot and root) and the total water consumption ($water_{cons}$) were used to calculate the water use efficiency (WUE) as follows:

$$WUE = \frac{\text{tot biom}}{water_{cons}}.$$

2.4. Statistical analysis

All response variables were compared between the two groups (MP add minus Ctr) using the package 'dabestr' [37] to generate unpaired mean differences via a bias-corrected and accelerated bootstrapped 95% confidence intervals (CIs). Graphical data representations were generated using the 'dabestr'. We supported our findings by analyzing the data with Kruskal–Wallis one-way ANOVA test.

We used this combined approach based on the expanding recognition of the limitation of using only the '*P*-value statistic' approach and to avoid dichotomous cutoffs [37, 38].

All analyses were performed using R version 4.0.2 [39].

3. Results

3.1. Exp I: polyester microplastic fibers effects on soil physical properties

In our experiments, we found a slight effect of PMF on soil physical properties. In particular, we found that polyester contamination slightly reduced the soil bulk density and microporosity (unpaired mean difference of −0.019 g cm^{-3} and −0.018 cm cm^{-3} for bulk density and microporosity, respectively; 95% CIs: −0.033 to −0.001 g cm^{-3} for bulk density and −0.029 to −0.007 cm cm^{-3} for microporosity; figures 3(a) and (d); *P*-value reported in table S1 available online at stacks.iop.org/ERL/17/054012/mmedia) while increased the soil macroporosity (unpaired mean difference of −0.252 cm cm^{-3}; 95% CIs: 0.12–0.379 cm cm^{-3}; figure 3(c); *P*-value reported in table S1). No differences were observed on the PAWCs between PMF contamination treatment and the uncontaminated control (on average 0.237 figure 3(b); *P*-value reported in table S1).

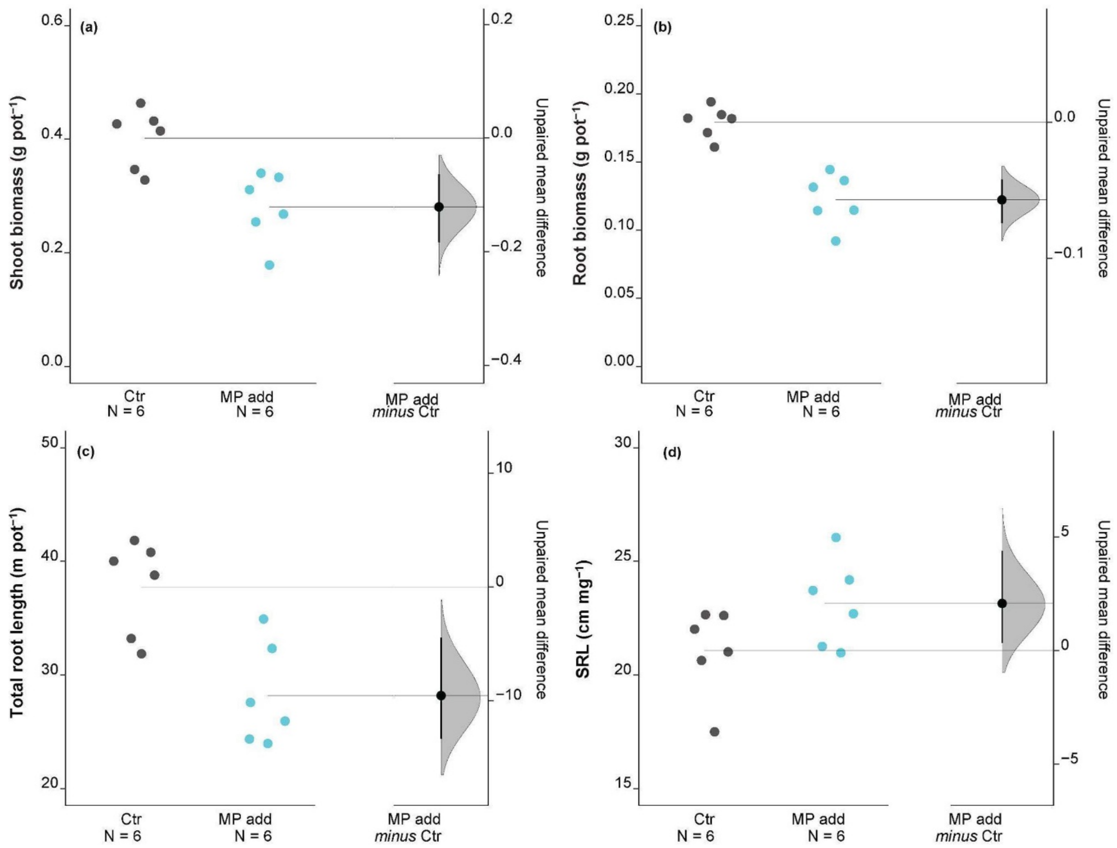

Figure 4. (a) Shoot dry weight; (b) root dry weight; (c) total root length; (d) SRL. Raw data of control (Ctr, grey dots) and PMFs addition treatment (MP add, green dots) are shown in the plot. The filled curve indicates the resampled distribution of unpaired mean difference, given the observed data. Horizontally aligned with the mean of the test group, unpaired mean difference is indicated by the black circle. The 95% CIs are illustrated by the black vertical lines.

3.2. Exp II: polyester microplastic fibers effects on plant performance and soil processes

PMFs contamination strongly affected plant biomass production and N uptake, for both shoot and root (figures 4(a) and (b); figures 5(a) and (b); table S1). In any case, polyester fibers contamination caused a strong detrimental effect leading to a reduction of circa 30% in plant biomass. It also led to a detrimental effect in total root length while it slightly increased SRL (unpaired mean difference of -9.57 cm pot^{-1} and 2.7 cm mg^{-1} for total root length and SRL, respectively; 95% CIs: -13.4 to -4.48 cm pot^{-1} for total root length and 0.338–4.39 cm mg^{-1} for SRL; figures 4(c) and (d); table S1). Soil contamination also substantially affected the N uptake with a decrease of circa 30% both in the above- and in the belowground plant fraction (figures 5(a) and (b); table S1). However, we did not observe the same effect on shoot and root N concentration (figures S1(a) and (b); table S2).

Concerning the efficiency in water utilization, we found that the contamination with PMF caused a detrimental effect of circa 26% (figure 5(c); table S1).

We found that PMF contamination increased the amount of leachate and N leached (both ammonium and nitrate forms) both in total and at each heavy rain event during crop growth (figures 6(a)–(c); figures S2–S4; tables S1 and S2). In total, we found that the PMF contamination increased the leachate amount by 38% (33.0 $\pm$ 6.1 vs 45.5 $\pm$ 8.9 ml per pot in the Ctr and in the MP add treatment, respectively; unpaired mean difference 12.6 ml; 95% CIs: 5.28–20.9 ml; figure 6(a)) and the total amount of N lost via leaching by 44.5%, without appreciable differences between the two investigated forms of N (0.18 $\pm$ 0.02 in Ctr vs 0.26 $\pm$ 0.02 in MP add and 0.21 $\pm$ 0.04 in Ctr vs 0.30 $\pm$ 0.05 in MP add mg of N leached per pot in ammonium and nitrate forms, respectively; figures 6(b) and (c)).

4. Discussion

In our experiment, we found that PMF contamination caused subtle effects on soil physical properties, but it strongly reduced plant growth, WUE, and the amount of N taken up by the maize crop; thus increasing the risk of release of the N supplied through the fertilizer in the environment.

Regarding the soil physical properties, we found that PMF contamination caused a slight decrease in soil bulk density. Studies investigating this soil property have not shown consistent results. For instance, Machado *et al* [13, 16] reported a decrease in bulk

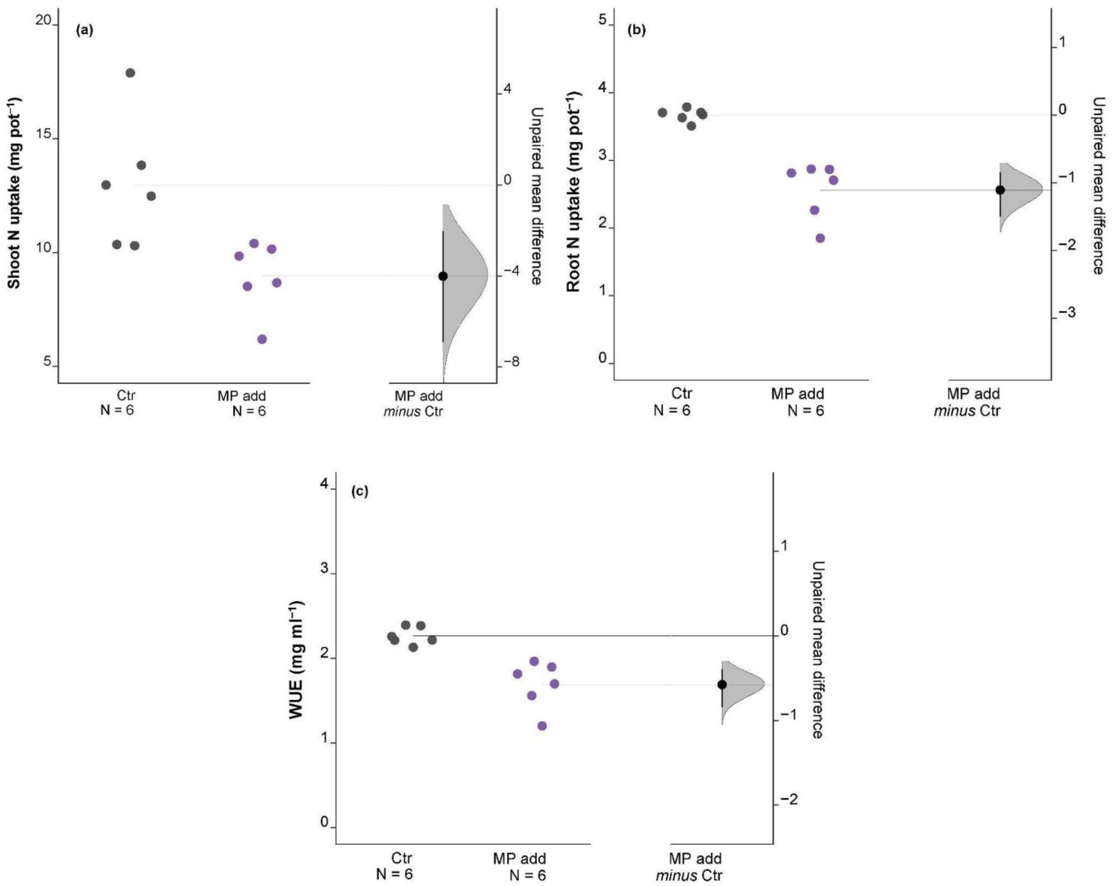

Figure 5. (a) Shoot N uptake; (b) root N uptake; (c) WUE. Raw data of control (Ctr, grey dots) and PMFs addition treatment (MP add, green dots) are shown in the plot. The filled curve indicates the resampled distribution of unpaired mean difference, given the observed data. Horizontally aligned with the mean of the test group, unpaired mean difference is indicated by the black circle. The 95% CIs are illustrated by the black vertical lines.

density in a loamy sandy soil, with stronger effects by increasing the contamination concentration (from 0.05% to 0.4% on soil dry weight in [13] and 0.2% on soil fresh weight in [16]), whereas Zangh *et al* [17] reported no effects due to the contamination of PMF at concentrations from 0.1% to 0.3% on soil dry weight in an experiment conducted in a clay loam soil.

In our experiment, we also found that PMF did not affect the PAWC and the total porosity, but slightly decreased the soil microporosity and increased the soil macroporosity compared to the uncontaminated control. Similar effects were also observed by Zhang *et al* [17] who ascribed the increment of soil macroporosity to an increased soil aggregation status that helped to entangle soil particles more efficiently to form aggregates. However, other experiments investigating this soil property have also reported contrasting results, suggesting that the soil type (soil texture, matrix from which soil particles originated) and other soil characteristics (e.g. organic matter content) can affect the interaction between the soil particles and the PMF [40]. In fact, as already suggested by Ingraffia *et al* [40], different soil particles may have a different affinity with the polyester fibers. Certainly, further research on such interactions is necessary to identify which factors and mechanisms can influence the structure and stability of soils contaminated with MP.

Concerning the plant performance, we found that the presence of PMF in soil drastically decreased maize biomass by circa 30%, both above- and belowground, as well as the WUE and the ability of plants to capture soil N. Our findings partially contrast with other authors who found that soil contamination with polyester fibers concentrations similar to that applied in our experiments increased the below- and aboveground biomass of *Allium cepa* and *Daucus carota* [16, 34, 41]. Certainly, the focal plant species (*Z. mais* in our experiment and *A. cepa* and *D. carota* in those of the cited authors) could have played a role in these contrasting results. Indeed, as reported by Lozano and Rillig [33], PMF can differentially affect different plant species, thus leading to shifts in plant community structure. The above-mentioned authors [16, 34, 41] have ascribed their results to a reduction of the soil bulk density and a concomitant increment in the water holding capacity, which may facilitate root biomass production and improve the nutrient and water supply of the plant. In our case, the effects of PMF on

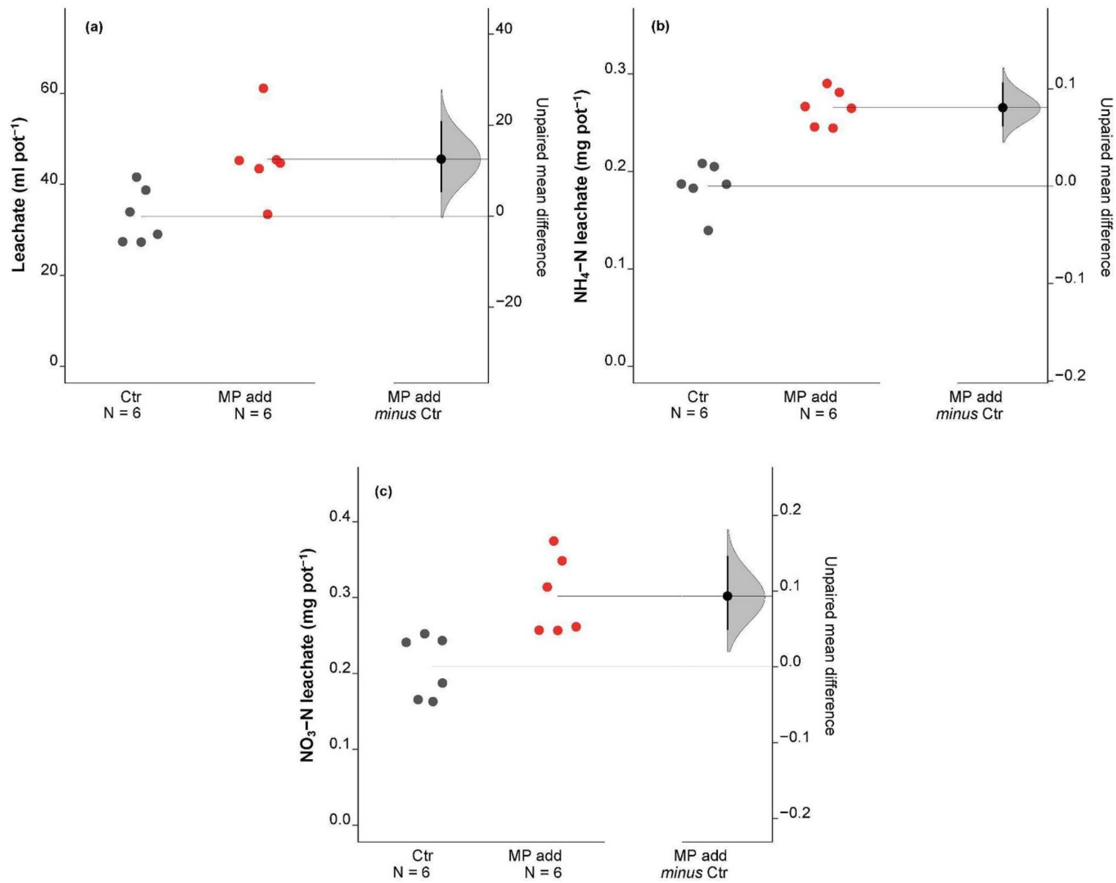

Figure 6. (a) Total leachate; (b) nitrogen leached in ammonium form; (c) nitrogen leached in nitrate form. Raw data of control (Ctr, grey dots) and polyester microplastic addition treatment (MP add, red dots) are shown in the plot. The filled curve indicates the resampled distribution of unpaired mean difference, given the observed data. Horizontally aligned with the mean of the test group, unpaired mean difference is indicated by the black circle. The 95% CIs are illustrated by the black vertical lines.

soil physical and hydrological properties were rather subtle, and we believe that such effects cannot explain entirely the pronounced differences that we observed in the plant performances grown in soil contaminated or not. In their review, Iqbal *et al* [16] hypothesized that the alteration of the soil environment due to the presence of microplastic can affect the activity of key N enzymes, soil N cycling processes (e.g. nitrification, volatilization), and affect the accumulation of dissolved N on the microplastic particles. These effects of microplastics could lead to a reduction in the availability of N with negative repercussions for plant growth. However, in our experiment, these factors certainly did not play a key role in the plant responses as the large amount of N-based fertilizer supplied guaranteed that N was not a limiting factor for plant growth. This was also confirmed by the similar values of N concentration in plant tissues observed in the two treatments (Ctr and MP). Therefore, we cannot exclude that other mechanisms have also contributed to our findings in terms of plant growth. For instance, direct effects of the PMF which could have acted as a physical barrier to the fine roots and thus limited the soil volume explored by the crop or other direct effects of the contaminant on soil chemical properties, chemical properties of the microplastic itself, and indirect toxicity effects on plant development via changes in soil microbial communities [40–42]. Indeed, the degradation of microplastic during the experimental period (we did a pre-sowing incubation period of circa 5 months) could have released nanoparticles that may have been absorbed by the plant root system and may have induced damage to plant tissues. Moreover, we cannot exclude that the microplastics used in the experiment did contain contaminants that may have affected the plant development (e.g. plasticizers and nonylphenol). However, these mechanisms are still very poorly understood and future investigations are needed to verify such hypotheses.

The reduced amount of N taken up and used by the crop in the contaminated soil suggests that soil N could have followed other pathways, including leaching and NH_3 emissions (as already observed by Sun *et al* [42]) and that in our experiment could have also been facilitated by the type of the applied fertilizer (50% ammonium and 50% nitrate). Indeed, in our study we found that PMF contamination increased the amount of leachate and N leached (both ammonium and nitrate forms) both in total and at each heavy rain event during crop growth; even if the loss of N through leaching was only a small fraction of the supplied N (circa 1%). Certainly, the observed increment of macroporosity due to soil PMF contamination could have provided preferential paths for water movement and thus leaching-related nutrient losses. Moreover, the reduction in plant root development and its ability to take up N have increased the pool of N potentially leachable in the contaminated soil, which was then leached during the extreme rain events.

5. Conclusions

Pathways to addressing key agricultural challenges, including maintaining and increasing food security and environmental protection, have been widely described in the literature. We here present empirical evidence that microplastic fibers contamination can affect soil physical properties and seriously undermine maize biomass production both qualitatively and quantitatively. If these findings were confirmed in larger-scale experiments, they would indicate that soil contamination with microplastic fibers could have significant implications on crop productivity. Moreover, despite the limits of the pot experiments, our results show that PMFs contamination could also be a potential hazard for natural environments due to an increment in the amount of leachate and N leached. Nevertheless, our data do not permit us to conclude with certainty that the increase of N leached due to soil contamination was caused by changes in soil physical properties since, at the same time, we observed a marked reduction in plant N uptake and an increase in potentially leachable nitrogen. Further experiments are needed to investigate more closely the interactions between PMF, soil, and plants in order to uncover the mechanisms underlying the observed responses. Overall, our results provide a new starting point to explore the effects of microplastics on the performances and environmental sustainability of agroecosystems. It is noteworthy to mention that we used only one microplastic type and one concentration; certainly various microplastic shapes and polymers may exert different effects on soil properties, and (agro)ecosystem functions and processes. In addition, different soil types and crop species may vary in their response to soil microplastic contamination. Therefore, testing the generality of our findings in various agroecosystem contexts should be a high priority, allowing more complete insights into the effects of this emergent anthropogenic global change factor in agroecosystems.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Author contributions

All the authors (R I, G A, A F, M I, M R, and D G) conceptualized the study. G A, A F, and D G acquired the funds to conduct the experiment. RI, AF, MI, and DG carried out the experiments. R I analyzed the data and wrote the first draft of the manuscript. G A, A F, M I, M R, and D G collaborated on the ideas and contributed critically to the drafts. All authors gave the final approval for publication.

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