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RESEARCH ARTICLE

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Effects of microplastics on crop nutrition in fertile soils and interaction with arbuscular mycorrhizal fungi

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Abstract

Introduction: Soil microplastic (MP) pollution has emerged as a main factor of global change, but its effects on soil nutrient availability and uptake by crops (macro and micronutrients) are largely unknown. Arbuscular mycorrhizal fungi (AMF) are regulators of nutrient availability and uptake and can interact with soil MP.

Materials and Methods: Building on previous studies, here we explored in a 50-days pot experiment the influence and interaction of MP fibres (0.4%) and commercial AMF in soil and onion chemistry, that is, in elemental composition of onion shoots and soils (C, N, Ca, Mg, K, P, S, Cu, Fe, Mn and Zn) and micronutrient soil availability (Cu, Fe, Mn and Zn).

Results: MP had detrimental effects on K, Mg and S, but increased the soil availability of Zn and shoot uptake. AMF inoculation buffered the effects of MP by balancing/enhancing nutrient availability and plant uptake. Particularly, the commercial AMF inoculum remarkably enhanced Mn uptake by onion.

Conclusion: Our results support the use of AMF to sustainably manage agricultural ecosystems contaminated with MP, buffering and counteracting the effects of MP by balancing nutrient availability and plant uptake.

KEYWORDS
macronutrients, micronutrients, microplastics, onion, soil

1 | INTRODUCTION

Microplastics (MP) are being recognized as an influential factor of anthropically-driven global environmental change, together with other factors including elevated atmospheric CO_2, drought, and warming.[1,2] MP are small particles (<5 mm) of any plastic material that has been produced in that small size (primary MP) or that has undergone physical fragmenting (secondary MP).[3] MP comprise a wide spectrum of materials with different chemical composition, additives, size and shapes,[4] which determine their ecological impact.

MP have been tested intensively from the ecotoxicological point of view, exposing different organisms to increasing doses of MP under controlled conditions and standard setups. But other aspects have been less intensely studied, such as the agro-ecological effects.[5]

Recently MP have been found to affect soil physical characteristics (e.g., soil aggregation and bulk density), chemical composition (e.g., organic matter content), and soil biota.[6] In the context of agriculture, MP have been shown to influence crops, by, for example, increasing plant growth and changing root architecture.[6–8] Despite a growing body of evidence of MP impacts on plant performance, their effects on element cycling are still obscure, especially in agricultural ecosystems. For instance, MP can affect dissolved organic C in the soil[9] or can sorb metals,[10,11] thus eventually altering mineralization in soil or nutrient uptake by plants. This suggests that MP contamination in soil has the potential to alter plant nutrition.

Plant nutrition and crop performance are often influenced by arbuscular mycorrhizal fungi (AMF), frequent symbionts of plants in agricultural ecosystems.[12] AMF are known to improve water[13,14] and nutrient acquisition,[15,16] and therefore they could modulate the impacts of MP on nutrients in agricultural ecosystems. Plants in general vary in their degree of mycorrhizal symbiosis in terms of root colonization with consequences for root morphology and anatomy.[17,18] Species that source out nutrient acquisition to mycorrhizal partners show larger fine-root diameters due to an increase of the cortical area—a pattern that also occurs plastically within species.[19] As AMF specifically enhances uptake of macronutrients as well as soil exploration efficiency,[20] effects on plant nutrition might be mediated by changes in soil chemistry or physical structure.

However, the interaction of these two agronomical factors, MPs and AMF, on crops remains underexplored. Filling this knowledge gap is critical because a wide part of the population is subjected to macro- and micronutrient deficient diets, while MP continue to accumulate in soils everywhere. Overall, a better knowledge of MP and AMF interactions will enhance our understanding of the indirect far-reaching consequences of MP accumulation, and may assist in a more sustainable management of agrosystems in upcoming years.

We therefore designed a greenhouse pot trial where we studied the potential impacts of MP and their interactions with AMF on soil chemistry and mineral composition of onion plants. We tested the hypothesis that MP change the occurrence and availability of nutrients in soil and have detrimental effects on mineral composition of onion, but that AMF may revert these detrimental effects.

2 | MATERIALS AND METHODS

We report data from a greenhouse experiment with a daylight period set to 12 h, 50 klx lighting, temperature 22/18°C (day/night) and relative humidity ~40%, for which plant growth data have previously been reported.[7] More details on the experimental setup are given in Lehmann et al.[7] The experiment had a fully factorial design with 10 replicates and two factors: MP addition (−/+) and AMF inoculation (−/+).

In short, the soil used for the experiment was collected from the top 30 cm of an Albic Luvisol and had sandy loam texture, a pH of 7.1, 1.9% total C content, 0.12% total N, 6.9 mg P/100 g and 5.0 mg/100 g K (calcium-acetate-lactate method).[21] The soil was sieved to <2 mm. The pots were filled with a mix of soil and <2 mm sand (1:1). Polyester microfibers (Paraloc rope, Mamutec, Switzerland, product number: 0025-00080-01-0, diameter of 30 µm) were manually clipped to obtain small fragments (1.7 ± 0.9 mm, *n* = 130, for more details on the shape of polyester microfibers (distribution of diameter and length), check Figure S1 in Lehmann et al.[7] MP was added to MP+ pots manually at a dose of 0.4% (w:w). Controls (MP−) were processed similarly to cause comparable disturbance. The prepared substrates were steam-sterilized at 100°C twice. We selected an AMF commercial inoculum (INOQ Agri; INOQ 120 GmbH) with three species (*Rhizoglomus irregularis*, *Funneliformis mosseae* and *Funneliformis caledonium*) on vermiculite as carrier. The inoculum was applied alive (AMF+) or sterilized (AMF−), after suspending it in water and following the instructions of the producer. A microbial soil and AMF carrier material wash was prepared to add microbial non-AM fungal communities to all treatments to avoid confounding factors related to the carrier of the AMF (changes in soil structure and nutrients induced by the carrier). Onion seeds, *Allium cepa* (variety 'Kaigaro', Albert 158 Treppens & Co Samen GmbH, EG Pflanzenpass Nr.: DE-BE1-29005 RP 25 003 253840), were germinated in the soil and cropped. The selection of onion was based on the response of this plant to AMF symbiosis, and also previous experiments with MPs used this plant.

After 50 days, soil and whole plants were carefully extracted from the pots. Plant material was washed and separated into below and aboveground tissues, dried, weighed, and ground into a fine powder. For analyses, only aboveground material (shoots) was used as estimation of plant element uptake. C and N contents in plants were determined by dry combustion with an Elemental Analyzer CN (Leco Truspec). Plant material was digested in nitric acid (70%), hydrogen peroxide (30%) and miliQ water in an autoclave (125°C) to analyse macro and micronutrients.[22]

Soil was dried before analysis. The available fraction of nutrients in the soil was extracted in 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine (TEA) (pH 7.3) (Lindsay and Norvel, 1979). In addition, the total nutrient fraction was extracted by nitric acid (70%) and hydrogen peroxide (30%) digestion in autoclave (125°C).[23]

Metals in plant and soil extracts were analysed by ICP-OES ICAP 6500 DUO/IRIS Intrepid II XLD system (Thermo Fisher Scientific). C and N in plants were analysed with an Elemental Analyst TruSpec CN628 (LECO).

Three parameters were calculated to help discuss nutrient uptake: the total amount of element taken up to the shoots (element uptake) as the concentration of elements in shoot times the shoot biomass; the available nutrient fraction as the ratio of available to total metal in soils; and the transfer factor as the ratio of element in shoot to element in soil. General Linear Model (GLM) analysis was used to identify the influence of MP, AMF and their interaction on soil and plant parameters, using IBM SPSS v 26.0.

Kolmogorov–Smirnov test was used to assess normality and Levene test to assess homoscedasticity, and the data were transformed if needed ($\log_{10}$ transformation). The data plotted in the figures are shown untransformed.

3 | RESULTS AND DISCUSSION

The soil used in our study did not exhibit any obvious limitation in nutrients according to the adequate plant growth and to the chemical analyses of nutrients. The occurrence of MP, the addition of AMF and the interaction of both factors did not significantly affect the total or DTPA-extractable concentration of macro- and micronutrients in soil (*p* values between 0.15 and 0.96; Figures S1 and S2).

The presence of MP increased the root and especially the shoot weight of onions grown without AMF (Table 1, Figure S3), as previously reported in Lehmann et al.[7] Positive effects of MP on some crops have been previously observed in a pot experiment and explained by an alteration of soil physical properties resulting in improved water holding capacity and aeration.[7] The addition of AMF tended to increase onion shoot biomass grown in soil without MP and decreased root biomass (Table 1, Figure S3), the latter most probably indicating a smaller investment in roots when plants establish symbiotic relationships with fungi that assist in nutrient and water acquisition.[24]

The concentrations of several macro- and micronutrients in onion shoots were affected by MP, AMF and their interaction (Figures 1 and 2, Table 1). In particular, MP significantly decreased the concentration of S (Figure 1, Table 1), whereas inoculation with AMF doubled the concentration of Mn in shoots compared with non-AMF plants (Figure 2, Table 1). This positive effect has not been described so far for this specific inoculum and is remarkable, as Mn is deficient in cropping systems in some specific soils and in human diets.[25,26] We also found marked interaction effects of MP and AMF on the concentration of macronutrients in onion shoots. In particular, MP caused a decrease in the concentration of C, N, K and Mg in onion shoots which is more evident in the absence of AMF (Figure 1, Table 1). The interaction of MP and AMF was particularly evident for Mg and Ca, where MP noticeably decreased shoot concentrations in the absence of AMF, but had minimal effects in AMF+. This highlights the potential buffering effects of AMF on detrimental impacts of soil MP, which may be related to the ability of AMF to efficiently provide nutrients to plants. On the contrary, in the presence of AMF macronutrient concentrations were generally lower, but MP had only minimal influence on nutrient concentration. Similarly, the presence of MP tended to decrease the concentration of Fe, Mn and Cu (all the micronutrients examined except Zn) in onion shoots, but the inoculation with AMF tended to mitigate this effect (Figure 2, Table 1). The decrease in K, Mg, Fe, Mn and Cu concentration in onion shoots in the presence of MP may be attributed to a dilution effect, as uptake (in elemental mass in onion shoots per pot) of these elements was not decreased (Figures S4 and S5, Table S1). However, uptake of Zn, N and P in the presence

TABLE 1 Results of General Linear Model analyses showing the effects of microplastics (MP) and arbuscular mycorrhizal fungi (AMF) on onion growth (shoot and root biomass, g; reported in Lehmann et al.[6]) and on nutrient concentration of onion aboveground tissues (% for macronutrients and mg kg^{-1} for micronutrients)

			Shoot		Root		C		N		Ca		K		Mg		P		S		Cu		Fe		Mn		Zn	
	hdf	**edf**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
MP	3	36	**6.6**	**0.015**	1.6	0.22	1.7	0.20	0.88	0.35	2.5	0.12	1.3	0.27	2.3	0.14	0.02	0.88	**9.2**	**0.004**	1.6	0.21	0.51	0.48	0.42	0.52	0.32	0.58
AMF	3	36	0.11	0.74	**4.5**	**0.041**	**5.4**	**0.026**	0.24	0.63	0.36	0.55	1.0	0.32	0.11	0.74	0.47	0.50	0.72	0.40	0.037	0.85	0.053	0.82	**23**	**<0.001**	0.20	0.66
MP:AMF	3	36	3.9	0.055	2.9	0.099	**4.8**	**0.035**	3.3	0.076	.002	0.97	**5.5**	**0.025**	**9.1**	**0.005**	0.75	0.39	0.79	0.38	3.4	0.073	1.6	0.21	0.28	0.60	0.20	0.65

Note: Hypothesis degrees of freedom (hdf) and error degrees of freedom (edf) are shown. *p* Values below 0.05 are given in bold.

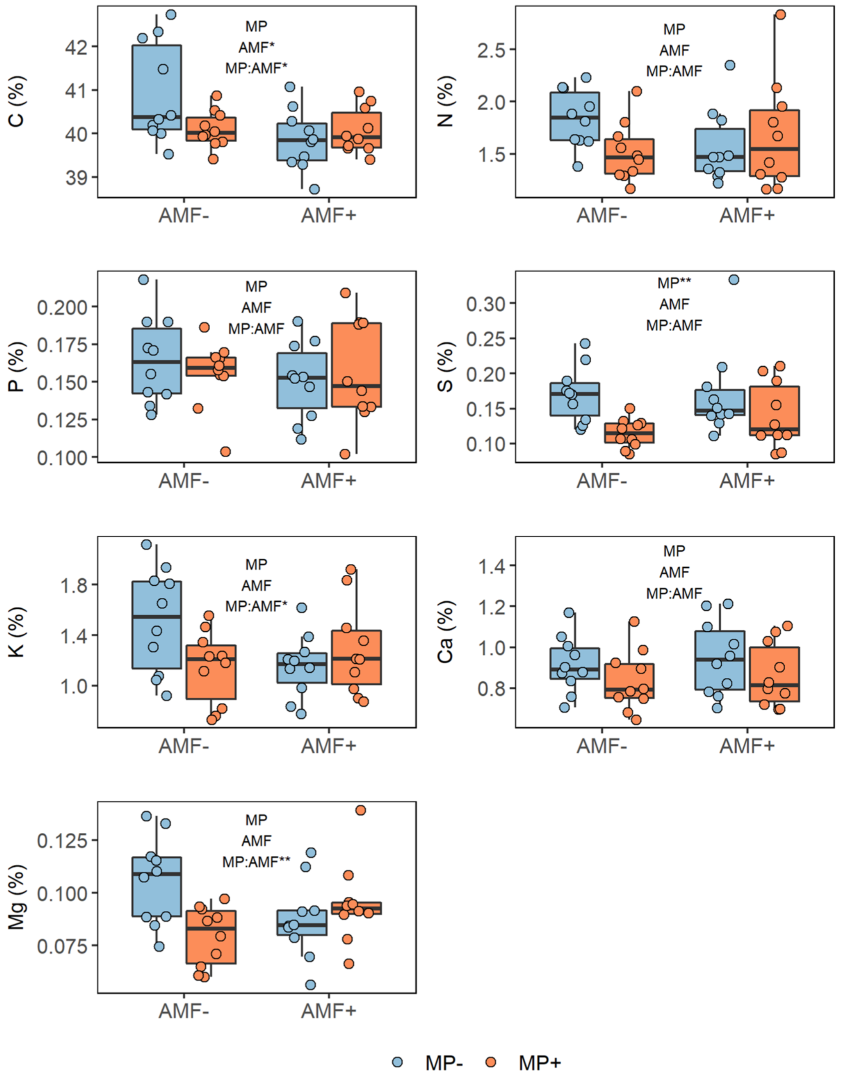

FIGURE 1 Concentration of macronutrients in shoots of onions as affected by microplastic fibres (MP− and MP+) and arbuscular mycorrhizal fungi (AMF− and AMF+). Single data points are shown in circles while their distributions are indicated in box-and-whisker plots (*n* = 10). AMF, arbuscular mycorrhizal fungi; MP, microplastic. *, ** and ***, significant at the 0.05, 0.01 and 0.001 probability level, respectively, General Linear Model analysis; absence of asterisks indicates no significant effects

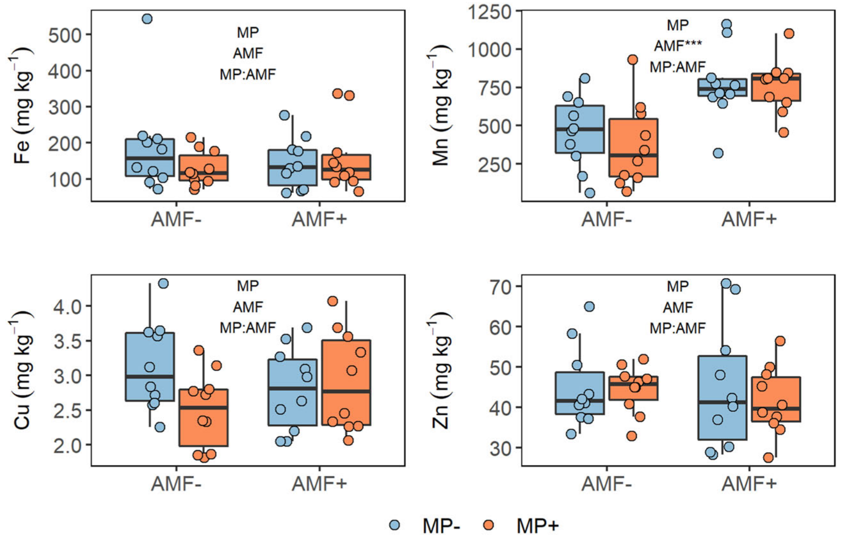

FIGURE 2 Concentration of micronutrients in shoots of onions as affected by microplastic fibres (MP− and MP+) and arbuscular mycorrhizal fungi (AMF− and AMF+). Single data points are shown in circles while their distributions are indicated in box-and-whisker plots (*n* = 10). AMF, arbuscular mycorrhizal fungi; MP, microplastic. *, ** and ***, significant at the 0.05, 0.01 and 0.001 probability level, respectively, General Linear Model analysis; absence of asterisks indicates no significant effects

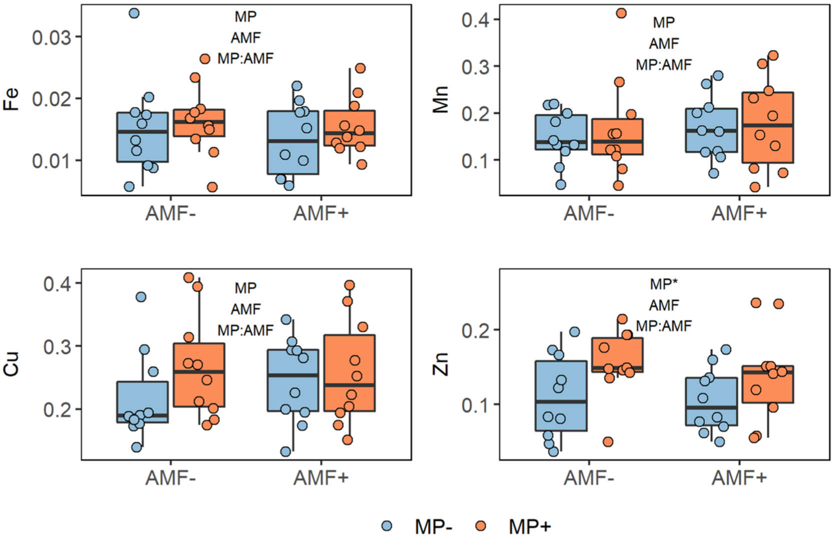

FIGURE 3 Available fraction of micronutrients (ratio of DTPA-extractable to total concentration; unitless) in soils as affected by microplastic fibres (MP− and MP+) and arbuscular mycorrhizal fungi (AMF− and AMF+). Single data points are shown in circles while their distributions are indicated in box-and-whisker plots (*n* = 10). AMF, arbuscular mycorrhizal fungi; MP, microplastic. *, **, and ***, significant at the 0.05, 0.01, and 0.001 probability level, respectively, General Linear Model analysis; absence of asterisks indicates no significant effect

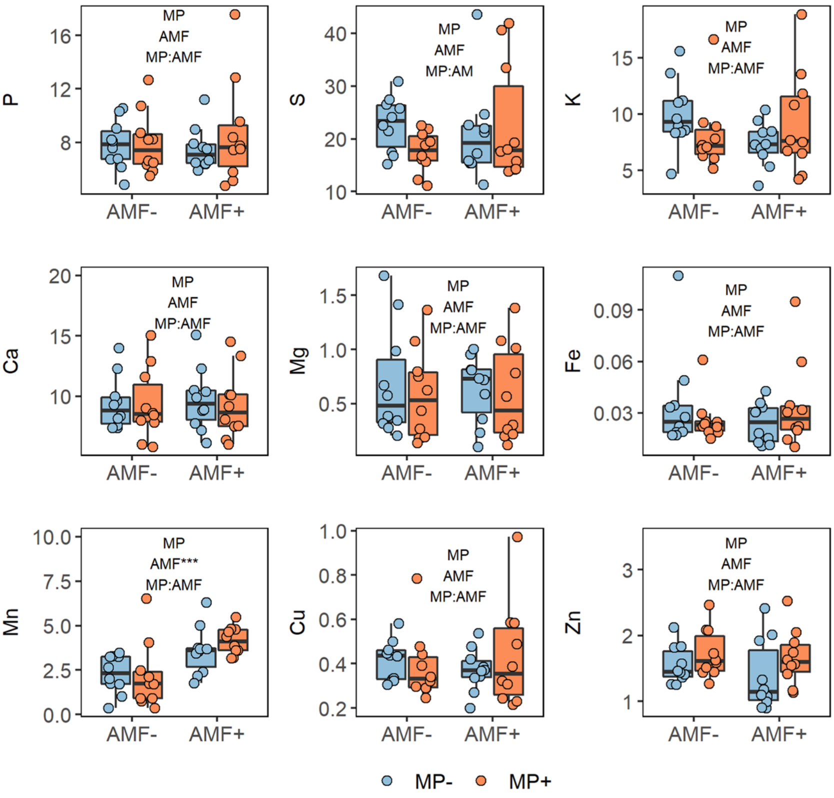

FIGURE 4 Transfer factor (ratio of element concentration in shoot to element concentration in soil; unitless) in shoots of onions cultivated affected by microplastic fibres (MP− and MP+) and arbuscular mycorrhizal fungi (AMF− and AMF+). Single data points are shown in circles while their distributions are indicated in box-and-whisker plots (*n* = 10). AMF, arbuscular mycorrhizal fungi; MP, microplastic. *, ** and ***, significant at the 0.05, 0.01 and 0.001 probability level, respectively, General Linear Model analysis; absence of asterisks indicates no significant effect

of MP was enhanced in non-AMF pots. MP have been previously found to increase the availability of P, N and Zn,[9,27] but such effect on plant elemental composition has not been reported before, to our knowledge. On the contrary, previous studies have frequently documented detrimental effects of MP on soil fertility through - indirect effects on microbial activity (reduction).[28,29] Our results illustrate that some elements are more readily taken up and accumulated when MP accumulate in the soil. MP can induce indirect (physical structure, microbial activity) and direct (C content, soil pH) changes in soils, which may alter elemental concentration and plant uptake, because they are very dependent on soil soluble pools of nutrients. Our results also suggest that the presence of AMF may mitigate the MP-induced alterations in plant concentrations and uptake of some nutrients. Mn uptake was greatly enhanced by AMF, in parallel to our findings in Mn concentration in shoots. In agreement with our results, AMF have been previously linked to improved Fe, Zn and P nutrition in other soil systems.[15,30,31] Altogether, our study broadly suggests that AMF may be useful to enhance plant nutrition (Mn) and subsequent nutritional status of food, but further research is needed to test this effect with different soils and crops and to ensure this effect is still observed under field conditions.

To evaluate the accessibility and transfer of elements from the soil to the plants, we used the ratio of DTPA-extractable to total concentration in soil (available fraction) and the ratio of element concentration in shoot to element concentration in soil (transfer factor) (Figures 3 and 4, Table S1). MP application significantly enhanced the available fraction of Zn in soils which resulted in an enhanced uptake but similar concentration of Zn in onion shoots. This unmatching trend of concentration and uptake of Zn reflects a dilution effect in MP+ plants rather than Zn uptake limitations in onion. Regarding the transfer factor, the only significant effect mirrored the strong positive influence of AMF on Mn in onion described above, highlighting the ability of AMF to enhance the acquisition of soil Mn, probably by active mechanisms or increasing soil volume exploration.[32]

MPs are widely recognized as a main factor of environmental change, particularly in soils, which have been shown to be highly vulnerable to MP accumulation in many aspects. Yet the singular issue of metals cycling and transfer to plants had remained unexplored to date. Our results indicated that in fertile soils MP have little effects on nutrients, decreasing the concentration of S, K and Mg in the absence of AMF and increasing the availability and uptake of Zn. All these effects are attributable to a mineral dilution effect, that is, more shoot biomass in the presence of MP. AMF can particularly enhance uptake of Mn and buffer some of the effects of MPs. These MP effects observed in a rather short-term experiment may be transient. Future research should examine potential negative changes in nutrient cycling in the longer term, particularly in nutrient-deficient soils, which were not studied here, to avoid further depletion in the nutritional quality of crops. This aspect is critical because many agricultural soils are limited in nutrients and support food of low nutritional value.

4 | CONCLUSIONS

MP fibre application to soils had specific negative effects on soil chemistry and nutrition, especially for K, Mg or S, although it increased Zn availability in soil and shoot uptake. Despite these effects not being dramatic in a relatively fertile soil, the changes could have more serious consequences in nutrient-limited soils and this needs to be further studied. AMF inoculation may buffer the effects of MP by balancing nutrient availability and plant uptake. The commercial AMF inoculum used in this study was able to remarkably enhance Mn nutrition in onion, which may be of interest in agriculture and food nutritional fortification. As a whole, the interactions between MP and AMF found in our study suggest that AMF can be used to sustainably manage agroecosystems polluted with MP.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

The authors confirm that they have adhered to the ethical policies of the journal.

AUTHOR CONTRIBUTIONS

Anika Lehmann designed (with Matthias C. Rillig) and performed the plant experiment, with the assistance of Linshan Feng, Eva F. Leifheit and Anja Wulf. Eduardo Moreno-Jiménez and César Plaza designed and performed the sample analyses. Eduardo Moreno-Jiménez and César Plaza drafted the manuscript. Anika Lehmann and Matthias C. Rillig refined the manuscript. All authors contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data are uploaded and available in the public repository Figshare (https://figshare.com/s/3ce78e3490b1f511ca11).

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SUPPORTING INFORMATION

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