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RESEARCH ARTICLE

Microplastics persist in an arable soil but do not affect soil microbial biomass, enzyme activities, and crop yield

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Abstract

Background: Microplastics (MP, plastic particles <5 mm) are ubiquitous in arable soils due to significant inputs via organic fertilizers, sewage sludges, and plastic mulches. However, knowledge of typical MP loadings, their fate, and ecological impacts on arable soils is limited.

Aims: We studied (1) MP background concentrations, (2) the fate of added conventional and biodegradable MP, and (3) effects of MP in combination with organic fertilizers on microbial abundance and activity associated with carbon (C) cycling, and crop yields in an arable soil.

Methods: On a conventionally managed soil (Luvisol, silt loam), we arranged plots in a randomized complete block design with the following MP treatments (none, low-density polyethylene [LDPE], a blend of poly(lactic acid) and poly(butylene adipate-co-terephthalate) [PLA/PBAT]) and organic fertilizers (none, compost, digestate). We added 20 kg MP ha^{-1} and 10 t organic fertilizers ha^{-1}. We measured concentrations of MP in the soil, microbiological indicators of C cycling (microbial biomass and enzyme activities), and crop yields over 1.5 years.

Results: Background concentration of MP in the top 10 cm was 296 ± 110 (mean ± standard error) particles <0.5 mm per kg soil, with polypropylene, polystyrene, and polyethylene as the main polymers. Added LDPE and PLA/PBAT particles showed no changes in number and particle size over time. MP did not affect the soil microbiological indicators of C cycling or crop yields.

Conclusions: Numerous MP occur in arable soils, suggesting diffuse MP entry into soils. In addition to conventional MP, biodegradable MP may persist under field conditions. However, MP at current concentrations are not expected to affect C turnover and crop yield.

KEYWORDS
field experiment, LDPE, microbial biomass, organic fertilizers, PLA/PBAT, plastics contamination, soil enzyme activity

1 | INTRODUCTION

Microplastics (MP) are commonly defined as plastic particles of various shapes and sizes between 100 nm and 5 mm (Okoffo et al., 2021; de Souza Machado et al., 2018). MP are suspected threats to soil organisms and functions (Helmberger et al., 2020; Pathan et al., 2020; Rillig et al., 2021; Q. Wang et al., 2022). Arable soils receive MP primarily due to amendment with sewage sludge, organic fertilizers, and plastic mulch (Corradini et al., 2019; Gui et al., 2021; van Schothorst et al., 2021; Vithanage et al., 2021; J. Wang et al., 2021; Weithmann et al., 2018; Yang et al., 2021). In addition, MP can enter soils through both wet and dry atmospheric deposition (Allen et al., 2019; Brahney et al., 2020; Kernchen et al., 2022). Soils receiving MP via sewage sludge application and plastic mulching have a global median background concentration of 1200 particles kg^{-1} soil (Büks & Kaupenjohann, 2020). Similarly, van Schothorst et al. (2021) found on average 888 particles kg^{-1} in soils that received annual compost inputs of 10 t ha^{-1} in the past 7–20 years. However, the reported uncertainties are large; robust estimates of MP loadings in soils due to organic fertilizer application are therefore not available (Büks & Kaupenjohann, 2020; Gui et al., 2021).

Biowaste as well as the composts and digestates derived thereof have been found to contain plastics and there is some evidence that plastic pieces can break down and form MP during biowaste processing (Judy et al., 2019; Gui et al., 2021; Rodrigues et al., 2020; Watteau et al., 2018; Weithmann et al., 2018). Composts contain plastics mainly from packaging and plastic bag residues, which are usually made up of low-density polyethylene (LDPE) (Bandini et al., 2020; Gui et al., 2021; Rodrigues et al., 2020; Weithmann et al., 2018). There have been attempts to tackle plastic contamination of composts and soils by replacing conventional plastics such as LDPE with biodegradable polymers (Agarwal, 2020; Liao & Chen, 2021; Qin et al., 2021). Polymer blends with poly(lactic acid) (PLA) and poly(butylene adipate-co-terephthalate) (PBAT) are biodegradable alternatives to LDPE (Agarwal, 2020; Liao & Chen, 2021; Musioł et al., 2018). LDPE is resistant to microbial degradation due to its stable carbon (C) backbone (Kumar Sen & Raut, 2015; Krueger et al., 2015). In contrast, PLA/PBAT blends are hydrolyzable through enzymes such as lipases, cutinases, and esterases, and thus potentially biodegradable in soil or compost (Freitas et al., 2017; Jia et al., 2021; Palsikowski et al., 2018; Tabasi & Ajji, 2015; Weng et al., 2013; Zumstein et al., 2018). However, there is significant uncertainty about the fate of biodegradable MP fragments originating from composts in arable soils. Indeed, there is some evidence for incomplete biodegradation of some biodegradable plastics, rapid fragmentation of biodegradable MP and thus more rapid in situ formation of MP in composts and soils compared with conventional polymers (Liao & Chen, 2021; Meng et al., 2022; Qin et al., 2021; Steiner et al., 2022). Biodegradable polymers could thus pose a greater risk of adverse effects on soil organisms and functions if they are not readily mineralized.

MP have many modes of action in soils. They can induce physicochemical changes in habitats by affecting soil porosity, bulk density, water holding capacity, and soil water repellence (X. Zhang et al., 2021), and form specific habitats for soil microorganisms, referred to as the plastisphere (Bandopadhyay et al., 2020; Rüthi et al., 2020; M. Zhang et al., 2019; Zhou et al., 2021). Less is known about the influence of MP on C cycling, but MP are C-rich substrates and have the potential to change soil organic C and thus C cycling (Meng et al., 2022; Rillig et al., 2021; X. Zhang et al., 2021). Soil C cycling involves the decomposition of organic compounds originating from plant, microbial, and animal residues. The degradation of different complex compounds (cellulose, chitin < xylan < lignin) is catalyzed by microbially produced enzymes (Burns et al., 2013). For example, ß-glucosidase and N-acetyl-glucosaminidase catalyze the final hydrolytic cleavage of cellobiose and chitobiose di- and oligomers after depolymerization of cellulose and chitin (Kandeler, 2015; Maillard et al., 2018), whereas ß-xylosidase hydrolyzes cleavage products, for example, xylobioses and other short xylooligosaccharides, from different hemicelluloses such as xylan (Dodd et al., 2011; Uffen, 1997). Phenoloxidases oxidize redox mediators initiating the depolymerization of lignin (Burns et al., 2013).

In a recent study under field conditions, increases of C cycling enzymes (α- and ß- glucosidase) were observed in response to LDPE-MP addition (Lin et al., 2020). A meta-analysis identified multiple negative impacts on plant growth including crop yield and plant height, resulting from pollution of croplands with plastic residues from mulch films (D. Zhang et al., 2020). Given the importance of agricultural soils for food production, understanding the loadings and the extent to which MP, and especially biodegradable MP, affect C cycling and crop yields in agroecosystems is crucial (Rillig et al., 2017; G. S. Zhang & Liu, 2018; X. Zhang et al., 2021).

This study aimed to better understand the fate of MP and effects of MP on microbial abundance and activity related to C cycling, as well as crop yields in arable soils. We established a field experiment (1) to investigate MP background concentrations, (2) to quantify concentrations of added conventional and biodegradable MP after one and 17 months of addition, and (3) to identify potential effects of MP and of MP-containing organic fertilizers on soil microbial abundance, activities of selected C cycling enzymes, and crop yields. We expected that (1) the arable soil shows a low but significant background MP loading (before setup), (2) biodegradable MP (PLA/PBAT) fragment in soil, (3) conventional MP (LDPE) persist and are not altered, (4) biodegradation of PLA/PBAT leads to increased activity of lipase in soil as this enzyme catalyzes ester bond cleavage, but microbial abundance, activities of enzymes catalyzing other reactions, and crop yields are not affected because breakdown of PLA/PBAT is slow and direct toxic effects MP on plants are unlikely, and (5) due to its persistence, LDPE has no impact on soil microbiological indicators of C cycling or crop yields.

2 | MATERIALS AND METHODS

2.1 | Microplastics

As biodegradable plastics, we used a blend of PLA (IngeoTM Biopolymer 7001D; NatureWorks LLC, Minnetonka, MN, USA) and PBAT (Ecoflex F Blend C1200; BASF SE, Ludwigshafen, Germany) in a mixing ratio of 80/20% w/w, which was compounded at the Institut für

Kunststofftechnik (University of Stuttgart, Stuttgart, Germany). LDPE (Lupolen 2420H; LyondellBasell Industries N.V., Rotterdam, Netherlands) served as the representative conventional MP. Polymer pellets were cryomilled (−196°C) with a speed rotor mill (Pulverisette, Fritsch GmbH, Idar-Oberstein, Germany) to obtain MP and subsequently fractionated using stainless-steel sieves to obtain two MP particle size fractions of <0.5 and 0.5–2 mm. Both fractions were then mixed in a 1:1 ratio (mass based).

2.2 | Organic fertilizers

Solid digestate (C/N: 11, dry mass: 22.2%, substrate: 48.8% plant residues such as silage, 51.2% animal by-products such as manure) was provided by the research station Unterer Lindenhof of the University of Hohenheim. Compost (C/N:17, dry mass: 61.8%, substrate: green cuttings) originated from Häckselplatz Möhringen in Stuttgart, Germany.

Since there were no detection methods for MP particles <1 mm in composts and digestates at the initiation of the experiment (Weithmann et al., 2018), we used the plastic loading of the fractions 1–5 and >5 mm in the compost and digestate as indicators of MP loading. The plastic loading of digestates and composts was determined after sieving and detection via attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy (cf. Section 2.4). The compost (one batch) contained three polypropylene (PP) particles in the fractions 1–5 mm per kg and three particles (50% were PP and 50% polystyrene [PS]) in the fraction >5 mm per kg. The digestate (mean of two batches) contained 11 particles in the fraction >5 mm per kg (25% were PE and 75% PP) and no particles in the 1–5 mm fraction.

2.3 | Study site characteristics, experimental setup, and soil sampling

The experiment was established on a conventionally managed agricultural field at the research station Heidfeldhof (University of Hohenheim, central point of the field: 9°11′22.984″ longitude, 48°43′11.137″ latitude, EPSG: 4326, WGS 1984). In the past, neither plastic mulch nor compost had been applied. In addition to the mineral fertilizers commonly used in conventional management, the field was sporadically fertilized with manure from the research station Meiereihof (University of Hohenheim). The soil is a Luvisol with texture silt loam (3.4% sand, 76.2% silt, 20.5% clay), total soil C and nitrogen (N) content of 1.19 and 0.13%, respectively (C/N ratio: 9), and pH of 6.3 (measured in 0.01 M $CaCl_2$). Weather conditions at the study site and farm management during the experiment are shown in Figure 1.

The experimental design included the factors MP (none, LDPE, PLA/PBAT) and organic fertilizer (none, compost, digestate) arranged in a complete randomized block design with four blocks (Figure 2). The area of one plot was 32 m^2 (length: 8 m, width: 4 m). To avoid carry-over effects from one plot to another by tillage, a 5 m-wide buffer area between the plots was established in the direction of machine travel. In consideration of German biowaste regulations that permits an application of max. 30 t compost ha^{-1} (note that all mass data are given on dry matter basis) over 3 years (BioAbfV, 2017), we applied 10 t ha^{-1} of compost and digestate. MP were applied at a concentration of 2 g m^{-2}. To homogeneously apply the MP, we weighted 10 kg soil randomly taken from the field per plot and added 68 g MP, then homogenized these MP–soil mixtures using a drilling machine with a stirring unit for 2 min in metal buckets (35 L). From these MP–soil mixtures, we took the amount required for two square meters, that is, 0.59 kg, added these to the plots (treatments without fertilizer) or mixed these with the amount of compost for two square meters, that is, 2 kg, using a drilling machine (treatments with compost). We chose these MP–soil mixtures and compost because they could be mixed, transported, and distributed well in the field. Due to the low bulk density of the digestate, it could not be mixed in the metal buckets with the MP–soil mixtures. Therefore, we applied the digestate and MP–soil mixtures (treatments with digestate and MP) separately to the field.

To investigate MP background contamination and determine soil properties, we took 15 randomly selected soil subsamples (Ap horizon, depth: 0–10 cm) on 32 m^2 ($n = 4$) from the plots without fertilizer and without MP using a soil core sampler (cross-sectional area: 9.53 cm^2) before the start of the experiment. To analyze MP particles added to the field and soil biological variables, before setup and 1 month (M1), 5 months (M5), and 17 months (M17) after setup, eight subsamples were taken from a 4 m^2 sampling square in the center of each plot (Ap horizon, depth: 0–10 cm) and pooled into composite samples of approximately 1 kg for each timepoint. Since the soil sampled in this way contained very few MP particles >0.5 mm at M1 and M5, we additionally sampled an area of 900 cm^2 per plot using a spade at the end of the experiment (M17).

Soil samples for soil biological analyses were stored at −20°C until analysis.

2.4 | MP analyses

To characterize the background contamination of the arable soil with MP and to investigate the fate of added MP particles, MP were extracted and measured according to Möller et al. (2022). In brief, soil samples were freeze–dried and sieved to 0.5 mm. All further analyses were done with aliquots of 250 g soil.

MP >0.5 mm were collected with tweezers and analyzed by ATR-FTIR spectrometry (spectrometer: Alpha ATR unit, Bruker 27; equipped with a diamond crystal for measurements). Spectra were taken from 4000 to 400 cm^{-1} (resolution 8 cm^{-1}, 16 accumulated scans; Software OPUS 7.5). Particles were identified by comparing the measured spectra against standard spectra from an in-house database described previously (Löder et al., 2015) and the database provided by the manufacturer of the instrument (Bruker Optik GmbH, Leipzig, Germany). An incident light microscope (microscope, Nikon SMZ 754T; digital camera, DS-Fi2; camera control unit, DS-U3; software, NIS Elements D) was used for visual documentation and size estimation of all synthetic plastic particles identified by ATR-FTIR.

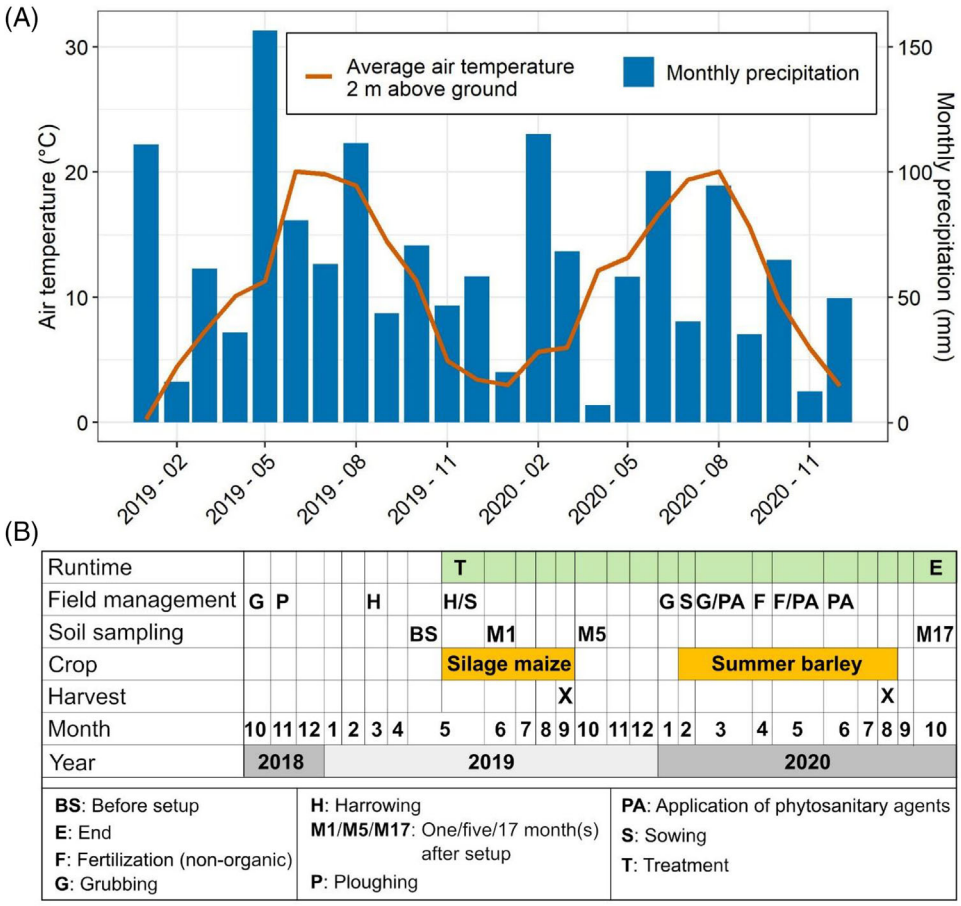
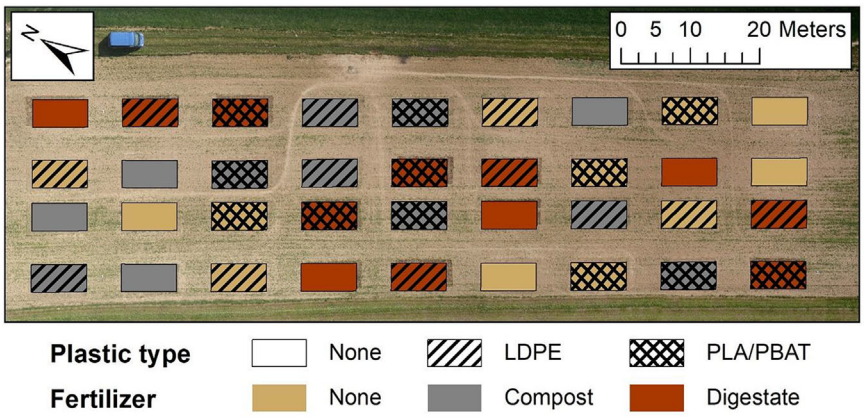


FIGURE 1 (A) Monthly average air temperature measured in 2 m above ground and monthly precipitation from nearby meteorological station and (B) overview of field management, soil sampling, and harvest. Meteorological data were obtained from LTZ (2021).

FIGURE 2 Experimental design of the field experiment. Plots were arranged in a complete randomized block design ($n = 4$).



Soil samples taken from the 900 cm^2 areas at M17 (corresponding to approximately 10 L soil) were analyzed in their entirety to detect large particles >0.5 mm. To this end, the soil samples were partitioned into 20 Fido jars (Bormioli Rocco, Fidenza, Italy; capacity 3 L each) and suspended with 2.5 L of water. The diluted samples were sieved at 2 mm and the retained particles were collected with tweezers (fraction >2 mm). All material <2 mm was sieved at 0.5 mm mesh size, and the retained particles were again collected with tweezers (fraction 0.5–2 mm).

According to Möller et al. (2022), MP <0.5 mm were extracted via density separation with a zinc chloride brine (ρ = 1.8 g cm^{-1}) and an enzymatic-oxidative purification step (Löder et al., 2017). Particles were then transferred onto an aluminum oxide sample carrier and analyzed by chemical imaging via Focal Plane Array-based μ-FTIR

spectroscopy (Löder et al., 2015). Identification of MP in the large chemical imaging data sets was performed with the help of an automated software solution based on Random Decision Forest Classifiers (Hufnagl et al., 2022). For quality control, the results of the automated MP classification was checked by trained experts. We only analyzed the samples from plots without organic fertilizers, that is, the samples from 12 out of 36 plots (see Figure 2), at M1 and M17 as well as the MP–soil mixtures that were added to the plots with MP treatment (in total: 12 + 12 + 8 = 32 samples). We had to limit the number of analyzed samples due to the extensive and time-consuming extraction and purification procedure (Möller et al., 2022). In addition, the high organic matter content of compost and digestate interferes with the treatment of the samples. Thus, these samples could not be analyzed. Due to high numbers of MP particles, deviating from the above-mentioned protocol, for the initial MP–soil mixtures that were added to the field, four subsamples of 5 g each were analyzed.

We calculated the initial MP concentrations in soil at the start of the field experiment (MP_{start}), assuming that the applied MP–soil mixtures were homogeneously mixed within the top 10 cm of soil (Equation 1):

$$c_{MP,i} = \frac{m_{mix} \times c_{mix}}{(d \times \rho_B)}, \quad (1)$$

where $c_{MP,i}$ is the initial MP concentration in the soil of the field experiment (particles kg^{-1}), m_{mix} is the mass of applied MP–soil mixtures per area (0.294 kg m^{-2}), c_{mix} is the measured MP concentration of the MP–soil mixtures (particles kg^{-1}), d is the depth of the soil layer (10 cm), and ρ_B is the bulk density of top soil (1400 kg m^{-3}).

Since soil samples were separated into two fractions due to sieving of 0.5 mm and these two fractions were analyzed differently as described above, we excluded particles >0.5 mm in the small fraction (5.1–38.5%) and particles <0.5 mm in the large fraction (0–2.1%), respectively (Table S1). Due to sieving of MP to 2 mm before use in our study, particles >2 mm were filtered from datasets (this applied only to MP–soil mixtures).

We derived particle size distributions of LDPE and PLA/PBAT particles as initially added to the soil based on MP particles detected in MP–soil mixtures (Figure S1). The median size of LDPE particles in the small and large fractions were 186 and 1092 µm, respectively (Figure S1). The median size of PLA/PBAT particles in the small and large fractions were 200 and 1013 µm, respectively (Figure S1).

2.5 | Soil microbiological indicators of carbon cycling

To assess effects of MP and organic fertilizers on the soil microbial abundance and activity, we used microbial biomass C and activities of enzymes involved in C cycling as soil microbiological indicators. These were measured before as well as 1 month (M1), 5 months (M5), and 17 months (M17) after the setup of the experiment.

Microbial biomass C (C_{mic}) and nitrogen (N_{mic}) were quantified via chloroform fumigation extraction according to Vance et al. (1987). For a description of the method, we refer to Blöcker et al. (2020).

We analyzed the activity of enzymes that catalyze the degradation of organic substrates of different complexities: we considered ß-glucosidase, N-acetyl-glucosaminidase, ß-xylosidase, and phenoloxidase as indicators of the degradation of the polymers cellulose, chitin, xylan (hemicellulose), and lignin. In addition, we analyzed the activity of lipase because of its possible involvement in the depolymerization of PLA/PBAT. The activities of ß-glucosidase, ß-xylosidase, N-acetyl-glucosaminidase, and lipase were measured using microplate assays with fluorogenic substrates (Cooper & Morgan, 1981; German et al., 2011; Marx et al., 2001). Lipase activity was determined based on an adapted protocol from Cooper and Morgan (1981). Substrates and standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). Standard stock solutions of 5 mM 4-methylumbelliferyl (MUF, M1381) were obtained by dissolving MUF in methanol and deionized water (1:1). Standard working solutions (10 µM MUF) were prepared in 0.1 M Tris–HCl buffer pH 6.8 (lipases) or MES buffer pH 6.1 (ß-glucosidase, ß-xylosidase, N-acetyl-glucosaminidase). For each soil sample, we prepared a standard curve with concentrations of 0, 0.5, 1, 2.5, 4, 6 µM MUF in soil suspension aliquots and buffer. Lipase substrate stock solutions (10 mM) were obtained by dissolving the substrates MUF heptanoate (M2514) in dimethyl sulfoxide (D8418). Working solutions (1 mM) were prepared by adding sterile 0.1 M Tris–HCl buffer pH 6.8. Substrate solutions of ß-glucosidase, ß-xylosidase, and N-acetyl-glucosaminidase were prepared and analyzed as outlined in Kramer et al. (2013).

Phenoloxidase activity was photometrically measured as described in Ali et al. (2015) with the following slight modifications. Before the measurement, we preincubated the microplates at 30°C and measured absorbance of the soil suspensions at a wavelength of 414 nm.

2.6 | Crop yields

Silage maize and summer barley were harvested in September of the first year (4 months after setup) and in August of the second year of the experiment (15 months after setup), respectively (Figure 1B).

To determine the biomass of the silage maize (*Zea mays*), we removed every second plant by cutting it 1 cm above its root system. We determined maize plant dry matter biomass (including cobs) after chopping the plants and drying them at 60°C and 110°C (for 3 days each). Two-step drying is common practice at the research station to accelerate drying to mass constancy at 110°C. We then multiplied mean silage maize biomass per plot by the number of plants per plot to obtain silage maize biomass yield per plot. Grain yield of summer barley (*Hordeum vulgare*) was determined from an area of 12 m^2 (1.5 m × 8 m) per plot and grains were sampled using a plot threshing machine. Crop yields were converted to t ha^{-1}.

2.7 | Data analyses

All data analyses and figures were carried out using the statistical software R 4.0.2 (R Core Team, 2020). In addition to the packages

explicitly mentioned in this section, we used: *broom.mixed* 0.2.6 (Bolker & Robinson, 2020), *broom* 0.7.0 (Robinson et al., 2020), *flextable* 0.5.10 (Gohel, 2020), *patchwork* 1.0.1 (Pedersen, 2020), *scales* 1.1.1 (Wickham & Seidel, 2020), and *tidyverse* 1.3.0 (Wickham et al., 2019).

The MP background concentrations (before setup) and concentrations of MP particles >0.5 mm were evaluated only descriptively because there were too few data for inferential statistical analysis. For particles <0.5 mm, differences in particle number between MP_{start}, M1, and M17 were tested using a linear mixed effects model with particle number as dependent variable, and timepoint (MP_{start}, M1, and M17) as the explanatory variable, while accounting for a random effect for plot (ID). Tukey contrasts were computed using functions from the *emmeans* 1.5.0 package (Lenth, 2020). Particle size distributions of particles <0.5 mm were compared by plotting empirical cumulative density functions and using the Kolmogorov–Smirnov test (*ks.test*), to test whether the MP particles in MP_{start}, M1, and M17, originated from the same distribution (van Schothorst et al., 2021). Empirical cumulative density functions were calculated based on pooled samples per treatment group ($n = 4$).

Crop yields were evaluated using a linear model with the crossed factors plastic type and fertilizer and accounting for a block effect. Soil enzyme activities, C_{mic}, and N_{mic} data were analyzed by means of linear mixed effects models. Therefore, the linear model used for crop yield data was extended by the initial state of the variable of interest as covariate to account for the field variability (Value_TMinus1). We integrated the repeated measures factor timepoint (i.e., M1, M5, and M17) by crossing it with the treatment structure, accounted for a block and block–timepoint interaction effect, and a random effect for the randomization unit (i.e., plot) (Piepho et al., 2004). The models were fitted to the data using functions from base R and the package *lme4* 1.1–23 (Bates et al., 2015). We used ANOVAs in the case of linear mixed effects models with the Kenward–Rogers approximation for the degrees of freedom using functions from the *lmerTest* 3.1–2 package (Kenward & Roger, 1997; Kuznetsova et al., 2017) to identify significant effects ($p < 0.05$) and subsequently compared estimated marginal means. If an interaction with timepoint was significant, we evaluated simple contrasts per timepoint level.

Model assumptions, that is, variance homogeneity and normal distribution of the residuals, were checked visually and met for all variables except for N-acetyl-glucosaminidase activity, for which the model assumptions were met after log-transformation.

3 | RESULTS

3.1 | Background loading of MP in the arable soil

The arable soil had a MP loading with nine different polymer types at a background concentration of 296 ± 110 (mean ± standard error) particles kg^{-1}. PP (108 ± 36 particles kg^{-1}), PS (76 ± 34 particles kg^{-1}), and polyethylene (PE, 60 ± 25 particles kg^{-1}) were the most abundant polymers and were found in all analyzed samples (Figure 3A). Other MP were polyacrylonitrile, PE terephthalate, polyvinyl chloride, polybutylene terephthalate, ethylene-vinyl acetate, and polysulfone.

PS particles were smallest with a median particle size of 60 µm (Figure 3B). PP and PE particles had median sizes of 156 and 146 µm, respectively. While the particle size distribution of PS MP was significantly shifted to lower particle lengths compared with PP ($p = 0.014$), the particle size distribution of PE MP was similar to that of PP and PS MP ($p = 0.187$ and $p = 0.188$).

3.2 | Fate of added MP <0.5 mm in soil

At the start, soil amended with LDPE and PLA/PBAT contained 1003 LDPE kg^{-1} and 134 PLA/PBAT particles kg^{-1} of MP <0.5 mm (MP_{start}; Figure 4A). After 1 month (M1), we detected on average 419 fewer LDPE particles kg^{-1} than at MP_{start} (not significant, $t_6 = -2.7$, $p = 0.082$). The mean number of LDPE particles 17 months after MP addition (M17) and PLA/PBAT particles at M1 and M17 did not differ significantly from MP_{start} (Table S2; Figure 4A). The particle size distribution of LDPE and PLA/PBAT MP at M1 and M17 did not differ from MP_{start} (Figure 4B).

3.3 | Fate of added MP >0.5 mm in soil

We found a total of 57 particles >0.5 mm (27 varnish, 13 PE, 16 PLA/PBAT, and one PP) at the final sampling (M17), in all soil samples taken together ($n = 36$). PLA/PBAT and LDPE particles (up to 2) were detected in soil samples from only two (PLA/PBAT) and three plots (LDPE) without fertilizer treatment, respectively. Due to this low recovery, a quantitative comparison of particles >0.5 mm with MP_{start} was not possible. PLA/PBAT particles occurred only in soil samples from plots where PLA/PBAT had been added (Figure 5A–C). All PLA/PBAT particles found looked similar (white and irregularly shaped) (Figure 5A–C) and like the originally added particles (Figure S2).

However, PE particles (Figure 5D–F) occurred not only in soil samples of plots, where PE had been added. They also had different shapes including plastic film residues (Figure 5D), fibers (Figure 5E), or irregularly shaped pieces (Figure 5F). PE particles found were distinct from the initially added PE particles (Figure S2D–F). All varnish particles were of the same type (Figure 5G–I).

3.4 | Soil microbiological indicators of carbon cycling and crop yields

We investigated the effects of adding 2 g MP m^{-2} on soil microbial abundance and activity related to C cycling and crop yields based on soil microbiological indicators (C_{mic}, N_{mic}, and activities of C cycling enzymes), biomass of silage maize, and grain yield of summer barley. Overall, MP from LDPE and PLA/PBAT did not cause changes of the soil microbiological indicators at 1, 5, and 17 months after MP addition, or

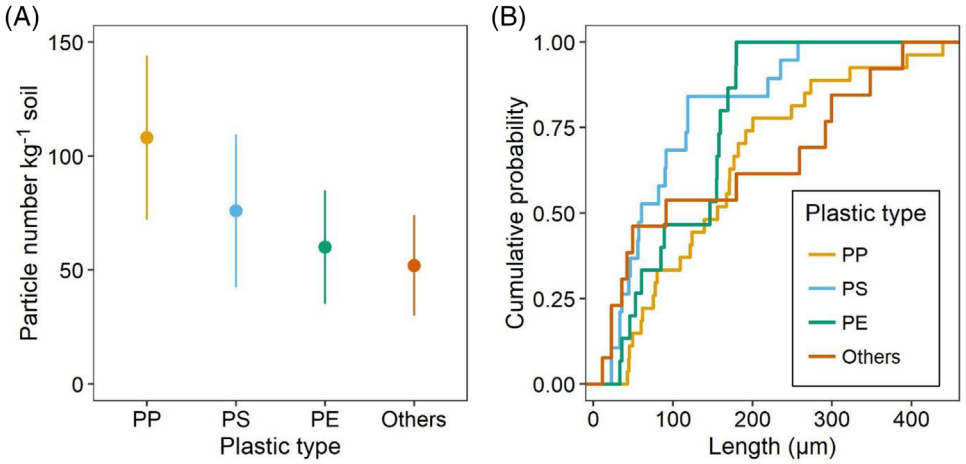


FIGURE 3 (A) Particle numbers of PP, PS, PE, and other polymers <0.5 mm. Data are presented as means and standard errors (error bars) ($n = 4$). (B) Empirical cumulative distribution function of pooled samples for PE (15 particles), PS (19 particles), PP (27 particles), and others (13 particles). Other MP were polyacrylonitrile, polyethylene terephthalate, polyvinyl chloride, polybutylene terephthalate, ethylene-vinyl acetate, and polysulfone.

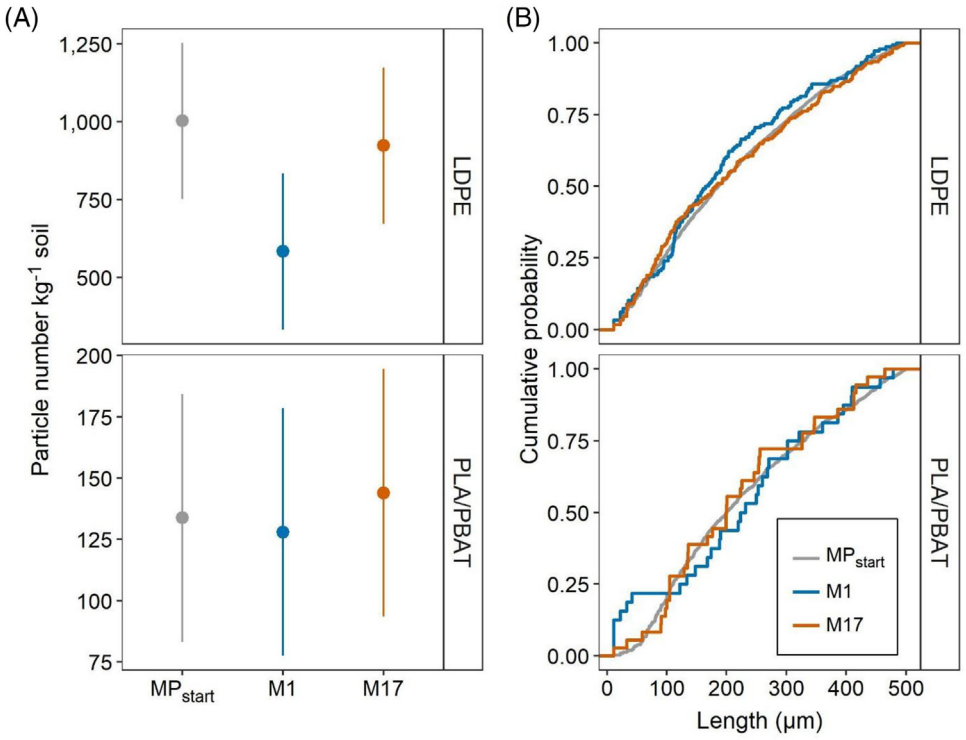


FIGURE 4 (A) Particle numbers of LDPE and PLA/PBAT particles after application of MP–soil mixtures as initially added to the plots (MP_{start}), after 1 month (M1), and after 17 months (M17). Data are presented as estimated marginal means with lower and upper 95% confidence intervals (error bars) ($n = 4$). Note that *y*-axis scales for LDPE and PLA/PBAT differ from one another. (B) Empirical cumulative density functions of number of LDPE and PLA/PBAT particles in MP mixtures as initially added to the plots (MP_{start}), at M1, and at M17, pooled by plastic type.

in crop yields compared with MP-free soil (Figure 6; Figures S3 and S4 and Tables S3 and S4). The exception was LDPE at M5, which reduced N_{mic} significantly by 36% compared with the MP-free soil (Figure S4A, Table S5).

No combined effects of MP with organic fertilizers were detected, but amendment of soil with composts and digestates affected the activity of C cycling enzymes in soil (Figure 6B, Figure S3, Tables S3–S6). Lipase activities responded to the addition of compost (M1 and M5) and digestate (M5) significantly increasing from 37 to 62% compared with fertilizer-free soil (Figure 6B, Table S5). ß-Xylosidase showed significantly enhanced activity in soil amended with digestate in comparison with the fertilizer-free soil at M5 (+60%) and M17 (+23%) (Figure S3B, Table S5). Both ß-xylosidase and ß-glucosidase activities increased by 47% in response to compost addition at M5 compared

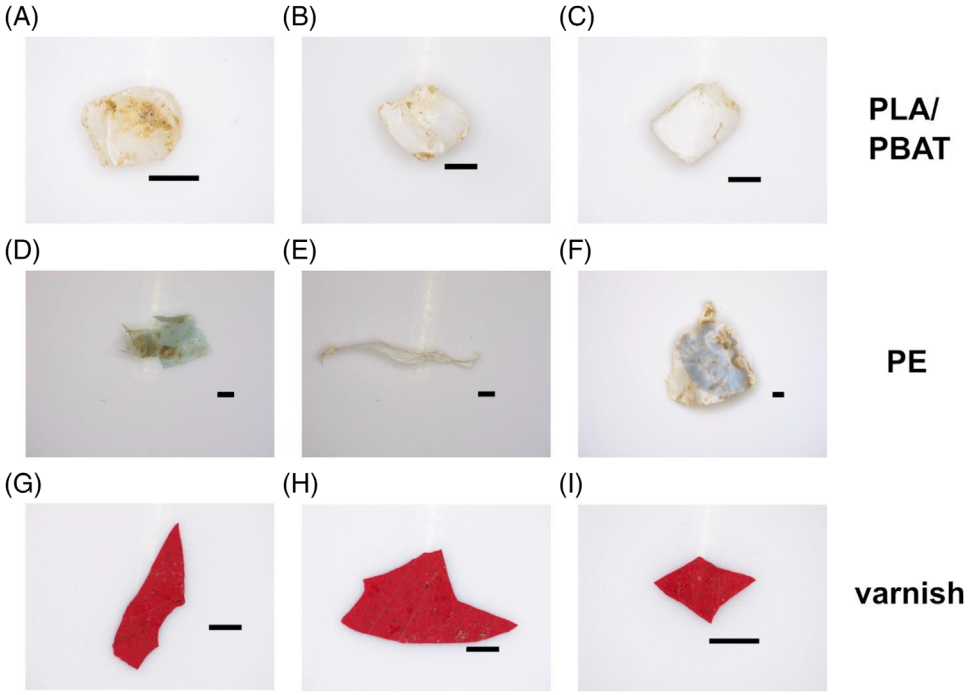


FIGURE 5 Representative microscopic images of MP >0.5 mm: (A–C) PLA/PBAT, (D–F) PE, and (G–I) varnish found after 17 months (M17). The scale bars indicate a length of 1 mm.

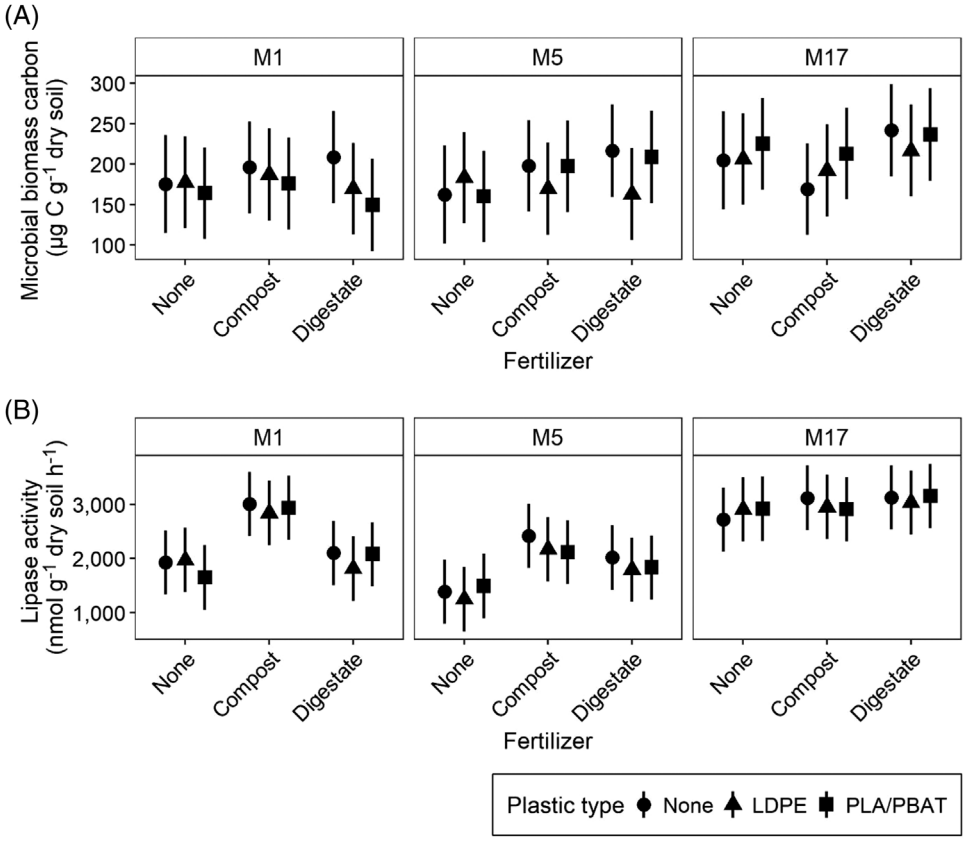


FIGURE 6 (A) Microbial biomass C and (B) lipase activity as a function of MP and organic fertilizers 1 month (M1), 5 months (M5), and 17 months (M17) after the addition of 2 g MP m^{-2}. Data are presented as estimated marginal means ($n = 4$) with lower and upper 95% confidence intervals (error bars).

with the fertilizer-free soil, but statistical uncertainties were large for ß-xylosidase ($p = 0.061$) (Figures S3A and S3B, Table S5). Compared with nonfertilized soil, N-acetyl-glucosaminidase activities increased 59% (significant) after digestate addition at M5 (Figure S3C and Table S6).

After 17 months, the activities of ß-xylosidase, N-acetyl-glucosaminidase, and ß-glucosidase were significantly higher in the soil amended with digestate compared with compost (Figures S3(A)–S3(C) and Table S5). Strikingly, this coincided with increased N_{mic} in the soil enriched with digestate compared with compost at M17 (+22%, $p = 0.026$) (Figure S4A, Table S5).

Independent of timepoint, phenoloxidase activity was 16.6% higher in soil amended with digestate in comparison with fertilizer-free soil (Figure S3D). However, statistical uncertainties were large ($p = 0.069$) (Table S7).

Biomass yields of silage maize (mean and standard error: 19.70 ± 0.48 t ha^{-1}) were not significantly higher on soil amended with compost and digestate in comparison with nonfertilized soil (Figure S4(B) and Table S4). However, grain yield of spring barley (estimated marginal mean: 6.95 t ha^{-1}) was larger (significantly) on soil amended with digestate compared with compost (6.31 t ha^{-1}) and larger (though not significantly) than on nonfertilized soil (6.46 t ha^{-1}) (Figure S4(C) and Table S7).

4 | DISCUSSION

4.1 | The arable soil was loaded with diverse MP types

The arable soil in our study contained 296 ± 110 (mean ± standard error) MP particles <0.5 mm kg^{-1} as background concentration. This concentration was lower than estimates for arable soils amended with compost (888 ± 500 particles kg^{-1} soil; van Schothorst et al., 2021), sewage sludge (930 ± 740 particles kg^{-1} soil for low-density plastics and 1100 ± 570 particles kg^{-1} for high-density plastics; van den Berg et al., 2020), or plastic mulch (18,760 particles kg^{-1} soil; G. S. Zhang & Liu, 2018).

The most common plastic types found in our soil were PP > PS > PE. These are among the most economically important polymers and are also those that have previously been most frequently detected in soil (PlasticsEurope, 2019; X. Zhang et al., 2021). In accordance with our results, Piehl et al. (2018) identified PP, PS, and PE as the most abundant MP particles (>1 mm) in a conventionally managed field that had not been amended with organic fertilizers or sewage sludges, and where no plastic mulches had been applied. Since the input of MP via the latter sources can be excluded in our study, the recovered MP presumably entered the soil by littering and atmospheric deposition (Allen et al., 2019; Dris et al., 2016; Kernchen et al., 2022; Scheurer & Bigalke, 2018). The relatively high number of extracted varnish particles (Figure 5) suggest that abrasion of protective coatings from agricultural machinery could be an important source of MP in arable soils (Figure S5).

We found that more than 75% of the PP, PS, and PE particles were smaller than 0.2 mm (PS: <117 µm, PE: <159 µm, PP: <196 µm), consistent with previous results from J. Wang et al. (2021). The current detection limit is 10 µm (Möller et al., 2020); we expect, therefore, that smaller particles occur even more frequently. This could have dramatic consequences for soil organisms because particles <10 µm can be ingested by key member species of the soil food web such as nematodes, resulting in intestinal damage and neurotoxicity (Fueser et al., 2019; Lei et al., 2018; Schöpfer et al., 2020). PS particles in particular pose a risk to soil animals; these were the smallest in our study (median of 60 µm). However, concentrations of small MP down to nanometer sizes are currently undetectable due to restrictions of analytical methods (Möller et al., 2020). Further progress in MP analytics is needed to better assess potential threats of small MP to soil organisms and their functions.

We can confidently state that the PLA/PBAT particles >0.5 mm we found at the last sampling of the experiment (M17) were the particles we had added. We found these exclusively in the PLA/PBAT treated plots but with no finds in the corresponding background loading. All PLA/PBAT particles looked similar and resembled the original particles. In contrast, we cannot rule out that a significant portion of the PE particles we found were part of the background loading. For one thing, LDPE particles also occurred in plots to which no LDPE had been added, and for another, the PE particles found had various shapes (Figure 5) and differed from the originally added LDPE particles (Figure S2).

At the last sampling, we found only very few particles >0.5 mm. We can exclude the possibility that the particles had been fragmented (with the exception of the fragmentation <0.01 mm, which we could not detect with our method) because this should have been detected via a clear shift in the size distribution of the particles <0.5 mm. The low recovery, we suggest, could be due to the possibility that the amount of soil or area sampled was insufficient or that the methodology for analyzing these large particles needs further development. Methodological limitations apply especially to the LDPE particles, which had a more fibrous shape than the predominantly irregularly shaped PLA/PBAT particles. The LDPE particles may have been more prone to fall through the sieve during MP analysis in wet sieving. It is also possible that a significant proportion of large particles were transported vertically or horizontally. A recent study provides evidence for horizontal transport of MP (irregularly shaped polymethyl methacrylate particles with a mean length of 1215 µm), which occurred along preferential pathways dictated by the micro- and macro-relief of the soil surface (Laermanns et al., 2021). However, more studies on the transport (including vertical transport) of particles in the field will be required to test our assumption.

4.2 | MP persisted in the arable soil

Both tested polymers persisted in the soil of the field experiment over 17 months. The number of added LDPE particles <0.5 mm (584–1003 particles kg^{-1}; Figure 4) in our study roughly represents the LDPE accumulation that can be expected after 7–20 years of compost

accumulation (van Schothorst et al., 2021). PE is highly resistant to microbial degradation in soil due to its large molecular size, lack of functional groups, and high hydrophobicity (Albertsson, 1978; Krueger et al., 2015), which explains the unaltered particle size distribution compared with the initial particles, indicating a lack of fragmentation in the studied soil. Surprisingly, S. Zhang et al. (2020) found that fertilization with N and phosphorous stimulates the fragmentation of LDPE. According to the authors, LDPE fragmentation was triggered by increased soil microbial diversity and abundance. This behavior and its mechanisms need to be confirmed by further studies.

Contrary to our expectations, we recovered the same number of PLA/PBAT particles <0.5 mm as initially added to the soil, most likely due to the lack of biodegradation (Figure 4). The few existing studies on the persistence of films of PLA, PBAT, and PLA/PBAT blends in soil under field conditions demonstrate their low biodegradability within the time period of our field experiment (Liao & Chen, 2021; Rudnik & Briassoulis, 2011; Sintim et al., 2020). PLA exhibited changes in mechanical properties after 11 months in a Mediterranean soil but was visually poorly disintegrated (Rudnik & Briassoulis, 2011). In another study, mass loss of 1–8% and 1–7% were observed for PLA and PBAT, respectively, after 6 months, whereas a PBAT/PLA blend (90/10% w/w) showed no significant degradation (Liao & Chen, 2021). A lower degradability of PLA/PBAT (75/25% w/w) blend compared with the sole polymers was also observed in a laboratory study (Palsikowski et al., 2018). While 21% of the PBAT-C and 16% of PLA-C were mineralized, only 10% of PLA/PBAT-C were mineralized after 180 days in soil. Liao and Chen (2021) attributed the poor degradation of the blend in their study to the blending of PLA with PBAT; blending would change physical properties and increase hydrophobicity, thus impeding microbial colonization and microbial degradation. This could explain, why no fragmentation of PLA/PBAT was observed in our study.

Based on our results, nonbiologically pretreated PLA/PBAT particles are likely to accumulate in the soil under field conditions, given the highly variable climatic conditions with extremes such as cold and drought that may slow the biodegradation of PLA/PBAT.

4.3 | MP did not affect soil microbial biomass, enzyme activities, and crop yields

We did not find any effect of LDPE on soil microbiological indicators of C cycling, likely due to its inert nature (Restrepo-Flórez et al., 2014). However, we found an effect of LDPE on N_{mic} (Figure S4A), but this occurred only sporadically (at one timepoint) and the measurement uncertainties were large (Table S5). In line with our results, Lin et al. (2020) did not observe significant changes in soil microbial biomass C and microbial community composition due to the addition of LDPE at concentrations 5, 10, 15 g m^{-2} (corresponding to 11,361, 23,789, and 39,172 particles kg^{-1}). In a recent field study, no effects of LDPE-MP on microbial abundance and composition were detected even at extremely high application rates up to 1000 g MP m^{-2} (Brown et al., 2022). However, Lin et al. (2020) found substantial increases in C cycling enzymes such as α-glucosidase and ß-glucosidase at all concentration levels between 36 and 86%, and an increase in L-leucine aminopeptidase, an N cycling enzyme, by 83–116%. They explained the enhanced enzyme activities by greater water availability due to a MP-induced increase of water holding capacity, which would positively influence enzyme activities. Compared with Lin et al. (2020), in our study, we used LDPE particles at a much lower concentration of 2 g m^{-2} MP (584–1003 LDPE particles kg^{-1}) and larger LDPE particles (Figure S1; 90th percentile of particles <0.5 mm and >0.5 mm of 430 and 1619 µm, respectively, compared with a 90th percentile of 68 µm in their study). Accordingly, particles in our study had a lower specific surface area with less potential to affect soil physical properties including water holding capacity (Ng et al., 2018).

As expected, the addition of PLA/PBAT particles did not affect any of the soil microbiological indicators of C cycling. However, contrary to our expectation, PLA/PBAT also did not increase lipase activity in soil. This was likely due to the lack of biodegradation of PLA/PBAT particles (see Section 4.2) in soil and to the fact that soil microorganisms were apparently not able to use the added PLA/PBAT blend as a C source. In another study, PBAT/PLA MP affected soil C and N pools (Meng et al., 2022). For instance, there were significantly higher dissolved organic C and N due to addition of 2 and 2.5% PBAT/PLA MP additions in comparison with the control. Again, the lower concentration of PLA/PBAT particles in our study could explain why we did not detect changes in soil microbiological indicators of C cycling.

We verified previous studies in which compost and digestate led to a stimulation of enzyme activities (Alburquerque et al., 2012; Crecchio et al., 2004; Vinhal-Freitas et al., 2010). Depending on the quality of the organic fertilizers, we found slightly different temporal patterns of degradation of high molecular weight organic compounds. The increased lipase activities in fertilized soil after one and five months of addition reflected the rapid breakdown of fats and oils contained in compost and digestate into free fatty acids, diacylglycerols, monoglycerols, and glycerol (Hanc et al., 2021). The more pronounced increase due to compost compared with digestate addition indicates a higher lipid content in compost than in digestate. Breakdown of other compost- and digestate-derived polymers (hemicellulose, cellulose, and chitin) were induced at a later timepoint. For example, the degradation of chitins in soil fertilized with digestate as well as the degradation of cellulose in compost-amended soil were only evident five months after addition. The degradation of hemicellulose derived from amendments was still visible after 17 months. Since we did not find any differences in microbial biomass under the two organic amendments, the observed increase in activities was likely due to higher enzyme production of already present microorganisms.

Crop yield, that is, silage maize biomass and grain yield of summer barley, was not affected by MP addition in our study. Direct effects due to uptake and accumulation in plants have been observed for MP <2 µm (Mateos-Cárdenas et al., 2021). Uptake by plants was unlikely in our study since MP were too large for uptake by plants. While additional mechanisms of MP effects on plant biomass remain unclear, changes in soil structure, bulk density, improved aeration, and microporosity, as well as rooting and nutrient immobilization, are discussed as possible results of both negative and positive effects of MP on plant

biomass (Boots et al., 2019; Lozano et al., 2021; Mateos-Cárdenas et al., 2021; Qi et al., 2018; Rillig et al., 2019). Such indirect effects are again likely to occur if MP concentrations exceed certain thresholds, which may be the case in fields with plastic mulch and sewage sludge application where MP loadings are particularly high (Büks & Kaupenjohann, 2020; G. S. Zhang & Liu, 2018; D. Zhang et al., 2020). However, Brown et al. (2022) did not observe growth and yield reductions of wheat plants even with loads of LDPE-MP >100 g m^{-2}. While these results, as in our case, indicate that MP might not pose a risk with respect to plant growth, this should be confirmed by investigations of other sites (with different soil types and climates) as well as plant species and MP types. Nevertheless, for fields with lower MP concentrations, such as in our study, no negative effects of MP on plant biomass can be expected.

5 | CONCLUSIONS

Our results highlight that diverse MP can be found in arable soils even without agricultural practices such as organic fertilization, sewage sludge addition, or plastic mulching. This indicates that there are significant diffuse MP inputs into soils through atmospheric deposition, littering, and, to our knowledge noted for the first time, due to the abrasion of coatings of agricultural machinery. In particular, small MP particles <0.2 mm were frequently found in the soil. Soil organisms can ingest such particles with to-date unknown long-term environmental risks. There remains much uncertainty regarding concentrations of small MP <0.01 mm and nanoparticles, and methods for their detection in soil are needed.

We provide evidence that conventional as well as biodegradable MP can persist and accumulate in soil under field conditions. Current MP loadings in arable soil under agricultural practices such as amendment with organic fertilizers have no detectable immediate negative consequences neither on soil microbial abundance and activity related to C cycling, nor on crop yields. However, due to regular MP inputs from diffuse sources and from organic fertilizers and sewage sludge contaminated with MP, as well as the high persistence of many polymers, long-term effects of MP on soil microbial abundance and activities related to C and nutrient cycling cannot be excluded. Additional long-term field studies examining different soil types and polymers will be crucial to assess the risks of environmental threats of MP to functions of agricultural soils.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available on Mendeley at http://doi.org/10.17632/8chdw8vgw9.2.

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