Supplementary Information (SI) for Environmental Science: Processes & Impacts.
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EM-ART-03-2026-000205

Supplementary Material

Microplastics affect belowground performance of winter wheat and microbial networks in the rhizosphere

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Soil characterization

The volumetric water content at saturation was 0.50 $cm^3\ cm^{-3}$, which is equivalent to a volumetric water-holding capacity (WHC) of 50% v/v, which was determined measuring soil water retention curves (SWRC). SWRCs of six soil samples, compacted to 1.2 $g\ cm^{-3}$, were recorded by using the evaporation method using a HYPROP® system (Meter Group, München, Germany) as described by Schindler et al. (2010) in combination with a dewpoint potentiometer (WP4®, Decagon Devices, Pullman, WA, USA). The volumetric WHC corresponded to a gravimetric WHC of 42% w/w (Eqn. 1), which was used to calculate the amount of water added to the plants during the experiment.

$$\text{grav. WHC} = \frac{\text{vol. WHC}}{\square_{soil}} \quad [1]$$

With grav. WHC = gravimetric water-holding capacity ($g\ g^{-1}$), vol. WHC = volumetric water holding capacity ($cm^3\ cm^{-3}$), $\square_{soil}$ = soil bulk density ($g\ cm^{-3}$).

It is not possible to definitively exclude the presence of micro- and/or nanoplastics in the soil, since the agricultural area from which the soil was collected may have been exposed to anthropogenic plastic inputs. Nonetheless, the site has no record of plastic mulching or sewage sludge fertilization. Any larger plastic debris discovered was promptly removed. In addition, the inclusion of control samples without added MP and the

relatively high MP concentration (0.4% w/w), a level not yet commonly observed in German farmland, mitigated any potential bias from prior contamination.

Microplastic characterization

The particle size distribution of the MP particles was measured with a laser diffraction particle size analyzer (HORIBA LA-950, Irvine, CA) by dispersing the respective MP powder in ethanol. The LDPE powder had the narrowest particle size distribution with a mean particle diameter of 444.6 +/- 1.1 μm, followed by PS (532.3 +/- 4.7 μm) and PP (548.0 +/- 4.7 μm).

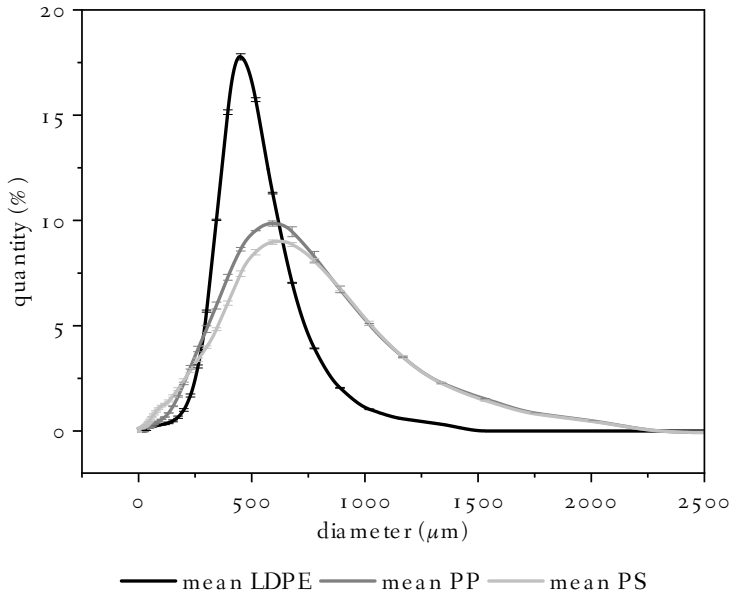

Figure S1. Particle size distribution of used microplastic (MP) particles. PS is polystyrene (in black), LDPE is low-density polyethylene (in grey), PP is polypropylene (in light grey). Each MP powder was measured two times.

The average fiber length was determined by manually measuring individual fibers (n = 50), while the diameter was determined by analyzing scans (EPSON, Expression, 12000xl, Meerbusch, Germany) of fibers via the software tool WinRHIZO (Regent Instruments Inc., Québec, Canada). The polymer structure was polyethylene terephthalate (PET) which was determined via Raman spectroscopy (Fig. S2). A piece of a PET

plastic bottle was used as a reference material for PET and its spectrum was compared with the spectrum of the fibers.

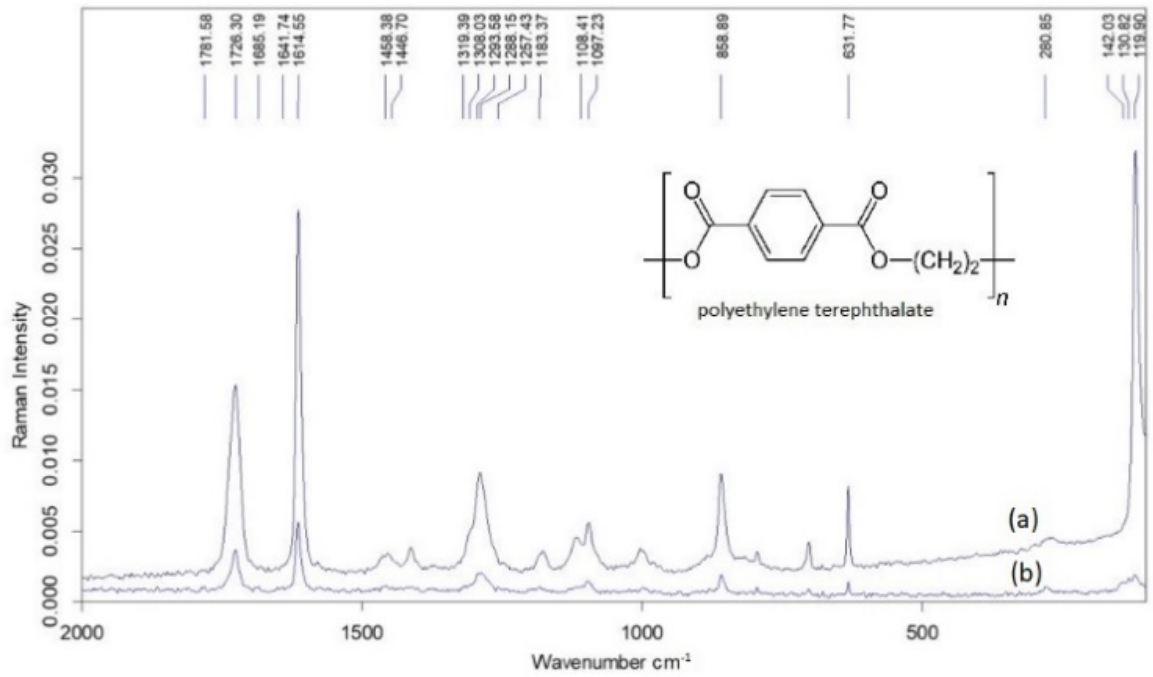

Figure S2. Raman spectra of a) polyethylene terephthalate (PET) reference material and b) polyester (PES) fibers. Spectrum (b) is comparable to spectrum (a) which indicates that the polymeric structure of PES fibers is identical with PET. Relevant assignments of PET Raman spectra are shown in Table S1.

Table S1. Relevant assignments of functional groups of PET in Raman spectra.[58,59]

Wavenumber	Assignment
cm^{-1}	
1726	Stretching C=O vibration
1614	Ring mode 8a (in Wilson′s notation)
1293	C(O)–O stretching
1183	Ring in plane C–H bond and C-C stretch
1096	a combination of C–O stretching, COC bending, CCO bending, and C–C stretching in the ethylene glycol segment

Extraction method and preparation of extraction media for soil analysis

For each extraction method, 5 g (+/- 10%) of fresh frozen soil samples were weighed into 50-mL centrifuge vials (Eppendorf AG, Hamburg, Germany), mixed with 40 mL of the respective extraction medium and then shaken horizontally for 1 h at 200 rpm. After centrifugation for 15 min at 3500 rpm, a syringe was used to collect 20 mL of the supernatant. The first 5 mL were discarded through a 0.45 μm filter (Sartorius AG, Göttingen, Germany), and the remaining 15 mL were collected and stored at -20°C for further analysis. The resulting concentration of soil in extraction medium was 0.1 g mL^{-1}.

Extraction medium for mineral plant-available nitrogen (N_{min}) analysis:

A 0.01 M calcium chloride ($CaCl_2$) solution was used by dissolving 7.35 g of $CaCl_2$ x 2 H_2O salt (Merck KGaA, Darmstadt, Germany) in 5 L deionized water.

Extraction medium for plant-available potassium (K_{av}) and phosphate (P_{av}) analysis:

The calcium acetate lactate (CAL) solution was prepared as 1 L stock solution by slowly dissolving 77.00 g of calcium lactate pentahydrate ($C_6H_{10}CaO_6$ x 5 H_2O) and 39.50 g of calcium acetate ($Ca(CH_3COO)_2$ x H_2O) respectively in 300 mL of hot deionized water while both solutions were stirred at 200 rpm. Both solutions were filled into a 1 L volumetric flask and were adjusted to 1 L after 90 mL of conc. acetic acid were added. The final stock solution was diluted to 5 liters.

Root analysis with WinRHIZO

Table S2. Parameter set for WinRHIZO analysis.

Criteria	Set parameter
Root and Background Detection	220
Debris Rough Edges	Area smaller than: 0.0025 cm^2
Rough Edges and Noise Removal	low

Diameter classes of winter wheat roots

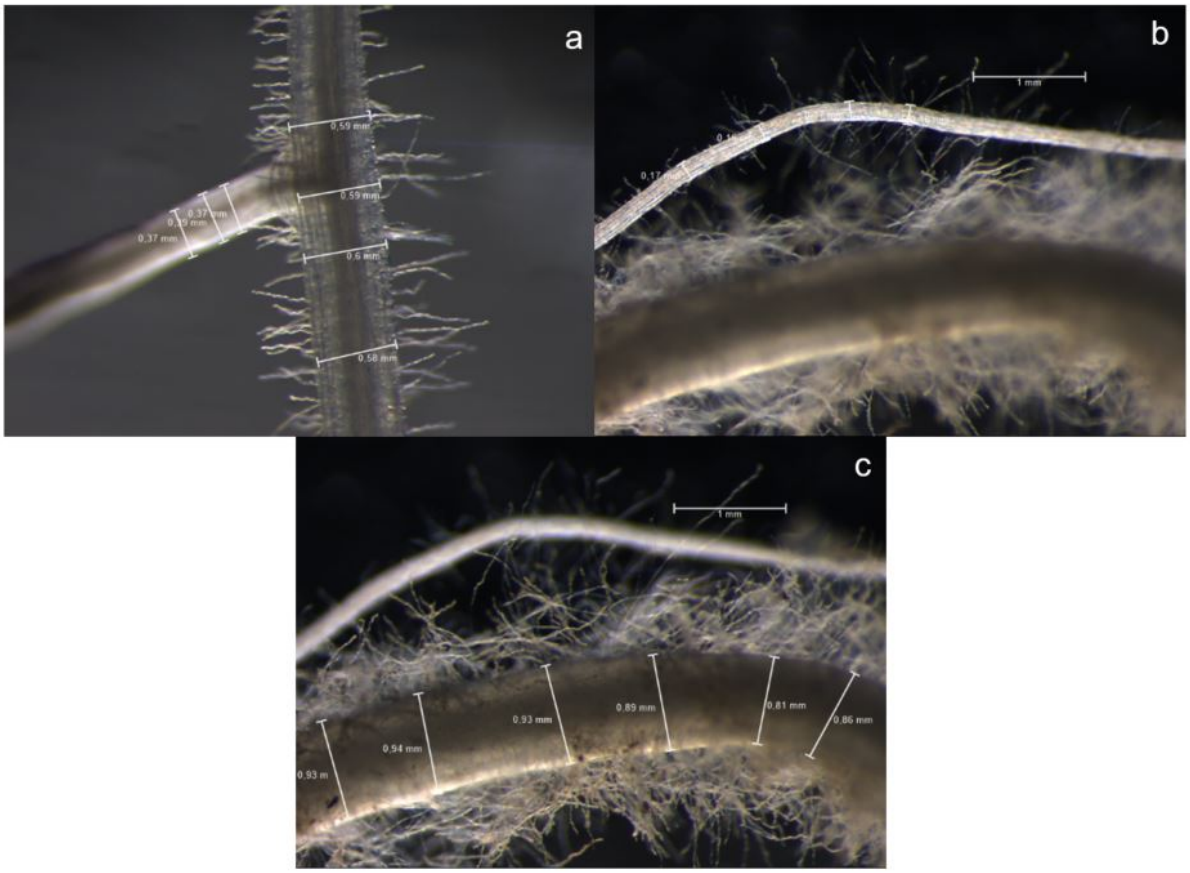

Figure S3. Stereomicroscopic pictures of winter wheat roots harvested after a growth period of 28 days. a) Primary root (width: 0.58-0.6 cm) and lateral root (width: 0.37-0.39 cm), b) Lateral root (width: 0.16-0.17 cm), c) Primary root (width: 0.81-0.94 cm).

Calculation of chlorophyll-a, -b, and carotenoid

The following equations as proposed by Lichtenthaler & Wellburn (1983) were used to determine the chlorophyll-a (chl_a), chlorophyll-b (chl_b) and the carotenoid (car) content of the extracts (Eqs. [2-[4). The values were further converted to μg per g leaf dry weight.

$$chl_a = abs_{663} \square 12.21 - abs_{646} \square 2.81 \quad [2]$$

$$chl_b = abs_{646} \square 20.13 - abs_{663} \square 5.03 \quad [3]$$

$$car = \frac{abs_{470} \square 1000 - chl_a \square 3.27 - chl_b \square 104}{229} \quad [4]$$

With chl_a = chlorophyll-a in μg mL^{-1}, chl_b = chlorophyll-b in μg mL^{-1}, car = carotenoid in μg mL^{-1}, abs_x = absorption at wavelength x nm.

Shoot analysis

After the growth period of 28 days, the shoots had a mean dry mass in the range of 390 to 570 mg (Fig. S10a), while their length ranged from 32 to 35 cm (Fig. S10b). Similarly, no significant difference in shoot length development over time was observed with the addition of MP (Fig. S12). The nitrogen (N) concentration ranged from 49 to 53 mg g^{-1} leaf mass (Fig. S10c), while the average total leaf N content of each plant (Fig. S10d) remained below 30 mg, and was not significantly different between the treatments.

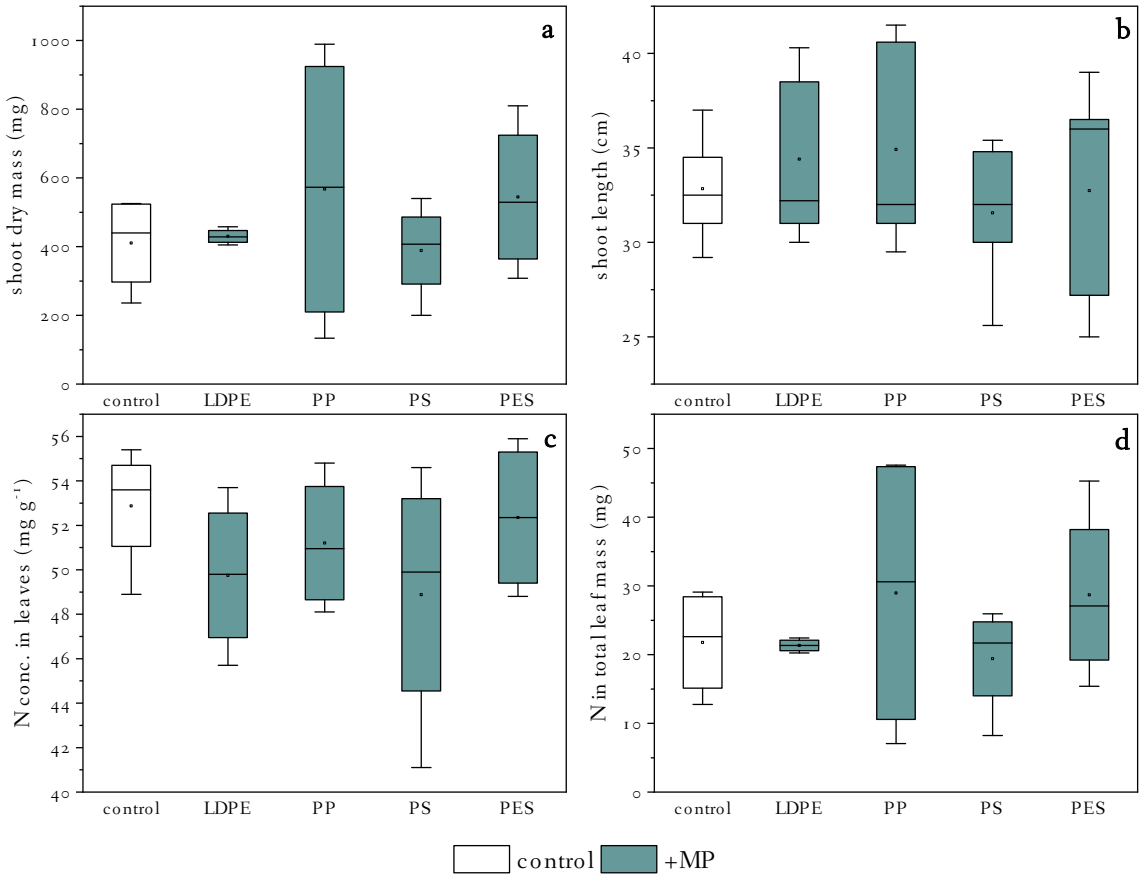

Figure S4. Shoot a) dry mass (in mg), b) length (in cm), c) nitrogen (N) concentration in leaves (in mg g^{-1}) and d) total leaf N content (mg) of winter wheat plants after the growth period of 28 days in rhizotrons. "+MP" represents microplastic (MP) addition to soil, while no MP was added to the soil of the control. The following MP types were added: LDPE = low-density polyethylene, PP = polypropylene, PS = polystyrene, PES = polyester fibers. Boxes are the interquartile range (IQR) from the 25th to 75th percentile, lines inside boxes indicate the median, white squares indicate the mean values and upper and lower whiskers are 1.5 times the IQR (n = 5).

The leaf chlorophyll content, chl_a (Fig. S5a) and chl_b (Fig. S5b), as well as the *car* content (Fig. S5c) of plants grown in MP-amended soil were not significantly different from the control. The chl_a values ranged between 3 to 3.6 $\mu g\ mg^{-1}$, for chl_b between 1 to 1.15 $\mu g\ mg^{-1}$ and for *car* 0.40 to 0.51 $\mu g\ mg^{-1}$.

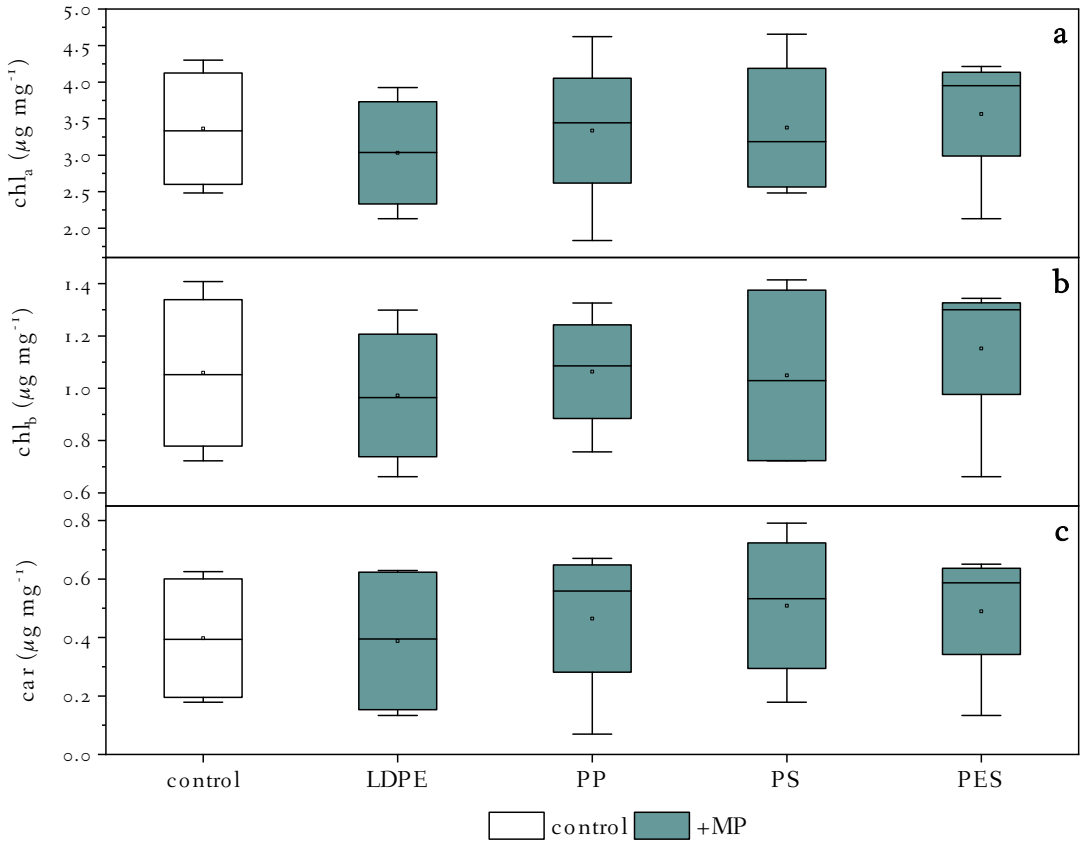

Figure S5. Chlorophyll, a) chlorophyll *a* (chl$_a$) and b) chlorophyll *b* (chl$_b$), and c) carotenoid (car) content (in μg mg^{-1}) of winter wheat plants grown for 28 days in rhizotrons. "+MP" represents microplastic (MP) addition to soil, while no MP was added to the soil of the control. The following MP types were added: LDPE = low-density polyethylene, PP = polypropylene, PS = polystyrene, PES = polyester fiber. Boxes are the interquartile range (IQR) from the 25th to 75th percentile, lines inside boxes indicate the median, white squares indicate the mean values and upper and lower whiskers are 1.5 times the IQR (n = 5).

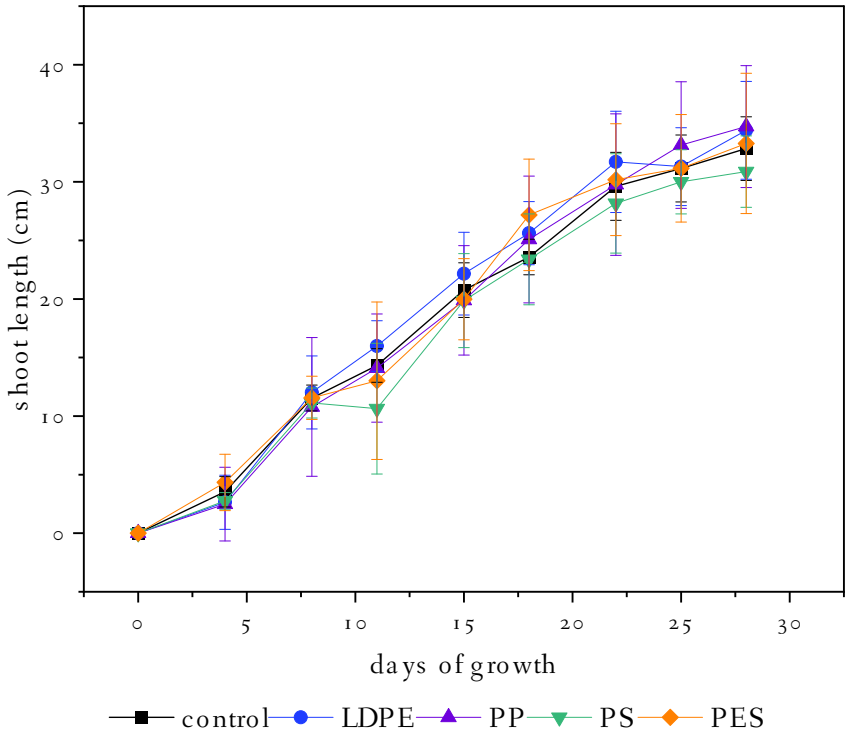

Figure S6. Shoot length (in cm) development over the growth period of winter wheat plants in rhizotrons for 28 days. Winter wheat plants grew without (control, in black squares) and with the addition of different microplastic (MP) types in soil (0.4% w/w). The following MP types were added: LDPE = low-density polyethylene particles in blue circles, PP = polypropylene particles in purple triangles, PS = polystyrene particles in green triangles, PES = polyester fibers in orange diamonds.

Soil nutrient analysis in $\mu g\ g^{-1}$

In general the soil nutrient analysis of plant-available mineral nitrogen species, e.g. NO_3^-, NH_4^+ and NO_2^- and K_{av} and P_{av} did not show large differences with MP load compared to the control (Fig. S7).

In the bulk soil of LDPE- and PP-amended soil it was noticeable that the NO_3^- content was slightly reduced with LDPE by 50% +/- 16% (p = 0.1) and with PP by 59% +/- 22% (p = 0.08). The NO_2^- concentration in rhizo soil was slightly enhanced with presence of PP particles (+33% +/- 34%, p= 0.08). With PS particles the soils' NH_4^+ content was enhanced in the rhizo soil by 62% +/- 68% (p = 0.07) with a weak

significance and significantly enhanced in bulk soil by 48% +/- 26% ($p = 0.03$). Moreover, the NH_4^+ content in the bulk soil of PES treatment was slightly enhanced (+71% +/- 90%, $p = 0.1$).

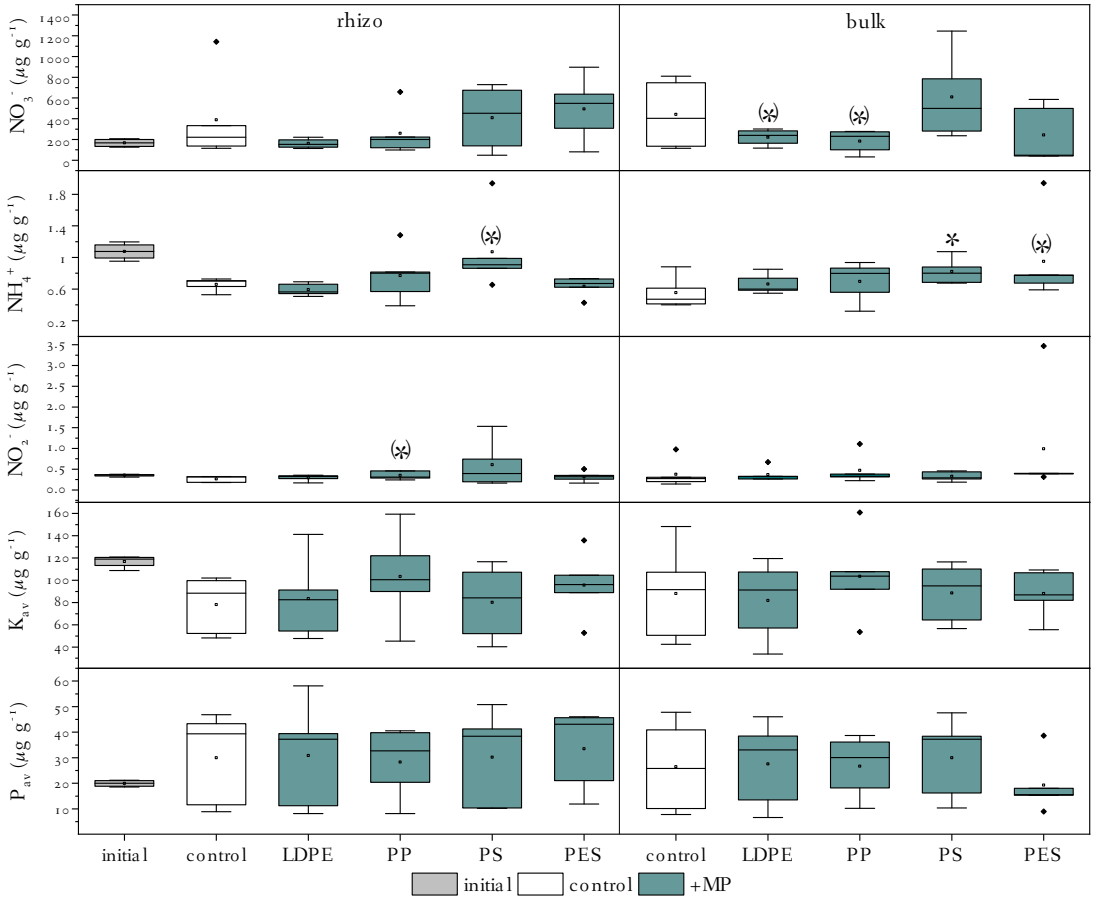

Figure S7. Soil nutrient analysis of plant-available nitrate (NO_3^-), ammonia (NH_4^+), nitrite (NO_2^-), potassium (K_{av}) and phosphate (P_{av}) (in $\mu g\ g^{-1}$) in rhizosphere (rhizo) and bulk soil before ("initial" in grey) and after the winter wheat growth period of 28 days in rhizotrons. The following microplastic (MP) types were added in soil (in dark green): LDPE = low-density polyethylene, PP = polypropylene, PS = polystyrene, PES = polyester fiber. Control (in white) resembles no MP addition in soil. Boxes are the interquartile range (IQR) from the 25th to 75th percentile, lines inside boxes indicate the median, white squares indicate the mean values, upper and lower whiskers are 1.5 times the IQR and diamond-shaped markers are outliers (n = 5). The asterisk show significant difference (p □ 0.05) towards the control and asterisks in parentheses show weak significant differences (0.05 □ p □ 0.1) to the control, tested with one-sided, heteroscedastic t-test.

Table S3. Two-way ANOVA testing the effect of root zone and microplastic (MP) type on the amount of soil nutrients (NO_3^- = nitrate, NH_4^+ = ammonium, NO_2^- = nitrite, K_{av} = potassium, P_{av} = phosphate) per root surface area ($\mu g\ cm^{-2}$), described by the test statistic (F) and critical test statistic ($F_{crit.}$) values, the p-value lower than 0.05, and the effect size eta-squared (□²).

	NO_3^-			NH_4^+			NO_2^-			K_{av}			P_{av}		
Source of variation	**F and $F_{crit.}$**	***p*-value**	**□²**	**F and $F_{crit.}$**	***p*-value**	**□²**	**F and $F_{crit.}$**	***p*-value**	**□²**	**F and $F_{crit.}$**	***p*-value**	**□²**	**F and $F_{crit.}$**	***p*-value**	**□²**
Effect of root zone	0.12 and 4.08	0.72	0.00	0.20 and 4.08	0.65	0	0.98 and 4.08	0.33	0.02	0.02 and 4.08	0.90	0.00	0.69 and 4.08	0.41	0.02
Effect of MP type	2.76 and 2.61	0.04*	0.20	3.65 and 2.61	0.01*	0.25	1.34 and 2.61	0.27	0.10	1.70 and 2.61	0.17	0.14	0.79 and 2.61	0.54	0.07
Interaction effect	1.09 and 2.61	0.38	0.08	0.63 and 2.61	0.64	0.04	1.26 and 2.61	0.30	0.10	0.17 and 2.61	0.95	0.01	0.26 and 2.61	0.90	0.02

*significantly different from control ($p < 0.05$).

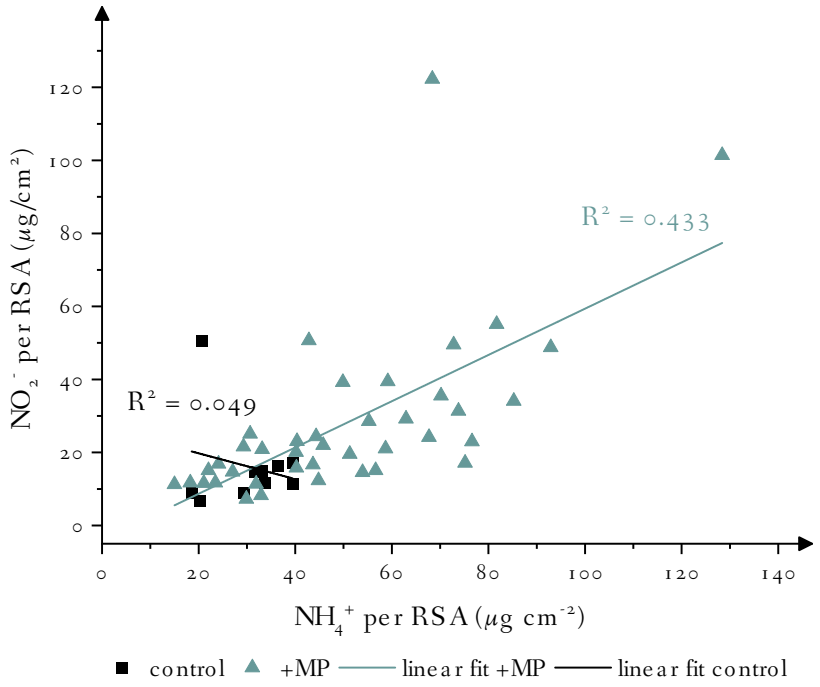

Figure S8. Plot of plant-available nitrite (NO_2^-) versus ammonium (NH_4^+) content of the soil per root surface area (RSA).

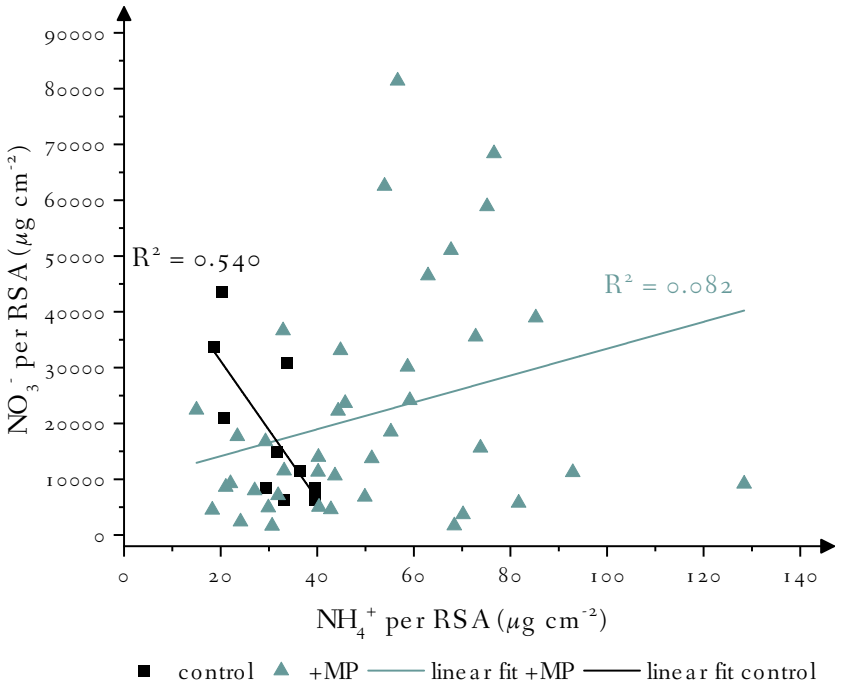

Figure S9. Plot of plant-available nitrate (NO_3^-) versus ammonium (NH_4^+) content of the soil per root surface area (RSA).

Microbiome analysis

Table S4. Statistical analysis of different alpha diversity measures. Statistical analysis was performed using Kruskal-Wallis and the Wilcoxon Rank-Sum test with Benjamini-Hochberg correction for multiple comparisons.

p-values	Richness (Rich)	Shannon	Simpson	Evenness (Even)
Soil	0.652	0.598	0.309	0.832
MP	0.721	0.897	0.705	0.769
Soil* MP	0.755	0.608	0.409	0.851

Table S5. Global network properties of the microbial co-occurrence network based on Pearson correlation for untreated (Control) and low-density polyethylene (LDPE) microplastics treated rhizosphere samples. Both treatments formed single-component networks with high clustering coefficients and edge densities. LDPE reduced vertex and edge connectivity by 50% and slightly lowered modularity. Average dissimilarity and path length remained comparable.

	Control	LDPE
Number of components	1.00000	1.00000
Clustering coefficient	0.94176	0.95691
Modularity	-0.00158	-0.00507
Positive edge percentage	49.5654	49.32838
Edge density	0.90178	0.91405
Natural connectivity	0.33447	0.34642
Vertex connectivity	28.00000	14.00000
Edge connectivity	28.00000	14.00000
Average dissimilarity*	0.65894	0.64355
Average path length**	0.97402	0.97445

*: Dissimilarity = 1- edge weight

**: Path length = Units with average dissimilarity

Table S6. Global network properties of the microbial co-occurrence network based on Pearson correlation for untreated (Control) and polyester (PES) fiber microplastics treated rhizosphere samples. Both treatments formed single-component networks with high clustering coefficients and edge densities. LDPE reduced vertex and edge connectivity by 50% and slightly lowered modularity. Average dissimilarity and path length remained comparable.

	Control	PES
Number of components	1.00000	1.00000
Clustering coefficient	0.94870	0.88754
Modularity	-0.00482	-0.00589
Positive edge percentage	49.46174	49.03877
Edge density	0.91874	0.82037
Natural connectivity	0.32672	0.28916
Vertex connectivity	28.00000	24.00000
Edge connectivity	28.00000	24.00000
Average dissimilarity*	0.65727	0.70149
Average path length**	0.97834	0.94937

*: Dissimilarity = 1- edge weight

**: Path length = Units with average dissimilarity

Table S7. Global network properties of the microbial co-occurrence network based on Pearson correlation for untreated (Control) and polypropylene (PP) microplastics treated rhizosphere samples. Both treatments formed single-component networks. The PES network showed reduced clustering coefficient, edge density, connectivity, and natural connectivity, indicating decreased network complexity and cohesion.

	Control	PP
Number of components	1.00000	1.00000
Clustering coefficient	0.91942	0.88415
Modularity	-0.00161	-0.00505
Positive edge percentage	49.77390	49.42149
Edge density	0.84706	0.82763
Natural connectivity	0.33241	0.28624
Vertex connectivity	19.00000	28.00000
Edge connectivity	19.00000	28.0000
Average dissimilarity*	0.67588	0.70113
Average path length**	0.96045	0.95186

*: Dissimilarity = 1- edge weight

**: Path length = Units with average dissimilarity

Table S8. Global network properties of the microbial co-occurrence network based on Pearson correlation for untreated (Control) and polystyrene (PS) microplastics treated rhizosphere samples. Both treatments formed single-component networks. The PP network showed a lower clustering coefficient, lower edge density, and natural connectivity compared to Control. Vertex and edge connectivity increased under PP.

	Control	PS
Number of components	1.00000	1.00000
Clustering coefficient	0.92931	0.92093
Modularity	-0.00372	-0.00591
Positive edge percentage	49.44391	49.71591
Edge density	0.86101	0.86676
Natural connectivity	0.33352	0.32253
Vertex connectivity	21.00000	23.00000
Edge connectivity	21.00000	23.00000
Average dissimilarity*	0.67057	0.67430
Average path length**	0.96231	0.95954

*: Dissimilarity = 1- edge weight

**: Path length = Units with average dissimilarity

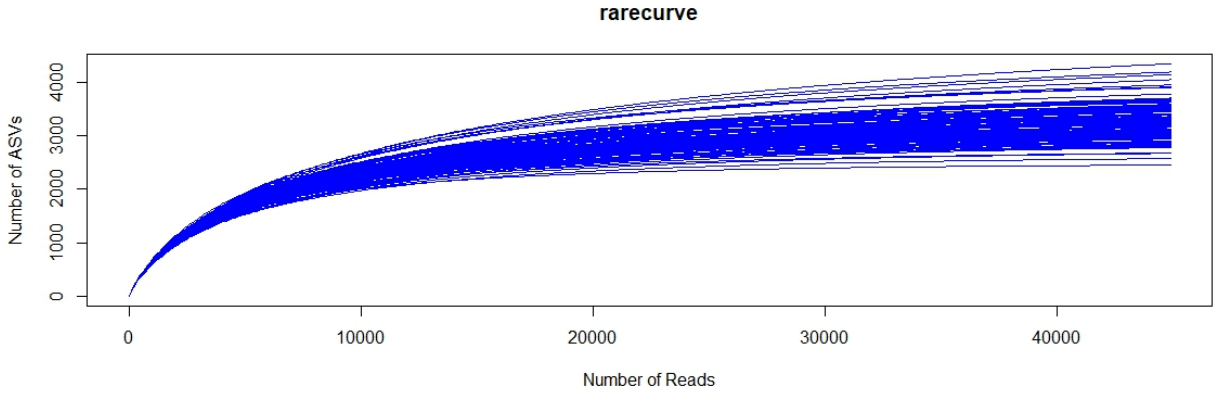

Figure S10. Rarefaction curve of amplicon sequence variants (ASVs) identified via 16S rRNA gene amplicon sequencing after subsampling to 44,905 reads per sample. The number of sequences ASVs reached a plateau for all samples, indicating a sufficient sequencing depth.

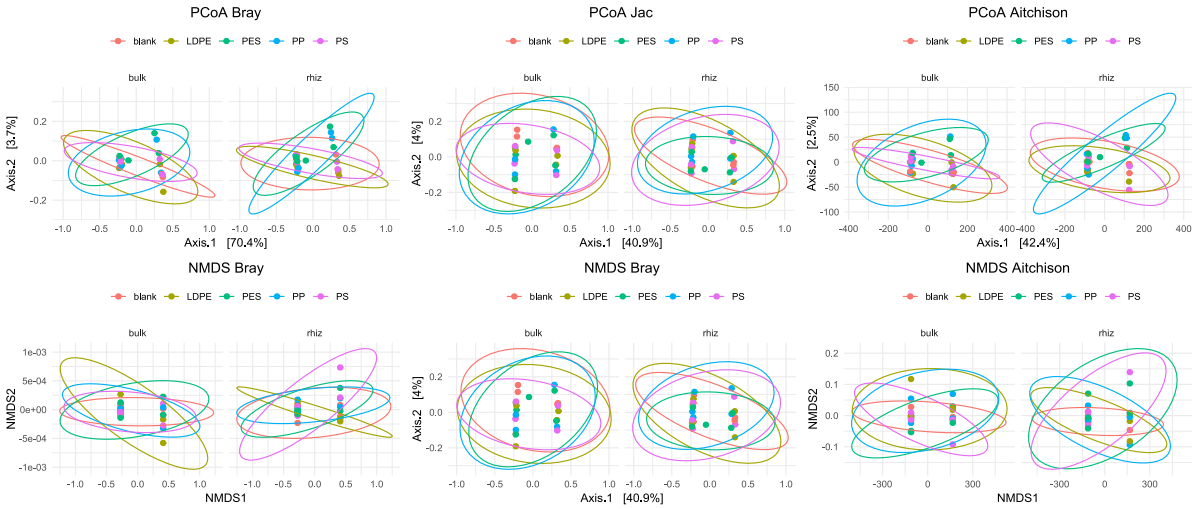

Figure S11. Community structure of bacteria in bulk soil (bulk) and rhizosphere (rhizo): Beta-diversity ordinations based on Bray–Curtis, binary Jaccard, and Aitchison distances.

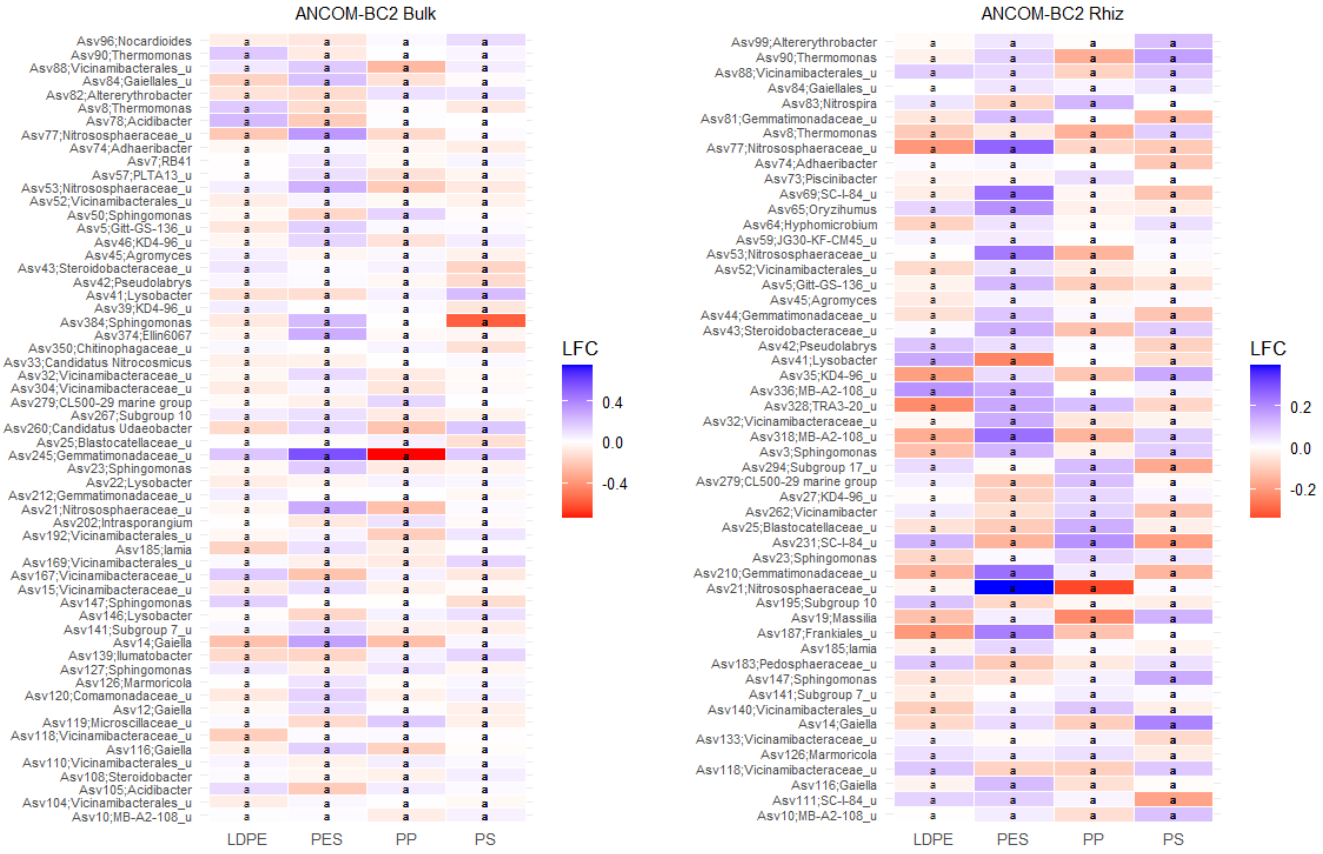

Figure S12. Community structure of bacteria in bulk soil (bulk) and rhizosphere (rhizo): ASV-level differential-abundance analysis using the compositionality-aware method ANCOM-BC2. A global test was first performed to identify candidate ASVs (unadjusted $p < 0.05$), followed by pairwise comparisons among MP treatments. No ASVs were identified as significantly differentially abundant in the global test.