

Supplementary material

Interactive impacts of microplastics and arsenic on agricultural soil and plant traits

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Supplementary methods

1. Physico-chemical analyses of soil and baseline microplastic pollution

Soil pH and electrical conductivity (EC) were determined at soil/water ratios of 1:2.5 and 1:2 respectively, using a glass electrode (Sparks et al., 1996). Cation exchange capacity (CEC) was measured with barium chloride (pH = 8.1) and titration with EDTA (0.05 N). Particle size distribution (sand, silt, and clay) was determined using the pipette method (Gee and Bauder, 1986). Total organic carbon (TOC), total carbon (C_{tot}) and total nitrogen (N_{tot}) were determined according to (Sparks et al., 1996) and using a FlashSmart Elemental Analyzer (Thermo Fisher Scientific, Milan, Italy). Available phosphorus (P_{avail}) was determined by colorimetry a solution of sodium bicarbonate, according to Olsen and Sommers (1992) and using a UV-Visible Spectrophotometer (Unicam- UV500 Thermospectronic, Cambridge, UK). The available potassium (K), calcium (Ca), sodium (Na), and magnesium (Mg) concentrations

were determined by ammonium-acetate exchange extraction and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES 5900 Agilent, Santa Clara, CA, USA) (Sparks et al., 1996). Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were measured after extraction with water at a ratio of 1:5 (w:v) for 1 h at room temperature using an ammonium-selective electrode (SevenMulti, Mettler Toledo) and a UV-Visible spectrophotometer, respectively (Sparks et al., 1996).

Briefly, samples of air-dried soil were weighed (5 g) and sequentially mixed with 20 ml of deionized water, 20 ml of saturated sodium chloride solution (NaCl , 5 M, $\rho = 1.20 \text{ g cm}^{-3}$), and 20 ml of concentrated zinc chloride solution (ZnCl_2 5 M, $\rho = 1.55 \text{ g cm}^{-3}$). At each addition, the mixture was subjected to magnetic stirring for 25 min, centrifugation at 2000 rpm for 15 min, and filtration on Whatman N°42 filter paper (retention $>2.5 \mu\text{m}$). Each step was repeated twice. The filter papers were visually examined under a Leica M205 C stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a Leica DMC4500 camera (Leica Microsystems GmbH, Wetzlar, Germany).

2. Chemical and thermal analyses of manufactured microplastics

The polymer composition of the plastic particles and the surface chemical modification were evaluated by ATR-FTIR spectroscopy following the procedure previously described. The possible modification caused by heating during grinding and by degradation processes occurring in soil during the experiment were assessed by differential scanning calorimetry (DSC) analysis using a Seiko SII ExstarDSC7020 calorimeter (Seiko, Chiba, Japan) in N_2 atmosphere. The thermal analyses by DSC on MPs were performed before (pellet) and after grinding ($< 250 \mu\text{m}$ and $250\text{-}300 \mu\text{m}$) to determine glass transition temperature (T_g), melting temperature T_m , crystallization temperature (T_c), measured melting enthalpy (ΔH_m), measured

crystallization enthalpy (ΔH_c) and degree of crystallinity (χ). Around 5.0 mg of plastic was weighed into an aluminum crucible and heated following the thermal programs (Table S2). The first temperature rise is used to eliminate the historical heat of the sample, and the second temperature rise is used to analyze the plastic. The Leica M205 C stereomicroscope was used to analyze the shape and size of the produced MPs as previously described.

References

- Gee, G.W., Bauder, J.W., 1986. Particle-size analysis, in: Klute, A. (Ed.), *Methods of Soil Analysis: Part 1d - Physical and Mineralogical Methods*. Soil Science Society of America, American Society of Agronomy, Madison, USA, pp. 383–411.
- Olsen, S.R., Sommers, L.E., 1992. Phosphorus, in: Klute, A. (Ed.), *Methods of Soil Analysis, Part 1*. ASA-SSSA, Madison, WI, pp. 403–430.
- Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., 1996. *Methods of soil analysis Part 3—Chemical methods*, Soil Science Society of America. Soil Science Society of America, Madison, USA. <https://doi.org/10.2136/sssabookser5.3.frontmatter>

Supplementary results

1. Microplastic background in soil

The ATR-FTIR spectroscopic analysis allowed to identify the MPs collected from the soil (background) as polyethylene (PE) and polypropylene (PP) (Fig. S1). The analysis was based on the comparison with commercial pellet spectra obtained with the same instrument. The infrared spectra showed the characteristic bands due to the vibration mode of PE (plastic 1) and PP (plastic 2). In the spectrum related to PE (Fig. 1a), in the range between 3000 and 2800 cm^{-1} , it was possible to observe strong intensity signals due to the asymmetric and symmetric stretching of CH_2 (2920 and 2853 cm^{-1} , respectively). In addition, the fingerprint region of the spectra, between 1500 and 650 cm^{-1} , was consistent with the identification of the collected MPs. Indeed, it was possible to distinguish the strong intensity signal assigned to the bending deformation at 1470 cm^{-1} and the medium intensity signal between 731 and 720 cm^{-1} assigned to the rocking deformation of CH_2 in the carbon backbone of PE.

The spectrum related to PP showed the characteristic vibration modes of polypropylene, consistently with the reference of commercial PP presented in Fig. 1b. Similar to PE, PP is made by polymerization of olefinic monomers where $\text{C}=\text{C}$ groups react forming chains following various possible mechanisms. A wide range of PE is available on the market, including, for example high-density polyethylene (HDPE) and LDPE. High-density polyethylene is characterized by linear chains (very low degree of branching) and therefore shows higher densities (0.940 - 0.965 g cm^{-3}) due to the greater interactions of the chains that lead to high degree of crystallinity (70% to 80% depending on molecular weight and thermal history). Low-density polyethylene is instead characterized by branches along the chains and this leads to a greater distance between the chains themselves and to a lower density of the

material ($0.91 - 0.925 \text{ g cm}^{-3}$) and lower degree of crystallinity than that found for HDPE (40% to 55% depending on molecular weight and thermal history).

The main structural difference between PE and PP is that the former contains no CH_3 groups (at least HDPE), while in the latter CH_3 is present as a pendant group bonded to the main chain. This difference was clearly evidenced by the region of the spectra between 3000 and 2800 cm^{-1} (Fig. S1b). Low-density polyethylene and other low-density PE have pendant groups with CH_3 , but the concentration of these groups is nevertheless low and not detected in the region between 2800 and 3000 cm^{-1} of the IR spectrum (Martínez-Romo et al., 2015). The strong intensity bands at 2953 and 2872 cm^{-1} are assignable to asymmetric and symmetric stretching of CH_3 groups, respectively. Similar to PE spectra, at 2920 and 2845 cm^{-1} CH_2 stretching modes were also detected. On the fingerprint region, the bands at 1372 and 1450 cm^{-1} were assigned to the bending modes of CH_3 groups of PP. In addition, the ATR-FTIR spectroscopic analysis revealed the possible occurrence of oxidative degradation reactions on both MPs types. Low intensity bands at 1640 and 1015 cm^{-1} for PE (Fig. S1a) and 1652 and 1030 cm^{-1} for PP (Fig. S1b) highlighted the presence of $\text{C}=\text{O}$ groups. These phenomena are typically accelerated by solar exposure and other environmental factors which enhance the reactions with atmospheric oxygen, eventually evolving towards the cleavage of polymer carbon backbone. The strong intensity bands at 1053 cm^{-1} and the relative shoulder at about 970 cm^{-1} observed in both spectra of MP found in soil can be assigned to Si-O and Si-OH vibrations of soil silicates. The IR bands characteristic of the groups of silicates and associated with oxidation phenomena were not found in the plastic used in the experiment. This difference can be explained considering that the plastics found in the soil were exposed to natural environmental conditions, whereas the plastics used in the present study are essentially raw materials.

2. Manufactured MPs

Differential scanning calorimetry (DSC) thermograms (Fig. S2) of virgin pellets and manufactured MPs of LDPE and PLA (<250 μm and 250-300 μm) suggested that the grinding process did not affect the general thermal behavior of the materials and the crystallinity, as shown in Table S3. The first heating of the LDPE and PLA samples revealed similar melting behavior among manufactured fractions of the same polymer (< 250 and 250-300 μm), with a slight increase in T_g and T_m (Table S4). However, the extent of the degradation phenomena during the grinding process can be considered negligible for the purposes of this work. Although the recrystallization phenomenon during the cooling process was not observed for the pellets of PLA and the PLA250-300 particles, the recrystallization occurred for the PLA250 (negligible entity) and for the virgin pellet and manufactured LDPE (both sizes). For these fractions the melting process was also observed during the second heating. The slight variations observed during the second heating (necessary to remove the thermal history from the first heating ramp) supported that the grinding process slightly influenced the general thermal behavior and crystallinity of the LDPE (both sizes) and PLA250. In virgin pellet and manufactured PLA (both sizes), the cold crystallization phenomenon was not observed. The degree of crystallinity of PLA and LDPE, calculated considering the theoretical values of the enthalpy of fusion of 100% crystalline PLA (ΔH_0 93 J g⁻¹) and 100% crystalline LDPE (ΔH_0 290 J g⁻¹) (De Monte et al., 2022), slightly decreased in all fractions due to the milling process. However, the magnitude of this variation can be considered negligible for the purposes of the present study.

References

Martínez-Romo, A., González-Mota, R., Soto-Bernal, J.J., Rosales-Candelas, I., 2015. Investigating the degradability of HDPE, LDPE, PE-BIO, and PE-OXO films under UV-B radiation. *J. Spectrosc.* 2015, 1–6. <https://doi.org/10.1155/2015/586514>

Supplementary tables

Table S1

Physico-chemical parameters of the agricultural soil used in the experiment. Data are the mean of three replicates \pm standard error.

Parameter ^a	
Soil texture ^b	Sandy loam
Clay (%)	8.3 \pm 0.09
Silt (%)	12.0 \pm 0.54
Sand (%)	79.7 \pm 0.45
pH	8.15 \pm 0.02
EC ($\mu\text{S cm}^{-1}$)	1289 \pm 13.4
CEC ($\text{Cmol}_{(+)}\text{ kg}^{-1}$)	15.3 \pm 1.08
TOC (%)	2.14 \pm 0.10
C _{tot} (%)	4.80 \pm 0.06
N _{tot} (%)	0.15 \pm 0.01
P _{avail} (mg kg^{-1})	16.3 \pm 0.24
Ca (g kg^{-1})	2.98 \pm 0.01
K (g kg^{-1})	0.19 \pm 0.00
Na (g kg^{-1})	0.18 \pm 0.00
Mg (g kg^{-1})	0.13 \pm 0.00
NH ₄ ⁺ (mg kg^{-1})	0.14 \pm 0.01
NO ₃ ⁻ (mg kg^{-1})	10.0 \pm 0.27

^a EC: electrical conductivity; CEC: cation exchange capacity; TOC: total organic carbon; C_{tot}: total carbon; N_{tot}: total nitrogen; P_{avail}: available phosphorus; K: potassium; Ca: calcium; Na: sodium; Mg: magnesium; NH₄⁺: ammonium; NO₃⁻: nitrate;

^b IUSS: International Union of Soil Sciences.

Table S2

Differential scanning calorimetry (DSC) thermal programs for low-density polyethylene (LDPE) and polylactic acid (PLA) used in this study.

Step	Temperature ramp (°C)	Heating speed (°C min ⁻¹)	Hold (min)
LDPE ^a			
1 st Heating	20 - 220	10	2
1 st Cooling	220 - 80	10	5
2 nd Heating	-80 - 220	10	2
2 nd Cooling	220 - 0	30	0
PLA ^a			
1 st Heating	20 - 180	10	1
1 st Cooling	180 - 0	10	2
2 nd Heating	0 - 180	10	2
2 nd Cooling	180 - 20	30	1

^a 5 mg of MPs were analyzed.

Table S3

Experimental design testing the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes], arsenic (0 and 60 mg As kg⁻¹) and their interaction on soil and plant parameters (lettuce, *Lactuca sativa* L.).

MPs	As (mg kg ⁻¹)	Code a
No MPs	0	CT
	60	CT+As
LDPE250	0	LDPE250
	60	LDPE250+As
LDPE250-300	0	LDPE250-300
	60	LDPE250-300+As
PLA250	0	PLA250
	60	PLA250+As
PLA250-300	0	PLA250-300
	60	PLA250-300+As

^a CT: control (no MPs, no As); CT+As (no MPs plus 60 mg As kg⁻¹); LDPE250 (LDPE with diameter <250 µm and no As); LDPE250-300 (LDPE with diameter 250-300 µm and no As); PLA250 (PLA with diameter <250 µm and no As); PLA250-300 (PLA with diameter 250-300 µm and no As); LDPE250+As (LDPE with diameter <250 µm plus 60 mg As kg⁻¹); LDPE250-300+As (LDPE with diameter 250-300 µm size plus 60 mg As kg⁻¹); PLA250+As (PLA with diameter <250 µm plus 60 mg As kg⁻¹); PLA250-300+As (PLA with diameter 250-300 µm plus 60 mg As kg⁻¹).

Table S4

Glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), measured melting enthalpy (ΔH_m), measured crystallization enthalpy (ΔH_c) and degree of crystallinity (χ) obtained from differential scanning calorimetry curves of conventional low-density polyethylene (LDPE) and biodegradable polylactic acid (PLA). The analyses were performed on three fractions, pellets and grinded and sieved at $<250 \mu\text{m}$ and $250\text{-}300 \mu\text{m}$ sizes. Five milligrams for each polymer and fraction were analyzed ($n=3$).

Fraction	T_g ($^{\circ}\text{C}$)	T_m ($^{\circ}\text{C}$)	T_c ($^{\circ}\text{C}$)	ΔH_m (J g^{-1})	ΔH_c (J g^{-1})	χ (%) ^a
First heating						
LDPE pellet	-	105.4 ± 0.1^b	-	93.0 ± 0.1	-	32.1 ± 0.0
LDPE250 ^c	-	103.4 ± 0.2	-	92.7 ± 0.0	-	31.9 ± 0.0
LDPE250-300 ^d	-	104.5 ± 0.1	-	95.4 ± 0.2	-	32.9 ± 0.1
Cooling						
LDPE pellet	-	-	89.1 ± 0.3	-	-126.0 ± 0.2	-
LDPE250	-	-	89.8 ± 0.0	-	-113.3 ± 0.1	-
LDPE250-300	-	-	88.9 ± 0.0	-	-123.5 ± 0.2	-
Second heating						
LDPE pellet	-	104.0 ± 0.2	-	126.0 ± 0.1	-	43.0 ± 0.1
LDPE250	-	102.9 ± 0.0	-	126.0 ± 0.0	-	43.0 ± 0.2
LDPE250-300	-	103.4 ± 0.1	-	128.2 ± 0.1	-	44.2 ± 0.2
First heating						
PLA pellet	60.1 ± 0.2	149.8 ± 0.2	-	35.6 ± 0.1	-	38.2 ± 0.0
PLA250 ^e	58.7 ± 0.1	148.3 ± 0.3	-	30.3 ± 0.3	-	32.5 ± 0.2
PLA250-300 ^f	60.2 ± 0.1	148.3 ± 0.1	-	34.2 ± 0.2	-	36.8 ± 0.2
Second heating						
PLA pellet	58.9 ± 0.1	-	-	-	-	-
PLA250	57.9 ± 0.3	149.7 ± 0.2	-	1.2 ± 0.3	-	1.3 ± 0.3
PLA250-300	58.4 ± 0.3	-	-	-	-	-

^a Degree of crystallinity (χ) was calculated as $(\Delta H_m/\Delta H_0)*100$. ΔH_0 = theoretical enthalpy of fusion for a 100% crystalline material, assumed equal to 290 J g^{-1} (LDPE) and 93 J g^{-1} (PLA);

^b Mean \pm SE

^c LDPE250: LDPE with diameter $<250 \mu\text{m}$;

^d LDPE250-300: LDPE with diameter $250\text{-}300 \mu\text{m}$;

^e PLA250: PLA with diameter <250 ;

^f PLA250-300: PLA with diameter $250\text{-}300 \mu\text{m}$.

Table S5

Germination Index (GI%) of *Lepidium sativum* L. following the phytotoxicity test with soil untreated and treated with microplastics [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes] and arsenic (As) (0 and 60 mg As kg⁻¹). The test was performed at the start of the experiment. Data are reported as mean \pm SE.

Treatments ^a	GI (%) ^b
-As ^c CT	100 \pm 3
LDPE250	104 \pm 5
LDPE250-300	105 \pm 2
PLA250	97 \pm 4
PLA250-300	99 \pm 8
+As CT	99 \pm 4
LDPE250	100 \pm 4
LDPE250-300	98 \pm 7
PLA250	100 \pm 2
PLA250-300	96 \pm 4

^a Codes of treatments are described in Table 1.

^b Four replicates per treatment.

^c The GI test was performed according to ISO 18763: 2016.

Table S6

P values of two-way ANOVA testing the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes] and arsenic (As) (0 and 60 mg As kg⁻¹) on the Germination Index (GI%) of *Lepidium sativum* L., following the phytotoxicity test.

Treatment ^a	GI
MP	0.837 ^b
As	0.439
MPxAs	0.891

^a Codes of treatments are described in Table 1.

^b *P* values according to the Tukey-B test.

Table S7

P-values of two-way ANOVA testing the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes], arsenic (0 and 60 mg As kg⁻¹) and their interaction on soil and plant growth parameters (lettuce, *Lactuca sativa* L.) at the beginning of the experiment (T0, before seeding) and at the end of the experiment (TF, 60 days).

Treatment	Soil pH	β -gluc ^a	NAG	Phosph	Buty Est	β -gluc	NAG	Phosph	Buty Est	Leaf FW ^b	Root FW	Plant FW	Chl ^c	Flv	Anth	NFI
	TF	T0				TF				TF			TF			
MP	<0.001^d	0.001	0.001	0.040	<0.001	0.018	<0.001	<0.001	0.022	0.003	0.485	0.020	0.034	0.012	0.470	0.248
As	<0.001	0.137	0.965	<0.001	<0.001	0.163	0.030	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	0.001	0.947	0.382
MPxAs	0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.598	0.001	0.117	0.022

^a β -gluc: β -glucosidase (EC 3.2.1.21); NAG: N-acetylglutamate synthase (EC 2.3.1.1); Phosph: acid phosphatase (EC 3.1.3.2); Buty Est: butyrate esterase (EC 3.1.1).

^b FW: fresh weight.

^c Chl: Chlorophyll; Flv: Flavonols; Anth: Anthocyanins; NFI: Nitrogen-Flavonol Index.

^d In bold, statistically significant values ($P < 0.05$) according to the Tukey-B test. Four replicates per treatment.

Table S8

Bioavailable arsenic (mg As kg⁻¹) at the end of the experiment (TF, 60 days) in soil untreated and treated with microplastics [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes] and As (0 and 60 mg As kg⁻¹). Data are reported as mean ± SE.

Treatment ^a	-As	+As ^b
CT	nd ^c	4.55 ± 0.06 a
LDPE250	nd	4.57 ± 0.06 a
LDPE250-300	nd	4.81 ± 0.05 b
PLA250	nd	4.90 ± 0.06 b
PLA250-300	nd	4.81 ± 0.05 b

^a Codes of treatments are described in Table 1.

^b Different letters within +As column indicate significant differences according to one-way ANOVA and Tukey-B test ($P < 0.05$). Four replicates per treatment.

^c nd: under the detection limit.

Table S9

P-values of two-way ANOVA testing the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes] at 60 mg arsenic (As) kg⁻¹ on As concentration and content in leaves, roots and whole plant of lettuce (*Lactuca sativa* L.) and on As translocation factor (TrF), As bio-concentration factor (BCF) in leaves and roots. Measures assessed at the end of the experiment (TF, 60 days).

Treatment	As concentration			As content			As TrF	As BFC	
	Leaves	Roots	Plant	Leaves	Roots	Plant		Leaves	Roots
MP	0.002 ^a	<0.001	0.042	0.003	0.078	0.153	<0.001	0.002	<0.001

^a In bold, statistically significant values ($P < 0.05$), according to the Tukey-B test. Four replicates per treatment.

Table S10

P-values of two-way ANOVA testing the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes], arsenic (0 and 60 mg As kg⁻¹) and their interaction on nutrient concentration in leaves and roots of lettuce (*Lactuca sativa* L.) at the end of the experiment (TF, 60 days).

Treatment	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
Leaves								
MP	0.491 ^a	<0.001	<0.001	0.142	0.002	0.010	0.222	<0.001
As	0.017	0.001	0.188	0.163	0.070	<0.001	<0.001	<0.001
MPxAs	0.684	0.089	0.001	0.460	0.193	0.031	0.206	<0.001
Roots								
MP	0.817	<0.001	0.020	<0.001	0.050	<0.001	<0.001	0.632
As	<0.001	<0.001	0.464	0.335	<0.001	0.187	<0.001	<0.001
MPxAs	0.181	<0.001	0.001	<0.001	0.017	0.001	0.004	0.015

^a In bold, statistically significant values ($P < 0.05$), according to the Tukey-B test. Four replicates per treatment.

Table S11

Results of permutational analysis of variance (PERMANOVA) used to test the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1% w/w) and polylactic acid (PLA, at 0.1% w/w) at two sizes], arsenic (0 and 60 mg As kg⁻¹) and their interaction on soil parameters (i.e., soil pH, enzyme activities,) and plants parameters (i.e., plant growth, As concentration and content in leaves and roots, nutrient concentrations in roots and leaves, physiological parameters). Lettuce (*Lactuca sativa* L.) was used as model plant and sampled at the end of the experiment (TF, 60 days).

	df	Pseudo- <i>F</i>	<i>P</i> (perm)	Variance explained
MPs ^a	1	56.43	0.001 ^b	11.95
As		46.37	0.001	46.65
MPs x As		50.57	0.001	20.86
Residual				20.56
<i>PERMDISP</i> ^c				
MPs			0.016	
As			0.885	

^a PERMANOVA was performed following a completely randomized design with MPs and As as fixed factors and with four replicate per treatment

^b In bold statistically significant values ($P \leq 0.05$)

^c Analysis of homogeneity of multivariate dispersion (PERMDISP) was performed to check the homogeneity of dispersion among groups (beta-diversity)

Supplementary Figures

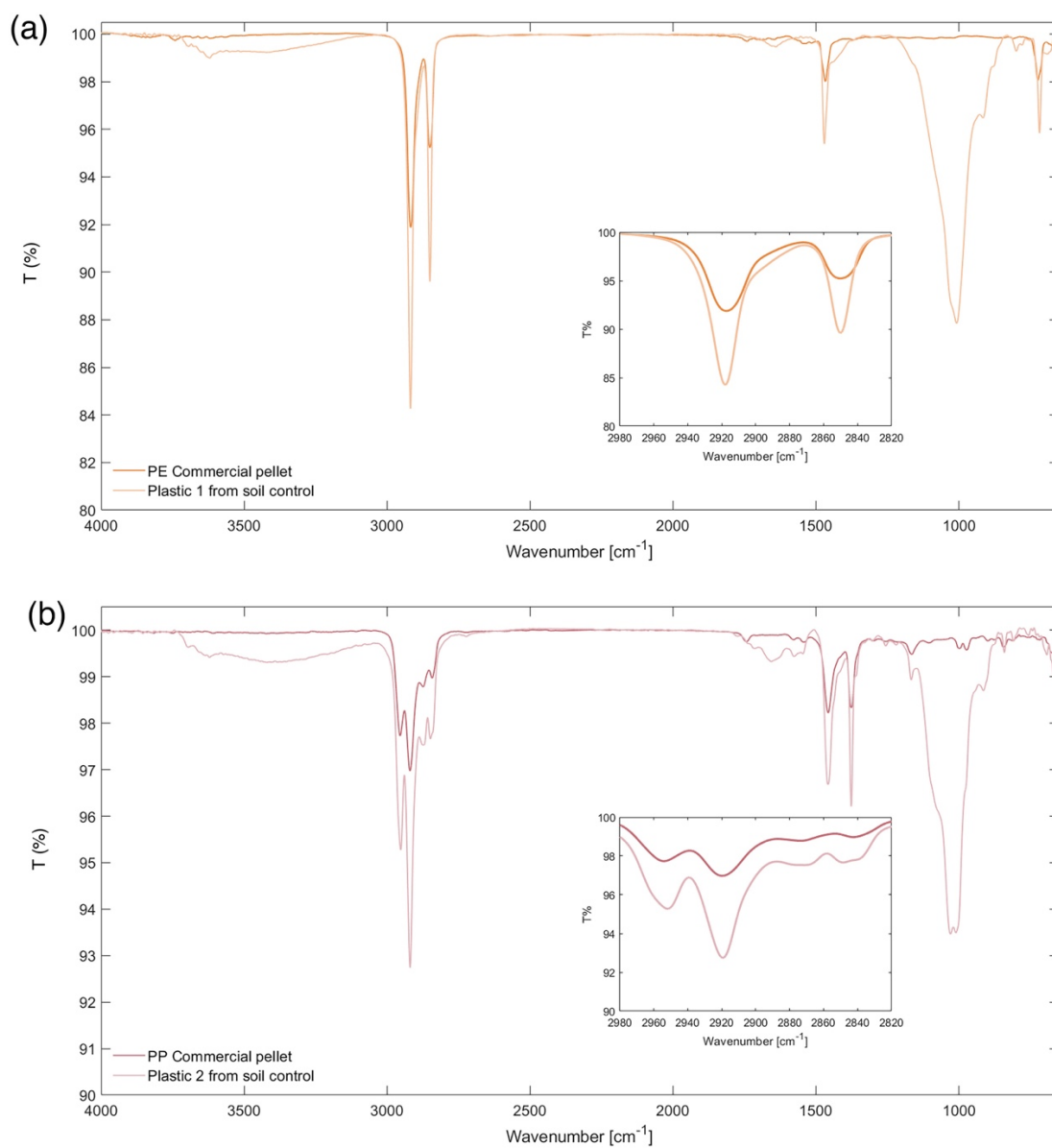


Fig. S1. ATR-FTIR spectra of the polyethylene (PE) (a) and polypropylene (PP) (b) microplastic found in the soil used in the experiment as substrate and their reference commercial pellet. The light lines represent the spectrum of collected plastics, while the dark lines represent the reference spectrum of the commercial PE and PP.

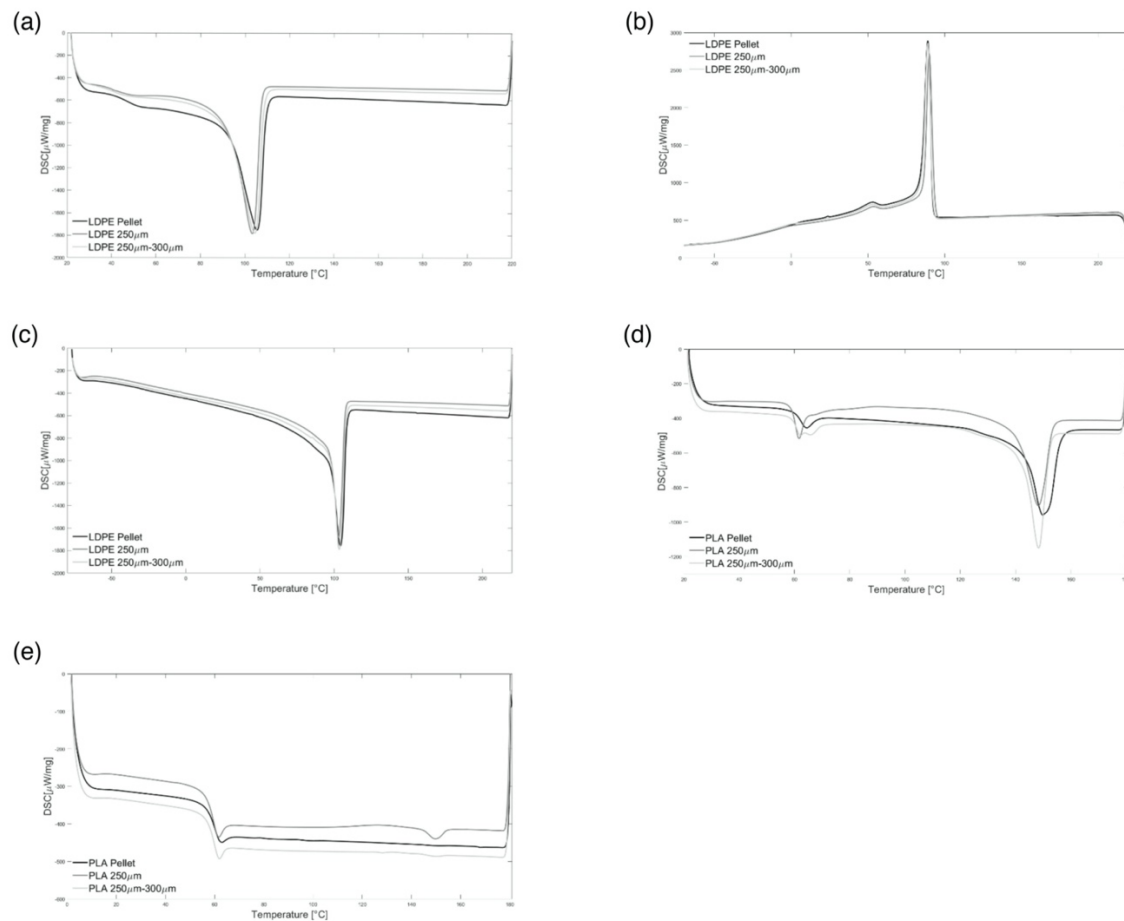


Figure S2. Differential scanning calorimetry (DSC) thermograms of low-density polyethylene (LDPE) (a-c) and polylactic acid (PLA) (d, e) in the form of pellets (black line) and microplastics in the fractions of <250 μm (dark grey line) and 250-300 μm (light grey line). DSC are detected before the set-up of the experiment. In particular, (a) represents the first heating step of LDPE, (b) the first cooling phase of LDPE, (c) the second heating step of LDPE, (d) the first heating step of PLA, and (e) the second heating step of PLA.