ACS Sustainable Chemistry & Engineering

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Perspective

Potential Use of Earthworms to Enhance Decaying of Biodegradable Plastics

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Cite This: *ACS Sustainable Chem. Eng.* 2020, 8, 4292−4316

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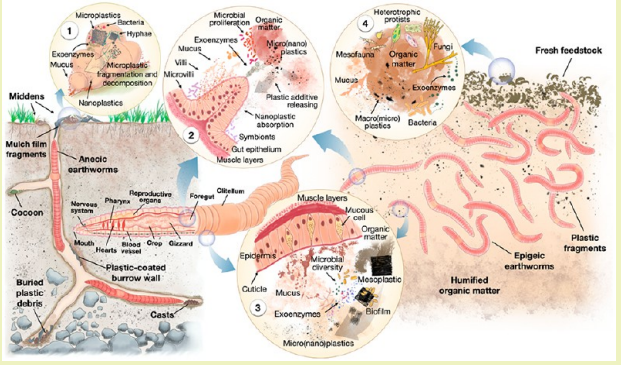
ABSTRACT: Biosolid application, wastewater irrigation, and plastic mulching technologies are major sources of plastic pollution in agroecosystems. Microplastics may interact with soil physicochemical properties and organisms and negatively affect plant growth. To alleviate environmental plastic pollution, synthetic and biobased biodegradable polymers are replacing nonbiodegradable polymers, but their biodegradation rate in the field is frequently lower than that estimated from standardized biodegradation testing. Plastic polymer biodegradation is a multistep process that involves plastic deterioration, microbial colonization, production of polymer-degrading exoenzymes, and mineralization. However, these physicochemical and biological processes are not always efficient because of unfavorable environmental conditions (e.g., temperature, soil moisture). We propose to use earthworms to increase the biodegradable polymer biodegradation rate by creating optimal habitats for microbial proliferation. Earthworm-induced processes that lead to soil alteration (bioturbation) and solid organic wastes decomposition (vermicomposting) are described to understand how earthworms may favor biodegradable plastic mineralization. Therefore, we suggest two practical sustainable bioengineering strategies: (1) enhancing bioturbation by inoculating agricultural soils with soil-dwelling earthworms, which is viable for horticulture where using biodegradable mulching films increases plastic debris in the soil and (2) vermicomposting with blended biodegradable plastic debris and solid organic wastes, which is complementary to industrial or home composting of single-use biodegradable plastics.



KEYWORDS: *Earthworms, Mulch films, Microplastics, Plastic biodegradation, Vermitechnology, Enhanced bioturbation*

INTRODUCTION

Plastics are ubiquitous in modern life, and their residues may generate microplastics, which are now recognized as an emerging environmental issue of global concern.[1,2] Microplastics commonly refer to manufactured polymers (primary microplastics) and plastic-derived fragments (secondary microplastics) ranging between 100 nm and 5 mm in size.[3] However, this definition remains ambiguous mainly because of the vast variety of plastic forms (e.g., spheres, fibers, films). Recently, Hartmann et al.[4] proposed a set of criteria to define and classify plastic debris in the environment, which includes chemical composition of the polymer, solid state, water solubility, size, shape, structure, color, and origin. For practical reasons, in this Perspective, we consider the plastic size only as the classification criterium as follows:[4] nanoplastics (1−100 nm), microplastics (1−1000 μm), mesoplastics (1−10 mm), and macroplastics (>1 cm).

Agricultural soils are among the most contaminated environmental compartments with plastic debris. It is estimated that the annual plastic input to land is about 4- to 23-times higher than the input to oceans,[5] which occurs via multiple routes.[2,6] The applications of treated sewage sludge (biosolids) and compost from municipal wastewater treatment plants to soil, wastewater irrigation, road runoff, atmospheric deposition, and plasticulture (i.e., use of plastic materials in agriculture) are the most significant direct routes of plastic contamination. For example, it is estimated that an annual input of 63,000−430,000 and 44,000−300,000 tons of plastic fragments (<5 mm) contaminate European and North American farmlands, respectively, via sludge applications.[7] Plasticulture uses plastic materials as diverse as mulch films, drip irrigation tapes, low tunnels, greenhouse covers, and solarization films.[8] However, mulch films represent the major source of plastic contamination in agricultural soil. Past studies

Received: September 13, 2019
Revised: January 18, 2020
Published: February 11, 2020

https://dx.doi.org/10.1021/acssuschemeng.9b05450
ACS Sustainable Chem. Eng. 2020, 8, 4292−4316

Table 1. Effects of Mesoplastics, Microplastics, and Nanoplastics on Soil Organisms

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
Earthworms					
Lumbricus terrestris	Low density polyethylene. Spherical shape particles (<150 μm)	7%, 28%, 45%, and 60% (dry mass) in plant litter	Test unit: container (300 mm × 405 mm × 30 mm) with sandy soil and 13 g of microplastic-litter mixture on top	Sublethal effects at the highest microplastic concentration (25% mortality and significant weight loss)	24
			N = 4 earthworms/treatment	No significant effect on reproduction rate.	
			Time of exposure: 14 d (mortality) and 60 d (weight change and reproduction rate)	High concentration of microplastics (50 μm) in casts	
			Soil temp.: 16−18 °C		
			Soil moisture: 20% (w/w)		
L. terrestris	Low density polyethylene pellets. Particles of different sizes: ≤50 μm (40%) and 63−150 μm (60%)	7%, 28%, 45%, and 60% (dry mass) in plant litter (*Populus nigra*)	Test unit: container (300 mm × 405 mm × 30 mm) with sandy soil and 13 g of microplastic-litter mixture on top	Earthworms exposed to 7% microplastics had a lower weight with respect to earthworms of other treatments.	121
			N = 4 earthworms/treatment	After 14 d incubation, the number of burrows created by earthworms significantly increased in the group treated with 7% microplastics compared to the other treatments.	
			Time of exposure: 14 d	Microplastic accumulation in burrow walls was higher in 7% and 60% treatments than in the other treatments.	
			Light:dark cycle: 8:16 h	Small-sized microplastics (≤50 μm) were mostly found in the burrow walls.	
			Soil temp.: 16.5 ± 1 °C		
			Soil moisture: 21% (w/w)		
L. terrestris	High-density polyethylene plastic bag fragments (0.92 ± 1.09 mm^2)	Zn-bearing microplastics (0.7 g) mixed in 200 g of air-dried soils (Zn concentrations: 236−4505 mg/kg)	Test unit: 260 g moist arable loam soil containing 0.35% Zn-bearing microplastics	No mortality and alteration of body weight in microplastic-exposed earthworms with respect to controls.	173
				Ingestion of Zn-bearing microplastics did not increase bioaccumulation of the metal.	
				Metal desorption from microplastics incubated in earthworm gut-simulated fluid suggested that Zn-bearing microplastics might facilitate metal uptake via the alimentary canal.	
L. terrestris	Spherical polyethylene microplastics (710−850, 1180−1400, 1700−2000, and 2360−2800 μm size)	750 mg microplastics added in soil surface (2.5 kg soil)	Test unit: plant pots of 3 L (19.2 cm height × 17.0 cm dimeter)	Earthworms transported microplastics from the soil surface to deep soil (10.5 cm); this effect was more pronounced with the smallest-sized microplastics.	30
			N = 5 earthworms/treatment	Mortality (33.3%, mean value of all treatments) and decrease in mean body weight (17.0%, mean value) observed although not linked to microplastic exposure.	
			Soil: Albic Luvisol, <5 cm sieved	Microplastics of 710−850 and 1180−1400 μm size were found in earthworm casts and adhered to worm skin. Ingestion and adherence of microplastics to skin mucus were the main route of vertical transport of microplastics.	
			Time of exposure: 21 d	Middens contained microplastics.	
			Light:dark cycle: natural (greenhouse)		
			Air temp.: 20 ± 2 °C		
			Soil moisture: not indicated, although soil received 100 mL of water every 2 days during the last 21 d incubation time		
L. terrestris	Polyester microfibers (361.6 ± 387 μm length × 40.7 ± 3.8 μm diameter)	0%, 0.1%, 1.0% w/w textile-derived microfibers	Test unit: plastic bags containing 300 g of air-dried soil	No earthworms dead after 35 d exposure, and they did not avoid microfiber-spiked soils.	174
			Soil: arable loam soil, <2 cm particle size	Highest microfiber concentrations caused a reduction in cast production, induction of metallothionein-2 expression, and an inhibition of the heat shock protein *hsp*70 expression.	
			N = 1 earthworm/treatment	Biomarker responses were not linked to metal (Cd, Cu, Pb, and Zn) concentration in both the soil and microfibers.	

https://dx.doi.org/10.1021/acssuschemeng.9b05450
ACS Sustainable Chem. Eng. 2020, 8, 4292−4316

Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
			Time of exposure: 35 d		
			Light:dark cycle: permanent dark		
			Temp.: 15 °C		
			Soil moisture: 25%		
L. terrestris	Low-density polyethylene microplastics, distributed in 250 μm−1 mm (50%), 150−250 μm (30%), and <150 μm (20%).	7% w/w mixed with *Populus nigra* dry litter and applied on topsoil	Test unit: columns (60 cm height × 12 cm dimeter) containing 7 kg of air-dried soil	Earthworm burrowing activity facilitated microplastic leaching.	175
			N = 2 earthworm/column	Earthworms transported microplastics along the soil column, with the lowest particles more abundant in the deepest soil layers.	
			Soil: sandy soil, <5 cm particle size		
			Time of exposure: 14 d		
			Light:dark cycle: no indicated		
			Temp.: 16 ± 1 °C		
			Soil moisture: 40 ± 5%		
L. terrestris	Field-weathered mulch films: synthetic (polyethylene) and biodegradable (Naturecycle).	10 pieces of mulch films (2 cm × 2 cm) deployed on soil surface	Test unit: glass terrarium (30 cm × 30 cm × 4 cm)	Earthworms dragged all pieces of the biodegradable mulch film within 1 week, whereas some polyethylene mulch film fragments remained on the soil surface.	31
			N = 2 earthworm/terrarium	Biodegradable and polyethylene mulch film pieces were found at different depths (16−20 cm). Fragmentation observed in the biodegradable mulch pieces was attributed to the dragging action of earthworms.	
			Soil: Skagit silt loam	Accumulation of mulch film fragments around the burrow entrances forming middens.	
			Time of exposure: 50 d		
			Light:dark cycle: 12:12 h		
			Temp.: 16 °C		
			Soil moisture: 25%		
Eisenia andrei (epigeic)	Low density polyethylene pellets (250−1000 μm particles)	0, 62.5, 125, 250, 500, and 1000 mg particles/kg of dry soil	Test unit: 1500 mL polypropylene pots containing 500 g of OECD artificial dry soil (control and microplastic-spiked soils)	No mortality and no significant changes in body weight over 28 d, irrespective of the treatment.	25
			N = 10 earthworms/treatment	No significant impact of microplastics on reproduction rate after 56 d of exposure.	
			Time of exposure: 28 and 56 d (reproduction rate)	Microplastics were found in earthworm gut content.	
			Light:dark cycle: 16:8 h	Histopathological alterations of gastrointestinal tissue, which depended on microplastic concentration.	
			Soil temp.: 20 ± 2 °C		
			Soil moisture: 40% of maximum water holding capacity (WHC)		
E. fetida (epigeic)	Low density polyethylene pellets (250−1000 μm particles)	0, 62.5, 125, 250, 500, and 1000 mg particles/kg of dry soil	Test unit: 500 g of OECD artificial dry soil (control and microplastic-spiked soils)	No mortality and no significant alteration in body weight over 28 d for all treatments.	26
			N = 10 earthworms/treatment	No relationship between the response of oxidative stress biomarkers and microplastic concentration.	
			Time of exposure: 28 d	Lipid peroxidation (a sign of cellular oxidative damage) significantly increased in earthworms exposed to 250−1000 mg/kg with respect to controls.	
			Light:dark cycle: 16:8 h		
			Soil temp.: 20 ± 2 °C		
			Soil moisture: 40% of maximum water holding capacity.		

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
E. fetida	Low density polyethylene pellets (0.25−1 mm, and 5 mm particle size)	Chlorpyrifos-contaminated microplastic particles (5 and 0.25−1 mm) deployed on soil surface (500 g soil)	Test unit: 500 g of OECD artificial dry soil (control and contaminated microplastic-spiked soils) N = 10 earthworms (300−600 mg weight) per treatment Time of exposure: 14 d Light:dark cycle: 16:8 h Soil temp.: 20 ± 2 °C. Soil moisture: 40% of maximum WHC	Chlorpyrifos was detected in soil-spiked microplastics (17.9 ± 4.5 to 2442 ± 238 ng/g dry soil) probably because of pesticide desorption from microplastics. Earthworms avoided soil surface containing pesticide-spiked microplastics. Higher inhibition of acetylcholinesterase activity in earthworms exposed to pesticide-spiked microplastic of 5 mm size with respect to earthworms exposed to 0.25−1 mm microplastics.	176
E. andrei	Three biodegradable plastic films: Mater-Bi DF04A, Mater-Bi EF04P, and Mater-Bi AF05S0	Plastics films (10 g) were separately incubated for 6 months in 800 g of artificial ISO soils (ISO 17556) supplemented with 10 g of cellulose at 28 °C and 16.9% moisture	Test unit: 500 g of soil 6-month incubated with biodegradable films N = 10 earthworms (400−500 mg weight) per treatment Time of exposure: 28 and 56 d Light:dark cycle: 16:8 h Soil temp.: 20 ± 1 °C	No lethal and sublethal effects were observed in earthworms incubated for 28 d (mortality percentage) and 56 d (cocoon production rate and viability) in the soils incubated for 6 months with the biodegradable plastic films.	177
E. fetida	Low-density polyethylene (≤300 μm) and polystyrene (≤250 μm) microplastics	0%, 1%, 5%, 10%, and 20% (w/w dry mass)	Test unit: glass beakers containing 200 g of soil N = 5 adult/beaker Soil: agricultural sandy loam Time of exposure: 14 d (microplastic ingestion and toxicity assays) and 28 d (bioaccumulation assay) Light:dark cycle: 16:8 h Temp.: 25 °C Soil moisture: 40%	Microplastics were found in earthworm casts, suggesting particle ingestion. Signs of oxidative stress (enzyme activities) and oxidative damage (lipid peroxidation) were detected in earthworms exposed to the highest concentration of both types of microplastics (20% w/w). Presence of microplastics in PAH- and PCB-contaminated soils decreased the bioaccumulation of the environmental contaminants.	27
E. fetida	High-density polyethylene (1.43 ± 0.55 mm diameter), polyethylene terephthalate (1.51 ± 0.46 mm), and polyvinyl chloride (1.18 ± 0.36 mm) microplastics	Test media: 0.5 g of microplastics/100 g of soil (three types of soils) containing 1 g of municipal waste compost, incubated for 0, 3, and 9 months (23.5 ± 2.8 °C, 60% maximum WHC)	Test unit: glass jars containing 500 g of test media for toxicity test. Two-chamber system (250 g test media/compartment) for avoidance response test N = 10 earthworms/treatment Soil: agricultural sandy loam Time of exposure: 24 h (avoidance test), 28 d (acute test), and 56 d (chronic test) Light:dark cycle: 16:8 h Temp.: 23.5 ± 2.8 °C Soil moisture: 60% MWHC	Earthworms did not avoid the microplastic-spiked soils, irrespective of both microplastic type and soil type. Earthworm survival, body weight, and reproduction rate did not differ statistically between control test media (compost-amended soils) and microplastic treatments. 0.5% microplastics in compost-amended soils were not toxic to *E. fetida*.	179
Metaphire californica (epigeic)	Polyvinyl chloride particles	Test media: 2000 particles/kg of dry soil containing 40 mg As (V) or metalloid-free soil	Test unit: polyethylene plastic containers (50 cm × 35 cm × 30 cm) containing 2.5 kg of test media Soil: field soil (<2 mm, size) Time of exposure: 28 d	Arsenic (As) concentrations were significantly lower in body tissues and gut of earthworms incubated in Asspiked soils with microplastics than in earthworms from microplastic-free, Asspiked soils. Earthworm exposure to microplastics alone did not change the bacterial community of earthworm gut. Microplastics mitigated	178

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
			Light:dark cycle: 12:12 h Temp.: 20 ± 2 °C Soil moisture: 30%	the negative impact of gut microbiomes probably inducing changes in As speciation.	
E. fetida	Polyurethane (<75 μm) particles containing PBDEs	Microparticles mixed in soil (1:2000 w/w) ΣPBDEs = 83 mg/kg dry wt	Test unit: glass jars with 10 individuals/600 g wet soil Soil: artificial soil (sand, kaolinite, peat moss and dolomite, 69:15:15:1 w/w) Time of exposure: 7, 14, and 28 d Light:dark cycle: 12:12 h. Temp.: 30 ± 3 °C. Soil moisture: 45%.	Accumulation of PBDEs in earthworm tissues, with penta-PBDE concentrations ranging between 1890 (7 d) to 3740 mg/kg lipid wt (28 d).	180
E. fetida	Low-density polyethylene microplastics (<400 μm)	0.1%, 0.25%, 0.5%, 1.0%, and 1.5 g/kg dry wt	Test unit: glass boxes (15 cm × 5 cm × 10 cm) with 1 kg soil *N* = 10 earthworms/box Soil: artificial soil (sphagnum peat, kaolinite, and industrial quartz sand, 10:20:70 w/w, containing 5% of organic matter) Time of exposure: 28 d Light:dark cycle: 8:16 h Temp.: 20 ± 2 °C Soil moisture: 20−40%	No dead earthworm. Dose-dependent variation of microplastics in casts after 14-d and 28-d incubation times. Damage of earthworm skin in 1.5 g/kg of microplastic treatment. No clear evidence for microplastic-induced oxidative stress (catalase activity and lipid peroxidation) or neurotoxicity (inhibition of acetylcholinesterase activity).	181
Enchytraeids					
Enchytraeus crypticus	Polystyrene (0.05−0.1 μm)	0.025%, 0.5%, and 10% dry oatmeal	Test unit: Petri dishes (3.5 cm diameter) with 20 mg of microplastic-spiked oatmeal *N* = 10 individuals/dish Time of exposure: 7 d Light:dark cycle: 8:16 h Temp.: 18.5 ± 2 °C Air relative humidity: 75%	Significant decrease in body mass of animals exposed to 10% microplastics. Reproduction rate (cocoon number) increased in animals exposed to 0.5% microplastics. Gut microbiota dysbiosis of animals exposed to 10% microplastics; decreasing microorganisms with an important role in N cycling and organic matter decomposition.	38
E. crypticus[a]	Nylon-6 polyamide (13−18 and 90−150 μm) microplastics	Ingestion test: 20 g/kg of soil for the three particle sizes Toxicity testing: 20, 50, 90, and 120 g/kg of soil	Test unit: polypropylene containers (96 cm × 45 cm) for microplastic ingestion assay or 50 mL polypropylene tube for toxicity assay. Both test units with microplastic-spiked artificial Lufa 2.2 soil Time of exposure: 20 h (particle ingestion assay) or 21 d (toxicity testing) Light:dark cycle: constant dark Temp.: 18 ± 1 °C Soil moisture: 50% of WHC	Enchytraeids ingested nylon-6 particles of all size fractions, although a higher number of particles were found in worms exposed to 13−18 μm particles. After 21 d of exposure, no effect of nylon-6 microplastics on worm survival, but reproduction (number of juveniles produced per worm) decreased.	42
E. crypticus[a]	Short (12 μm−2.87 mm) and long (4−24 mm) polyester fibers	0.02%, 0.06%, 0.17%, 0.5%, and 1.5% w/w	Test unit: 100 mL jars with microplastic-spiked artificial Lufa 2.2 soil	Survival was slightly decreased after 21 d exposure to long polyester fibers at 0.17% and 0.5% w/w.	182

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
			Time of exposure: 21 d	Reproduction rate significantly decreased in worms exposed to long-sized fibers at all concentrations except in the 0.06% treatment group.	
			Light:dark cycle: 16:8 h Temp.: 20 ± 2 °C Soil moisture: 50% of WHC	Short fibers did not affect both survival and reproduction rate at any concentration.	
Isopods					
Porcellio scaber	Short (12 μm−2.87 mm) and long (4−24 mm) polyester fibers	0.02%, 0.06%, 0.17%, 0.5%, and 1.5% w/w	Test unit: 200 mL jars with microplastic-spiked artificial Lufa 2.2 soil, and 5 isopods	Short- and long-sized fibers did not affect the survival, biomass, and feeding activity of isopods.	182
			Time of exposure: 28 d Light:dark cycle: constant dark Temp.: 20 ± 2 °C Soil moisture: 40% of WHC	No significant variations were found in total concentration of proteins, carbohydrates, and lipids in fiber-exposed isopods.	
P. scaber	Plastic bag films (183 ± 93 μm) and facial cleanser microplastics (137 ± 51 μm)	4 mg/kg of food pellet (dry wt)	Test unit: Petri dishes (9 cm diameter) with a moist filter paper on the bottom (1 individual/dish) and microplastic-spiked food pellets Time of exposure: 14 d Light:dark cycle: 16:8 h Temp.: 21 ± 1 °C Air relative humidity: >80%	No significant effects of microplastics on body mass, mortality, food ingestion rate, defecation rate, food assimilation rate and efficiency, and energy reserves (proteins, carbohydrates, and lipids) after 14 d of exposure to microplastic-spiked food (replaced by fresh contaminated food three times per weeks).	183
Snails					
Achatina fulica	Polyethylene terephthalate fibers (1257.8 μm length × 76.3 μm diameter)	6.4% microfibers mixed with food (feeding study only)	Test unit: glass containers with field soil and 24 individuals (feeding assay) or 10 snails (toxicity testing) Time of exposure: 5 h (feeding assay) or 28 d (toxicity testing)	Snails progressively accumulated microfibers along digestive tract. Significant fragmentation and deterioration in microfibers collected from both digestive tracts and excrements.	184
		0.014, 0.14, and 0.71 g/kg of dry soil (toxicity testing)	Light:dark cycle: 16:8 h Temp.: 25 ± 1 °C Soil moisture: 60%.	No significant impact of microfibers on snail growth, but dose-dependent disruption of excretion, gut tissue damage, and oxidative stress in the hepatopancreas (0.71 g/kg).	
Mites					
Oppia nitens	Polyester fibers (4−24 mm)	0.5% w/w (soil or food)	Test unit: 100 mL jars with microplastic-spiked artificial Lufa 2.2 soil. For food exposure, fibers were mixed in baker's yeast and water. Time of exposure: 28 d Light:dark cycle: 16:8 h Temp.: 20 ± 2 °C Soil moisture: 50% of WHC	No significant effect of polyester-contaminated soil or food on mite survival and reproduction.	182
Hypoaspis aculeifer *Damaeus exspinosus*	Polyvinyl chloride (80−250 μm)	5000 microplastics per test container (Petri dish)	Test unit: plastic Petri dishes (9 cm diameter) containing microplastics at the center of the dish *N* = 30 mites/dish. Time of exposure: 7 d	Mites dispersed microplastics via pushing, crawling, and particle attachment to the cuticle. *D. exspinosus* showed a higher capacity to disperse microplastics in the plates than *H. aculeifer*.	28

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
			Light:dark cycle: 8:16 h Temp.: 20 ± 2 °C Air relative humidity: 75%		
Collembolans					
Folsomia candida	Short (12 μm−2.87 mm) and long (4−24 mm) polyester fibers	0.02%, 0.06%, 0.17%, 0.5%, and 1.5% w/w	Test unit: 100 mL jars with microplastic-spiked artificial Lufa 2.2 soil. For food exposure, fibers were mixed in baker's yeast and water. Time of exposure: 28 weeks Light:dark cycle: 16:8 h Temp.: 20 ± 2 °C Soil moisture: 50% of WHC	No significant effect of polyester-contaminated soil or food on springtail survival and reproduction.	182
F. candida *Proisotoma minuta*	Urea−formaldehyde micoplastics (<100 μm and 100−200 μm)	2.5 mg (<100 μm) 5 mg (100−200 μm)	Test unit: specimen cups with 5 mg or 2.5 mg of particles in the middle *N* = 25 individual/cup Time of exposure: 5 d Light:dark cycle: 16:8 h Temp.: 20 ± 2 °C Soil moisture: 60%	Both species distributed microplastics of both sizes on the surface, although *F. candida* moved a higher number of particles than *P. minuta*.	29
F. candida	Polyethylene beads (<500 μm)	0.5 and 1.0% w/w for avoidance behavior response (ABR) test	Test unit: cylindrical glass containers (7 cm diameter x 6 cm in depth), divided into two equal section in the case of ABR test.	Springtails avoided microplastic-contaminated soils (83% and 88% of total individuals found in the control soil).	185
		0.005%, 0.02%, 0.1%, 0.5%, 1.0% w/w for reproduction test	Soil: artificial soil (sphagnum peat, kaolinite clay, and quartz sand, 7:2:1 mass ratio, containing 6.1% of organic matter) Time of exposure: 24 h (ABR test) 28 d (reproduction test) Light:dark cycle: constant dark Temp.: 20 ± 1 °C Soil moisture: 50% of WHC	Reproduction rate (number of juveniles) decreased dose-dependently as microplastic concentration increased (70.2% decrease at the highest concentration), and the estimated EC_{50} was 0.29% microplastics. Gut dysbiosis in the springtails exposed to microplastic-spiked soils with respect to controls.	
F. candida	Polyvinyl chloride (80−250 μm)	5000 microplastics per test container (Petri dish)	Test container: plastic Petri dishes (9 cm diameter), containing microplastics at the center of the dish, and 30 springtails. Time of exposure: 7 d Light:dark cycle: 8:16 h Temp.: 20 ± 2 °C Air relative humidity: 75%	Springtails were able to disperse microplastics in the plates (0.54% of added microplastics). However, they avoided microplastics with increased time of exposure, thus aggregating in microplastic-free areas of the plates.	28
Lobella sokamensis	Polystyrene (0.47−0.53 μm, beads) Polystyrene (<45, 45−200, and >200 μm, fragments) Polyethylene (27−32 and 250−300 μm beads)	4 and 8 mg/kg 1000 mg/kg 1000 mg/kg	Test unit: glass slide containing 0.4−0.5 g of microplastic-contaminated soil *N* = 1 individual/slide Soil: artificial Lufa 2.2 soil Soil moisture: 80% of WHC Time of exposure: 3 min, under optical fluorescence microscope	Behavior of springtails in contaminated soils caused an accumulation of microplastics around their cuticle. Decreased mobility of springtails in soils contaminated with 1000 mg/kg of microplastics (beads and fragments) and 8 mg/kg of polystyrene microplastics.	186

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
Nematodes					
Caenorhabditis elegans	Polystyrene nanoplastics (100 and 500 nm)	1.0 mg/L	Test unit: nematode growth medium plates, containing microplastics	Survival of microplastic-exposed nematodes was particle size-dependent.	187
	Polystyrene microplastics (1.0, 2.0, and 5.0 μm)		N = 30−50 nematodes/plate	Lowest survival rate (32.2%) and body length were observed with 1.0−μm microplastics.	
			Time of exposure: 3 d	Microplastic-exposed nematodes also showed damage in the cholinergic neurons (0.5 and 1.0−μm treatments).	
			Temp.: 20 °C	Changes in gene expression related to motor behavior and oxidative stress.	
C. elegans	Polystyrene nanoplastics (50 and 200 nm)	17.3 and 86.8 mg/L for both particle sizes	Test unit: nematode growth medium plates (9 cm diameter), containing microplastics	Reduced locomotion and reproduction of nematodes exposed to polystyrene nanoplastics.	188
			Time of exposure: 24 h	Metabolomic study revealed significant changes in metabolites, amino acids, and precursor of neurotransmitters.	
			Temp.: 20 °C	Exposure to 86.6 mg/L of nanoplastics (50 nm) caused oxidative stress.	
Plants					
Allium fistulosum	Polyester fibers (5 mm length × 8 μm diameter), polyamide beads (15−20 μm, diameter), high density polyethylene (643 μm fragments), polyester terephthalate (187 μm fragments), polypropylene (624 μm fragments), and polystyrene (492 μm fragments)	0.2% w/w fresh weight (polyester fibers)	Test unit: 200 mL glass beaker containing 200 g of microplastic-spiked soil	Some microplastics (polyester and polystyrene) stimulated plant root biomass, and all microplastic types increased total root length and decreased root diameter.	19
		2.0% w/w (rest of the microplastics)	Soil: loamy sandy soil (<5 mm)	Microplastic impact on *A. fistulosum* was due to indirect effects of microplastics on soil physicochemical properties (soil bulk density, soil aggregate, and water dynamics).	
			Time of incubation: approximately 2 months (no plants) and after 1.5 months (with plants)		
			Light:dark cycle: constant dark (no plants) and after natural photoperiod in greenhouse (with plants)		
			Air temp.: 21 ± 1 °C		
			Soil moisture: 90% of WHC (no plants) and 60% of WHC (with plants)		
Vicia faba	Polystyrene fluorescent microplastics (5 μm and 100 nm)	10, 50, and 100 mg/L of both plastic sizes	Test unit: soaking of seeds with 20 mm root length in 3 mL microplastic solutions	Microplastics (5 μm) caused a dose-dependent decrease in both root length and weight compared to effects from nanoplastic (100 nm) exposure, where adverse effects were observed at 100 mg/L only.	46
			Time of incubation: 48 h	Nanoplastics caused higher genotoxic and oxidative damage than microplastics.	
			Air temp.: 24.5 °C	Nanoplastics accumulated in roots, probably reducing nutrient uptake.	
Lepidium sativum	Fluoro-Max Green Fluorescent Polymer microspheres (50, 500, and 4800 nm).	10^3−10^7 particles/ml (distilled water).	Test unit: Petri dishes (9 cm diameter), containing 10 seeds each plate, and 5 mL of microplastic concentration.	Transient dose-dependent decrease of germination rate with all three microplastic sizes.	47
			Time of incubation: 72 h.	No significant differences were observed in both root length and shoot length between microplastic treatments and controls after 72 h of exposure.	
			Light:dark cycle: constant light (6000 lx)	Reduced germination rate was probably caused by physical blockage of the seed capsule pores.	
			Air temp.: 24 °C		
			Air relative humidity: > 80%		

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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Table 1. continued

Test species	Polymer type (and size)	Microplastic concentration	Exposure conditions	Main findings	ref.
Lactuca sativa[a]	Polyethylene (~23 μm)	0.25, 0.5, and 1.0 mg particles/mL of nutrient solution	Test unit: Plant supporting trays containing lettuce seedlings irrigated with microplastic-contaminated or microplastic-free (control) nutrient solution	Morphological parameters (leave and root weights, plant height, leaf number, and root length) were negatively impacted by 14 and 28 d exposure to microplastics.	15
			Time of incubation: 14 and 28 d	Photosynthesis was adversely impacted at all microplastic concentrations.	
			Light:dark cycle: 12:12 h Air temp.: 25 ± 2 °C Air relative humidity: 60%	Microplastics increased oxyradical production in leaves and roots following 14 d exposure but decreased 2 weeks later. This oxidative response was concomitant with the response of enzymatic antioxidant mechanisms.	
Triticum aestivum[a]	Low-density polyethylene mulch film (6.92 ± 6.10 mm macroplastics and 1−0.25 mm microplastics)	1% w/w for macroplastics and microplastics of both polymer types	Test container: 2 L pots (18 cm high ±10 cm bottom diameter ±13 cm upper diameter) containing 1.5 kg of soil spiked with 15 g of macroplastics or microplastics and 3 seedlings.	Macroplastics and microplastics of polyethylene and biodegradable mulch films had a negative impact on both above- and below-ground parts of the wheat.	52
	Starch-based biodegradable mulch film (6.89 ± 6.01 mm macroplastics and 1−0.25 mm microplastics)		Time of incubation: 2 and 4 months	Starch-based biodegradable fragments showed stronger negative effects on plant growth and development than those of polyethylene-based fragments.	
			Light:dark cycle: 14:10 h Air temp.: 22 (day and 17 °C (night) Air relative humidity: 70% Soil moisture: 12−18% w/w	Impact of the size of both mulch films on plant growth and development was insignificant.	

[a]Other polymer types (e.g., PVC), polymer concentrations, treatments (e.g., microplastics and organic pollutants), or toxicity endpoints (e.g., microplastic ingestion assay) tested in those studies were not considered in this review for simplification.

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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reported that about 0.7 million tons of plastic mulch film are used annually in agriculture worldwide,[1,9] and this amount is increasing each year.[10] For example, China is the country with the highest mulch film use, with an annual increase rate of 7.1%,[11] which seriously threatens soil quality and crop production.[12] Despite these data, mulching technology is still a desirable option in intensive agriculture because of the benefits to crop production such as conditioning of soil moisture and temperature under unfavorable environmental conditions, reduction of weed growth, decreased agrochemical inputs, increased crop yield, shortened harvesting time, and soil erosion prevention,[13−15] although the latter depends on plastic mulch film dimensions.[16]

Polyethylene-based polymers are the most used film in mulching technology because of their cost-effective production, physical properties, reduced weight, and resistance to biodegradation. However, the end-of-life management of polyethylene-based films is an environmental challenge because of the high cost of removal after harvesting.[1,17] Therefore, frequent incomplete recovery after the crop season leads to accumulation of polyethylene film debris in the soil,[17,18] which negatively impacts the soil quality and has potential adverse consequences to plant growth.[19] Although alternative approaches such as copyrolyzing spent plastic mulch film and animal manure to produce value-added biochar have shown promising results in laboratory-scale experiments,[20] landfilling, incineration, and recycling are still the practical options for polyethylene-based film disposal.[17,21] Using biodegradable polymers (BPs) is an emerging strategy to reduce soil pollution by nonbiodegradable plastic debris. However, field biodegradation of BPs frequently does not take place at the predicted rate that is indicated in standardized laboratory testing. Many environmental (e.g., temperature, pH, and moisture) and enzymatic (e.g., enzyme preference for plastic polymers, regulation of enzyme gene expression, requirement for multiple enzymes for polymer breakdown, and nutrient deficiency for enzyme synthesis) limitations make microbial degradation of BPs in the field complex and unpredictable.[22,23]

In this Perspective, we propose to use earthworms to promote biodegradation of BPs through creating favorable habitats for microbial plastic degraders. We first summarize the potential effects of plastics on soil organisms. Then, we discuss the use of bioplastics as an ecofriendly option to reduce the environmental contamination by nonbiodegradable plastics and the uncertainties around their environmental fate and impact. Second, we introduce the following two practical scenarios whereby earthworms can function as biological vectors of plastic biodegradation: (1) enhanced bioturbation via inoculation of soil-dwelling earthworm species in agricultural soils and (2) vermicomposting with biodegradable plastics mixed with solid organic wastes. The first scenario would decrease the BP residence time in the soil and counterbalance the potential negative effects on soil fertility that are derived from intensive plastic mulching. The second scenario is a complementary disposal route for BPs that produces environmentally safe value-added products (biofertilizers). To understand how these two options may favor plastic biodegradation, we briefly describe the biological processes that occur in both systems.

EFFECTS OF PLASTIC DEBRIS ON SOIL ORGANISMS

In the past few years, an increasing number of studies have investigated microplastic and mesoplastic effects on soil organisms. Many of these investigations have used earthworms as a model organism and nonbiodegradable plastics such as polyethylene, polystyrene, or polyvinyl chloride as target polymers (Table 1). Changes in body mass, reproduction rate, and mortality have been the main endpoints for assessing microplastic and mesoplastic exposure and toxicity. For example, an average mortality rate of 25 ± 43% was recorded in *Lumbricus terrestris* after 60-days of incubation in soil with plant litter on top spiked with low-density polyethylene (LDPE) microplastics (60% w/w, dry mass).[24] This occurred concurrently with a significant decrease in earthworm body mass. Although researchers in that study demonstrated that earthworms ingested microplastics, the reason for earthworm mortality was not clarified. Contrasting results were reported by Rodriguez-Seijo et al.,[25] who showed no significant changes in both the body weight and reproduction rate of *Eisenia andrei* incubated for 28 days and 56 days in artificial soils contaminated with 62.5 and 1000 mg kg^{-1} dry soil of LDPE microplastics (250−1000 μm, range of size), respectively. Despite the insignificant alterations in body weight and reproduction rate, the researchers found clear signs of tissue damage by microplastics in some earthworms that were exposed for 56 days to microplastics, which varied from gut inflammation and fibrosis (125 mg kg^{-1}) to detachment of the gut epithelium from the circular muscle layer (500 mg kg^{-1}).[25] In a similar study, the same researchers observed signs of oxidative damage (lipid peroxidation) in *Eisenia fetida* exposed to 250−1000 mg kg^{-1} dry mass of LDPE microplastics (<1 mm);[26] this finding was corroborated in the same earthworm species that was exposed to 20% (w/w, dry mass) polyethylene and polystyrene microplastics.[27] Other studies have described microplastic-associated behavioral effects such as dispersion of microplastics by collembolans[28,29] or vertical transport of microplastics in the soil column by anecic earthworms (*L. terrestris*).[30,31] Anecic species are large earthworms that construct permanent, long, vertical burrows (up to 3 m deep) and feed on decaying organic resides that are dragged into their burrows.[32] This ecological group of earthworms differs from endogeic earthworms, which construct nonpermanent burrows in the uppermost 10−15 cm of soil without preferential orientation and ingest a large amount of soil to obtain nutrients.[33] Therefore, because of the direct and intensive action of both anecic and endogeic earthworms on the soil structure and physicochemical properties, these two ecological groups of earthworms are ideal models to investigate the environmental fate of mesoplastics and microplastics in soil.

Since early reports on the occurrence of microplastics, mesoplastics, and macroplastics in the stomach of marine organisms,[34] plastic ecotoxicology has focused on the potential adverse effects on digestive function. The effects include, among others, disruption of feeding activity because of obstruction of the digestive canal, enhanced energetic costs resulting from digestion of microplastics, nutrient deficiency because of food dilution by a high microplastic/energy source ratio, damage (abrasion) of digestive epithelium, and gut microbial imbalance or dysbiosis.[35−38] However, recent studies have shown that microplastic exposure also leads to an

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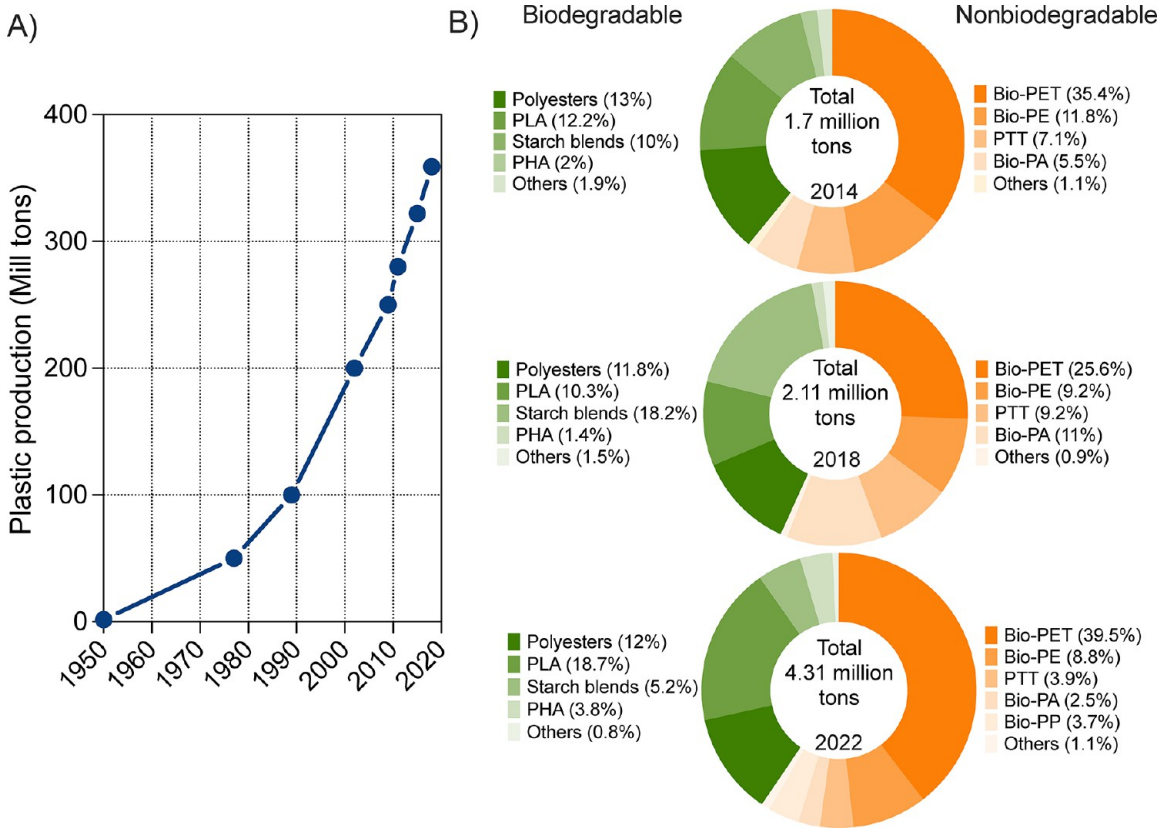

Figure 1. (A) World plastics production during 1950−2018. Based on data from PlasticsEurope (www.plasticseurope.org). (B) Global production of bioplastics by polymer type. Biodegradable bioplastics: polyesters (polybutyrate adipate, polybutylene succinate, and polycaprolactone), PLA (polylactic acid), starch blends, PHA (polyhydroxyalkanoate), and others (include biodegradable cellulose esters and compostable hydrated cellulose foils). Nonbiodegradable bioplastics: Bio-PET (polyethylene terephthalate with biobased ethanol), Bio-PE (biobased polyethylene), PTT (polytrimethylene terephthalate), Bio-PA (biobased polyamides), Bio-PP (biobased polypropylene), and others. Based on data from *Biopolymers Facts and Statistics: 2018* (www.ifbb-hannover.de/en/facts-and-statistics.html) and ref 56.

enhanced immune response, DNA damage, altered gene expression, enhanced detoxifying enzyme activities, neurotoxicity, and more frequently oxidative stress.[39−42] Despite all these adverse effects, there is still a limited understanding of the toxic action mechanism of microplastics and nanoplastics.[43] Furthermore, it is still unclear to what extent current environmental concentrations of plastic particles represent a risk to environmental health. For example, Revel et al.[44] found no significant effects of polyethylene and polypropylene microplastics (<400 μm) on tissue integrity, cellular oxidative status, DNA integrity and immune system in the Pacific oyster *Crassostrea gigas* following 10 days of microplastic exposure, despite particles being detected in the oyster excrements. Similarly, a laboratory study with *E. fetida* suggested that environmentally realistic concentrations of polyethylene and polystyrene microplastics (<300 μm) probably have no significant toxic effects in this species.[27] However, investigations on the chronic effects of long-term exposure to low levels of microplastics are still required to clarify the potential adverse effects of environmental concentrations of these pollutants.

Indirect effects from the interaction of microplastics with soil microbial communities and root symbionts could also have potential effects on plants. Rillig[45] suggested that plants may be adversely or beneficially impacted by microplastics via mechanisms such as nutrient immobilization by microplastics, microplastic-associated transport or adsorption of soil contaminants, direct toxicity from plastic polymers (e.g., release of plastic additives while polymer degradation, uptake of nanoplastics by plant root), and indirect effects derived from the interaction of microplastics with soil microbial communities and root symbionts. However, studies summarized in Table 1 suggest that the effects of microplastics on plant health are also caused by the interaction of these materials with physical (e.g., aggregate formation, water holding capacity) and chemical (e.g., carbon content) properties of the soil.[45−47] Therefore, the use of nonbiodegradable plastics in plasticulture as well as the occurrence of microplastics and nanoplastics in biosolids, composts, and wastewater irrigation may threaten the quality of agricultural soils and plant health. Thus, as pointed out by Calabrò and Grosso,[48] the strategy to reduce the input of nonbiodegradable plastics in the environment must be built on developing or improving systems that increase interception of discharged plastic waste and on substituting nonbiodegradable oil-based polymers with BPs. However, although still premature, some ecotoxicological studies have shown that BPs may be equally as harmful to aquatic organisms[49−51] and terrestrial plants[52] as nonbiodegradable plastics. Thus, more research is needed to understand the environmental fate and potential adverse effects of BPs in soil.

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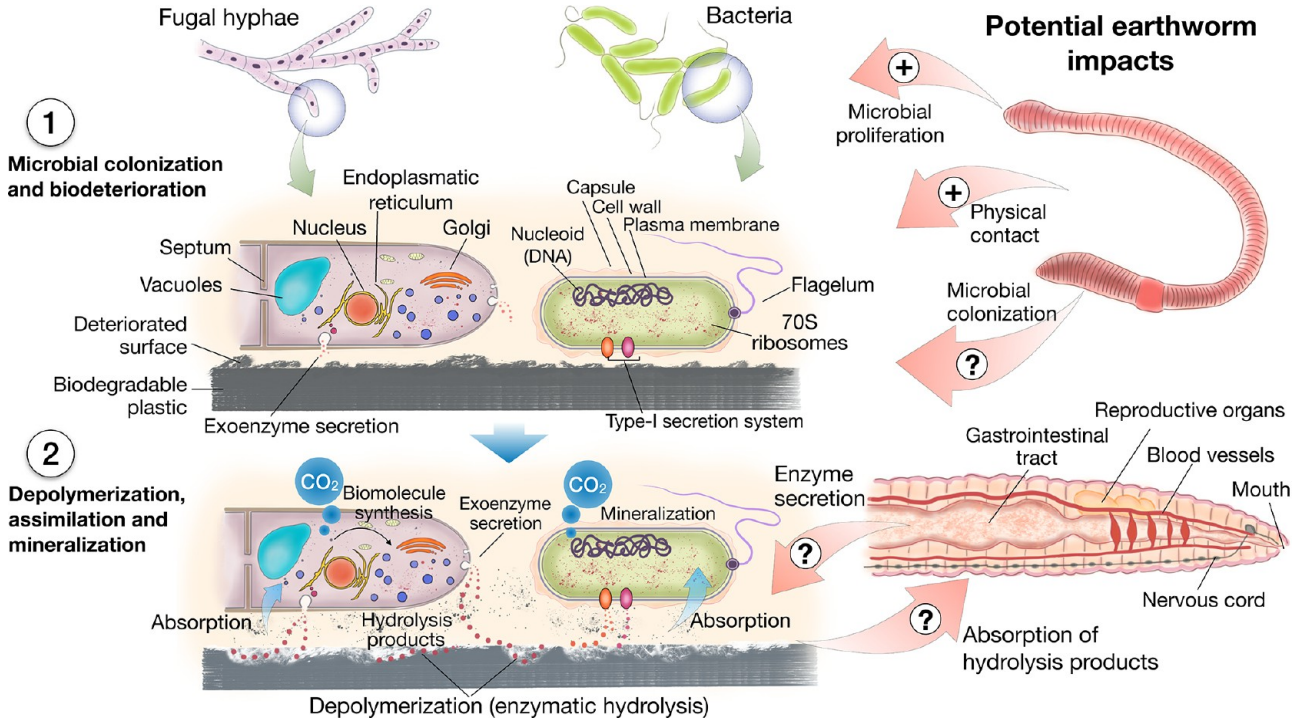

Figure 2. Main steps for polymer biodegradation in soil. First, microbial colonization of the plastic surface by polymer-degrading fungi (purple color) and bacteria (green color). Deterioration of plastic surfaces by abiotic factors and the action of soil organisms (biodeterioration) may facilitate microbial colonization. Second, secretion of exoenzymes from fungi (e.g., endoplasmatic reticulum/Golgi machinery of secretion) and bacteria (e.g., type-I secretion system) initiates the chemical decomposition of polymers (depolymerization) into hydrolysis products (oligomers and monomers), which are assimilated for energy production (releasing CO_2) and biomolecule synthesis. The potential impacts from earthworm activity on plastic biodegradation are indicated by red arrows. Although earthworms stimulate microbial activity and facilitate the contact of plastic debris with microbial populations, other earthworm-linked processes such as potential monomer absorption or exoenzyme secretion via earthworm casting (question marks) remain to be elucidated (based on refs 55, 65, 66, 189, and 190).

BIODEGRADABLE PLASTICS TO ALLEVIATE SOIL PLASTIC POLLUTION

Plastic biodegradation is defined as the capacity of a microorganism or microbial consortium to use the polymer as a sole source of carbon and energy.[53] This term must be differentiated from "degradation", which means chemical changes in a polymeric material that result in undesirable changes in the values of in-use properties of the material, and occasionally accompanied by a decrease in molecular weight.[54] Therefore, fragmentation or deterioration of plastics does not mean biodegradation,[55] and biodegradable plastics should be fully degraded to CO_2 and microbial biomass.[56] Biodegradable plastics can either be petroleum-based (e.g., polybutylene adipate terephthalate and polycaprolactone) or synthesized plastics using biological polymers as raw materials (e.g., polylactic acid, thermoplastic starch, cellulose esters, and polyhydroxyalkanoate).[23] The latter are also named bioplastics or biobased polymers, which are not necessarily biodegradable. Some biobased polymers such as polyethylene-2,5-furandicarboxylate are resistant to microbial biodegradation.[53] However, biobased polymers represent an ecofriendly alternative to using petroleum-based, nonbiodegradable plastics. The global demand for biobased polymers is continuously growing, as shown in Figure 1, although it only represents 1% of the total plastic production of 359 million tons[57] (www.plasticseurope.org).

Typical BPs that are used in mulch film manufacturing are polybutylene adipate-*co*-terephthalate, polybutylene succinate-*co*-terephthalate, polybutylene succinate, poly(butylene succinate-*co*-adipate), polypropylene carbonate, polylactic acid, polyhydroxyalkanoate, polycaprolactone, starch-based polymers, cellulose-based polymers, and blends of these BPs.[17,58,59] These materials must meet the following two essential conditions: (1) satisfy comparable agronomic benefits such as those from conventional nonbiodegradable plastic mulch films[60,61] and (2) be fully biodegraded *in situ* after harvesting. Accordingly, biodegradable plastic mulch films are designed to be tilled into the soil after harvesting, where microorganisms are expected to decompose them to CO_2 within a reasonable period of time and thus ensure the absence of microplastic and nanoplastic accumulation and toxic effects. Some studies have shown that incorporation of biodegradable plastic mulch fragments into soil increases soil microbial activity and alters the microbial community composition.[58]

Recently, the European Committee for Standardization (CEN) released the European Standard EN 17033 (2018) *Plastics — Biodegradable mulch films for use in agriculture and horticulture — Requirements and test methods* for certification of plastic mulch films as being biodegradable in agricultural soil.[62] The standard considers a polymer to be biodegradable when ≥90% of the polymer is transformed into CO_2 within 2 years at 20−28 °C. However, some researchers have highlighted issues that need to be taken into account when polymers are designated as biodegradable.[63] For example, laboratory trials need to be corroborated with field experiments because the EN 17033 standard recommends the use of a standard soil (ISO 17556) consisting of a mixture of industrial quartz, kaolinite clay, natural soil, and mature compost, which cannot be

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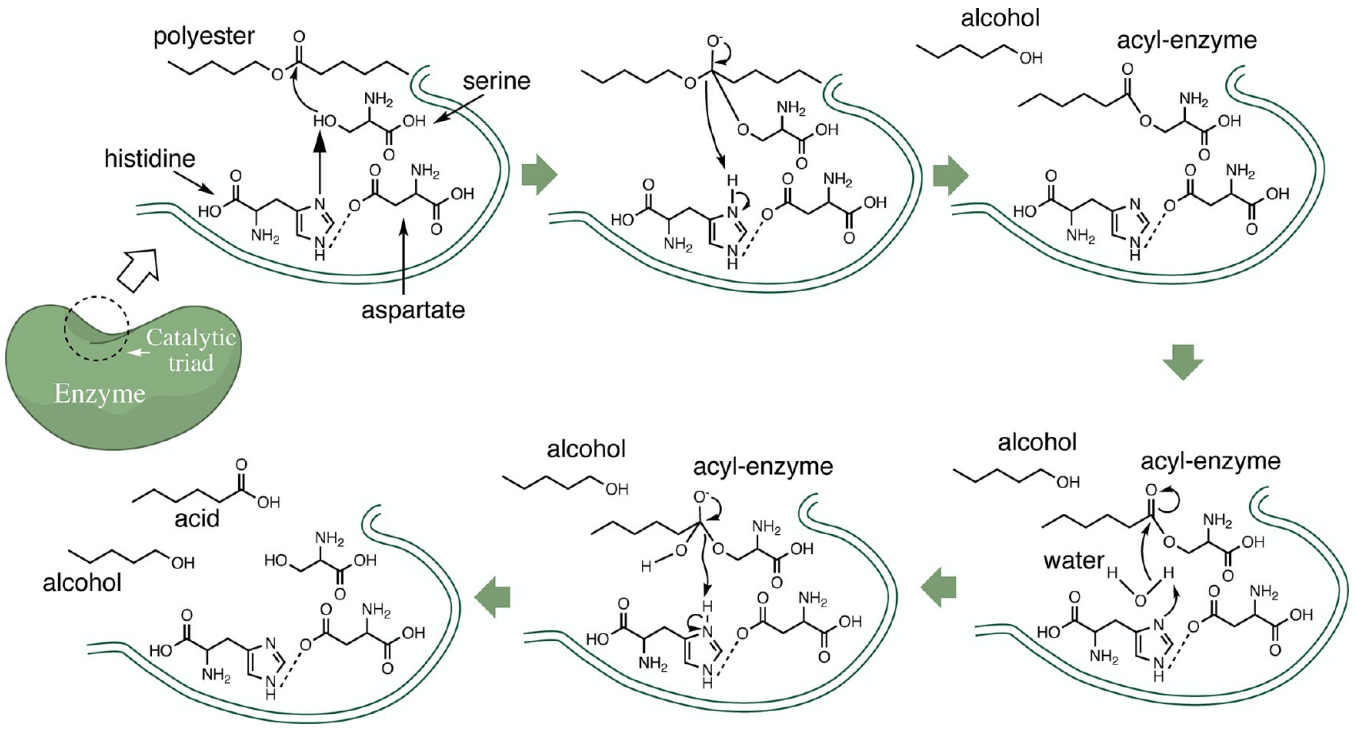

Figure 3. Mechanism of enzymatic hydrolysis of polyesters leading to the formation of the corresponding alcohol and carboxylic acid. The active site of the enzyme involves the amino acids serine, aspartate, and histidine (adapted with permission from ref 66).

considered to be a field soil.[63] In addition, the standard suggests the use of a fine powder of plastic mulch films, which is not the actual scenario in the field. Therefore, longer times are probably needed under field conditions for satisfactory biodegradation (≥90%) compared to those predicted using the EN 17033 standard.[63] Additionally, biodegradability defined as the property of a material to be degraded by a biological entity[64] of BPs is environmentally dependent because many intrinsic (chemical additives, crystallinity, presence of chemical functional groups) and extrinsic variables (temperature, moisture, pH, microbial communities) affect the BP biodegradation rate.[23,53]

In an aerobic environment, polymer biodegradation can be summarized as follows[65]

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{biomass} + C_{residue} \quad (1)$$

where $C_{polymer}$ represents the carbon in the polymer, $C_{biomass}$ is the carbon fraction that is assimilated by microorganisms, and $C_{residue}$ is the carbon fraction that remains in the residual polymers and monomers in the case of incomplete biodegradation.[65] The chemical reaction (eq 1) can be described in three steps: (1) microbial colonization and biodeterioration, (2) enzymatic depolymerization (i.e., process of converting a polymer into its monomer or a mixture of monomers[54]), and (3) assimilation and mineralization (Figure 2).[66] The first step consists of surface erosion and fragmentation of the BP by the combined action of microorganisms and decomposer fauna (e.g., heterotrophic protists, collembolans, mites, among others). The increased surface of the newly formed fragments and the eroded surface may facilitate biodegradation because of an increased amount of surface that is exposed to microbial colonization.[67] Biodeterioration is characterized by the growth of microbial consortia or biofilm on the plastic surface.[66] Exposure of plastics to solar radiation, temperature, and chemicals (e.g., atmospheric pollutants, H_2O, and O_2) also contributes to deterioration of biodegradable plastic, but a discussion about the impact of these abiotic factors on the biodegradation process is beyond the scope of this paper. Depolymerization initiates when the polymer is broken into low-molecular weight monomers and oligomers by the action of exoenzymes. Many microorganisms have been identified as potential BP degraders,[68−70] and the exoenzymes that are involved in depolymerization mostly correspond to serine hydrolases (esterases, lipases, cutinases, and proteases),[66,71−75] which have been shown to break down synthetic aliphatic polyesters and aliphatic−aromatic copolyesters (e.g., polyethylene terephthalate, polyurethane, polybutylene succinate, polyethylene succinate).[71,74,76,77] The ester bond of polyester polymers is susceptible to enzymatic attack by serine esterases.[78] The active site of these enzymes involves a catalytic triad consisting of the amino acids serine, histidine, and aspartate, which catalyze the hydrolysis of the polyester to yield the corresponding alcohol and carboxyl end groups (Figure 3).[66] Assimilation is the last step of polymer biodegradation, and it describes the uptake of monomers by microorganisms and their use to maintain cellular function, which ultimately produces CO_2 and H_2O (and CH_4 in anaerobic environment) and synthesizes biomolecules (Figure 2).

UNCERTAINTY OF THE ENVIRONMENTAL IMPACT OF BIODEGRADABLE PLASTICS

Biodegradation criteria are generally assessed in laboratory standardized conditions, which poorly reflect the fluctuating environment conditions.[10,79,80] Temperature is a key environmental variable that affects BP biodegradability.[81] Some studies question the BP biodegradation rate in the environment. For example, Narancic et al.[79] examined the biodegradation rate of six BPs and some of their blends, using standard environments

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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(industrial and home composting and anaerobic digestion) and natural environments (soil, marine water, freshwater, and aquatic anaerobic digestion). The results of this study showed that, except for polyhydroxybutyrate and thermoplastic starch, the other tested BPs (e.g., polylactic acid, polyhydroxyoctanoate, polybutylene succinate, and polycaprolactone) did not degrade in the aquatic environment. Moreover, polylactic acid, polybutylene succinate and polyhydroxybutyrate as well as their blends did not meet the biodegradation criteria of the standards ISO 17556 (soil biodegradation) and ISO 14855 (industrial and home composting). Similarly, Napper and Thompson[82] investigated the deterioration of plastic bags (both pieces and whole bags) that were manufactured from oxo-biodegradable, biodegradable, compostable, and conventional polymers in soil, the marine environment, and laboratory conditions. These researchers found that oxo-biodegradable and biodegradable plastic bags persisted for 3 years in the soil and marine environments with apparently intact physical properties, although the tensile stress (ultimate strength) decreased over time. Moreover, compostable plastic bags were found in soil after 27 months of incubation, although their physical properties were significantly deteriorated. These two examples suggest that outcomes from standard tests for biodegradation must be taken with caution probably because differences in conditions and microbial consortia between the standard test and the environment, which determine the discrepancy in biodegradation capacity.

Although biodegradation of plastic polymers entirely results from microorganisms, other soil fauna components may also contribute directly or indirectly to their biodeterioration and simultaneously promote microbial polymer degraders (Figure 2).[83] Among them, earthworms are one of the most active promoters of biodegradation because of their strong impact on the soil microbiota[84] and intense soil bioturbation (which is defined as the biological reworking of soils and sediments by all kinds of organisms, including microbes, rooting plants, and burrowing animals).[85] Earthworms alter physicochemical and biological features of soil,[86,87] with large beneficial impacts on plant growth.[88] Their presence is considered to indicate healthy soils.[89] Earthworm feeding and casting significantly contribute to the promotion of soil nutrient cycling[84] and increased soil fertility.[90] Together with nematodes and protozoa, earthworms are also regarded as "trucks" that disperse microorganisms in the soil.[91] Their gastrointestinal and skin mucous secretions are important nutrient sources that exert a strong simulating impact on soil microorganisms. In addition, earthworms indirectly stimulate the production of microbial exoenzymes[92,93] that are capable of depolymerization of plastic polymers.[71] Therefore, we suggest that earthworms could create habitats for increasing the biodegradation rate of BPs. These habitats are the drilosphere (soil environment under the influence of earthworms) and vermicompost (finely divided, porous peat-like material derived from the oxidative decomposition of solid organic wastes and characterized by a high water-holding capacity, high content in humic substances and nutrients, well-established bacterial and fungal communities, and high extracellular enzyme activity).

POTENTIAL OF EARTHWORMS AND THEIR DRILOSPHERE FOR DEGRADING BIODEGRADABLE PLASTICS

Earthworms play the role of ecosystem engineers because of their capacity to modify soil structure through burrowing activity.[94−96] This bioturbation changes the distribution of soil aggregate fractions[97] and increases soil aeration and water filtration.[33] As a consequence of their strong physical impact on soil, earthworms trigger multiple indirect chemical and biological effects that are evident in the burrow walls,[93,98] middens (small mounds built around the burrow entrance by the accumulation of plant litter mixed with casts),[99,100] and casts (feces).[101] These biostructures are defined by the term drilosphere[102] and include the soil fraction that is in contact with the earthworm skin, the luminal microenvironment of the alimentary canal, casts, middens, and burrow walls.[86]

The drilosphere's components are hotspots of fauna diversity, microbial proliferation, and extracellular enzyme (or exoenzyme) production. Middens made by anecic earthworms are nutrient-rich microenvironments that hold a rich mesofauna diversity (e.g., springtails, enchytraeids, mites, nematodes and millipedes)[103−105] and microorganisms.[106,107] These structures even attract other earthworm species.[103,108] Collembolans and mites are, however, the two microarthropod groups that mostly benefit from earthworm middens,[109] although the type of organic residue used for constructing middens significantly affects the abundance and richness of invertebrate fauna.[103] The burrow system created by anecic and endogeic earthworm species as well as their casts are also biostructures of high microbial and enzymatic activity.[98,105,110−114] Casts should not only be viewed as surface biostructures but also as subsurface deposition because most of them are produced in the burrow system, being crushed along the walls when worms reuse the burrow.[110] The earthworm gut is also an important drilosphere element in decomposition and mineralization of soil organic matter.[86] Although the gut epithelium secretes many different digestive enzymes (e.g., proteases, esterases, lipases, chitinases, phosphatases, cellulase and glucosidases),[115−117] gut symbionts also contribute to the luminal pool of digestive enzymes,[118] which are modulated by intestinal mucus secretion.[86]

Recent microbiology research has shown that the phylum Actinobacteria was the most represented in the drilosphere that was created by *L. terrestris*.[113] However, fresh casts of *Aporrectodea caliginosa* contained a higher relative abundance of Bacteoidetes and Proteobacteria (62% of combined relative abundance) and contained, to a lesser extent, Actinobacteria, Acidobacteria, Chloroflexi, Planctomycetes, and Verrucomicrobia (36% of combined relative abundance).[114] Many of the members of these phyla were also the most abundant in the soil that was incubated for 90 days with polyethylene mulch films[119] or in soil microbial suspensions that were incubated for 30 days in the presence of polyethylene mulch film fragments.[120] Collectively, these studies suggest that the drilosphere holds a microbial community that is potentially relevant to BP biodegradation. However, earthworms should facilitate the physical contact of plastics with these highly active biostructures. Pioneering research by Huerta Lwanga et al.[24,121] revealed that *L. terrestris* (Figure 4A) interacts with mesoplastics and microplastics in a manner that is similar to its interaction with tree leaves.[122] The earthworm was able to actively transport LDPE particles (≤150 μm in size) to the subsoil by the following two processes: (1) dragging litter mixed with microplastics and (2) ingesting microplastic-contaminated litter, which has a microplastic content that was accumulated in the burrow depth by casting.[24] Microplastics less than 50 μm in size were mostly found in casts (90% of total microplastic fractions) at a concentration that

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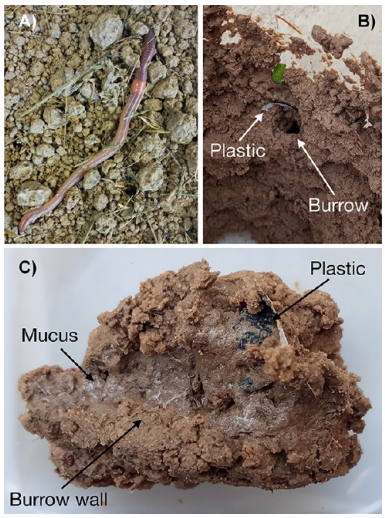

Figure 4. Pictures showing the accumulation of mulch film mesoplastics by the earthworms *Lumbricus terrestris* (A) on the burrow walls (B) during a 1 month microcosm experiment. Plastic fragments can be found mixed with casts and mucus on the burrow wall (C). Photos by Juan C. Sanchez-Hernandez.

was 2 times higher than that in leaf litter. Further studies have also shown that *L. terrestris* transported both engineered spherical microplastics[24] and mulch film-derived macroplastics (2 cm × 2 cm in size) toward the burrow depth.[31] Earthworms also ingested large-sized mulch film fragments (1.5 cm × 1.5 cm) when these mesoplastics were previously conditioned via composting or environmental weathering (6−12 months of soil incubation).[31] Both ingestion of mesoplastics and microplastics by *L. terrestris* and adherence of microplastics to the mucous skin were suggested to be the most plausible mechanisms for the vertical transport of mulch film fragments and microplastics.[30] Therefore, soil bioturbation by anecic earthworms could facilitate bioaccessibility of biodegradable plastics to microbial communities and soil mesofauna that are established in the drilosphere (Figure 5).

The earthworm bioturbation defines four potential microhabitats for plastic biodegradation. The first microhabitat is developed on the soil surface via midden formation. *Lumbricus terrestris* and most anecic species are able to accumulate plastic fragments together with litter and casts that are deployed on the soil surface (Figure 5, microhabitat 1). Mesofauna associated with middens could interact with microplastics, thus facilitating plastic surface biodeterioration and microbial colonization (Figure 2).[123] Some studies have demonstrated that collembolans actively disperse microplastics on the surface, even although such an interaction largely depends on the species and the microplastic type and size (Table 1).[29] Similarly, these organisms may fragment microplastics by scraping or chewing activities, as suggested by Rillig,[124] thereby increasing the surface area for microbial attack. The second microhabitat is the burrow wall where mesoplastics and microplastics can be accumulated by anecic earthworms (Figure 5, microhabitat 2, and Figure 4B). Secretion of mucus that earthworms use to facilitate displacement through the burrows and increase their stability[125] could be a nutritious substrate for microbial biofilm development on plastic surfaces. Therefore, accumulation of organic composites made from mucus, organic matter (cast and leaf litter), and plastic fragments on burrow walls may provide favorable conditions for polymer biodegradation by microorganisms (Figure 4C). The third microhabitat is represented by the earthworm casts that are deposited either on the soil surface or in burrow walls (Figure 5, microhabitats 1 and 3). The studies by Huerta Lwanga and co-workers[24,121] showed that *L. terrestris* casts are significant reservoirs of small-sized microplastics (<50 μm), so we postulate that specific microorganisms and exoenzymes could participate in the depolymerization of ingested biodegradable microplastics and nanoplastics. The last microhabitat for plastic biodegradation would occur in the gastrointestinal lumen of earthworms (Figure 5, microhabitat 4). A study by Huerta Lwanga et al.[126] suggested that *L. terrestris* gut microorganisms degrade microplastics during their gastrointestinal transit. The researchers found that the gut luminal content of microplastic-exposed earthworms contained Gram-positive bacteria belonging to Actinobacteria and Firmicutes, which degraded around 60% of the initial LDPE microplastics after 21 days of incubation. Detection of volatile long-chain alkanes seemed to suggest a depolymerization process by these bacteria. However, mineralization (CO_2 formation) was not measured in that study, so LDPE biodegradation by earthworm gut symbionts remains to be demonstrated. Other researchers have described a high activity of carboxylesterase in the gastrointestinal luminal content of *L. terrestris*[127] and *Aporrectodea caliginosa*,[128] which could also indicate potential depolymerization of ingested biodegradable microplastics and nanoplastics. However, the efficacy of carboxylesterases to hydrolyze polyester polymers depends on structural properties of the active sites of the enzyme[129] and on the polyester structure (e.g., presence of side chains and high-molecular weight polymers).[55] Despite these findings, further studies are required to elucidate the possible involvement of earthworm gut carboxylesterases in the BP hydrolysis. Standard methods for measuring plastic biodegradation such as CO_2 production or the use of ^{13}C-labeled polymers are strong tools to demonstrate that the earthworm gastrointestinal environment may contribute to plastic biodegradation.[68]

INOCULATING HORTICULTURE SOILS WITH EARTHWORMS: AN EXAMPLE OF ENHANCED BIOTURBATION EFFECTS

It is well known that plastic mulch films provide multiple agronomic benefits such as warming the soil, extending the growing season, controlling insect pests and weeds, conserving water, and reducing herbicide use.[9] Despite these advantages, the technology has also adverse side effects such as decreasing soil biodiversity and fertility.[21] For example, plastic mulching may lead to soil nutrient exhaustion because of microbial proliferation, which is, in turn, exacerbated by the mulching-induced favorable environmental conditions (high soil temperature and moisture in film-covered soil with respect to uncovered soil).[21,130] Although the current tendency is substituting nonbiodegradable mulch films for biodegradable mulch films, some studies have shown that the latter may accumulate in the soil as a result of successive crop seasons and unfavorable conditions for satisfactory biodegradation.[22] It has been estimated that biodegradable mulching films may reach a plateau phase of soil accumulation of 281.3 kg ha^{-1} in successive crop seasons.[131] At this level of plastic accumulation, the emergence rate of cotton seeds and cotton production seems to be affected.[11] A viable strategy for reducing soil persistence of BPs could be the introduction of

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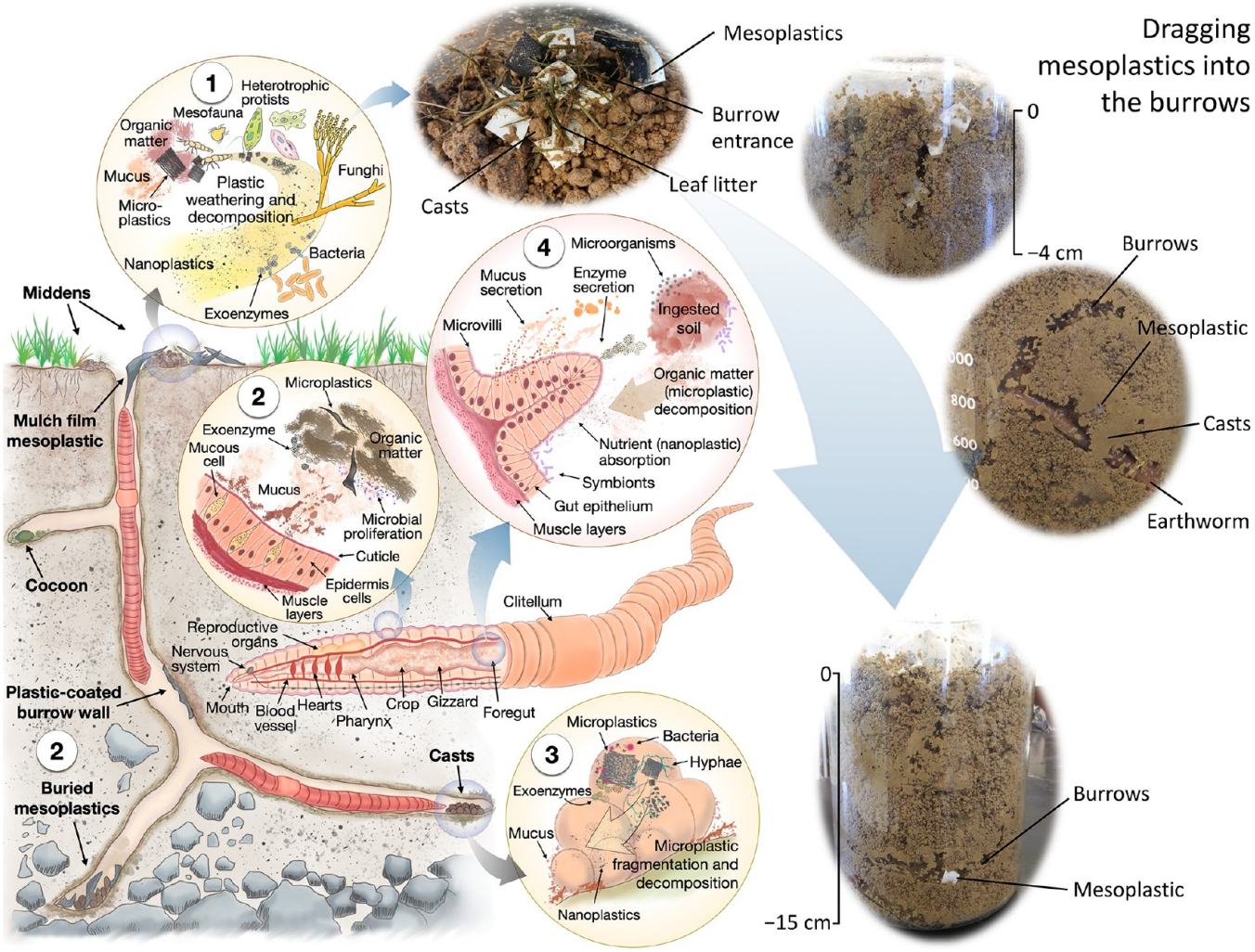

Figure 5. Pictorial representation of the earthworm impact on the environmental fate of mesoplastics, and microplastics, and potential microhabitats for polymer biodegradation. Earthworms may contribute to plastic biodegradation by creating four optimal conditions, as follows: (1) middens formed on soil surface, which may contain plastic fragments of different sizes, (2) burrow linings with a high content of mucus, casts, organic residues, and eventually plastic fragments that are buried by earthworms, (3) casts internally deposited in the burrow or externally on the soil surface, which may contain microplastics and nanoplastics, and (4) the gastrointestinal tract of earthworms, in which the activity of symbionts and digestive enzymes may contribute to depolymerization of biodegradable plastics. Photos by Juan C. Sanchez-Hernandez.

anecic and endogeic earthworms, where indigenous earthworms are scarce (Figure 6). This practical approach would improve the soil quality under plastic mulching and accelerate the biodegradation rate of film fragments.

The beneficial effects of organic mulching (i.e., covering the soil surface with a layer of organic material such as plant litter or animal wastes)[14] on the earthworm population have already been studied in the past century.[132] Since then, many studies have corroborated the increased earthworm population and the positive impact of earthworm burrowing and feeding activities that were observed in organic mulched soils. Such effects result from the increase in food provision (organic mulch), the role of the mulch as a shelter for earthworms against predators such as birds, and the higher and consistent soil humidity below the mulch, which favors earthworm survival and activity.[132−136] However, the effects of plastic mulching technology on earthworms has not been explored. We hypothesize that earthworms may also provide multiple direct and indirect beneficial effects in plastic mulched soils so long as they have an organic cover on the topsoil (Figure 6). In this conceptual model, earthworms may exert the following two main chemical effects in plastic mulching technology: increase the nutrient availability and remove soil organic contaminants and pathogens. The former is achieved by increasing the exoenzyme activities in earthworm-treated soils (e.g., phosphomonoesterases, urease, arylsulfatase, and glucosidases, among others),[112] which enhances nutrient cycling. Organic matter translocation by anecic earthworms together with the creation of macropores (burrow system) indirectly facilitate the development of the plant root.[88] Many studies have demonstrated that earthworm burrowing activity, which largely depends on food availability[137] and quality,[138,139] significantly increases plant growth and health.[137,140] Likewise, it is well known that earthworms significantly alter the environmental fate of organic contaminants and mycotoxins.[141,142] Earthworm-assisted degradation of environmental contaminants occurs via stimulating microbial degraders, increasing the bioavailable fraction of contaminants, and modifying soil conditions (e.g., pH, moisture, and aeration) that favor abiotic degradation.[143,144] Moreover, earthworms also uptake organic

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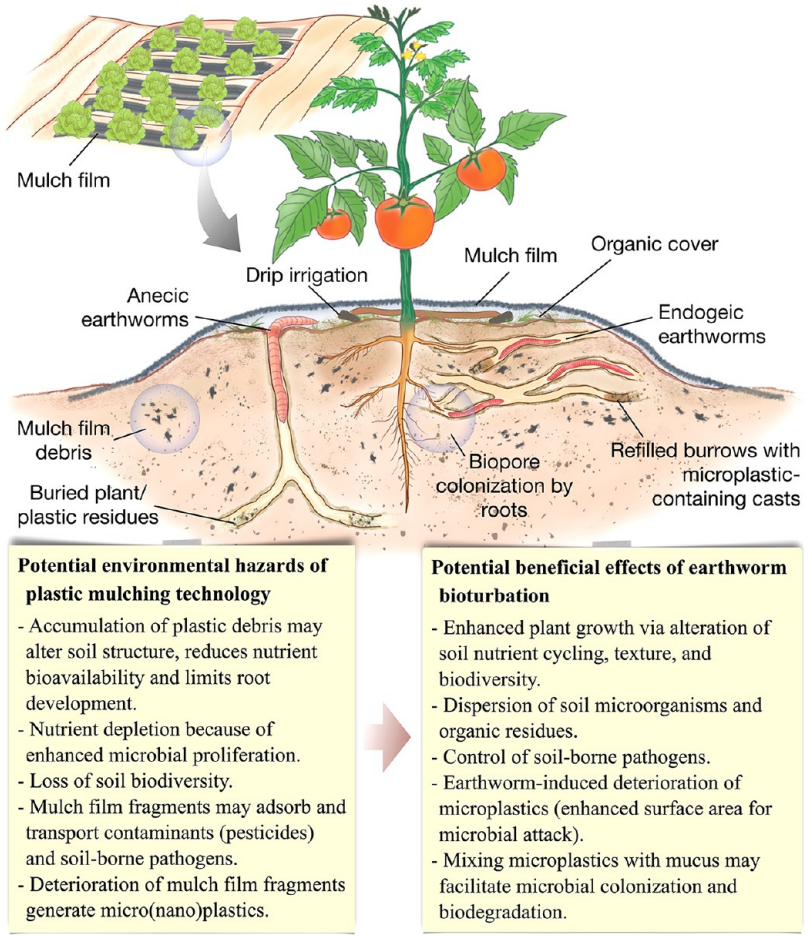

Figure 6. Potential environmental hazards derived from plastic mulched soils and main effects of soil-dwelling earthworms on soil quality and environmental fate of microplastics.

pollutants through their skin and alimentary canal,[145,146] which may later be metabolized by detoxifying-enzymatic systems,[147] and thereby, the earthworms directly contribute to contaminant dissipation. Earthworms also reduce soil mycotoxins. For example, soils covered with wheat straw residues infected with the fungus *Fusarium culmorum* showed that when inoculated with *L. terrestris*[141] or *A. caliginosa*[142] the presence of this phytopathogenic fungus and the concentration of the mycotoxin that it produces (deoxynivalenol) were significantly reduced. In addition, earthworms are able to uptake deoxynivalenol through their gastrointestinal system and accumulate it in body tissues.[148]

Although soil inoculation with earthworms was shown to be a viable option for ecosystem restoration,[95] its application in agriculture has been rarely explored with the noticeable exception of Australia and New Zealand.[149] Perhaps the main limiting factor for applying such biotechnology is the absence of readily available techniques for earthworm rearing and production, although conditions for culturing some species are well known.[150] In addition, some techniques for the controlled release of earthworms in agricultural plots have been described and tested.[95,149−155] However, agricultural practices such as repeated tillage cause an important reduction in the earthworm population,[156] which are incompatible with our practical approach of enhancing soil bioturbation via earthworm inoculation. The system would be workable as long as earthworms are inoculated at the beginning of the crop season where they can benefit from the soil conditions (e.g., consistent moisture through drip irrigation), and seedlings and plants can benefit from earthworm bioturbation.

POTENTIAL OF VERMITECHNOLOGY IN PLASTIC BIODEGRADATION

Composting is defined as the biological decomposition of organic waste under thermophilic conditions in an aerobic environment, in which microorganisms decompose the organic matter to release H_2O and CO_2.[157] Biodegradable plastic materials (e.g., plastic bags and bottles, food service and packaging materials, and medical service materials) are designed to be easily biodegraded using composting facilities. Production of CO_2, spectroscopy analysis (e.g., infrared, nuclear magnetic resonance), mass loss, and visual inspection of plastics (e.g., signs of deterioration such as discoloring, thickness, consistency, etc.) are the methodologies that are commonly used to assess BP biodegradation in compost.[158] Theoretically, the result of composting biodegradable plastics must be a stable, humus-rich, environmentally safe product to be used as a soil amendment. However, some studies have shown that composting is insufficient to fully degrade the BPs, and it may even produce compost with residual mulch film additives. For example, Sintim et al.[159] reported that composting of two biodegradable mulch films (a copolyester containing polybutylene adipate-co-terephthalate and a polylactic acid-polyhydroxyalkanoate blended polymer) resulted in

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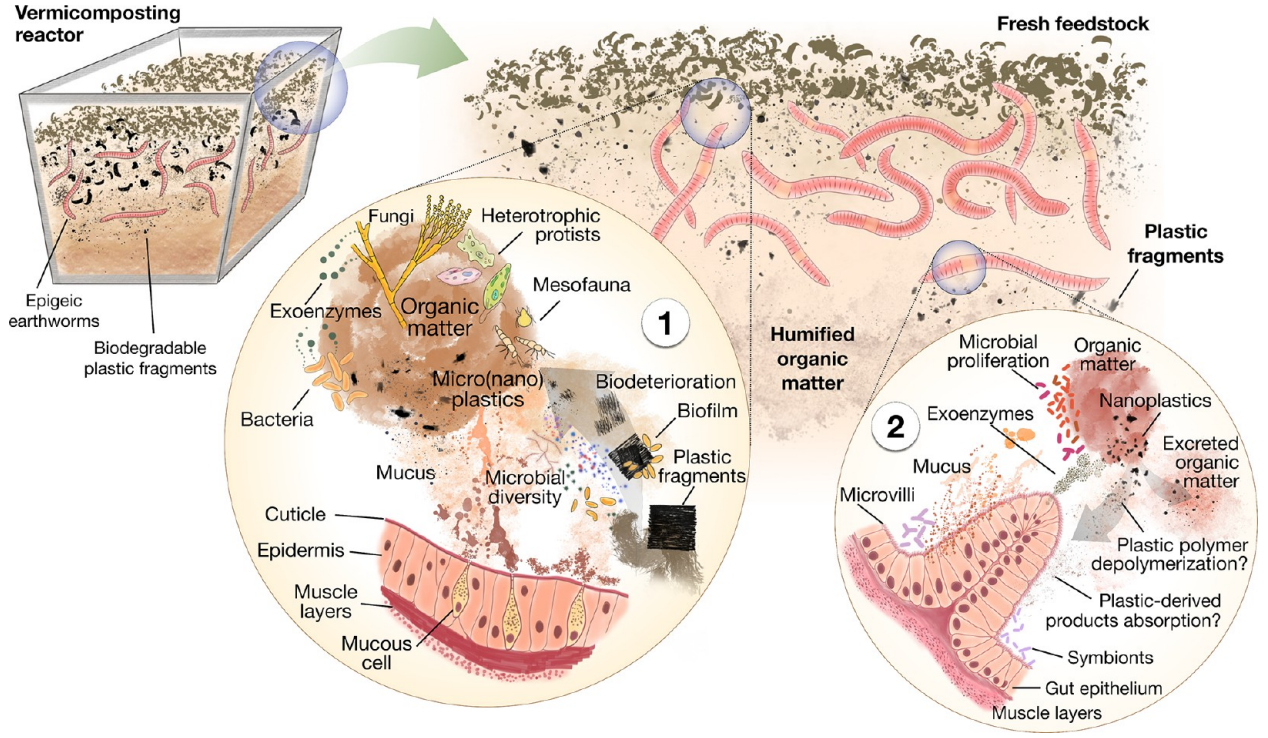

Figure 7. Hypothesized model on plastic biodegradation in vermitechnology. Vermicomposting is described as a two-step process that involves cast-associated (sphere 1) and gut-associated biological (sphere 2) processes. The high microbial and enzymatic activities in both microenvironments could facilitate the biodegradation rate of biodegradable plastics added to the vermicomposting reactor.

an accumulation of residues of carbon black pigment in the final compost, although its impact on compost quality remains unknown. Similarly, an investigation reported that home composting (ISO 14855 at 28 °C) of the polylactic acid, polybutylene succinate and polyhydroxyoctanoate polymers, and their blends, did not meet the standard criteria for biodegradable plastics.[79] Thus, these studies suggest that industrial and home composting facilities may not be sufficient for complete BP biodegradation. One promising strategy to accelerate the biodegradation of these polymers in composting was the addition of specific exogenous microbial strains (i.e., bioaugmentation).[160] In this context, the second strategy we propose to promote BP biodegradation is vermicomposting with blended solid organic waste and biodegradable plastics.

Vermicomposting is defined as an aerobic, mesophilic process in the presence of earthworms, by which organic matter is transformed into vermicompost.[161] Typical earthworms that are used in vermicomposting are *E. fetida*, *E. andrei*, or *Lumbricus rubellus*, which belong to the ecological group of epigeic earthworms. These are surface-dwelling species that feed on the organic matter that is accumulated on the soil surface, and they rarely burrow into the soil and ingest soil.[32] During vermicomposting, earthworms trigger biological processes via stimulating microbial proliferation and distributing microorganisms in the feedstock through casting and mucus secretion.[162] Therefore, vermitechnology is a cost-effective, energy saving, and faster technique to generate organic fertilizer from organic solid feedstocks compared to composting.[163] To understand how vermicomposting could facilitate BP biodegradation, the biological transformations that occur during vermicomposting are described below (Figure 7). Vermicomposting takes place in two processes: the earthworm gut-associated processes (GAPs) and the cast-associated processes (CAPs).[164,165]

During GAPs, the fresh organic matter ingested by earthworms undergoes physical (e.g., grinding in the gizzard) and biochemical transformations that are mediated by enzymes released to the luminal environment by both symbiont microorganisms and the earthworm gut epithelium.[163] Nutrients are absorbed through the gut epithelium, whereas secretion of compounds such as mucus, urea, ammonia, and enzymes will form the chemical composition of the egested material or casts. In GAPs, the initial microbial composition and activity of the ingested material changes during the gastrointestinal transit. Several studies have examined the succession of a microbial community during vermicomposting of vegetable wastes,[166−168] sewage sludge, and cattle dung.[169,170] Members of the phyla Actinobacteria, Bacteroidetes, Proteobacteria, Actiomycetes, and Acidobacteria are the most abundant during vermicomposting and in the final vermicompost. Some microorganisms that are abundant in vermicompost from these feedstocks have also been found to participate in the biodegradation of biobased polymers (e.g., species of the genera *Streptomyces*, *Paecilomyces*, *Trichoderma*, and *Paenibacillus*).[56]

The CAPs occur in the earthworm casts, and microorganisms and other decomposer fauna (collembolans) actively participate in further decomposition of more recalcitrant organic wastes such as lignin, cellulose, and hemicellulose. The high organic matter content of casts (recalcitrant molecules of feedstock plus the earthworm gastrointestinal secretions of organic molecules) provides a nutritive cocktail for microorganisms and decomposer fauna. Therefore, CAPs prolong the decomposition of the feedstock although earthworms move away seeking fresh and non-

https://dx.doi.org/10.1021/acssuschemeng.9b05450
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ingested organic waste or they are intentionally removed from the vermicomposting system (maturation phase). Changes in the enzymatic profile, microbial composition, and nutrient concentration still occur in the maturation phase (earthworm free) of vermicomposting.[171,172]

We postulate that vermicomposting creates two microbial and enzymatic-rich microhabitats that could accelerate BP biodegradation. The first microhabitat would correspond to CAPs and the fresh feedstock, where a vast variety of decomposer organisms other than microorganisms could participate in the BP biodegradation (Figure 7). The second microhabitat would reflect the GAPs, where secretion of serine hydrolases by the earthworm gut epithelium and symbionts could contribute to BP depolymerization. In this microhabitat, uptake of polymer-derived monomers by the earthworm gut epithelium would be an additional process of assimilation besides that of microorganisms (Figure 2).

CONCLUSIONS AND FUTURE PROSPECTS

Over the past decade, many studies have shown the magnitude and severe threat of plastic pollution in many marine and freshwater ecosystems. However, the new challenge is now the terrestrial system, which receives a higher amount of plastic debris and microplastics than that estimated for oceans. The use of biodegradable plastics seems to be a viable option to reduce the high input of nonbiodegradable microplastics into the environment. However, recent studies show that the biodegradation rate of these polymers is not as high as predicted from standardized laboratory testing. In this Perspective, we propose the use of earthworms to accelerate the biodegradation rate of BPs via the following two practical strategies: (1) enhancing bioturbation by inoculating soil with earthworms and (2) vermicomposting with blended plastic debris and solid organic wastes. The stimulating effects that soil-dwelling earthworms (option 1) and composting earthworms (option 2) exert on microbial communities and mesofauna suggest that the persistence of biodegradable plastic debris in soil, industrial, or home composting could be reduced. However, this Perspective leaves some open questions that deserve further research. For example, how would earthworms and microbial populations collectively respond to the presence of BPs that may contain contaminant residues (e.g., metals, herbicides, insecticides) that are adsorbed onto their surfaces or that may release additives while they degrade? Could earthworms absorb plastic-derived monomers, thereby contributing to polymer mineralization? What would be the physicochemical benefits of vermicompost obtained from blending solid organic wastes with biodegradable plastics? Finally, studies of microplastic ecotoxicology have involved three earthworm species, i.e., *L. terrestris*, *E. fetida*, and *E. andrei*. We encourage future investigations using other earthworm species of ecological and agronomic interest such as endogeic species (e.g., *Aporrectodea* spp.), which are among the most abundant species in agroecosystems. Because anecic and endogeic earthworms inhabit agricultural soils together, their synergistic effects on the fate of plastics and biodegradation offer an interesting topic for future research.

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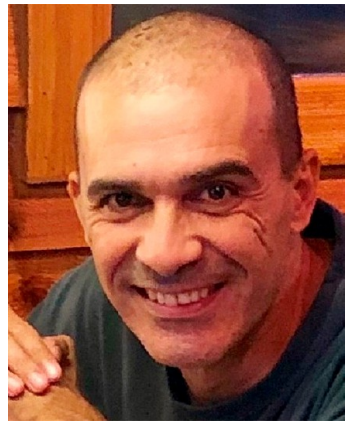
Notes

The authors declare no competing financial interest.

Biographies



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https://dx.doi.org/10.1021/acssuschemeng.9b05450
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■ ACKNOWLEDGMENTS

This research was supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (Grant PGC2018-098851-B-I00) and the USDA-ARS National Programs 212. J.C.S.-H. acknowledges receipt of a fellowship from the OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems in 2019 (Grant TAD/CRP JA00101046). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). We gratefully acknowledge four anonymous reviewers for their valuable comments on our manuscript.

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