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## Review

## Effect of (bio)plastics on soil environment: A review

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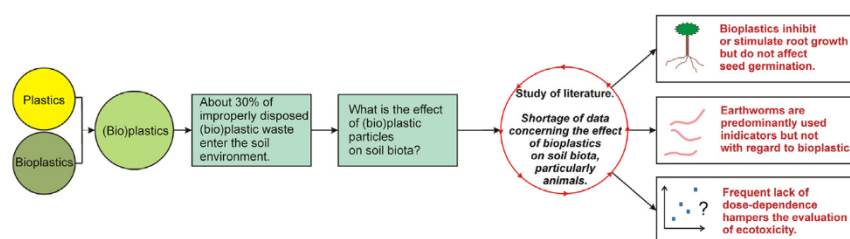
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## HIGHLIGHTS

- (Bio)plastics, i.e. petroleum-derived plastics and bioplastics, affect soil biota.
- Effect on soil biota of bioplastics rarely studied than petroleum-derived plastics.
- Earthworms are predominantly used to test petroleum-derived plastics toxicity.
- Hardly any data about the impact of bioplastics on earthworms and other soil fauna.
- (Bio)plastics cause either inhibition or stimulation of root growth.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The contribution of improperly disposed plastic wastes is globally evaluated at the level of 30% and these wastes make a particular threat to all living creatures. Thus, the evaluation of the possible impacts of plastic particles on the biotic part of ecosystems has become increasingly important in recent years. As a result, the growing number of publications concerning this subject has been observed since 2018. This paper aims to review the advances in studies on the effect of petroleum-derived plastic and bioplastic particles, taken together in the term (bio)plastics, on the terrestrial ecosystem, particularly on soil biota. It is the first review, in which both petroleum-derived plastics and bioplastics were analysed regarding their potential impacts on the soil compartment. Petroleum-derived plastics were more frequently studied than bioplastics and among analysed papers about 18% concern bioplastics. It was found that (bio)plastics did not affect the germination of seeds. However, they might contribute to the delay in germination processes. Both inhibitory and stimulating effects were observed in relation to the growth of roots and stems. (Bio)plastic microparticles did not inhibit the biochemical activity of nitrifiers and transformation of carbon compounds. Earthworms were predominantly used organisms to test the effect of petroleum-derived plastics on soil biota but there are hardly any data about bioplastics. Petroleum-derived microplastics present in soil at concentrations up to 1000 mg kg<sup>-1</sup> usually neither cause to the mortality of earthworms nor affect their reproduction. Micro- and nanoparticles of petroleum-derived plastics could be accumulated in the earthworm intestine and transferred in the food chain. Summarizing, a high variability of results and often appearing lack of dose-dependence relationships hamper the final evaluation of the ecotoxicity of (bio)plastics simultaneously creating a need to develop the ecotoxicological studies on (bio)plastics, especially including these on the effect of bioplastics on soil animals.

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**Abbreviations:** HDPE, high-density polyethylene; LDPE, low-density polyethylene; LLDPE, linear low-density polyethylene; PA, polyamide; PAC, polyacrylic; PAN, polyacrylonitrile; PC, polycarbonate; PBAT, poly(butylene adipate-co-terephthalate); PBS, poly(butylene succinate); PCL, poly( $\epsilon$ -caprolactone); PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate; PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PE, polyethylene; PEHD, polyethylene high-density; PES, polyester; PET, poly(ethylene terephthalate); PLA, polylactide, poly(lactic acid); PMMA, poly(methyl methacrylate), acrylic glass; PP, polypropylene; PS, polystyrene; PTT, poly(trimethylene terephthalate); PU, polyurethane; PUF, polyurethane foam; PUR, polyurethane resin; PVC, polyvinyl chloride.

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## 1. Introduction

Contamination of the environment with plastic debris is one of the major environmental problems that nowadays influences on the ecosystems including the human-beings (Koelmans et al., 2019). Plastics are typically hydrocarbons of polymeric structure enriched with additives for the purpose of enhancement of the specific properties. Massive production of (bio)plastics began in the 1940s and 1950s and it is still increasing reaching almost 368 million tons globally in 2019 (PlasticsEurope, 2020). Almost 1% of this production (2 million tons) in 2019 made up bio-based plastics (European Bioplastics, 2020). Bio-based plastics are polymers synthesized from the renewable resources like starch, sugar, natural fibres or other organic components in varying composition (Niaounakis, 2013). The production of polymers from the renewable resources is regarded as one of the fastest growing materials sectors and it is expected that the contribution of bio-based plastics reached about 2% of the plastics introduced on the market worldwide in 2025 (Niaounakis, 2013; European Bioplastics, 2020). Bio-based plastics do not need to be biodegradable. It was estimated that almost half of bio-based polymers were not susceptible for the biological decomposition in the environment (European Bioplastics, 2020). Both bio-based and biodegradable plastics can be classified as bioplastics. According to the definition proposed by European Bioplastics “a bioplastic is either bio-based, biodegradable or features both these properties” (<https://www.european-bioplastics.org/bioplastics/>).

Petroleum-derived plastics as well as bioplastics that are introduced on the market usually contain additives and/or modifiers to improve the physicochemical properties of the pure polymers. The additives increase pliability, resist ultraviolet radiation, reduce flammability or impart other preferred physical characteristics of the final product (Lambert et al., 2014). These are for example plasticizers, dyes, pigments, antioxidants, light and UV stabilizers. Additives or modifiers may be incorporated into a polymer in any proportions during any stage of polymerization or processing (Niaounakis, 2013). These compounds change not only the physicochemical properties of polymers but also their biological properties including biodegradability and toxicity.

After use both petroleum-derived plastics and bioplastics became a waste that should be properly managed. In 2018 about 250 million tons of plastic waste was globally generated (Coversio Market&Strategy, 2020). Most of them, i.e. about 70%, was collected, and then directed to the managed landfills, recycling sites or energy recovery units (Coversio Market&Strategy, 2020). The rest of plastic waste (about 30%) was disposed improperly (Coversio Market&Strategy, 2020). In practice they entered the terrestrial and/or aquatic ecosystem directly. Considering how much plastic waste is generated, it is not surprising that plastic particles are regarded as the most abundant type of debris encountered in the environment (Chae and An, 2018). It was estimated that about 32% of all plastics produced might remain in the continental systems and agricultural soil might store even more microplastics than oceanic basins (Nizzetto et al., 2016; de Souza Machado et al., 2018). It obviously

makes a serious threat to the terrestrial ecosystems. At the same time soil is an essential, biologically active component of the environment that delivers water and nutrients for living creatures and participates in the cycling of carbon and other elements through the global ecosystems.

Petroleum-derived plastics and bioplastics that enter the soils are subjected to many biological, chemical and physical processes. Biological transformations are mainly connected with the activity of earthworms, bioturbation by plant roots and microbiological decomposition (Rillig et al., 2017a, 2017b; Zhang and Liu, 2018; Li et al., 2020). Physicochemical processes comprise adsorption, desorption, sedimentation, incorporation into soil aggregates, chemical interactions with water, humic-like compounds and some other components present in the soil (Guo et al., 2020; Li et al., 2020). As a result of these various transformations and weathering processes (e.g. UV radiation, rainfall) plastics are fragmented into macroplastic (<150 mm), microplastic (<5 mm) and nanoplastic (< 100 nm) particles. The disintegrated and/or degraded plastics are often called as secondary plastics. At the same time primary plastics are the raw material that is directly used in industry, medicine and other branches of human activities (Guo et al., 2020).

The petroleum-derived plastics and bioplastic particles, particularly the smaller ones (micro- and nanoparticles), can be easily transported vertically and horizontally in the soil matrix and some of them may reach the aquifer contributing to the contamination of groundwater. As a result of transportation, the plastic particles are widespread distributed and they are found not only in the industrial areas but also in the non-urban locations and even on the shorelines of the most remote islands (Barnes et al., 2009; Liu et al., 2017; Guo et al., 2020). Scheurer and Bigalke (2018) analysed samples from 26 floodplain sites in Switzerland and found that 90% of floodplain soils contained microplastics. European farmlands receive an annual input of 63,000–430,000 tons of microplastics, while North American farmlands receive 44,000–300,000 tons (Gionfra, 2018; Ju et al., 2019). It is mainly caused by the application of sewage sludge or irrigation with wastewater in the farmland or degradation of plastic mulch on the agricultural lands in the semiarid regions (GESAMP, 2015; Steinmetz et al., 2016; Huerta Lwanga et al., 2017a). In sewage sludge the number of plastic particles varies from 1000 to more than 20,000 particles per kg of dry mass (Hohenblum et al., 2015).

As it is shown in Table 1 the content of microplastics in soil has been so far expressed either in the number of particles per the mass of dry soil or in the mass (usually milligrams) of plastics per mass of dry soil. It makes the comparison of the level of contamination of different areas by plastic particles very difficult. For example Zhang and Liu (2018) reported that microplastics concentrations in the farmland in China varied from 7100 to 42,960 particles  $\text{kg}^{-1}$ , while Fuller and Gautam (2016) found that the concentrations of microplastics in the industrial area in Australia were from 300 to 67,500  $\text{mg kg}^{-1}$ . One of exceptions is the work of Scheurer and Bigalke (2018), who used both types of units and informed that the concentrations of microplastics in the floodplain achieved up to 55.5  $\text{mg kg}^{-1}$ , which corresponded to 593 particles  $\text{kg}^{-1}$ .



**Table 1**  
Concentrations of plastics in soil.

Location	Soil type	Size of plastic particles	Abundance	Reference
Switzerland 29 sites all over Switzerland	Floodplain soil	<2 mm	55.5 mg kg <sup>-1</sup>	Scheurer and Bigalke (2018)
Australia, Sydney	Industrial soil	<1 mm	300–67,500 mg kg <sup>-1</sup>	Fuller and Gautam (2016)
Chile, Mellipilla	Agricultural field	< 1 mm	0.57–12.9 mg kg <sup>-1</sup>	Corradini et al. (2019)
China, Loess plateau	Fruit field	< 5 mm	8 ± 25–540 ± 603 mg kg <sup>-1</sup>	Zhang et al. (2018)
Germany Middle Franconia, southeast Germany	Agricultural field	< 5 mm	0.34 ± 0.36 particles kg <sup>-1</sup>	Piehl et al. (2018)
China Dian Lake, southwestern China	Farmland	<10 mm	7100–42,960 particles kg <sup>-1</sup>	Zhang and Liu (2018)
China, Xinjiang	Cotton field	< 5 mm	70 ± 0.86–1724 ± 68.3 particles kg <sup>-1</sup>	Hu et al. (2021)

The pollution of the terrestrial compartment by petroleum-derived plastics as well as bioplastics directly contributed to the changes in the chemical composition of soil and furthermore it influenced soil structure and functions including the effect on soil organisms. The fate and behaviour of plastic particles in the terrestrial compartment depend on many factors including their susceptibility to microbiological decomposition. The reviews that have been published so far did not comprise bioplastics and did not differentiate between petroleum-derived plastics and bioplastics (Chae and An, 2018; Guo et al., 2020; Li et al., 2020). Its possible reason is that the number of papers concerning the impact of bioplastics either on physicochemical properties of soil or on soil biota is very limited. Taking into account the increasing contribution of bio-based and biodegradable plastics in the global plastics market there is a need to include them in the review of literature data concerning ecological risks of plastics in soil. It allows us to examine the interactions between bioplastics and soil and as a consequence to identify the research gaps.

In this work the effect of petroleum-derived plastic and bioplastic particles taken together by the concept of (bio)plastics on the terrestrial ecosystem was analysed. The analysis was made upon the existing literature data, in particular the most recently (2018–2021) published data, which were summarized and finally presented in the form a review. Although abiotic and biotic parts of the terrestrial ecosystem were taken into consideration, the special attention was paid to soil biota. The comparison and summary of previously reported data allow for better understanding of the phenomena associated with the pollution of soil by (bio)plastics and should be useful in the mitigation of the impact of (bio)plastics on the terrestrial ecosystem in the future.

## 2. Methodology of this review

Literature referred to soil pollution by (bio)plastics was thoroughly reviewed. The analysis of literature data comprised the sources, transformations and concentrations of (bio)plastics in the terrestrial compartment as well as the effect of (bio)plastics on soil physicochemical properties and biota. After analysis the following approach focusing on two issues was applied: (1) to describe briefly the changes of physical and chemical features of soil and the transformations in the abiotic part of soil ecosystem resulting from the plastics pollution, (2) to examine carefully the effects of (bio)plastics particles on soil organisms distinguished by plants, microorganisms, earthworms and other soil fauna (e.g. springtails, nematodes).

Basically two databases, i.e. EBSCO host Web and Web of Science, were searched in order to review the literature. Additionally, Google Scholar was used mainly to find the full texts of the selected papers or other documents subjected to analysis. In each database the mode “Advanced search” was used in order to narrow the search to the specific criteria. The keywords “plastic”, “bioplastic”, “toxic”, “ecotoxic”, “soil”, “terrestrial ecosystem” were selected to search for the relevant data. Two or three of these keywords were joined with the help of Boolean “AND” and used in each query in each database. The basic keyword used each time was either “plastic” or “bioplastic”, whereas the other keywords mentioned above were the accompanying keywords to the

basic one. The keywords used in the query were sought in the field “Abstract”. Other criteria used in searching the databases were as follows. Timespan was set from 2010 and 2021. All types of documents but written only in English were searched. The number of results varied usually from 0 to about 850 for the basic keyword “plastic” and from 0 to about 90 for the basic keyword “bioplastic” dependent on the query and database. The results obtained in each query were initially reviewed upon the abstracts in order to find, whether their content fits to one of two aforementioned issues, i.e. either effect of (bio)plastics on the abiotic part of soil ecosystem or effect of bioplastics on the soil biota. Finally, about 70 papers published from 2010 to 2021 were selected, analysed and then described. Several older publications and up-to-date web pages of international organisations were also subjected to analysis if it was required.

The authors of the papers analysed in this review represented the countries from Europe, Asia, North and South America, Australia and Oceania. The highest contribution in the description of the impact of (bio)plastics on soil ecosystems had the authors from the following countries: China (~23%), Germany (~18%), the Netherlands (~13%), the United States of America (~10%), Mexico (~9%) and Australia (~7%).

## 3. Impact of (bio)plastics on abiotic part of terrestrial ecosystems

The occurrence of (bio)plastics in the terrestrial environment changes the chemical composition of soil and lead to the interactions between soil components and (bio)plastics particles as well as other chemicals that pollute the soil compartment. (Bio)plastic particles are extremely diverse materials, composed of many different polymers at different weathering states, and of different shapes and sizes (Browne, 2015; GESAMP, 2015; Koelmans et al., 2019).

Fibres are considered as the predominant microplastic form (up to about 92%), as followed by fragments (4.1%) (Zhang and Liu, 2018; Guo et al., 2020). Regarding the chemical composition the following plastic fibres, i.e. polyethylene (high, low, and linear low density: HDPE, LDPE, LLDPE), PP, PS, PVC, PET, PUR, PES, PA and PMMA, represent 92% of plastics ever made (Birch et al., 2020; Geyer et al., 2017). At the same time the most common types of petroleum-derived plastics reported for the environmental studies were PE, PP, PS, PVC, and PET (Rochman et al., 2013; SAPEA, 2019; Birch et al., 2020).

Taking bioplastics into account the following materials were globally produced in the highest amounts in 2020: starch blends (18.7%), PLA (18.7%), PBAT (13.5%), PA (11.9%), PE (10.5%), PTT (9.2%), PET (7.8%) and PBS (4.1%) (European Bioplastics, 2020). Most of them were biodegradable (58.1%), while still significant part (41.9%) of bioplastics was not susceptible for the biological decomposition (European Bioplastics, 2020). Starch based bioplastics belonging to the most often produced are complex blends of starch with compostable plastics such as PLA, PBAT, PBS, PCL and PHAs (Ravindra et al., 2018; Venkatachalam and Palaniswamy, 2020). Data concerning the abundance of bioplastics in the soil compartment have not been available yet. The fate of bioplastics residues depends on the methods of collection and processing of bioplastic waste, i.e. whether they are collected separately or incorporated into biowaste fraction or whether they go to the plastics fraction of waste.



With regard to processing of bioplastics waste, it should be taken into consideration that bioplastics suitable for the industrial composting (as defined according to the EN 13432 standard) are fit for the conditions in composting plants, but not for those outside in nature (<https://www.european-bioplastics.org/bioplastics/waste-management/>).

Plastics are basically carbon compounds and a hypothesis appeared that in a long perspective carbon from polymers might become a relevant pool of carbon in soils contributing to the selective pressure for soil microbes (Rillig et al., 2019). Apart from carbon atoms some types of (bio)plastics contain such elements as nitrogen (polyacrylonitrile) or fluorine (polytetrafluoroethylene) that might be included in the biogeochemical cycle (de Souza Machado et al., 2019).

As it was mentioned above (bio)plastics contain various additives that may be incorporated into the polymer in any proportions at any stage of polymerization or processing (Niaounakis, 2013). Nizzetto et al. (2016) estimated that additives make up 70% of mass of plastics. So they can be also introduced into the soil matrix and may influence physical and chemical properties of soil. The chemical composition of different types of additives was described in many handbooks and reports (e.g. Niaounakis, 2013; EPA, 2016).

In spite of the direct changes of the chemical composition of soil caused by the presence of petroleum-derived plastics and bioplastics, there are also indirect ones. Liu et al. (2017) found that the higher level (28% w/w) of PVC microparticles significantly increased the nutrient (carbon, nitrogen and phosphorus) contents of the dissolved organic matter. Moreover, the microplastic PVC facilitated the accumulation of high-molecular weight humic-like materials and fulvic acids (Liu et al., 2017). The accumulation of fulvic acids may favour the transformations, bioavailability and mobility of contaminants in soil (Liu et al., 2017; Li et al., 2018a; Guo et al., 2020).

Bioplastics susceptible for the microbiological decomposition may contribute to the decrease of nutrients content in soil (Rillig et al., 2019). Microorganisms need nitrogen, phosphorus and other elements for their metabolism to undergo biodegradation processes and as a result of these processes the deficiency of these substances might appear.

Plastics are able to adsorb hazardous contaminants, including toxic organic chemicals, heavy metals (e.g. Zn, Pb), and antibiotics such as amoxicillin, tetracycline (Wang et al., 2015; Hodson et al., 2017; Laganà et al., 2018; Li et al., 2018b; Guo et al., 2020). As a result of both adsorption properties of plastics and their mobility in soil and aquatic environments they may contribute to the changes of the antibiotics resistance profiles of bacteria. Laganà et al. (2018) reported that plastics could serve as vectors for the spread of multiple resistances to antibiotics (belonging to various groups, i.e. cephalosporins, quinolones and  $\beta$ -lactams) across Antarctic marine environments.

The changes of the chemical composition of soil caused by the presence of (bio)plastic particles induce the changes of physical soil properties and affect various soil parameters and processes like, for example, pore space, capillarity, wetting processes, bulk density, soil moisture and evapotranspiration (de Souza Machado et al., 2018; Guo et al., 2020). It is associated with the fact that micro- and nanoplastic particles can be loosely or tightly incorporated into soil matrix (Ng et al., 2018; Guo et al., 2020). The smaller the particle, the larger its surface-to-volume ratio and its reactivity, as a consequence the more dynamic behaviour of nanoparticles is observed (Wang et al., 2016; Ng et al., 2018). It was shown that the shape of plastics should be also taken into account. Fibres, particularly microfibres, affect physical properties of soil stronger than beads do (de Souza Machado et al., 2018; Rillig et al., 2019). Zhang et al. (2019) found the efficient interactions between polyester microfibres and fine soil particles in the formation and stability of macro-aggregates. At the same time Lozano et al. (2021) observed generally the decrease in soil aggregation by about 25% irrespective of the shape of microplastics (fibres, films, foam, fragments) added to the soil. However, soil aggregation was higher with PET fragments compared to PET films, and it was also higher with PP films compared to PP fibres (Lozano et al., 2021).

The soil bulk density varies with the soil type and the compaction degree (USDA, 2013). Sandy soils usually have higher bulk densities ( $1.3\text{--}1.7\text{ g cm}^{-3}$ ) than fine silts and clays ( $1.1\text{--}1.6\text{ g cm}^{-3}$ ) because they have larger, but fewer, pore spaces. The soils rich with organic compounds may have densities lower than  $1\text{ g cm}^{-3}$  (McKenzie et al., 2002; USDA, 2013). At the same time the bulk density of (bio)plastics varies widely from  $0.140$  to  $2.3\text{ g cm}^{-3}$  (<https://omnexus.specialchem.com/polymer-properties/properties/density>). PLA density is around  $1.24\text{ g cm}^{-3}$  (Yang et al., 2015; Abdullah et al., 2019). The density of the commonly produced starch-based bioplastics with the addition of PLA varies from  $1.2$  to  $1.3\text{ g cm}^{-3}$  (Abdullah et al., 2019). So the effect of (bio)plastics on soil bulk density depends on types of plastics and their concentration as well as on the type of soil. Zhang et al. (2019) reported that polyester microfibres significantly increased the volume of  $>30\text{ }\mu\text{m}$  pores and reduced the volume of  $<30\text{ }\mu\text{m}$  pores. This phenomenon may cause the changes in bulk density of soil in the presence of any plastic materials. A few studies that have been performed in the context of the effect of (bio)plastics on soil bulk density so far revealed that the presence of petroleum-derived plastics in the soil either decreased or did not alter soil bulk density (de Souza Machado et al., 2018, 2019; Zhang et al., 2019). Zhang et al. (2019) found that there were no detectable changes in the soil bulk density containing polyester microfibres at concentrations from  $0.01$  to  $0.3\%$  w/w. At the same time de Souza Machado et al. (2018) tested the soil bulk density with regard to four materials, i.e. polyacrylic fibres, polyester fibres, polyamide beads and polyethylene high-density fragments. Polyacrylic and polyester fibres were added to the soil at the concentrations from  $0.05$  to  $0.4\%$  w/w, whereas polyamide and polyethylene particles were added at the concentrations from  $0.25$  to  $2\%$  w/w. All tested materials affected soil bulk density and the dose-response relation was found for polyester fibres (de Souza Machado et al., 2018). Polyacrylic fibres and polyethylene fragments did not trigger so marked decreases in bulk density as polyester fibres did, despite these polymers possess lower density than polyester (de Souza Machado et al., 2018). In the case of polyethylene no clear trend was found. Next study published by de Souza Machado et al. (2019) showed that soil bulk density was decreased by PEHD, PES, PET, PP, and PS. All microplastics tested in this work were added at  $2.0\%$  w/w to fresh soil excluding PES that was added at  $0.2\%$  w/w to soil fresh (de Souza Machado et al., 2019). The decrease of soil bulk density may act positively leading to better soil aeration and improvement of soil productivity (Rillig et al., 2019; Lozano et al., 2021).

The presence of (bio)plastics in soil influenced water saturation and transformations in soil, and finally on water cycle in the terrestrial compartment. Wan et al. (2019) observed that plastics increased the rate of soil water evaporation by creating channels for water movement. It is in a line with the findings of de Souza Machado et al. (2019), who proved that evapotranspiration was increased by about 35% and 50% due to the presence of PA and PES, respectively. In the case of PEHD, PET, PS smaller increases in evapotranspiration were reported (de Souza Machado et al., 2019). The increase in evapotranspiration contributed to the water loss from the terrestrial compartment and finally to soil drying.

de Souza Machado et al. (2018) showed that PES fibres affected water holding capacity (WHC) of the soil, particularly compared to other microplastics studied in this work, i.e. polyacrylic fibres, PA beads and PEHD fragments. Moreover, the increase in concentrations of PES fibres in soil significantly enhanced this parameter. In another work de Souza Machado et al. (2019) confirmed that PES-treated soils substantially enhanced WHC and kept water saturation higher for longer periods. Other microplastics tested in this work, i.e. PA, PEHD, PET, PP and PS contributed to the increase of WHC as well but it was smaller in comparison to PES (de Souza Machado et al., 2019).

Regarding the effect of (bio)plastics on water cycle in the terrestrial compartment the role of plants should be also taken into account. It was found that most plastics tested by de Souza Machado et al. (2019)



**Table 2**  
Studies on the effect of (bio)plastics on plants.

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
PHBV	Commercial product. The strips of the length about 1 cm and with average thickness of $0.06 \pm 0.01$ mm were used.	<i>Lepidium sativum</i>	1 g of soil containing PHBV (1 g of PHBV) was added to 400 g of soil and subjected to biodegradation. It was mixed with water 1:5 w/w and filtered aliquot was tested.	48 h	No soil. Filtered aliquot obtained from the mixture of soil and PHBV that were initially subjected to biodegradation was tested.	OECD Test no. 208	Germination ratio	PHA biodegradation products had no influence on germination activity in soil.	<a href="#">Arcos-Hernandez et al. (2012)</a>
LDPE Starch-based biodegradable plastic (Bio)	Commercial products; LDPE MaP the average length $6.92 \pm 1.47$ mm, the average width $6.10 \pm 1.39$ mm; Bio MaP the average length $6.98 \pm 1.61$ mm, the average width $6.01 \pm 1.31$ mm LDPE MP and Bio MP the diameter from 50 to $\mu\text{m}$ to 1 mm	<i>Triticum aestivum</i>	15 g of plastic material add to 1500 g of soil	61 d 139 d	Sandy soil from the agriculture in Wageningen (the Netherlands)	The experiments in pots. Three factors were tested: types of plastics, size of plastics, presence/absence of earthworms <i>Lumbricus terrestris</i>	Plant height; Number of tillers and fruits; Plant biomass and its allocation; Number of leaves; Leaf area; Relative chlorophyll content; Stem diameter	Biodegradable plastic residues showed stronger negative effects on wheat growth than polyethylene. The presence of earthworms had an overall positive effect on the wheat growth.	<a href="#">Qi et al. (2018)</a>
HDPE Materbi® (MB)	Commercially available plastic bags. They were cut into pieces of approximately $1 \text{ cm}^2$ .	<i>Lepidium sativum</i>	Liquid (water) to solid (plastic) ratios: 100, 10 and 5.	72 h	No soil. Leachates from plastic materials and cellulose filter paper were used.	Experiments made in Petri dishes located in the culture chamber at $24 \pm 1$ °C.	Total germination; Radicle length, Hypocotyl length	Leachates from both types of materials did not affect seed germination. But, a significant number of seedlings showed developmental abnormalities or reduced seedling growth. The hypocotyl was the most sensible seedling organ to HDPE bag leachates while the radicle was the most vulnerable to MB ones.	<a href="#">Balestri et al. (2019)</a>
Green fluorescent plastic (Fluoro-Max Green Fluorescent Polymer Microspheres)	Commercially available. Nominal sizes of particles: 50; 500; 4800 nm	<i>Lepidium sativum</i>	$10^3$ , $10^4$ , $10^5$ , $10^6$ , and $10^7$ particles $\text{ml}^{-1}$	72 h	No soil. Suspension of plastic particles in distilled water	Experiment in the Petri dishes at 24 °C, at a relative humidity of >80%, and constant 6000 lx top illumination germination checked after 8 h, 24 and 72 h	Relative seed germination; Relative root growth; Relative shoot growth	No difference in germination rate occurred after 24 h of exposure but significant reduction was found after 8 h; No differences in root growth after 48 and 72 h of exposure; Reduction of	<a href="#">Bosker et al. (2019)</a>

(continued on next page)

Table 2 (continued)

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
PA, PEHD, PES, PET, PP, PS	Commercial products of different sizes: PA - 15 – 20 µm; PES - the average length 5000 µm, diameter 8 µm; PEHD - the average dimension 643 µm; PP - 647 – 754 µm; PS - 547 – 555 µm; PET - 222 – 258 µm	<i>Allium fistulosum</i> (spring onion)	0.2% of PES in the soil fresh weight; 2% in the case of all other plastic in the soil fresh weight	About 2 months (April 11–June 29, 2017) + additional 1.5 months (until August 9, 2017)	A loamy sandy soil collected at the experimental facilities of Freie Universität Berlin, Germany	Experiments in glass beakers of total volume 200 ml placed in the greenhouse.	(1) Physical soil parameters; (2) Root and leaves parameters incl. Root biomass; Leaf biomass; Root diameter and area; Root tissue density; Root colonization by mycorrhizal fungi; (3) General plant fitness	shoots at two the highest concentrations tested irrespective of the size of particles PES fibres and PA beads triggered the most pronounced impacts on plant traits and functions. Plastic particles with different properties (i.e., much larger, much smaller, or distinct constitution) might cause to very different responses in soils and plants.	de Souza Machado et al. (2019)
HDPE, PET, PVC	HDPE from shopping bags; PET from drinking bottle; PVC from tablecloth; < 2 mm	<i>Triticum aestivum</i>	HDPE, PET: 0.1, 0.25, 0.5 and 1% w/w of MWO; PVC: 0.01, 0.1, 0.25, 0.5 and 1% w/w of MWO	14 d	Three types of soil from agricultural region in New South Wales, Australia; Mixed Waste Organic Output (MWO) was added to soil	OECD Test 208	Germination index	No significant negative effect on wheat seedling emergence, wheat biomass production,	Judy et al. (2019)
PLA	Commercially available PLA	<i>Triticum aestivum</i>	From 0.1 to 5% w/w in the commercial compost	60 d	Loess soil from Huldenberg (Belgium)	Experiments made in 18-l pots located in a climate-control cell	Grain number Plant dry matter	Plant growth and seed production was not affected by PLA mixed with compost.	Huerta-Lwanga et al. (2021)
LDPE, PA, PC, PES, PET, PP, PS, PU	Commercially available microplastics of different shapes: fibres, films, foams, and fragments	<i>Daucus carota</i> (wild carrot)	From 0.1 to 0.4% w/w	28 d	Sandy loam soil from a dry grassland located in Dedelow, Germany	Experiments made in pots located in the greenhouse chamber with a daylight period set at 12 h, temperature regime at 22/18 °C day/night with a relative humidity of about 40%.	Root biomass Shoot biomass	All shapes increased plant biomass. Shoot mass increased by about 27% with fibres, ~60% with films, ~45% with foams, and by ~54% with fragments.	Lozano et al. (2021)

interacted with the plants to either increase (e.g. PES) or decrease (e.g. PA) evapotranspiration. What is more, the increases of evaporation were smaller than those of water holding capacity. Therefore, water availability was generally higher in soils treated with microplastics, which was attenuated by plants (de Souza Machado et al., 2019).

To sum up, the effect of (bio)plastics on physicochemical soil properties is a multidimensional issue that includes changes in soil chemical composition and structure (pore formation) as well as turbulences in water balance and cycle in the ecosystems. The course and results of these changes depend on many factors (e.g. chemical composition of (bio)plastics, concentration of (bio)plastics, type and composition of soil), and it is difficult to generalize them.

#### 4. Impact of (bio)plastics on terrestrial organisms

Soil biota consists of the microorganisms (bacteria, fungi, archaea and algae), plants (monocotyledoneae and dicotyledoneae) and soil animals (protozoa, nematodes, mites, springtails, spiders, insects, earthworms). Organisms representing species from different taxonomic and functional groups, for example *Lepidium sativum*, *Vibrio fischeri*, nitrifying bacteria, *Eisenia fetida*, *Folsomia candida* were employed for the evaluation of the impacts of (bio)plastics particles on terrestrial ecosystems.

The duration of ecotoxicity tests that have been made for (bio)plastics varied usually from 24 h to 60 days what allowed for the evaluation of



**Table 3**  
Studies on the effect of (bio)plastics on microorganisms and enzyme activity.

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
PHBV	Commercial product. The strips of the length about 1 cm and with average thickness of $0.06 \pm 0.01$ mm were used.	<i>Vibrio fischeri</i>	1 g of soil containing PHBV (1 g of PHBV) was added to 400 g of soil and subjected to biodegradation. It was mixed with water 1:5 w/w and filtered aliquot was tested.	48 h	No soil. Filtered aliquot obtained from the mixture of soil and PHBV that were initially subjected to biodegradation was tested.	Microtox according to ISO 11348-3	EC10 and EC50	Soil extracts examined during polymer degradation were non-toxic to <i>Vibrio fischeri</i> .	<a href="#">Arcos-Hernandez et al. (2012)</a>
PS	Commercial product. Polystyrene nanoparticles (PS-NPs).	Soil microbiota	10; 100 and 1000 ng PS-NP g <sup>-1</sup> dry weight	28 d	Soil collected at Helenenberg, NW of Trier, Germany	Experiment made in glass beakers incubated at 18 °C in the dark.	Biomass concentration; Metabolic quotient; Basal respiration; Dehydrogenase activity; Activity of enzymes representing major pathways of C-, N-, and P-cycling in soil	PS-NPs exhibit antimicrobial activity in the soil environment.	<a href="#">Awet et al. (2018)</a>
HDPE PET PVC	HDPE from shopping bags; PET from drinking bottle; PVC from tablecloth; < 2 mm	Soil microbial functions	HDPE, PET: 0.1, 0.25, 0.5 and 1% w/w of MWO; PVC: 0.01, 0.1, 0.25, 0.5 and 1% w/w of MWO	28 d	Three types of soil from agricultural region in New South Wales (Australia); Mixed Waste Organic Output (MWO) was added to soil	OECD Test 216 OECD Test 217	Carbon or Nitrogen transformation, i.e. Substrate Induced Respiration (SIR) and Substrate Induced Nitrification (SIN), respectively.	A little effect of microplastics on SIR and SIN. The only decrease in SIR relative to control was observed in the 0.25% w/w HDPE treatment. A high degree of variability with regard to the SIN values.	<a href="#">Judy et al. (2019)</a>
PP	Commercial product; <180 µm	Enzymes activity: Fluorescein diacetate hydrolase (FDAse); Phenol oxidase (PO); Chemical composition of soil	7% w/w 28% w/w	30 d	Soil was collected in Ansai County (China)	Experiments in the climate-controlled chamber	Enzyme activity expressed in mg kg <sup>-1</sup> h <sup>-1</sup>	Microplastic stimulated the activities of both enzymes (FDA and PO). Microplastic addition stimulated enzymatic activity, activated pools of organic C, N, and P, and was beneficial for the accumulation of dissolved organic C, N and P.	<a href="#">Liu et al. (2017)</a>
Mater-Bi DF04P	Material based on corn starch and biodegradable copolyester (Novamont). The form of material used in the tests was powder received by cryogenic grinding of mulch film.	Mixed cultures of nitrifiers	10 g of polymer added to 800 g of soil	29 d	Soil from agricultural field in Albegna (Italy). Soil was prepared in agreement with ISO 17556	ISO 14238 (2012)	Concentrations of ammonium (N-NH <sub>4</sub> <sup>+</sup> ) and nitrates (N-NO <sub>3</sub> <sup>-</sup> ) nitrogen in time	No inhibition of the nitrification potential of the soil.	<a href="#">Bettas Ardisson et al. (2014)</a>

(continued on next page)

Table 3 (continued)

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
PLA	Commercially available. Processed to receive the film of the thickness $0.02 \pm 0.01$ mm	Mixed cultures of nitrifiers	8 g of PLA added to 400 g of soil	29 d	Garden soil from Michigan State University (US)	ISO 14238 (2012)	Concentrations of ammonium ( $\text{N-NH}_4^+$ ), nitrites ( $\text{N-NO}_2^-$ ) and nitrates ( $\text{N-NO}_3^-$ ) nitrogen in time	No effect of PLA on the soil nitrification	Satti et al. (2018)

potentially adverse effects of (bio)plastics on exposed organisms (Tables 2, 3, 4 and 5). Most of them were the ecotoxicological laboratory tests being a fundamental tool for the assessment of the ecological risks posed by (bio)plastics polluting the soil environment, although typically in the laboratory conditions the effects on the exposed organism are more severe than those observed in the field conditions. Ecotoxicological studies on (bio)plastics have been mainly made towards individual species, whereas multispecies tests are much less frequently met. In order to describe and quantify the impacts of (bio)plastics on soil biota a variety of endpoints were applied. These were lethality, growth, reproduction, enzyme activity and other metabolic and behavioural responses. The ecotoxicity experiments were performed according to four different scenarios as it was shown in Fig. 1. In scenarios 1 and 2 direct impact of (bio)plastics particles or the products of their degradation on soil organisms were tested respectively, whereas in scenarios 3 and 4 the impact of leachates obtained from (bio)plastics and leachates containing products of (bio)plastics degradation on soil biota was studied (Fig. 1). Below the results of the studies on ecotoxicity of (bio)plastics towards soil organisms that were published from 2012 to 2021 are presented.

#### 4.1. Effect of (bio)plastics on plants

Evaluation of phytotoxicity of chemicals is a crucial component of the ecological risks assessment because primary producers form the essential trophic level of any ecosystem (Hoffman et al., 2003). Studies concerning the effect of (bio)plastics on terrestrial plants were performed towards monocotyledoneae (*Triticum aestivum*, *Allium fistulosum*) and dicotyledoneae (*Lepidium sativum*) plants (Table 2). The plants used as indicators grew in the soil or were cultivated in hydroponic cultures.

Neither the presence of (bio)plastics microparticles in soil nor the products of leaching of (bio)plastics had any effect on the germination of seeds (Qi et al., 2018; Balestri et al., 2019; Judy et al., 2019). For example, Judy et al. (2019) reported that the addition of one out of three microplastics (HDPE, PET, PVC) to the soil did not have an effect on the emergence of the wheat seedlings (Table 2). Germination ratio of the seedlings was close to 100% irrespective of the treatment (Judy et al., 2019). The same was also observed for the biodegradation products of (bio)plastics. Arcos-Hernandez et al. (2012) found that the germination ratio was at the level from 95 to 100% in the case of cress (*Lepidium sativum*) seeds exposed to the biodegradation products of PHA for 48 h (Table 2). So it was documented that this stage of plant growth was independent of the external substrate (Milberg and Lamont, 1997; Balestri et al., 2019). Simultaneously, it was observed that up to 40% of the seeds of *Lepidium sativum* germinated in the presence of plastic leachates showed such developmental abnormalities as a short or stubby radicle, twisted hypocotyl and malformed and/or supernumerary cotyledons (Balestri et al., 2019). It concerned both types of (bio)plastics tested, i.e. HDPE and compostable plastics based on starch and vinyl-alcohols copolymers (Balestri et al., 2019). Bosker et al. (2019) reported that green fluorescent plastic particles significantly reduced germination of *Lepidium sativum* seeds in the first hours of the experiment. This phenomenon was observed after 8 h of exposure for all three sizes of plastics (50, 500 and 4800 nm) and the adverse effect

increased with the increase of plastics sizes (Bosker et al., 2019). However, no difference in germination rate occurred after 24 h of exposure, regardless of the size of the plastics used. These data indicate that it was worth observing the early stages of the germination of seeds exposed to (bio)plastics. The effect on the early stages of the germination was most probably caused by the physical blockage of the pores in the seed capsule by microplastics, which was confirmed by microscopic observations with the use of confocal microscope (Bosker et al., 2019).

The impact of (bio)plastics on growth of plant's organs (roots, stems etc.) and total plant biomass varied depending mainly on the chemical composition of the material tested. De Souza Machado et al. (2019) found that all tested microplastics (PA, PEHD, PES, PET, PP and PS) contributed to the increase in total root length and simultaneously decreased the average root diameter (Table 2). In this study PES made 0.2% of the soil fresh weight, whereas in the case of all other plastics it was 2% (de Souza Machado et al., 2019). PS and PES triggered the significant increase in root biomass of spring onion (de Souza Machado et al., 2019). PA also caused to the increase in root biomass but to a lower extent than PS and PES did (de Souza Machado et al., 2019). At the same time PEHD, PET, and PP exerted a weak effect on the root biomass and the values of root biomass obtained in the tests with these microplastics were comparable to the ones from the control tests (de Souza Machado et al., 2019).

Qi et al. (2018) studied two materials, i.e. LDPE and starch-based biodegradable plastics, at the concentration in the soil equal to 15 g per 1500 g (Table 2). LDPE and starch-based biodegradable plastics tested in two sizes, i.e. micro- and macroparticles, contributed to the decrease of root biomass of wheat (*Triticum aestivum*), which was particularly visible at the 2 months harvest (Qi et al., 2018). What is interesting, the presence of earthworms increased the root biomass and diminished the differences in root biomass between the tests with plastics and without plastics addition (Qi et al., 2018). Shoot biomass was significantly lowered by the presence of starch-based plastics, particularly at the 2 months harvest, whereas in the case of LDPE (micro- and macroparticles) no significant differences compared to controls were found (Qi et al., 2018). It shows that biodegradable plastics may inhibit stronger the shoots growth than the conventional petroleum-derived one (LDPE).

Regarding the plant height it was found that the presence of either LDPE or starch-based biodegradable plastics in soil did not affect this parameter at the 4 months harvest (Qi et al., 2018). However, both micro- and macroparticles of starch-based biodegradable plastics inhibited the wheat growth (plant height) during the tillering stage of growth (from around 14 day to 40 day of harvesting), while LDPE micro- and macroparticles did not show any effect on it (Qi et al., 2018). At the same time total plant biomass of wheat was reduced by the addition of plastics (LDPE or starch based plastic) micro- or macroparticles and the lowest values of this parameter were found in the experiments with microparticles of starch-based biodegradable plastics (Qi et al., 2018). The presence of earthworms contributed to the increase in total plant biomass for wheat by 20.9% at the 2 months harvest and 26.2% at the 4 months harvest (Qi et al., 2018). At the same time none of microplastics (PA, PEHD, PES, PET, PP, PS) decreased total plant biomass of spring onion (de Souza Machado et al., 2019). Most of these



microplastics caused to the increase in total plant biomass (de Souza Machado et al., 2019). The most significant increase was noticed in the case of PA and PES (de Souza Machado et al., 2019). Judy et al. (2019) also found that the wheat biomass was unaffected by the presence of the microplastics (HDPE, PET, PVA) in soil (Table 2). The similar finding was reported by Huerta-Lwanga et al. (2021) for PLA (Table 2). Lozano et al. (2021) observed the increase in plant biomass in the experiments with *Daucus carota* exposed to microplastics of different shape, i.e. fibres, films, fragments, foams for 28 days (Table 2). What is more, it was stated that the increase of plant biomass was independent of microplastics shape (Lozano et al., 2021). No clear relationship between the concentrations of microplastics studied in the range from 0.1 to 0.4% w/w and shoot or root biomass was determined (Lozano et al., 2021). Lozano and Rillig (2020) found that root and shoot mass increased in the presence of microfibrils, which was most probably connected with the reduction of soil bulk density, improvement of soil aeration, and better penetration of roots in the soil.

Balestri et al. (2019) reported that leachates from plastics tested (HDPE or compostable plastics) contributed to the reduction of radicles as well as hypocotyl length of garden cress (Table 2). It was observed regardless of plastic type, exposure and pollution degree (Balestri et al., 2019). The hypocotyl was the most sensible seedling organ to HDPE bag leachates, while the radicle was the most vulnerable to the ones from the compostable plastic bags. Analysing the effect of (bio)plastics leachates it is important to take care about the products that are released from plastics to liquid phase. They may contain additives, by-products as well as the products of degradation. Balestri et al. (2019) identified twelve compounds in the virgin plastic bag leachates. These were inter alia bisphenol A and linear long-chain alkanes and alkenes in HDPE leachates, and 1,6-dioxacyclododecane-7,12-dione and free butane-1,4-diol in the leachates from the compostable plastics. Thus, in order to obtain the full picture of the possible impacts of (bio)plastics on plants, the effect of (bio)plastics' leachates on plants growth should be tested as well.

Bosker et al. (2019) found that the clusters of plastic particles were especially accumulated on the root hairs but they were also found on the leaves and epidermis. Nevertheless, it did not cause the reduction of root growth of *Lepidium sativum* exposed to green fluorescent plastic particles (50 nm, 500 nm and 4800 nm) for 48 h or 72 h (Bosker et al., 2019). After 24 h of the exposure the significant differences in root growth were observed, i.e. the decrease in root growth when exposed to 50 nm particles, and the increase when exposed to 500 nm plastic particles (Bosker et al., 2019). Shoots growth was inhibited at two highest concentrations ( $10^6$  and  $10^7$  particles  $\text{mL}^{-1}$ ) in particular for the particles of 500 nm and 4800 nm (Bosker et al., 2019).

The results of phytotoxicity tests were often inconsistent and devoid dose-response relationships. Nevertheless, it occurred that both petroleum-derived plastics and bioplastics including biodegradable ones represent a potential threat to the primary producers. More studies regarding particularly biomicroplastics and the comparison of their effect on plants growth with conventional microplastics are required.

#### 4.2. Effect of (bio)plastics on soil microorganisms and their activity

Biochemical activity of soil is directly related to the number and activity of the soil microbiota (e.g. microbial biomass) as well as it is associated with the decomposition of organic compounds present in soils and the release of nutrients (Trasar-Cepeda et al., 2008). Thus, both the number and activity of microorganisms, and the activity of enzymes produced by microorganisms were used in order to describe the effect of (bio)plastics on soil microbiota (Table 3). Soil enzymes play a key role in controlling the cycling of soil nutrients such as C, N and P (Trasar-Cepeda et al., 2008; Liu et al., 2017). In particular soil microbial nitrification is widely used and it is regarded as a good bioindicator in the evaluation of the impact of chemicals on soil (Table 3).

Fluorescein diacetate hydrolase (FDAse) represents overall microbial metabolic activity and is an effective indicator of short-term changes of soil quality (Muscolo et al., 2014; Liu et al., 2017). In general, the addition of microplastics (PP) to the soil at concentrations 7% w/w or 28% w/w caused to the increase of FDAse activity (Liu et al., 2017). At the lower tested (7% w/w) concentration of microplastics in soil no significant effect on the destruction of dissolved organic matter (DOM) during the first seven days was observed, but between days 7 and 30 the rate of DOM decomposition decreased what contributed to the increase in the nutrient contents (Liu et al., 2017). At the higher concentration of microplastics (28% w/w) the nutrients content of the dissolved organic matter (DOM) solution increased (Liu et al., 2017).

Judy et al. (2019) used substrate-induced respiration (SIR) and substrate-induced nitrification (SIN) in order to test the effect of microplastics (HDPE, PET, PVC) on carbon and nitrogen transformations in soil (Table 3). Three soils of different composition and properties from three locations were used (Judy et al., 2019). The exposure time was 28 days (Judy et al., 2019). Both SIR and SIN revealed inconsistency in trends and the high degree of variability and as a result no dose-dependence in the relationship between the amounts of microplastics spiked into the soils and the effect on SIR or SIN was found (Judy et al., 2019). It was concluded that there was a weak effect of microplastics (HDPE, PET, PVC) on SIN or SIR (Judy et al., 2019). Microbial community diversity was not significantly affected by the addition of microplastics to the soil either, although the results of the analysis of microbial community were highly variable (Judy et al., 2019).

Awet et al. (2018) tested the effect of plastic (PS) nanoparticles at concentrations 10, 100 and 1000  $\text{ng g}^{-1}$  soil dry weight on microbial biomass and activity in soil environment for 28 days (Table 3). PS nanoparticles (PS NPs) revealed the potential antimicrobial activity, however the obtained results occurred to be inconclusive. PS NPs negatively affected the activities of enzymes involved in C, N and P transformations in soil and, to a low extent, microbial biomass, while the basal respiration rate and metabolic quotient increased (Awet et al., 2018).

Biodegradable plastics such as PLA or materials based on corn starch were also tested towards their effect on soil microorganisms. These studies mainly focused on soil microbial nitrification. Satti et al. (2018) observed that ammonium was depleted at the equal rate in the nitrification tests irrespective of PLA addition to the soil (8 g of PLA to 400 g of soil). The rates of formation of nitrates were also similar in all soils tested (with and without PLA) (Satti et al., 2018). The amount of newly formed  $\text{N-NO}_3$  was fairly consistent with the amount of  $\text{N-NH}_3$  added initially which indicated almost complete quantitative conversion of ammonium to nitrate (Satti et al., 2018). Thus, it was concluded that PLA biodegradation did not affect soil microbial nitrification (Satti et al., 2018). Bettas Ardisson et al. (2014) studied a biodegradable plastic material based on corn starch and biodegradable copolyesters (Table 3). This material was added to the soil (8 g of biodegradable plastics to 800 g of soil) and mixed cultures of nitrifiers were exposed to it for 29 days (Bettas Ardisson et al., 2014). It occurred that the ammonium was completely converted irrespective of the addition of biodegradable plastics to soil, and the depletion rate of ammonium nitrogen in soil that had been supplemented with the biodegradable plastics was even higher than that in the control tests (Bettas Ardisson et al., 2014). The amount of newly formed  $\text{N-NO}_3$  was fairly consistent with the amount of  $\text{N-NH}_4$  added to the soils, indicating that the added ammonium nitrogen was totally transformed into  $\text{N-NO}_3$  by means of nitrification (Bettas Ardisson et al., 2014). It proved that the addition of biodegradable plastics did not exert an inhibitory effect on the nitrification activity of soil (Bettas Ardisson et al., 2014). Arcos-Hernandez et al. (2012) studied the rates of biodegradation of PHBV in a 48 h soil test and the effect of their degradation products on microbial activity (Table 3). Soil extracts examined during the degradation of PHBV were not toxic towards bacteria *Vibrio fischeri* (Arcos-Hernandez et al., 2012).

**Table 4**  
Studies on the effect of (bio)plastics on earthworms.

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
PUF Poly-brominated diphenyl ether (PBDE)	Commercial, microparticles <75 µm	<i>Eisenia fetida</i>	1:2000 w/w of PUF in artificial soil	7, 14 and 28 days	Artificial soil	Direct exposure. Experiment in jars.	Bioaccumulation; Bioturbation; Accumulation Factor (BSAF)	<i>E. fetida</i> in contact with soil containing PUF accumulate substantial amount of PBDEs	Gaylor et al. (2013)
LDPE	Commercial, microparticles from <50 to 150 µm	<i>Lumbricus terrestris</i>	7; 28; 45; 60% w/w of LDPE in plant litter	4, 14 and 60 days	Plant litter and sandy soil	Direct exposure. OECD guidelines (1984)	Mortality (LD50), Growth rate ( $k_{gr}$ ); Ingestion rate (IR <sub>g</sub> ); Cast concentration factor (CF)	Combined effect Increase of mortality and reduction of growth rate with the increase of LDPE concentration. LDPE was concentrated in cast especially at the lowest of concentrations tested. The highest BE at the concentration of 7% of LDPE. MPI increased with the increase of LDPE concentration. No effect on burrow length and volume	Huerta Lwanga et al. (2016)
LDPE	Commercial, microparticles from <50 to 150 µm	<i>Lumbricus terrestris</i>	7; 28; 45; 60% w/w of LDPE	14 days	Plant litter and sandy soil	Direct exposure. Mesocosm test	Growth rate ( $k_{gr}$ ); Burrow formation; Burrow volume; Microplastics incorporation rate (MPI); Bioturbation efficiency ratio (BE)		Huerta Lwanga et al. (2017a)
LDPE	Plastic waste from home/garden; Microplastic (MP) <5 mm and macroplastics (MaP) from 5 to 150 mm	Earthworms and <i>Gallus domesticus</i>	74.4 ± 20.4 of PE bottles m <sup>-2</sup> and 7.4 ± 6.5 particles m <sup>-2</sup> of MaP on the surface	About 8 months	Garden soil	Study site: home gardens of 50 × 50 m in Mexico Samples were taken from soil, earthworms and chicken feces and chicken crop	MP concentration ratios soil/chicken feces (C <sub>CS</sub> ); MP concentration ratio soil/chicken gizzard (CMP <sub>g</sub> ); MaP concentration ratio soil surface/chicken gizzard and crop (CMap <sub>g</sub> )	MP concentration increased from soil to earthworm cast to chicken feces. MP and MaP were capable of entering terrestrial food webs.	Huerta Lwanga et al. (2017b)
PE	Commercial product spherical microparticles of different sizes: 710–850 µm, 1180–1400 µm, 1700–2000 µm and 2360–2800 µm	<i>Lumbricus terrestris</i>	750 mg of PE added to 2.5 kg of soil	21 d	Soil from a meadow in Berlin (Germany)	Experiments were carried out in pots located in an air-conditioned greenhouse with a temperature of 20 °C (±2 °C).	Earthworms mortality and change in body mass; Transport of microplastic particles	Earthworms transported microplastics in soil via burrows, egestion and adherence to the earthworm exterior. Smaller PE particles were transported downward to a greater extent. No effect on body mass and earthworms mortality.	Rillig et al. (2017a)
PE	Commercial product. Pellets of diameter of 5000 µm fragmented to the size from 250 to 1000 µm	<i>Eisenia andrei</i>	62.5; 125; 250; 500 and 1000 mg kg <sup>-1</sup> of dry soil	28 d 56 d	OECD artificial soil	OECD 222 guidelines (2004)	Number, weight and reproduction ability of earthworms.	No significant effects were recorded on survival, number of juveniles and, in the final weight of adult earthworms after 28d of exposure	Rodriguez-Seijo et al. (2017)
HDPE PET PVC	HDPE from shopping bags; PET from drinking bottle; PVC from tablecloth; < 2 mm	<i>Eisenia fetida</i>	HDPE, PET: 0.1, 0.25, 0.5 and 1% w/w of MWOO; PVC: 0.01, 0.1, 0.25, 0.5 and 1% w/w of MWOO	48 h – avoidance test; 28 d – mortality test; 56 d – reproduction test	Three types of soil from agricultural region in New South Wales (Australia); Mixed Waste Organic Output (MWOO) was added to soil	ISO method 17512–1 OECD Test 222	Avoidance of earthworms (%); Mortality and reproduction of earthworms (no. of juveniles)	No significant negative effect on earthworm growth, mortality or avoidance behaviour.	Judy et al. (2019)
LDPE PS	Commercial products; LDPE<300 µm; PS ≤ 250 µm	<i>Eisenia fetida</i>	1; 5, 10 and 20% w/w plastics in the soil (ingestion tests); 0.1; 1; 5 and 10%	14 d 28 d	A clean agriculture soil (sandy loam) from Southern California (US)	Ingestion and bioaccumulation experiments in glass beakers of total volume 200 ml.	Presence of LDPE/PS particles in the earthworms excrete; Enzymes activity; The tissue concentrations of PAH and PCB	Ingestion of PE and PS particles by <i>E. fetida</i> was confirmed. No oxidative stress observed at the amendment rates of LDPE or PS to the soil at the level ≤ 10% w/w. Microplastics did not act as a carrier to	Wang et al. (2019)



LDPE	Commercial product; <150 µm;	<i>Lumbricus terrestris</i>	w/w in the soil (bioaccumulation tests) 1%, 3% and 7% dry weight	14 d	Sandy soil (26.6% brown sand, 24% silver sand and 50% loamy silt with 0.2% organic matter) Glyphosate was added to soil.	Tests were made in the mesocosm box (300 × 405 × 30 mm). OECD Test no. 207	Gallery volume and weight; Glyphosate content at different soil layers.	Earthworm gallery weight was negatively affected by the combination of glyphosate and microplastics. Earthworm activities strongly influence pollutant movement into the soil, which potentially affects soil ecosystems	Yang et al. (2019)
PS	Fluorescent polystyrene microplastics (PS-MPs) of size 100 nm and 1300 nm	<i>Eisenia fetida</i>	100 and 1000 mg kg <sup>-1</sup> dry weight	14 d	Artificial soil	Tests were made in 2 l glass beakers incubated at 20 ± 2 °C	Mortality; Accumulation in the earthworms intestines; Histopathological changes and DNA damage.	Average accumulated concentrations in the earthworm intestines were higher for 1300 nm PS-MPs. The exposure to PS-MPs induced DNA damage in earthworms. 1300 nm PS-MPs showed more toxic effect than 100 nm PS-MPs on earthworms.	Jiang et al. (2020)
PLA	Commercially available PLA	<i>Lumbricus terrestris</i>	From 0.1 to 5% w/w in the commercial compost	14 d 60 d	Loess soil from Huldenberg (Belgium)	Modified OECD 222 Modified OECD 207	Mortality; Biomass change	Mortality found at the concentrations 0.75% and 1% w/w. No significant differences in biomass changes	Huerta-Lwanga et al. (2021)

The studies carried out so far have shown that (bio)plastics usually did not affect biochemical activity of microorganisms, in particular nitrifying bacteria. However, the number of tested materials, both petroleum-derived plastics and bioplastics, is very limited (Table 3). Hence, more data in this area of ecotoxicological studies on (bio)plastics are required.

#### 4.3. Effect of (bio)plastics on earthworms

Earthworms are soil ecosystems engineers taking part in soil formation and decomposition of organic matter. They are regarded as key mediators of such soil functions as production, support and regulations. Therefore, earthworms have been predominantly used in the ecotoxicity tests aiming at the evaluation of the effect of (bio)plastics on soil biota. The most often used test species are *Eisenia fetida*, *Eisenia andrei* and *Lumbricus terrestris*. Chae and An (2018) found that the majority of studies (about 60%) concerning the adverse effect of various microplastics on soil organisms were performed with earthworms as test species. Until the half of 2021 the contribution of studies about the impact of (bio)plastic particles on earthworms was decreased to about 39% (11 out of 28). Earthworms are very appropriate organisms for studying the impact of macro-, micro- or nanoparticles on soil animals because they are able to ingest, accumulate and transport them in soil ecosystems. The role of earthworms in the transportation of plastic particles and the mechanisms responsible for this process as well as the effect of plastics on earthworms' mortality and growth were deeply studied (Table 4). In most works commonly produced polymers, i.e. PE or LDPE, being also the most commonly encountered in the environment, were used (Table 4). At the same time there are hardly any data, only one study published by Huerta-Lwanga et al. (2021), concerning the bioplastics and their impacts on earthworms.

Rillig et al. (2017a) designed experiments aiming at the evaluation of potential transport of surface-deposited microplastic (PE) particles of various sizes by the activity of *Lumbricus terrestris* (Table 4). PE particles were transported by anecic earthworms downward into a soil profile from the surface to a depth of 10 cm and the smallest particles were found the most in deeper layers (Rillig et al., 2017a). The mechanisms of transport were dependent on particles size and comprised attachment to the outside of the earthworm, movement down the burrows with water, casting activity, and movement by the earthworm following crossing through the intestine (Rillig et al., 2017a). Smaller particles (from 710 to 1400 µm) were present in the casts, whereas the higher ones (1700–2800 µm) were not noticed there (Rillig et al., 2017a). Smallest particles of microplastics were moved proportionally the most. Huerta Lwanga et al. (2017a) made similar observations. They found that smaller particles (≤50 µm) were more mobile, bioavailable and efficient in taking up toxic chemicals and transferring those to the food web than the bigger ones of size 63–150 µm (Huerta Lwanga et al., 2017a). It proves that the fragmentation of (bio)plastics promotes their transport and spread in the soil compartment.

Biogenic transportation and concentration of microplastics (LDPE) in the casts were observed by Huerta Lwanga et al. (2016) too (Table 4). Ingestion rate of LDPE particles by *Lumbricus terrestris* increased with the increase of LDPE concentration and was higher in treatment with 45% compared to 7% microplastics (Huerta Lwanga et al., 2016). Also the incorporation rate of LDPE microparticles by *L. terrestris* increased with the increase of microplastics concentration in the litter from 7% to 60% w/w (Huerta Lwanga et al., 2017a). At the same time concentration factor (CF) expressed as the ratio of microplastics in the casts to microplastics in the litter, was 2 for the casts from earthworms exposed to the litter with the concentration 7% microplastics, while at higher concentrations of microplastics (28%, 45%, and 60% w/w) it was significantly lower and did not exceed 1.2 (Huerta Lwanga et al., 2016). Bioconcentrated and expelled in the casts plastic particles were forming the burrow walls (Huerta Lwanga et al., 2017a). Bioturbation by *L. terrestris* in the soil defined as the

**Table 5**

Studies on the effect of (bio)plastics on soil fauna other than earthworms.

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
PS	Commercial products. Nanoparticles (NPs) of size 100 and 500 nm; Microparticles (MPs) 1, 2 and 5 $\mu\text{m}$	<i>Caenorhabditis elegans</i>	1 mg l <sup>-1</sup>	3 days	No soil	Survival assay; Lifespan assay; Motor behaviour assay; Cholinergic neuron assay; Oxidative damage assay; Antioxidant experiments	Survival rate; Body length; Lifespan; Body bends; Head trashes; cholinergic neurons; Oxidative damages	The moderate-sized, i.e. 1.0 $\mu\text{m}$ polystyrene particles, resulted in the biggest toxicity on the survival, development and motor-related neurons in nematodes. The exposure to NPs and MPs accelerated the frequency of body bending and head thrashing, and increased crawling speed	Lei et al. (2018)
PVC	Commercial product from 80 to 250 $\mu\text{m}$ in diameter	<i>Folsomia candida</i>	1 g kg <sup>-1</sup> of dry soil	28 d 56 d	Natural soil	Experiments in the glass cylinders. Preincubation procedure for collembolans applied.	Growth, reproduction and isotope composition and the gut microbiota of the collembolans	Collembolan growth and reproduction were significantly inhibited by 16.8 and 28.8%, respectively.	Zhu et al., 2018
PE	Commercial products of the size from below 50 to 500 $\mu\text{m}$	<i>Folsomia candida</i>	0.5 and 1% w/w (the avoidance experiments) 0.005, 0.02, 0.1, 0.5 and 1% w/w (reproduction experiments)	28 d	Artificial soil	ISO 17512-2 ISO 11267	Avoidance rate; Mortality rate; Reproduction	Springtails exhibited avoidance behaviours at 0.5% and 1% MPs, and the avoidance rate was 59% and 69%, respectively. Reproduction was inhibited when the concentration of MPs reached 0.1%. EC50 for reproduction was 0.29%.	Ju et al. (2019)
HDPE PET PVC	HDPE from shopping bags; PET from drinking bottle; PVC from tablecloth; < 2 mm	<i>Caenorhabditis elegans</i>	HDPE, PET: 0.1, 0.25, 0.5 and 1% w/w of MWOO; PVC: 0.01, 0.1, 0.25, 0.5 and 1% w/w of MWOO	24 h – mortality 72 h – reproduction	Three types of soil from agricultural region in New South Wales (Australia); Mixed Waste Organic Output (MWOO) was added to soil	ISO method 10,872	Mortality and reproduction of nematodes	No significant negative effect on nematode mortality or reproduction.	Judy et al. (2019)
PE PS	Commercial products subjected to laboratory treatment. Two forms were studied: Plastic macro beads (PMB) of sizes 0.47–0.53 $\mu\text{m}$ , 27–32 $\mu\text{m}$ , and 250–300 $\mu\text{m}$ and plastic microfragments (PMF) with particle sizes of <45 $\mu\text{m}$ , 45–200 $\mu\text{m}$ , and > 200 $\mu\text{m}$ .	<i>Lobelia sokamensis</i>	4 mg kg <sup>-1</sup> ; 8 mg kg <sup>-1</sup> and 1 g kg <sup>-1</sup> in the plastic contaminated soil. 10 mg l <sup>-1</sup> and 20 mg l <sup>-1</sup> in a plastic solution.	3 min for the observations made in the plastic contaminated soil; 10 s for the observations made in a plastic solution.	LUFA (Germany) standard soil type no. 2.2.	Experiments in glass slides or glass Pasteur tube. Microscopic observations of springtails behaviour.	Movement index; Movement velocity	Behaviour of plastic particles in the soil not only disrupts the movement of springtails but also has wider implications for effective management of soils	Kim and An (2019)
PS	Commercial products. Nanoparticles of size 50 and 200 nm	<i>Caenorhabditis elegans</i>	17.3 mg l <sup>-1</sup> and 86.8 mg l <sup>-1</sup> for both sizes, selected after the calculation of LC50 by the lethality test.	24 h 5 days	No soil	Lethality tests. Nematodes exposed to nanoplastic solutions for 24 h at 20 °C. Reproduction assay (5 d). Reactive oxygen species	LC50; the total progeny; the average progeny; the body band; the oxidative images; identification of metabolites	Locomotion and reproduction of <i>C. elegans</i> were decreased by exposure to nanopolystyrene particles. The nanoparticles also induced oxidative stress. These results	Kim et al. (2019)



Table 5 (continued)

Tested material		Species	Experimental conditions			Method	Endpoint	Results	Reference
Polymer type	Origin, form and size		Conc.	Duration	Media				
HDPE LDPE PAN PET PP PS	Most of them available commercially; Microparticles of different size (<250, 250–630, and 630–1000 $\mu\text{m}$ )	<i>Caenorhabditis elegans</i>	From 0.1% to 1% w/w	24 h	Soil collected from Linde, Märkisch Luch, Germany	assay; Locomotion assay.  Methodology based upon ISO 10872:2010 and ASTM 2001: E2172–01	Number of offspring (% control)	suggested that nanopolystyrene had toxic effects on <i>C. elegans</i> , including the disruption of energy metabolism and induction of oxidative stress. PAN and PET showed the highest toxicity, while HDPE, PP and PS induced relatively less adverse effects on nematodes, LDPE induced no acute toxicity at the tested range of concentrations.	Kim et al. (2020)
PET	Commercial product. The average length and diameter of microfibres (MFs) were 1257.8 $\mu\text{m}$ and 76.3 $\mu\text{m}$ , respectively.	<i>Achatina fulica</i>	0.01 – 0.71 $\text{g kg}^{-1}$	28 d	Cultivation soils collected from the campus of East China Normal University (China) mixed 2:1 with sand and the aerated water.	Digestion kinetics experiments in glass containers. Assay for food intake, excretion and shell changes.	Accumulation of MFs, The length and diameter of shells; The daily averages food intake and excrement amount	Digestion kinetics experiments on 24 snails showed that MFs can be ingested and excreted within 48 h. Prolonged exposure to 40 snails showed that 0.14–0.71 $\text{g kg}^{-1}$ MFs caused an average reduction of 24.7–34.9% food intake and 46.6–69.7% excretion. MFs have adverse impacts on the fitness of soil organisms, and highlight the ecological risks of microplastic pollution in terrestrial ecosystems.	Song et al. (2019)

ratio of the amount of microplastics (mg) present on the soil surface to the amount of microplastics (mg) present inside the burrows was significantly higher in the experiment at the concentration of 7% surface microplastics (LDPE) compared to those with 28, 45 and 60% microplastics on the surface (Huerta Lwanga et al., 2017a). The reason why at the concentration of 7% microplastics, the bioconcentration of LDPE particles in the casts and bioturbation were higher than it was observed at other concentrations of microplastics (28%, 45%, 60% w/w) requires still explanation.

Biogenic transportation of (bio)plastic particles may affect the movement of other pollutants present in soil compartment. Yang et al. (2019) proved that LDPE microplastics might influence pollutant movement into the soil (Table 4). However, the contribution of microplastics to the transport of pollutant (glyphosate) still requires studies, particularly in the conditions of high levels of microplastics added (Yang et al., 2019).

Microplastics are also accumulated and transferred in the food chain. Jiang et al. (2020) demonstrated that the size of microparticles added to soil at concentrations 100 or 1000  $\text{mg kg}^{-1}$  soil dry weight influenced the accumulation of plastics in the earthworms. PS particles of 1300 nm were accumulated in the earthworms' intestine at higher concentrations than PS particles of 100 nm (Jiang et al., 2020). Huerta Lwanga et al. (2017b) examined the consumption of PE microparticles and macroparticles by earthworms and chicken (*Gallus Gallus*

*domesticus*) in ten home gardens located in Mexico (Table 4). Microplastics concentrations increased from soil ( $0.87 \pm 1.9$  particles  $\text{g}^{-1}$ ), to earthworm casts ( $14.8 \pm 28.8$  particles  $\text{g}^{-1}$ ), to chicken feces ( $129.8 \pm 82.3$  particles  $\text{g}^{-1}$ ) (Huerta Lwanga et al., 2017b). Macroparticles of PE (1–10 mm) were also found in the chicken organs (i.e. gizzards) that might pose a risk to human health (Huerta Lwanga et al., 2017b). These results proved that micro- and macroplastics could be incorporated into the terrestrial food web.

Wang et al. (2019) reported the ingestion of microplastic particles, i.e. LDPE and PS, by earthworms, too (Table 4). In this study *Eisenia fetida* that is much smaller in comparison to the large earthworm species *Lumbricus terrestris* was used as test species (Wang et al., 2019). It proved that other small soil invertebrates, such as enchytraeids, nematodes or even termites might be able to ingest and transport micro- and nanoplastics (Maaß et al., 2017; Wang et al., 2019). Gaylor et al. (2013) showed that the presence of PUF microparticles in soil might enhance accumulation of polybrominated diphenyl ether (PBDE) flame retardants in the organisms ingesting soil (*Eisenia fetida*).

Mortality, reproduction and growth of earthworms depend primarily on the concentration of plastic particles in soil. In general at concentrations of microplastics up to 1000  $\text{mg kg}^{-1}$  no effects on mortality and reproduction were reported. Rillig et al. (2017a) observed no effect of microplastics (PE) on the survival as well as on the weight of *Lumbricus terrestris* exposed to PE (750 mg of PE added to 2.5 kg of soil) for 21 days

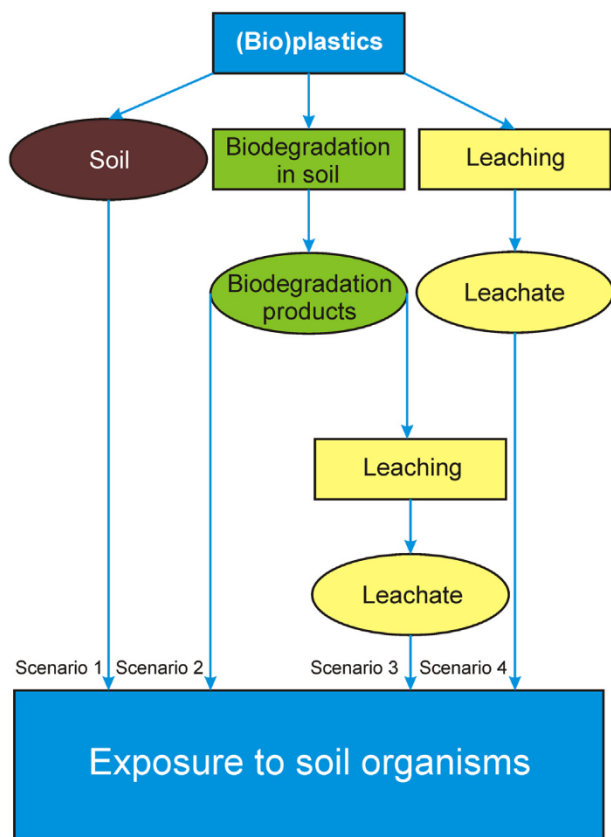


Fig. 1. Main scenarios of the ecotoxicity studies of (bio)plastics towards soil organisms.

(Table 4). PE pellets at sizes between 250 and 1000  $\mu\text{m}$  did not cause to the mortality or to the decrease in number of juveniles or weight of *Eisenia fetida* adults exposed to microplastics at concentrations from 62.5 to 1000  $\text{mg kg}^{-1}$  (Rodríguez-Seijo et al., 2017). Huerta Lwanga et al. (2016) found that the lower the concentration of plastic microparticles in the litter was, the more time was required to kill *L. terrestris*. At concentration of 7% microplastics were not harmful for *L. terrestris* in 60 days, whereas at the higher concentrations of microplastics (28%, 45% and 60% w/w) the mortality at the level of 8–25% was observed (Huerta Lwanga et al., 2016). At the same time PLA in composts (1% w/w) caused the mortality of earthworms (*L. terrestris*) at the level of 16.7% but the standard deviation was 28.9% (Huerta-Lwanga et al., 2021). What is more, the mortality of *L. terrestris* in the control runs was also at the level 16.7% (standard deviation 14.4%) (Huerta-Lwanga et al., 2021). Thus, these results should be handled with the care and they require confirmation in other studies. Earthworms exposed to microplastics lost weight however no dose-dependent relationship was proved (Huerta Lwanga et al., 2017a). Wang et al. (2019) characterized the effect of microplastics on earthworms fitness by measuring the activity of enzymes involved in the antioxidise system (Table 4). No adverse effects on the antioxidase system in *Eisenia fetida* exposed to soil containing PE or PS at concentrations up to 10% w/w were observed (Wang et al., 2019). Also no significant difference was reported for the relative growth rate of *E. fetida* in the soil amended with microplastics (at concentrations 1, 5, 10 and 20% w/w) compared to the control (Wang et al., 2019). In contrast Jiang et al. (2020) demonstrated that the oxidative stress was induced after 14 days exposure of *E. fetida* to PS microparticles at low concentrations of plastics, i.e. 100 and 1000  $\mu\text{g kg}^{-1}$ . Based upon the results of comet assay they observed DNA damage in earthworms exposed to PS microparticles (Jiang et al., 2020). Toxic effect of PS microparticles was stronger in the case of particles of 1300 nm compared with those of 100 nm (Jiang et al., 2020).

Earthworms play a key-role in the biogenic transportation of plastic particles. It most probably concerns both petroleum-derived plastics and bioplastics, although for bioplastics it has not been clearly reported so far. Fragmentation of petroleum-derived plastics favours their mobility in the soil compartment. No unequivocal relationship between the size of petroleum-derived plastic particles and their harmful effect on earthworms has been found till now. The presence of petroleum-derived plastic particles in soil at concentrations up to 1000  $\text{mg kg}^{-1}$  did not usually affect earthworms' mortality and reproduction. Due to the scarce data concerning the effect of bioplastics on earthworms, it is impossible to compare the impact of bioplastics and petroleum-derived plastics on these soil organisms.

#### 4.4. Effect of (bio)plastics on other soil fauna

Earthworms are the most frequently tested soil animals with regard to the effect of chemicals on soil fauna. Apart from them also other animals, i.e. primarily springtails and nematodes, were used as bioindicators in the ecotoxicity studies referring to the terrestrial ecosystem (Table 5).

*Folsomia candida* is a springtail belonging to family *Isotomidae* and it is regarded as a model organism in the soil toxicity tests. Petroleum-derived microplastics present in soil at concentration of 0.1% w/w reduced growth and reproduction of *F. candida* (Ju et al., 2019; Zhu et al., 2018). Zhu et al. (2018) observed low mortality (<8%) of collembolan exposed to microplastics (PVC) and the significant decrease in their body weight and reproduction, i.e. 16.8% and 28.8%, respectively (Table 5). Ju et al. (2019) found that the presence of microplastics (PE) at concentration 1% w/w contributed to the reduction of reproduction of *F. candida* by 70.2% during 28 days experiments (Table 5). The value of the effective concentration EC50 of PE microparticles was estimated at the level of 0.29% w/w for the reproduction of *F. candida* (Ju et al., 2019). It was found that the exposure to petroleum-derived microplastics altered the microbiota in the collembolan gut (Ju et al., 2019; Zhu et al., 2018). At the same time the presence of microplastics in soil might either increase bacterial diversity in collembolan gut (Zhu et al., 2018) or decrease it (Ju et al., 2019). Microplastics (PVC) influenced on the elemental composition of collembolan tissues (Zhu et al., 2018). The content of carbon and nitrogen in the collembolan tissues was higher in the experiments with PVC microparticles compared to the control test despite the fact that microplastics were not ingested by the collembolan (Zhu et al., 2018). It is most probably connected with the habitat change and effect on the nutrient consumption resulted from the presence of microplastics in the soil ecosystem (Zhu et al., 2018). The springtails moved to avoid trapping, and as a result of this behaviour the bio-pores in the soil were created. Kim and An (2019) used this behavioural response of the springtails *Lobelia sokamensis* in order to study the effect of plastics on soil-dwelling organisms (Table 5). It occurred that the springtails showed lower mobility in the plastics-contaminated soils even at concentration of microplastics 8  $\text{mg kg}^{-1}$  (Kim and An, 2019).

*Caenorhabditis elegans*, a free-living soil nematode, was employed to study impact of plastic particles on this group of worms. The studies with the use of *C. elegans* as the bioindicator were performed with regard to plastic micro- or nanoparticles. Lei et al. (2018) tested size-dependent adverse effects of both nanoplastics (100 and 500 nm) and microplastics (1, 2 and 5  $\mu\text{m}$ ) of PS (the concentration 1  $\text{mg l}^{-1}$ ) on mortality, body length and lifespan of *C. elegans* (Table 5). PS nano- and microparticles contributed to the decrease of survival rate of nematodes, however their influence on body length was not observed despite the fact that the particles of 1  $\mu\text{m}$  caused to shortening of body length of *C. elegans* (Table 5). Regarding lifespan it was reported that two out of five size groups of PS particles, i.e. 1.0 and 5.0  $\mu\text{m}$ , resulted in the significant decrease in average lifespan (Lei et al., 2018). In overall, the results presented by Lei et al. (2018) showed that nematodes exposed to the 1  $\mu\text{m}$  PS particles had the lowest survival rate, the largest



decrease in body length and the shortest average lifespan in nematodes. They demonstrated that PS nano- and microparticles accelerated the frequency of body bending and head thrashing, and increased crawling speed of nematodes (Lei et al., 2018). Kim et al. (2019) proved that PS nanoparticles (50 and 200 nm) added to soil at concentrations 4, 8 and 1000 mg kg<sup>-1</sup> influenced the movement as well as reproduction of *C. elegans*, but the smaller particles affected them to the greater extent compared to the bigger ones (Table 5). Simultaneously, no size-dependent effects of microparticles of different chemical composition (HDPE, PET, PS) on nematodes reproduction were found (Kim et al., 2020). Reduction of nematodes movement increased with the increase of concentration of nanopolystyrene particles from 1 µg l<sup>-1</sup> to 86.8 mg l<sup>-1</sup> (Lei et al., 2018). Similar phenomenon was observed with regard to the reduction of the number of progeny (Kim et al., 2020; Kim et al., 2019). The dose dependent relationship was found between HDPE, PET and PS concentration and reduction in nematode reproduction (Kim et al., 2020). Generally the highest of the tested concentration of microplastics (1% w/w) occurred to be the most toxic and contributed to the decrease of the number of offspring to 78–80% (for PP and PAN) and 56–68% (HDPE, PET, and PS) compared with that for the control group (Kim et al., 2020). Additionally, Kim et al. (2019) reported that the toxic effect of nanopolystyrene particles on nematodes might comprise the disruption of energy metabolism and induction of oxidative stress.

Terrestrial snails belong to the most widely distributed invertebrates around the world (Lange and Mwinzi, 2003). They inhabit soil surface and are exposed to plastic particles during their activity. A land snail *Achatina fulica* was used for the examination of the possible toxic and oxidative stress effects of PET microfibres on snails (Song et al., 2019). All snails survived four weeks exposure to PET microfibres (Song et al., 2019). The growth of snails expressed by the shell diameter and length was not affected by PET as well (Song et al., 2019). At the lowest concentration of microfibres in soil (0.01 g kg<sup>-1</sup>) the reduction in food intake was not significant, compared with the unexposed snails, whereas at concentrations 0.14 g kg<sup>-1</sup> and 0.71 g kg<sup>-1</sup> it decreased on average by 24.7 ± 7.0% and 34.9 ± 6.7%, respectively (Song et al., 2019). The excretion was substantially disrupted by PET microfibres in a dose-dependent manner (Song et al., 2019). Prolonged exposure of snails to PET microfibres caused significant damage in the gastrointestinal tract (Song et al., 2019). It was also proved that oxidative stress was involved

in the toxic mechanism in the case of *Achatina fulica* (Song et al., 2019). In this context the evaluation of biochemical toxicity of PLA and PE microparticles towards dragonfly larvae revealed that PLA increased the oxidative stress processes to higher extent than PE did (Chagas et al., 2021).

The studies on the effect of (bio)plastics on springtails, nematodes and other soil fauna were limited to petroleum-derived plastics. Therefore, further ecotoxicological works in this area should necessarily be extended to bioplastics.

## 5. Conclusions and recommendations for future research

(Bio)plastics affect both abiotic and biotic part of the terrestrial ecosystem (Fig. 2). They cause changes in soil chemical composition and structure, and consequently may contribute to the disturbances in water balance and cycle in the soil environment (Fig. 2). Such changes in soil physicochemical properties act (directly or indirectly) on soil biota. Thus, ecotoxicological studies on (bio)plastics in the soil compartment comprise the direct impact of (bio)plastics particles (scenario 1) or the products of their degradation (scenario 2) on soil organisms as well as the impact of leachates obtained from (bio)plastics (scenario 3) or leachates containing products of (bio)plastics degradation (scenario 4) on soil biota. The majority of these studies concerns the direct influence of (bio)plastic particles on the biotic part of terrestrial ecosystems (scenario 1). In spite of that testing of the products released from (bio)plastics to the liquid phase remains of high-importance and should be made in parallel.

Dominating types of (bio)plastics tested are petroleum-derived plastics, in particular different types of PE (HDPE, LDPE, LLDPE, PE), PET and PS, whereas bioplastics still require deeper insights and evaluation with regard to their effect on soil organisms. The studies, in which bioplastics have been tested, constitute about 18% of all analysed papers. With regard to the size of particles tested, the majority of studies have dealt with microparticles of (bio)plastics followed by nanoparticles and macroparticles.

Terrestrial organisms representing species from different taxonomic and functional groups, i.e. plants (mono- and dicotyledonous), microorganisms (pure and mixed cultures), earthworms, springtails, nematodes and snails have been employed for testing of the potential toxicity of plastics in the terrestrial ecosystems. Regarding bioplastics

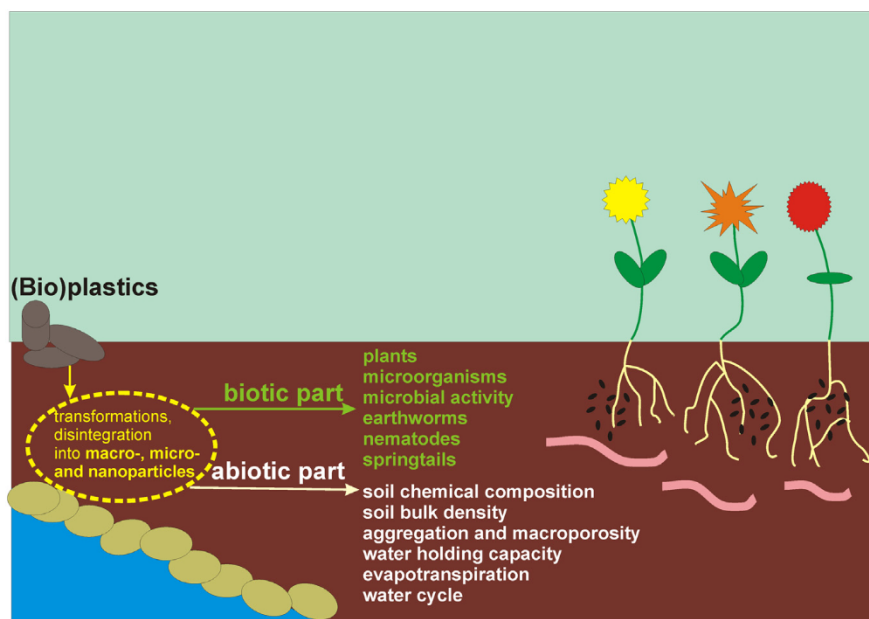


Fig. 2. Potential impact of (bio)plastics on abiotic and biotic part of terrestrial ecosystems.

only plants and microbiota have been used as bioindicators. Any data about the potential impact of bioplastics on soil fauna are in scarce.

(Bio)plastics do not affect the germination of seeds but may contribute to the delay in germination. Abnormalities in morphology of radicles and hypocotyls may be observed but it requires deeper insight and additional tests. Both inhibitory and stimulating effects are observed in relation to roots and stems growth. Nanoparticles of (bio)plastics are able to accumulate in plant organs.

Inhibition of soil microbial nitrification has been the most commonly used indicator of the effect of (bio)plastics on microbiota. (Bio)plastics microparticles do not inhibit the biochemical activity of nitrifiers. Also the activity of other microbial enzymes including these responsible for the transformation of carbon compounds is not affected in the presence of (bio)plastics microparticles. At the same time the nanoparticles of petroleum-derived plastics may decrease the biochemical activity of soil microorganisms. The scope of ecotoxicity tests of (bio)plastics using the soil microbiota should be extended to other species of microorganisms including both pure cultures and microbial communities.

Earthworms are predominantly used organisms in testing of the effect of (bio)plastics on soil biota. They are actively involved in the biogenic transport and distribution of (bio)plastics in the terrestrial ecosystems. Plastic particles present in soil at concentrations up to 1000 mg kg<sup>-1</sup> usually do not either cause to the mortality of earthworms or affect their reproduction. Micro- and nanoparticles of plastics can be accumulated in the earthworm intestine and transferred in the food chain. A major gap in this area is the scarce data about the effect of bioplastics on earthworm's mortality, reproduction, metabolism and behaviour.

Microparticles of petroleum-derived plastics contribute to the reduction of growth and reproduction of springtails. They may also cause to the decrease of the movement of collembolan. Nanoparticles and microparticles of petroleum-derived plastics influence on the survival and behaviour of nematodes. They usually decrease nematodes movement, survival rate and reproduction ability. Bioplastics should be tested towards their potential impact on springtails and nematodes.

Ecotoxicological data on bioplastics are limited to their effect on plants and microorganisms. These data indicate that bioplastics exert similar or in some cases even stronger effect on plant growth than petroleum-derived plastics do. With regard to nitrifying bacteria neither bioplastics nor petroleum-derived plastics inhibit them. Due to the shortage of data it is difficult to unequivocally evaluate the environmental safety of bioplastics.

The results of ecotoxicity tests of the (bio)plastics towards soil organisms often show a high degree of variability and lack of dose-dependence relationships. Therefore, their interpretation and formulation of conclusions is a hard task. Simultaneously, the variability of results creates a need for the continuation and development of ecotoxicological studies on petroleum-derived plastics and bioplastics to soil biota.

Summing up, on the basis of this literature review the gaps in research on the impact of (bio)plastics on the soil environment, particularly on its biotic part, were identified. In order to fill these gaps the following main directions of further ecotoxicological studies on (bio)plastics are suggested:

- evaluation of the effect of bioplastics on earthworms and other soil fauna,
- extension of the scope of ecotoxicity tests of (bio)plastics towards plants and microorganisms aiming at more detailed comparison of the impact of petroleum-derived plastics and bioplastics on these soil biota,
- development of field ecotoxicity tests of bioplastics and petroleum-derived plastics towards soil organisms,
- evaluation and comparison of the effect of bioplastics and petroleum-derived plastics on soil biota at the community level (multispecies tests),

- comprehensive assessment of both direct effect of (bio)plastics on soil organisms as well as the indirect effect, i.e. effect of leachates containing the products released from (bio)plastics to liquid phase, on soil organisms,
- development of the environmental safety assessment for replacing petroleum-derived plastics with bioplastics.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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