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### Review

### Soil under stress: The importance of soil life and how it is influenced by (micro)plastic pollution



### L. Joos<sup>a</sup>, C. De Tender<sup>a,b,\*</sup>

<sup>a</sup> Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 96-109, 9820 Merelbeke, Belgium <sup>b</sup> Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 71, 9052 Zwijnaarde, Belgium

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### ABSTRACT

Soil organisms and specifically microorganisms are indispensable to life on Earth. They regulate essential ecosystem functions from carbon sequestration to primary production. These organisms often experience stress when the balance of the soil system is disrupted by agricultural practices and environmental disturbances. A new stressor is plastic, which can be found in soils, in and around soil-dwelling organisms, and close to plants. The presence of plastic can affect soil chemistry, plant growth and the survival of higher-order organisms. Microbial organisms respond sensitively to these changes in their surroundings and will thus be (in)directly affected by plastic. Eventually, this results in a different microbial activity, composition and reduced diversity. Plastic might even serve as a specific habitat for microorganisms, generally referred to as the plastisphere. In this review, we make predictions based on the observed effects of (micro)plastics and the potential impact on the plant-soil-microbiome system. We use prior knowledge of other disturbances (e.g. tillage and pesticides) which have been studied for many years in relation to the soil microbial community. Further research is needed to develop standardized methods to study smaller plastic particles (micro- and nanoplastics) as these play the most dominant role in terrestrial ecosystems.

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Abbreviations: AMF, arbuscular mycorrhizal fungi; DOC, dissolved organic carbon; FDA, fluorescein diacetate hydrolase; HDPE, high density polyethylene; LDPE, low density polyethylene; MP, microplastic; NP, nanoplastic; PBAT, polybutylene adipate-co-terephthalate; PES, polyethersulfone; PET, polyethylene terephthalate; PCPF, plant growth promoting fungi; PGPR, plant growth promoting rhizobacteria; PLA, polyactic acid; PP, polypropylene; PS, polystyrene; PVC, polyvinylchloride; SOC, soil organic carbon; SOM, soil organic matter.

<sup>\*</sup> Corresponding author at: Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 96–109, 9820 Merelbeke, Belgium. *E-mail address:* caroline.detender@ilvo.vlaanderen.be (C. De Tender).

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### 1. Introduction

Soils are a heterogeneous habitat formed by a complex mixture of minerals, organic matter and a network of water- and air-filled pore spaces [1] (Fig. 1). This habitat is the home of a wide abundance and diversity of soil organisms performing key soil processes and functions. The most dominant players in all of these processes are the microorganisms residing in the bulk soil and/or rhizosphere (the soil surrounding plant roots) [2]. Microorganisms belong to the most abundant and diverse groups of soil organisms, representing an estimated total biomass of 3–4% on earth (up to 20 Gt carbon (C)) [3]. They contribute to key biological processes by interacting with their surroundings, other organisms living in the soil, and plants. Specifically, 80–90% of the soil processes are mediated by the soil microbiome including bacteria, fungi, archaea, viruses and protista, for which they form the main focus of this review [4].

Microorganisms mediate a multitude of soil processes and thus contribute directly to key ecosystem processes such as: (1) C sequestration, (2) nutrient cycling, and (3) the formation of soil structure [5] (Fig. 1).

After the oceans, soil is the second largest C sink, sequestering around 80% of the global terrestrial C underground. It is estimated that 58% of the soil organic C is contained in soil organic matter (SOM) [6–9]. Soil bacteria and fungi contribute substantially to the flux of the below- and aboveground C through respiration and decomposition from the root deposits and plant litter.

Nutrient (C, nitrogen (N) and phosphorus (P)) cycling directly affects soil dynamics and ecosystem services such as plant growth [5]. The decomposition of organic material as well as the input of root exudates contributes to nutrient cycling [10]. Macrofauna, especially earthworms, break down the organic material into smaller pieces, thereby increasing the surface area available for colonization by microorganisms or by redistributing the organic material [11]. Especially bacteria and fungi transform the complex chemical compounds using enzymatic activity into simpler compounds and molecules that can be absorbed by plants, thus providing indirect feedback for plant productivity [12]. Positive direct effects of microbially-mediated nutrient inputs for plants are associated with symbiotic relationships. Well-studied examples are the N-fixing bacteria and archaea that convert atmospheric N into ammonium-N [13,14] and the increased P uptake by plants via arbuscular mycorrhizal fungi (AMF) [15]. Overall, the rate of organic matter decomposition and subsequent nutrient cvcling depends on the composition of the microbial community and environmental conditions (e.g. temperature, pH, soil moisture, etc.).

Soil structure refers to the size and arrangement of soil particles and associated pore spaces that create room for water, nutrients, gases and soil organisms [16]. The soil particles and pore spaces are arranged into aggregates of different sizes; their stability is an important aspect of the soil structure [17]. Several studies revealed not only that soil structure is a major driver for the adaptation of soil organisms, but also that microorganisms contribute to the soil structure, e.g. by adding organic matter through decomposition and conversion of organic material [18–21]. The role of earthworms is also unmistakable for the soil structure, as they grind and remold ingested particles into new aggregates and pores, thereby both compacting and loosening the soil [21,22].

In addition to their prominent role in key ecosystem processes, microorganisms play a vital role in the overall growth, productivity and health of crops. Plants actively attract microorganisms towards the root, by which microbial species will reside in the rhizosphere [23,24] (Fig. 1). Plant species can shape the microbial composition and activity in their rhizosphere via differences in root architecture [25] and rhizodeposition [26,27]. Although rhi-



Fig. 1. Illustration of the vital roles of organisms in soil. Numbers indicate the main soil processes to which microorganisms contribute. (1) carbon (C) sequestration: C enters the soil mainly via plants uptake and organic material. It is the second largest C sink, sequestering around 80% of the global terrestrial C, of which 58% is contained in the soil organic matter. Soil microorganisms contribute to this C cycle through respiration and decomposition from root deposits and plant litter (2) nutrient cycling; Macrofauna, such as earthworms, breaks down the larger organic material into smaller pieces, making it available for microorganisms which can either consume or degrade these smaller pieces. This breaks down the complex chemical compounds into more simple compounds that can again be taken up by plants. (3) soil structure; the soil structure consists of air and water-filled pore spaces created by organisms such as earthworms. Roots can reach these water-filled spaces, and these spaces are also inhibited by hydrophilic organisms. Fungi are able to bridge air-filled pore spaces with their hyphae. Microorganisms themselves contribute to the soil structure by converting organic material. The rhizosphere is magnified in black square and highlights the role of plant-growth promoting rhizobacteria and fungi (PGPR and PGPF) for the growth, productivity and health of crops. These microorganisms can also suppress pathogens by their biological control activity. PGPR plant-growth promoting rhizobacteria; PGPF plant-growth promoting fungi; AM arbuscular mycorrhizal fungi; SOC soil organic carbon; C carbon; N nitrogen; P phosphorus; CO<sub>2</sub> carbon dioxide; CH<sub>4</sub> methane; N<sub>2</sub> dinitrogen; N<sub>2</sub>O nitrous oxide; NO3 nitrate; NH4 ammonium; SOM soil organic matter.

zodepositions cost C for the plant, the C exuded by the roots serves as a major energy source that nurtures a diverse and abundant microbial population originating from the bulk soil [28]. Beneficial microbial organisms can colonize the root surface and the inner root tissues [29]. Some of these colonizers have the potential to enhance plant development, biological control activity and stress tolerance; these are referred to as plant-growth promoting rhizobacteria and -fungi (PGPR and PGPF, respectively) [30] (Fig. 1).

The diversity and composition of the soil microorganisms in the bulk soil are primarily driven by (1) soil characteristics such as pH, organic matter content [31,32] or moisture content [33,34], (2) soil structure and (3) the soil food web (Fig. 1). Bacteria (specifically Gram-negative bacteria) are more sensitive to moisture fluctuations due to their thinner cell wall and lost capacity to sporulate, than Gram-positive bacteria, archaea and fungi [35]. Within the root microbiome, specific increases of Gram-positive Actinobacteria and Firmicutes were observed after an extended drought per-

iod, while Gram-negative bacteria declined [35]. Fungi can more easily reach water-rich environments using their hyphae [36,37]. In addition, soil structure can influence the ability of soil organisms to sense food sources or prey [38]. The appearance of other soil organisms (micro-, meso- and macrofauna) and the interaction of all organisms in the soil food web mediates both the abundance and activity of microorganisms in soil. The soil food web represents possible feeding connections in a soil ecosystem by clustering organisms in trophic levels [39]. The biomass of each level is controlled by other trophic levels, either bottom-up or top-down. The interactions between all these trophic levels are major determinants of soil processes, notably in litter decomposition [40,41] and nutrient turnover [42,43].

Soil microorganisms are also influenced by the soil composition and global and local patterns of the soil. Multiple studies have contributed to the general understanding of the biogeography of the abundance and biomass of different organisms, including bacteria [44–50], fungi [34,44,50,51], earthworms [52], nematodes [53– 56], protists [50,57,58] and microbial biomass [33,59,60]. From these studies, patterns of distribution are related to climatic conditions such as annual moisture availability (protists, fungi and nematodes) and pH (bacteria).

Because some soil processes are carried out by a variety of microorganisms, the loss of a few species is assumed to have limited impact on the soil system. This phenomenon is called functional redundancy and reflects the potential robustness of terrestrial ecosystems [61]. The buffering capacity of soil has its limits however, which should not be explored too much as it can lead to degraded soils [62]. Human-induced changes, particularly those brought by intensive agriculture and global warming, have led to the modification of soil structure and physicochemical properties [62,63]. This has resulted in tremendous changes in the microbial community composition and diversity, which in turn alters the dynamics of the soil food web [64].

Soil disturbances linked to agricultural intensification include mechanical impacts (tillage and compaction), monoculture, and fertilizer and pesticide application [1]. These frequently applied practices, used to maximize crop vield, cause a loss of microbial diversity and alter the microbial composition, resulting in unbalanced ecosystem functions and services and worsening soil health [65–69]. For instance, tillage practices can affect the soil organisms by making significant modifications to the soil's physical and chemical properties [70]. Conventional tillage disrupts soil aggregates, exposing soil microorganisms to an increased risk of desiccation and restricted access to food sources [71]. This type of tillage typically results in a bacterial-dominated community [72,73], with a higher abundance of protists and bacterivores thriving under the increase of the bacteria [74-77]. In contrast, less disturbed soils often have a higher fungal and predatory nematode biomass [76,78,79] and are correlated with lower N leaching and higher C sequestration [80,81]. Soil compaction caused by passage of agricultural machinery affects the physical structure of the soil by reducing the porosity and increasing soil bulk density [82]. This leads to a slower soil water infiltration rate and negatively affects air diffusion [83]. Compaction prevents roots from penetrating the deeper soil layers, resulting in shallow root growth [84]. Additionally, lower biomass of bacteria, fungi, nematodes and microbial activity and smaller earthworm populations are found in more compacted soils [85,86].

Soil microorganisms are not only sensitive to agricultural practices; they also respond to environmental disturbances (e.g. moisture availability and temperature). Most microorganisms are dependent on the water films surrounding soil particles as a resource for microbial cellular function and transport medium [87]. Changes in moisture availability affect the soil microbiome through osmotic stress and fluctuating accessibility to nutrients via the water film [88]. Next to bacteria, nematodes are sensitive to moisture fluctuations as they are restricted to live in water matrixes. They can survive desiccation however by going dormant [89,90].

Seasons are correlated with ambient temperature fluctuations and therefore also soil temperature. The higher temperatures during summer are correlated with an increased microbial respiration and activity of the plant roots, resulting in a higher loss of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O from the soils [91,92]. Higher temperatures as a result of climate change will only increase these respiration rates. The soil microbiome will be affected by more extreme weather events such as more frequent, longer and more intense heat waves. It is therefore thought that by 2050 soils will no longer serve as a Csink, but will become a Csource [93]. In contrast, cold stress during winter periods affect the soil microbiome and rhizobiome, with increased abundances of PGPR in the plant root that can protect the plants from chilling temperatures [94].

The soil and the soil microbiome are therefore under continuous pressure by the changing climate and pollution due to human activities. A more recent stressor on the soil ecosystem is the presence of (micro)plastic pollution. In this review, we will focus on how plastic pollution, with specific attention to microplastics (MPs), i.e. plastics with a diameter smaller than 5 mm, disrupts the reciprocal interactions between the plant, the soil and the microbial community. In the first part, we will focus on the accumulation and distribution of (micro)plastic in soil and its effect on the soil structure and plant development. Second, we elaborate on the disruptions in community activity, complexity and composition MP pollution causes on the microbial community. In the last part of this review, we highlight the role of plastic as a microbial hotspot within the soil ecosystem.

### 2. A new stressor in the field: plastic pollution

Mass production of plastic dates from the early 1950s [95]. Since then, the production rate of plastic has grown to over 300 million tons per year (Fig. 2). The popularity of plastics is easy to explain: they are cheap, lightweight, strong and durable [96]. In an ecological context, plastics can be distinguished based on either form (filaments, beads, sheets, foams, etc.) or polymer (the most common are polyethylene terephthalate (PET), high density polyethylene (HDPE) and polypropylene (PP)) [97]. The widespread use of plastics has led to plastic pollution in nearly every conceivable environment, even in the deepest marine trenches [98]. We focus here on the impact of plastic pollution in terrestrial (agricultural) environments.

### 2.1. Plastic pollution in the terrestrial ecosystem

The first appearances of plastic were made in seabird guts and alongside plankton in oceanographic sampling nets in the 1960's and early 1970's, respectively [99–101], whereas the appearance of MPs was reported for the first time on New-Zealand beaches in 1978 [102] (Fig. 2). It has been estimated that up to 5.25 trillion plastic particles are now scattered in the marine environment [103].

Most (up to 80%) of the plastic particles found in aquatic environments were produced, used, and disposed of on land. Terrestrial plastic pollution is therefore expected to be 4- to 23-fold larger than that of marine environments [104]. Agricultural soils alone may contain more (micro)plastics than oceanic basins [105]. Despite these expectations of intense plastic pollution on land, only limited research has been performed on plastic contamination in soils. Especially the smaller particles, nanoplastics (NPs;  $\leq 1 \mu m$ ) and MPs ( $\leq 5 mm$ ), are not currently being studied, probably



**Fig. 2.** Annual plastic production and overview of the number of research papers on Web of Knowledge (on 21/5/2021) regarding microplastic pollution in terrestrial, freshwater or marine environments. Annual plastic production is displayed by a black line starting from 1862. The search included the following keywords: terrestrial (soil + MP OR terrestrial + MP), freshwater (river + MP, freshwater + MP), marine (marine + MP, aquatic + MP, sea + MP). All reviews were excluded from the literature search.

because advanced techniques and methodologies for sampling, extraction and detection have not vet been developed [106,107]. The extraction of MP filaments from water is comparatively easy compared to extraction from a complex organo-mineral soil matrix [108]. Nevertheless, it is expected that the smaller particles can cause the most damage to biodiversity and the food web. Despite the call for research on MP contamination in terrestrial ecosystems dates back from 2012, it took over four years before the first research article was published, studying the effects of MP contamination on the growth rate and mortality of earthworms [108,109]. To date, only very limited data on the occurrence of MP particles in terrestrial systems are available. A search on Web of Knowledge shows that from 2012 until May 2021, only 10% of all research articles on MP pollution focuses on terrestrial ecosystems (Fig. 2; used keywords: terrestrial (soil + MP OR terrestrial + MP), freshwater (river + MP, freshwater + MP), marine (marine + MP, aquatic + MP, sea + MP)). The current focus on MPs in marine environments is probably due to researchers' tendency to build on previous studies, but this leaves an enormous knowledge gap regarding MP pollution in other ecosystems.

### 2.2. Sources and accumulation of terrestrial (micro)plastic

Plastic contamination in soil is both intentional and unintentional [110] (Fig. 3). Examples of intentional plastic addition to soil are plastic mulching and addition of plastic to fertilizers [104,105], while unintentional contamination can occur via composts and fermented organic waste products [111], sewage sludge [112] or irrigation with water from contaminated lakes or rivers [111]. For example, Nizzetto et al. (2016) estimated that between 125 and 850 tons MPs per million inhabitants are added annually to European agricultural soils through sewage sludge or processed biosolids [105]. An annual sludge application on croplands can lead to 2or 3-fold increases in MP concentration in soil [113–115]. In addition, MPs enter the soil ecosystem through atmospheric deposition such as tire abrasion [116] and disposal of industrial and consumer plastic waste [117]. The high level of plastic input results in high accumulation levels in the soil. In Europe, concentrations range from 0.3 and 3.4 mg MPs per kg soil near Malmö, Sweden [118] up to 55.5 mg MPs per kg soil on floodplain sites in Switzerland [119]. Industrial soils in Sydney, Australia were documented with a MP contamination in excess of 500 mg per kg soil [106]. In southwestern China, the abundance of plastic particles was found to range from 7,100 to 42,960 items per kg soil [120]. All of these studies were conducted on soils with a history of sewage sludge application, plastic mulching or a geographic location near an industrial site. The only study that reportedly studied uncontaminated farmlands (located in Southern Germany) found concentrations of only 0.31 items per kg soil [111,121]; however, this study did not account for particles smaller than 1 mm, which were the majority of MPs in other studies [111].

# 2.3. Disturbance of the soil and plant system due to microplastic pollution

To protect the current status of the soil, models indicate that the number of MPs should not exceed 2,128 mg per kg soil or 14,435 mg per kg soil to maintain 50% of the currently present soil biota or soil properties, respectively. For a protection of 95%, values should stay even below 520 and 655 mg per kg soil [122]. As MP contamination of soils will only increase in the near future, pre-sumable effects on the soil chemistry [123], plant performance [124,125] and soil biota are to be expected. So far, it is known that MPs interferes with the soil physicochemical composition by: (1) decreasing soil bulk density, (2) changing the water availability, (3) increasing soil pH, and (4) increasing dissolved organic matter (Fig. 3).

First, it has been shown that the incorporation of MP particles such as polystyrene (PS), PP, PET, polyethersulfone (PES) or HDPE at concentrations up to 2% (w:w) can decrease soil bulk density [126,127]. In addition, Lehmann et al. (2019) showed that the incorporation of generally large (5 mm) microfibers at concentrations over 0.2% (w:w) reduces the stability of soil aggregates [128]. Microfibers thus have the potential to alter the soil structure



Fig. 3. The known and potential effects of micro- and nanoplastics on the soil physicochemical and microbial characteristics. Proven effects of microplastics are indicated in black boxes: first it has been shown that the presence of MP changes the soil bulk density and is able to increase the dissolved organic carbon (DOC) in the soil. This is related to changes in the water availability (increase) and evaporation (increase). Effects on plant development have also been noted. These effects are still uncertain either (increase or a decrease) and are therefore indicated with a crossed-out tilde MPs also affect the soil food web. Decreases in the survival and reproduction of earthworms and nematodes have been noted and also an active uptake of MPs by these organisms has been shown. The combination of these effects can explain at least in part the effect on the microbial community, with changes in the microbial activity, composition and a decrease in the microbial diversity. In addition, we added some hypothetical effects (in orange) plastics might cause. The degradation of plastic might result in an increase in the carbon:nitrogen (C:N) ratio. Also, differences in the rhizosphere microbiome are expected as the root architecture of the plant is different in plastic-polluted soil. The presence of plastic might also increase the abundance of plastic degrading organisms, either in the soil or residing on the plastic. Plastic can thus serve as a vector; however, it remains unclear if this will be more for pathogens or beneficial microorganisms as indicated by exclamation point and check mark, respectively. DOC dissolved organic carbon; MP microplastics; C carbon; N nitrogen; H<sub>2</sub>O water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[128,129], which is at least in part mediated by the presence of soil microorganisms [128]. For other polymer shapes like foams and particles, the polymer type is an important factor that co-modulates the soil physicochemical properties [130].

Second, for water availability, contradictory results have been noticed. Soil contamination with polyester fibers can lead to increasing water availability in the soil [126,127], which might affect soil moisture and evapotranspiration. On the other hand, the study of Wan et al. (2019) showed that the addition of plastic films (fragment sizes 2, 5, 10 mm and an average concentration of 0.5% or 1%) leads to increased water evaporation and desiccation

cracking [131]. This can lead to higher water losses and soil drying with negative consequences for both the soil microbiome [132] and plant performance [133].

Third, tire debris application in soil led to an increase in pH in several soils [134].

Fourth, addition of PP in extremely high concentrations (7% w: w to 28% w:w; particle size < 180  $\mu$ m) to Chinese loess soil increased soil enzymatic activity, with an accumulation of dissolved organic C, N and P as a result [123]. In contrast, studies making use of MP concentrations as found in industrialized soils (5% to 7% w:w) did not have a significant effect on dissolved organic C (DOC) in the short term [106,123,135]. On the other hand, detrimental effects on Potassium (K), Magnesium (Mg) and Sulfur (S) were noted in the presence of MP fibers (0.4% w/w), while the bioavailability of Zinc (Zn) increased [136].

The number of studies focusing on the effect of MPs on plant growth and plant development are limited and show contradictory results (Fig. 3; Table 1). The growth of wheat was retarded by the presence of MPs (concentration 1% w:w) [137], but in spring onion, both positive and negative effects were noted depending on the polymer type (concentration 2% w:w) [127]. Exclusively positive effects were noted for Daucus carota, when MPs in different shapes and concentrations were added, ranging from shoot mass increases of 27% up to 60% [129]. In roots, the presence of MPs increased the total root length and root area in spring onion, whereas it decreased root growth of cress seeds and the average root diameter of spring onion [127,138]. MPs are also able to accumulate in seed capsules of cress seeds, which can cause delayed germination [138]. Recently, the smallest plastic particles (NP) were even found to accumulate in edible fruits and vegetables. On average 223,000 and 97,800 NP particles per gram were found in fruits and vegetables, respectively, with the highest concentrations found in apples and carrots [139].

### 3. Can plastic disrupt the soil and plant microbiome?

The disturbance of soil chemistry and plant development by MP pollution will disturb the interactions between the soil, the plant and the microbial community. Here we summarize studies that describe the disruption on the soil food web, with a focus on the soil microbiome, due to MP contamination.

## 3.1. Plastic decreases growth rates and increases mortality of invertebrates

Invertebrates, specifically earthworms, have been the major focus for researchers in terms of MP pollution in soil. Whereas they cannot be classified as microorganisms, earthworms play an important role in the soil food web [11]. The effect of MPs has been studied on several species, such as Lumbricus terrestris [109,140,141], Eisenia fetida [142–145] and Eisenia andrei [146,147]. Earthworms can ingest and digest MPs [109,148] and can also transport and incorporate MPs into the soil matrix [140,148] (Fig. 3). Decreases in growth rate and higher mortality (28 to 60%) of L. terrestris when MPs were introduced in the soil have been noticed [109]. In contrast, no adverse effects on survival, reproduction or body weight of the earthworm E. andrei was observed [147]. In addition, MPs can cause skin lesions and reduce reproductive rates in earthworms [149], the latter potentially caused by damage to male reproductive organs [146]. The uptake of MPs by earthworms can lead to the production of NPs through activity of the earthworm's gut microbiome, which will release smaller plastics in the environment [146,148]. Upon excretion, these particles become available to other soil organisms, e.g., smaller decomposers, such as microarthropods. Indeed, springtails can

#### Table 1

Information of the effect of specific polymers on the shoot and root of certain plant species. Data represents the plant species (+reference), the type of polymer (*PA* Polyamide, *PEHD* polyethylene high-density, *PP* Polypropylene, *PS* polystyrene, *PET* polyethylene terephthalate, *PE* Polyethylene, *PU* Polyurethane, *PC* polycarbonate), the concentration in plastic weight over soil weight, the polymer size (med. = median size of the fragments) and the effect on shoot and root development.

Plant species Reference	Polymer	Conc. (w/w)	Polymer size	Effect shoot	Effect root	
Allium fistulosum (spring onion)	PA	2.0%	15–20 μm	Decrease dry biomass onion Increase total biomass	No reported effects Increase root biomass	
[127]	PES fibers	0.2%	Length: 5000 µm Diameter: 8 µm	Increase total biomass		
	PEHD	2.0%	med. 643 μm	No reported effects	Trendwise increase root biomass	
	PP	2.0%	med. 624 μm	No reported effects	Trendwise increase root biomass	
	PS	2.0%	med. 492 μm	Increase total biomass	Increase root biomass	
	PET	2.0%	med. 187 μm	Increase total biomass	Trendwise increase root biomass	
Daucus carota (carrot) [129]	PP (fibers, film, foam or fragments)	0.1% 0.2% 0.3% 0.4%	max. 5 mm	Increase shoot biomass of 53.1% (hbers), 64.2% (films) and 56.3% (fragments)	Increase of root biomass of 71.5% (fragments)	
	Polyester fibers	0.1% 0.2% 0.3% 0.4%	max. 5 mm	No reported effects	No reported effects	
	PA fibers	0.1% 0.2% 0.3%	max. 5 mm	No reported effects	No reported effects	
	PE films or foams	0.4% 0.1% 0.2% 0.3% 0.4%	max. 5 mm	Increase shoot biomass of 43.7% (films) and 64.6% (foams)	Increase of root biomass of 79.9% (films) and 40.4% (foams)	
	PET films or fragments	0.1% 0.2% 0.3% 0.4%	max 5 mm	Increase shoot biomass of 72.4% (films) and 51.1% (fragments)	Increase of root biomass of 70.0% (films) and 38.0% (fragments)	
	PS foams	0.1% 0.2% 0.3% 0.4%	max. 5 mm	No reported effects	No reported effects	
	PU foams	0.1% 0.2% 0.3% 0.4%	max. 5 mm	Increase of shoot biomass of 50.6%	Increase of root biomass of 160.3%	
	PC fragments	0.1% 0.2% 0.3% 0.4%	max. 5 mm	Increase of shoot biomass of 54.6%	Increase of root biomass of 42.6%	
Lepidium sativum (cress) [138]	Not defined		50 nm 500 nm 4800 nm	No reported effects	Increase (50 nm), decrease (500 nm) or no effects (4800 nm) on relative root growth	
Triticum aestivum (wheat) [137]	LDPE	1%	12.5% [501 μm- 1 mm] 62.5% [251 μm- 500 μm] 25% [50 μm-	Decrease shoot biomass	No reported effects	
	Starch-based biodegradable plastic (37.1% Pullulan, 44.6% PET, 18.3% PBT)	1%	250 μmj 12.5% [501 μm– 1 mm] 62.5% [251 μm– 500 μm] 25% [50 μm– 250 μm]	Decrease shoot biomass	Decrease root biomass at 2 months harvest,	

experience critical damage (e.g. reduced mobility, mortality) by MP exposure [150,151] and transport the MPs to deeper soil layers [152]. The isopod *Porcellia scaber* was however not affected at all

[153]. In conclusion, we can say that the presence of MPs in soil will have an effect on the invertebrate community, but that the sensitivity of each of the species will be different.

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### 3.2. Negative effects on the survival and reproduction of nematodes by soil MP pollution

The species Caenorhabditis elegans is the most commonly studied nematode in terms of MP toxic effects. It has been shown that MPs will not only be ingested by C. elegans, but can also affect reproduction, survival and behavior [154-160]. Particle ingestion is limited to the particle-to-mouth size ratio, and therefore will be species dependent. For the species *C. elegans*, the maximum particle size to be ingested is estimated to be  $4.4 \pm 0.5 \mu m$  [156]. The negative effects on the survival and reproduction of MPs on nematodes are dependent on the polymer type, particle size and MP concentration [154,155]. The exposure of micro-sized low density polyethylene (LDPE), polylactide polymers (PLA) and polybutylene adipate-co-terephthalate (PBAT) will lead to fewer offspring (up to 22.9%) of C. elegans [154.155.158.159]. The behavior of C. elegans changes as well: the frequency of body bending and head thrashing accelerated and crawling speed increased, indicating that MPs can induce size-dependent excitatory toxicity on locomotor behavior [157]. The mechanism behind these effects is currently unknown, but might be related to (1) MP uptake, as smaller and higher concentration of MPs have higher toxic effects [154,155] and (2) chemical additives on the plastic particles [159]. We hypothesize that the uptake of MP particles can interrupt the digestive tract of nematode species, leading to growth reductions. In addition, the observed relation between extractable additives and the MP toxicity on nematodes indicates toxic effects of chemical additives bound to MP particles [159]. In addition, it has been shown that bulk density, cation exchange capacity, and clay and sand content are dominant factors influencing the toxicity of PS particles on nematodes [159].

MPs thus seem to have a major effect on nematodes. Three remarks should be made, however. First, a recent study of Mueller et al. (2020) indicated that the nematodes Acrobeloides nanus and Plectus acuminatus responded differently after long term exposure to MPs [156]. Although P. acuminatus was not affected by the MP treatment, A. nanus populations developed significantly faster 31 days after MP treatment. More studies should thus be undertaken to verify the effects of MPs on nematode species other than C. elegans, particularly because nematodes play a pivotal role in many trophic levels [161]. Second, most of these studies were performed using non-soil media or spherical beads [159]. To elucidate the effect in terrestrial ecosystems, more research should be undertaken in a soil matrix and with more representative concentrations of MP. Last, all studies conducted focused on one to maximum three nematode species. We suggest that effects on the total soil nematode community should also be evaluated to elucidate the effect on the soil food web and soil health.

## 3.3. Interruption of the soil microbial activity and composition by MP soil pollution

After an initial focus on invertebrates and nematodes, studies looking into the effects of MPs on the soil microbiome are now emerging. Most of these studies focus on microbial activity rather than composition. So far, effects on fluorescein diacetate hydrolase (FDA), phosphatase activity, dehydrogenase activity, soil microbial respiration, and enzymes involved in the C, N or P cycles have been observed (Table 2).

For FDA, a measure for total microbial activity, both positive and negative effects have been observed by the presence of plastic. In the presence of plastic mulch residues, FDA in soil decreases

Table 2

Information of the effect of specific polymers on the microbial activity. Data represents the microbial activity (+reference), the soil type and composition, the type of polymer (*PA* Polyamide, *PVC Polyvinyl chloride*, *PP* Polypropylene, *PS* polystyrene, *PET* polyethylene terephthalate and *PE* Polyethylene), the polymer size and increase or decrease of the activity (% decrease or increase added when available in the original manuscript).

Microbial activity Reference	Soil type	Soil composition	Polymer type	Polymer size	Increase/Decrease activity
FDA [123 126 162]	Unpolluted shrub field China	Not defined	PVC	$20 \text{ mm} \times 20 \text{ mm}$	Decrease
1511[123,120,102]	onponuteu sin ub neiu, ennu				(-1.6%  up to  -30.7%)
	Top loess soil, China	18.4% clay	PP	180 µm	Increase
	-	25.0% silt			
		55.9% sand			
	Experimental site, Freie	Sandy loam	PA,	Beads: 8–20 µm	Decrease for PA and PES
	Universität Berlin		Polyester, PE	Fragments:	
Debudar and [102, 104]	Use allots data and faild China	Net J.C. J	DUC	643 μm	Deeman
Denydrogenase [163–164]	Unpolluted shrub field, China	Not defined	PVC	$20 \text{ mm} \times 20 \text{ mm}$	Decrease $(14.0 \text{ up to } 50.0\%)$
	Not defined	Silt loam	PS	$695 \pm 0.5$ nm	(-14.9 up to -59.0%) Increase until day 14 Decrease
	Not defined	Sht Ioani	15	05.5 ± 0.5 mm	at day 28
N-(leucineaminopeptidase) cvcle [164]	Not defined	Silt loam	PS	69.5 ± 0.5 nm	Decrease
C-(β-glucosidase and cellobiohydrolase)	Not defined	Silt loam	PS	69.5 ± 0.5 nm	Decrease
cycle [163–164]	Top loess soil, China	18.4% clay	PP	$20 \text{ mm} \times 20 \text{ mm}$	No consistent effect
		25.0% silt			
		55.9% sand			
P-(alkaline-phosphatase) cycle [164]	Not defined	Silt loam	PS	69.5 ± 0.5 nm	Decrease
Urease activity [163,165]	Cinnamon soil, China	35.7% clay	PE	$2 \text{ mm} \times 2 \text{ mm}$	Increase
		46.8% silt			(+175% up to +234%)
	<b>T</b> 1 1 01 1	17.5% sand	55		
	Top loess soil China	18.4% clay	PP	$20 \text{ mm} \times 20 \text{ mm}$	No consistent effect
		25.0% SIII			
Catalase activity [165]	Cinnamon soil China	35.9% Saliu 35.7% clav	PF	2 mm × 2 mm	Increase
	chinamon son, china	46.8% silt	1 L	2 11111 ~ 2 11111	(+139%  µp to  +149%)
		sand 17.5%			(*135% up to *115%)
		sand			
Invertase activity [165]	Cinnamon soil, China	35.7% clay	PE	$2 \text{ mm} \times 2 \text{ mm}$	No consistent effect
		46.8% silt			
		17.5% sand			
Phosphatase activity [163]	Top loess soil China	18.4% clay	PP	$20 \ mm \times 20 \ mm$	Increase
		25.0% silt			
		55.9% sand			

[162], whereas relatively high concentrations of PP (7% to 28% w: w) in Loess soil increases FDA activity [123]. A significant relation between MP concentration (0.05% to 2%) and FDAse activity was observed for various polymer types including polyamide beads, PE fragments, polyactic acid (PLA) and polyester fibers [126]. Likewise, phosphatase activity increases in the presence of MPs (7% to 28% w:w), whereas it decreases in the presence of low amounts of NPs (10 to 1000 µg per kg soil) [163,164]. Both the incorporation of NPs (10 to 1000 µg per kg soil) and MPs (7% to 28%) have shown to decrease the dehydrogenase activity [162,164] and temporarily (less than 60 days) enhance the soil microbial respiration [163,164]. In addition, decreases in most enzyme activities involved in C (B-glucosidase and cellobiohydrolase), N (leucineaminopeptidase) and P (alkaline phosphatase) cycles have been noted 28 days after exposure of low concentrations (0.1-1 mg per kg dry matter of soil) of NPs [164]. Also LDPE  $(2 \text{ mm} \times 2 \text{ mm} \text{ fragments}, 0.076 \text{ g per kg soil})$  altered the soil microbial activity, with increases of urease and catalase activities, and no effect on soil invertase activity [165].

It can be suggested that the altered microbial activities may reflect an altered microbial community composition or diversity, which eventually will impact the soil food web (Fig. 3). An initial study using PCR-denaturing gradient gel electrophoresis showed that the incorporation of a PBAT film in soil resulted in enrichments of the fungal phylum Ascomycota after seven months of exposure, whereas the bacterial community stayed relatively stable [166]. More recent studies using rRNA gene metabarcoding did show differences in bacterial and fungal community composition upon MP addition, however [127,135]. The incorporation of PE led to a significantly higher abundance of OTUs assigned to the orders Flavobacteriales and Longimicrobiales and a family belonging to the Betaproteobacteria. In addition, PES treatment significantly enhanced the colonization of spring onion roots by soil bacteria and fungi and increased the abundance of AMF hyphae [127].

Several studies indicated a decrease in soil microbial biodiversity or biomass when MPs or NPs were present [135,162,164,166] (Fig. 3). In contrast, it has also been shown by serial dilution that the biennial plastic mulching can significantly increase the population of the soil microbial physiological groups [167]. Null effects of MP incorporation (PE, 2 mm  $\times$  2 mm fragments, 0.076 g per kg soil) on the microbial biodiversity and richness have also been shown after 90 days of exposure [165]. The observed differences in effect are probably related to the size of the plastic: Ren et al. (2020) showed that larger MPs decreased the fungal richness and diversity, whereas smaller MPs appeared to increase richness and diversity of both bacterial and fungal communities [135]. The mechanism behind these effects is so far unknown, however could be related to the relation of soil particle size fractions and microbial diversity: small size fractions generally yield higher microbial diversity [168].

Whereas the mechanisms of MPs on the changes of the soil microbial activities and community composition have not been elucidated so far, we can hypothesize the following (Fig. 3).

First, the small size and high surface area-to-volume ratio of MPs and NPs allow it to closely interact with the microbial cell and sub-cellular structure, which might lead to antimicrobial activities and an overall decrease in enzyme activities (FDA, phosphatase, dehydrogenase and C, N and P cycles) [169]. Second, in relation to the nematode community, the presence of toxic compounds on MPs could have contributed to a decline in microbial activity and diversity [170]. Recently, it has been shown that the changes induced in N cycling by MP addition (polyvinylchloride (PVC)) was mainly a function of the phthalate residing on the plastic, whereas the pure PVC was relatively inert under the studied conditions [171]. This is an important message that special atten-

tion should be given to chemical additives on the plastic surface in terms of toxicity. In aquatic environments it has already been shown that (micro)plastics can serve as a vector of chemical contaminants [172]. This raises the question whether a similar process occurs in soil ecosystems. For example, pesticides and mineral fertilizers residing in the soil, might attach to plastic particles, resulting in local, high concentrations of the chemical substances. Pesticides can kill or inhibit soil microorganisms as well as pests and pathogens, resulting in a lower microbial diversity, biomass and/or soil respiration [173]. It was observed that the number of viable *Rhizobium* sp. decreased after fungicide treatment [174] and a reduction in N-fixation was noted after application of herbicides [175].

Third, the presence of MPs can change the soil bulk density and porosity [126–130]. The change in soil porosity can result and explain the increase of the soil microbial respiration. Furthermore, we hypothesize that the observed decrease in soil bulk density by MP pollution can eventually lead to lower soil compaction as described in the introductory section of this review. An increased soil aggregate stability and therefore an increase of pore spaces in which the microbial community can move is to be expected [176]. This increase in microbial habitat, will result in the increase in microbial activity and degradation of organic matter, releasing more plant- and microbial-available nutrients and thus potentially leads to an increase in microbial biomass, which should be the focus of future studies.

Based on the observed effects on soil chemistry and plant performance, we predict that the addition of MP particles can also lead to an increase in the C:N ratio, a higher fungal biomass in the soil, and a change in the rhizosphere community of the plant.

Plastic particles have a very high C content [177]. It has already been suggested that plastic material can be degraded (slowly) in soil ecosystems, and therefore might interfere with the C:N ratio. This might lead to microbial immobilization [124], but might also shift the microbial community towards a higher abundance of plastic-degrading microorganisms.

In addition, it can be expected that the fungal biomass will increase for three main reasons. First, for tillage it has been shown that less disturbed soil with higher C:N ratios contain a higher fungal biomass (see introduction). Second, plastic interfere the soil moisture fluctuations, potentially influencing the composition of the microbial community [88,132]. With the observed increase in soil water availability and evaporation, we expect higher abundances of drought-tolerant organisms, such as fungi, but potentially also sporulating bacteria, in plastic polluted soils. Third, an elevated soil pH is noted in the presence of tire particles. As described earlier, bacteria are sensitive to pH fluctuations and thus the bacterial community is expected to change drastically. Fungi will be less affected by this change. However, as most fungi favor slightly acidic environments, an increase in pH might lead to a decrease in fungal biomass. The elevated pH will also indirectly disturb plant and microbial growth. Increases in pH to levels above 7 will lead to a drastic decrease in the bioavailability of many essential metal ions. For example, it has been shown that the solubility of Cd is reduced 8.8-fold by an increase in pH from 6 to 7 [178].

Furthermore, the observed change in root structure by the presence of MPs will lead to other microorganisms being attracted to the plant's rhizosphere (see introduction), either directly through the change of the root architecture [25] or by the release of root exudates [26,27], which will attract mycorrhizae, N-fixers or PGPR (Fig. 3). It is unclear whether this will have a negative result for the plant.

Last, in aquatic environments, it has been shown that especially smaller organisms such as microalgae, Bryozoa, insects, macrobenthos, bacteria and fungi might benefit from the presence of plastic particles, as they can use them as a water vehicle to travel to new foreign habitats, among others [179–183]. In comparison to soil ecosystems, aquatic environments contain however a low amount of particles. Nonetheless, plastic might still play a role as transport vehicle as well in soil ecosystems, as other organisms might be attracted towards the plastic compared to the soil particles [165]. This may enable these microorganisms to travel into deeper soil layers or even into groundwater. This is hard to verify for microbes; the first step will be to determine whether plastic carries any of these organisms.

Most of this information is still preliminary; more research regarding the effects of MPs on the soil-plant-microbiome interplay is needed. The results described in this review give a first impression on how the soil-plant-microbiome interactions can be interrupted. Results are often contradictory, however. We suggest that this can be attributed to: (1) the lack of standardized methods, and (2) the focus on macro- and larger MPs in soil systems. The first concern is that different polymer types, soils and plants are used, as well as different concentrations and sizes of MPs. Several publications have shown that at least the type of polymer, particle size, shape and probably concentration will result in different effects on the soil microbiome and in relation the soil physicochemistry and plant growth. In addition, soil type might play a role. For instance, Wan et al. (2019) showed that the reduction of soil water evaporation by plastic addition is not only related to smaller plastic sizes, but was much more pronounced if the soil matrix consisted of clay minerals compared to silt or sand [131]. The lack of information on MP contamination in soil makes it, however, difficult to set up good standardized experiments. In-depth studies are urgently needed throughout all different soil ecosystems to determine target concentrations of MPs. In addition, these target concentrations of MPs should be based not only on the current, but presumably higher future levels of contamination as well [184]. Second, most studies still focus on bigger (micro)plastics as these are "easier" to use in studies. We expect major consequences for the tiniest fractions (NPs and MPs) however for three main reasons: (1), it has been shown that these can change the soil structure and the soil permeability: (2) these can be ingested by larger soil biota such as earthworms and nematodes, thereby affecting the soil food web and soil compaction; (3) they can be taken up by the plant [185,186], potentially harming plant development.

#### 4. The plastisphere: a microbial environment on its own

The presence of microorganisms on plastic was reported for the first time in a marine environment. In the early 1970s, Carpenter and Smith reported the presence of diatoms, bacteria and hydroids on the surfaces of plastic debris in the Sargasso Sea [99,100]. By using Scanning Electron Microscopy, Sieburth (1975) was able to make images of pennate diatoms, filamentous cyanobacteria, coccoid bacteria and bryozoans on HDPE plastic bottles [187]. These were the first glimpses of what we now refer to as the "plastisphere" [188], a name originally assigned to the diverse assemblage of taxa that inhabit the thin layer of life on the outer surface of plastic debris, but which now more broadly refers to life on the surface of plastic litter in general.

Previous studies in aquatic environments have shown that microbial biofilms on MP surfaces are formed within one to two weeks and that the plastisphere selects for particular microbial communities that are distinct from those of the surrounding environment, e.g. water and sediment [179,189–192] and other inert surfaces, e.g., glass [192]. Data on the plastisphere microbiome in terrestrial ecosystems are scarce. In relation to aquatic environments, the microbiome of MPs contains a significantly smaller

number of bacteria and fungi and microbial diversity compared to its environment, the bulk soil [165,193]. Whereas the complex microbial assemblage of the plastisphere differs significantly from those of the bulk soil [193–195], this effect seems to diminish over time. Puglisi et al. (2019) showed that the microbial communities on more degraded plastics present for a longer time in landfills become more similar to the surrounding soil [196]. Analysis of the bacterial communities indicated that MPs might serve as a "special microbial accumulator" in farmland soil, enriching taxa that might degrade the plastic, such as Pseudomonas sp., Streptomyces sp. and Leptothrix sp. [194]. These effects are influenced by the type of soil [193], but also the polymer type [193,196] used in the study. In addition, it has been hypothesized that biodegradable plastics like PLA show larger changes in microbial community structure in the plastisphere compared to non-degradable fragments like PE [193]. We believe this is related to higher metabolic activity (due to biodegradation), which will release metabolites and attract other species towards the plastisphere community. Recently, it has been shown that the presence of biodegradable polyhydroxyalkanoates did not only alter the soil microbial community, but increase the activity of the microbial biomass in the plastisphere with higher activities of β-glucosidase and leucine aminopeptidase and lower enzyme affinity. The authors claimed that this was the driving factor of higher C and nutrient turnover in the soil [197].

These first studies reveal results similar to the studies that show how plastic serves as a new microbial habitat [165,193–196]. It remains unknown whether this community will differ between environments, will influence the surrounding environment, or will interfere with soil–plant-microbiome interactions; these subjects should be the focus of future studies.

### 5. Summary and future perspectives

In this review we have shown the importance of the microbial community in the soil ecosystem. Microbes are largely responsible for the soil physicochemistry dynamics, as they mediate C sequestration and play a role in the formation of the soil structure. In addition, the growth and defense response of plants is influenced by the microbial community as well, as microbes play a dominant role in nutrient cycling and can be actively attracted to the plant root. Human interference is the main reason the interaction of soil microbial communities, soil processes and plant development are disrupted. Soil research has primarily focused on agricultural processes such as tillage, compaction, monoculture and the addition of soil amendments, fertilizers and pesticides. Human-caused pollution, of which plastic is the most common and well-known, will disrupt the microbial community as well. We indicated that the decrease in soil bulk density, the fluctuations in soil moisture content and the change of the soil pH might form the basis of the decreased microbial activity and microbial biomass and the increase in soil microbial respiration by the presence of MP particles. In addition, we addressed that the presence of chemical additives compounds (e.g. pesticides and mineral fertilizers) on the surface of plastic particles might be even more important to explain the toxic effects of MP particles on the soil microbiome. Furthermore we present the hypothesis that MP incorporation will lead to an increase in fungal biomass by the decrease of the soil pH and C:N ratio, a larger abundance of plastic degraders, and a change in the rhizosphere microbiome due to the changed root architecture of MP-exposed plants. Due to the importance of microorganisms in soil ecosystem functioning, we therefore urge the research community to focus on terrestrial and not exclusively marine MP pollution, and to focus on the effect of MP pollution on the microbial community in future experiments. In addition, we

urge the research community to develop standardized methods to study the smaller plastics (MP and NP) environmentally realistic MP concentrations, as the concentration of these small plastic particles will strongly influence the observed effects.

### **CRediT authorship contribution statement**

**L. Joos:** Conceptualization, Writing – original draft. **C. De Ten-der:** Conceptualization, Supervision, Writing – review & editing.

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#### **Declarations of interest**

None.

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