

1 **Implication of microplastics on soil faunal communities – identifying gaps of knowledge**

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8 **Abstract**

9 There is mounting evidence that plastic and microplastic contamination of soils can affect
10 physico-chemical processes and soil fauna, as has been excellently summarised in many
11 recently published meta-analyses and systematic reviews elsewhere. It has become clear that
12 impacts are highly context dependent on e.g., polymer type, shape, dose and the soil itself.
13 Most published studies are based on experimental approaches using (semi-)controlled
14 laboratory conditions. They typically focus on one or several representative animal species
15 and their behaviour and/or physiological response – for example earthworms, but rarely on
16 whole communities of animals. Nevertheless, soil animals are rarely found in isolation and
17 form part of intricate foodwebs. Soil faunal biodiversity is complex, and species diversity and
18 interactions within the soil are very challenging to unravel, which may explain why there is
19 still a dearth of information on this. Research needs to focus on soil animals from a holistic
20 viewpoint, moving away from studies on animals in isolation and consider different trophic
21 levels including their interactions. Furthermore, as evidence obtained from laboratory studies
22 is complemented by relatively few studies done in field conditions, more research is needed
23 to fully understand the mechanisms by which plastic pollution affects soil animals under
24 realistic field conditions. However, field-based studies are typically more challenging

logistically, requiring relatively large research teams, ideally of an interdisciplinary nature to maintain long-term field experiments. Lastly, with more alternative, (bio)degradable and/or compostable plastics being developed and used, their effects on soil animals will need to be further researched.

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30 **Introduction**

Research on how plastic (especially microplastic) pollution affects soil ecosystems has increased greatly over the last decade, with many comprehensive systematic reviews and meta-analyses recently published on the topic (e.g. [1-9]). Recently a comprehensive database has been compiled, amalgamating data research on the toxicity of microplastics in a wide range of habitats [10]. It is widely accepted that biologically healthy soils are crucial for ecosystem functioning [11, 12] and the myriad of services they provide [13] (Figure 1). With the increase in (micro)plastics pollution in soils, however, ecosystem services and associated faunal communities are considered to be under threat [14]. The aim of this paper is not to systematically review the current state of knowledge, as that has recently been extensively done elsewhere, but to identify specific gaps of understanding of how (micro)plastic pollution affects soil faunal communities, i.e. effects on higher levels of biological organisation, to direct future research.

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44 **Interactions between plastics and soil fauna at higher levels of organisation.**

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It is thought that the majority of plastic pollution in soil ecosystems can be attributed to agriculture and poor waste management, but there are many other pathways for plastics to enter soil, as recently reviewed by [15], for example car tyre particles from road wear [16],

49 clothing fibres from washing [17] and application of wastewater or sludge to soils [18]. In
50 some cases, plastics are added onto soil deliberately; an obvious example being the
51 application of plastic mulches in agriculture [19] to create favourable microclimates for crops
52 to grow [20], but with the potential of plastic fragments remaining in the soil [21-23], even
53 though there are plastics marketed as biodegradable or compostable e.g. made of
54 biodegradable polymer. However, with many types of plastics designed to last, and thus
55 degrade poorly under natural conditions in the soil [24], there is concern for the accumulation
56 of plastics in soils [25], including as microplastics [26], currently still defined as particles <
57 5mm or < 1mm in size [27]. Since raising concerns of microplastic contamination of soils a
58 decade ago by [28], there has been an increased effort to understand their impacts on
59 terrestrial ecosystems, with studies exploring effects on ecosystem functioning [29] and soil
60 biota [7]. When deposited on the soil, microplastics are translocated in the soil profile through
61 bioturbation by soil animals (e.g. earthworms), agricultural activities such as ploughing, but
62 also natural physical soil processes such as slaking [30] and precipitation [31], and certain soil
63 animals can contribute to the further fragmentation of plastic particles into microplastics [32].
64 There is mounting evidence for the effects of microplastics on soil physico-chemical processes
65 [33], which appear to depend on the dose, shape, size and chemical composition of the plastic
66 particle [29, 34]. For example, soil porosity and associated water movement and capacity can
67 change with certain types of microplastics [33, 35,36], but also evaporation increases with the
68 presence of plastic particles in the soil [37]. Furthermore, soil pH – a crucial factor for many
69 (bio)chemical processes can also be altered when microplastics are in soil [38], for with
70 cascading effects on other processes such as nitrogen cycling [39]. Most of these processes
71 are associated with soil microbes and their activity, in particular bacteria and fungi [3], which
72 have been the focus of many studies concerning the effects of microplastics on soil

biodiversity. There is evidence that microplastics can alter the composition and functioning of microbial communities [3], and there is evidence, based on genetic sequencing, that microplastics themselves may have different microbial communities from the surrounding bulk soil (e.g. [40]). The relatively greater research focus on microbial communities (compared with larger soil biota such as worms and microarthropods) is possibly due to the relative ease to gather phylogenetic information from microbes by exploring eDNA with well-defined universal primers (such as those amplifying 16S rRNA) combined with metagenomic analyses and associated putative functional profiles [41]. Such an approach using eDNA is challenging for soil animals, which may explain why soil faunal communities are currently under-studied compared to microbial communities. Furthermore, it can be challenging to experimentally reconstruct representative soil faunal communities in the laboratory or field. As such, most studies considered how single, or an assemblage of a few animal species are affected by microplastics (as reviewed by e.g. [2, 42-44]). Earthworms, in particular, have been studied in the context of microplastic research. They can be considered important in the soil as ecosystem engineers [45] and are relatively easy to maintain under controlled laboratory conditions. For example, Jiang et al. (2020) [46] found that tiger worms (*Eisenia fetida*) responded negatively to the presence of polystyrene microplastics, with several biochemical changes at the cellular level. Similar results were found by Sobhani et al. (2022) [47] who exposed *E. fetida* to microplastic made of polyethylene. Other species of earthworms have been reported to be negatively affected by exposure to microplastics, for example, the endogeic rosy-tipped worm (*Aporrectodea rosea*) had reduced growth when exposed to different types of microplastics in a mesocosm study by Boots et al. (2019) [48]. A seminal study on the anecic earthworm *Lumbricus terrestris* by Huerta Lwanga et al. (2016) [49] found that their growth also was negatively impacted, including their survival, by the presence of

LDPE microplastics in a simulated litter layer. A subsequent publication by Huerta Lwanga et al. (2017) [50] reports that microplastics can be translocated into the soil matrix through burrowing activities by *L. terrestris*, thereby potentially exposing other soil fauna to microplastics. Another study with *L. terrestris*, by Zhang et al. (2018) [51], explored their potential involvement in incorporating plastics into soil when exposed to larger pieces from biodegradable and conventional plastic mulches, and found that the earthworms incorporated both types of plastics into the soil as well. When in the soil, these plastics may further degrade and or be redistributed through the soil profile. Other soil animals may also contribute to the redistribution of plastics within the soil. For example, the collembola *Folsomia candida* and *Proisotoma minuta* were studied by Maaß et al. (2017) [52] to understand their role in the translocation of particles and fibres. They found that the two species interacted differently with the microplastics suggesting that they could affect the distribution of microplastics differently within the soil matrix. However, Kim et al. (2019) [53] reported that the collembolan *Lobella sokamensis* was negatively affected when microplastics were present due to their propensity to block micro-cavities within the soil matrix where the animals are typically found thereby restricting their movement. The potworm *Enchytraeus crypticus* has been shown to avoid microplastics obtained from HDPE bottle caps when in the soil, moving to areas with no or lower concentrations [54]. Lahive et al. (2019) [55] explored how *E. crypticus* responded to the presence of different size and shapes of microplastics in soil, and they found that *E. cryptus* ingested nylon fibres and that their reproduction was reduced. In another controlled mesocosm-based study, Selonon et al. (2020) [56] explored the response of *E. crypticus*, *F. candida*, the oribatid mite *Oppia nitens* and the isopod *Porcellio scaber* to polyester fibres either mixed in soil or leaf litter. They also reported that *E. crypticus* ingested the fibres and displayed reduced reproduction, but the other test organisms showed

121 marginal responses. The animals, however, were studied in separate mesocosms which did
122 not allow the exploration of community-level effects of the microplastics. Most studies
123 appear to have been done on specific taxa, such as worms, perhaps because they are relatively
124 easy to maintain, can be considered model organisms representing different functional
125 groups [57]. Other soil organisms are also often used in soil ecotoxicological studies, such as
126 isopods [58], yet there is limited information on these taxa when exposed to
127 microplastics. Even so, soil animals rarely occur alone as single species in soil, which can
128 contain great diversity and abundance of animals from different taxonomic groups,
129 comprising an extremely complex foodweb of multiple trophic levels of detritivores, grazers
130 and predators at the micro-, meso- and macroscale (e.g. [59]). Even though there is ample of
131 evidence for single-species effects when exposed to the myriad of microplastics (e.g. shapes,
132 polymers, sizes, doses) as reviewed elsewhere, similar studies considering multi-species at
133 higher levels of organisation are not very common in the literature [60]. There are very few
134 studies reporting the effects of microplastics on soil faunal communities, including those done
135 under field conditions. For example, a seminal observational field study by Huerta Lwanga et
136 al. (2017) [61] explored trophic translocation from soil containing microplastics which were
137 also found at increased levels in earthworm casts and subsequently increased in chicken
138 faeces, but they were also detected in chicken and crop plants. It is not clear however, how
139 the presence of microplastics affected the highest trophic level, nonetheless this raised
140 concern that microplastics can move up the food chain. A more recent, 287-day long study by
141 Lin et al. (2020) [62] showed that when LDPE microplastics are experimentally applied to the
142 topsoil layer in a field, microarthropod and nematode communities were affected. In that
143 study [62], animals were classified by functional groups (e.g. feeding types of nematodes) or
144 order (e.g. ants as Hymenoptera) and they found that there was an overall decrease in

abundance of organisms with microplastics present, but also the community composition shifted with increasing density of microplastics. At 287 days, the study reported in [62] is relatively long-term, but to fully understand how soil fauna responds to microplastic pollution, more long-term research is needed. This is especially important for agricultural settings so that different crop growth seasons will be encompassed. Long-term experiments, however, are challenging and require continuous funding to maintain and sampled typically by interdisciplinary research teams. A study by Hernández-Gutiérrez et al. (2021) [63] explored how soil invertebrates responded to microplastics under agricultural mulching (plastic LDPE sheets), but also the use of glyphosate. Mulching was applied to the field from 2009 and the study was conducted in 2019. They found that there were more microplastics in the soil with mulching and they reported that there were more individuals and taxonomic orders of above-ground invertebrates captured with pitfall trap associated with the mulched soils. It remains unclear, however, if the microplastics in the soil caused these differences. Maintaining long-term and field-based studies, especially of a manipulative nature is challenging and requires relatively large research teams. Ideally the team should consist of several scientific disciplines so that a holistic picture can be sketched of how soil biodiversity and associated ecosystem functioning can be affected.

Moving forward: focus for research

Soils are extremely heterogeneous systems which make studying mechanistic pathways challenging [64] and, therefore, generalisation is very difficult. To make generalisation feasible, more empirical data is needed which encompass the many different biological, chemical and physical processes within soils. As these processes rarely occur in isolation, researching them in tandem (i.e. reciprocal effects on physico-chemical parameters and soil

biodiversity) will provide a more holistic picture of how microplastics can affect soil ecosystems. Moving on from general observational studies, there is a need to shift to studies designed to unravel hypothesised mechanistic pathways, for example related to the shape, size and chemical composition of the plastics. Mechanistic pathways can be direct (e.g. physical contact) or indirect (e.g. via affecting a trophic level), but unravelling these pathways at the community level requires very careful experimental designs to ensure confounding variables are minimised. Furthermore, effects on animals at the molecular level (genes, enzymes), such as oxidative-stress related responses [65, 66] could help further explain how soil animals are being affected, including sub-lethal but chronic effects. To bring this further, as also suggested by So et al. (2022) [67], it is important to explore how microplastic pollution affects soil ecosystems under more realistic conditions, ideally in the field, with realistic doses of microplastics applied [68]. Laboratory-based studies are valuable, and they suggest that soil biological and physico-chemical characteristics respond differently to the characteristics of plastic contamination, however, more information is required under different field settings to understand how microplastics affect soil fauna at the community level. Combined with trait-analysis techniques (e.g. [69]), potential community level (e.g. foodweb) effects can be explored. To achieve this, simplified model communities of soil fauna could be constructed representing several trophic levels (including primary producers). Field-based experiments, however, typically require more maintenance, effort and costs, and they introduce more noise than controlled or semi-controlled approaches. There could be an ethical issue regarding the deliberate addition of an emerging pollutant to field plots which will need to be considered. Nevertheless, by moving from lab-based studies to long-term field settings, we can fully understand the impact of microplastics in soil ecosystems, including soil fauna.

Furthermore, there are still many groups of soil fauna which remain relatively understudied. In 2018, research on soil protists was already identified as lacking [70], such as amoeba which are important members of the soil foodweb, and there is still a dearth of information on this group of soil fauna. Soils can contain a great diversity of soil micro- and mesofauna, including pathogens and parasites, which all contribute to the health of soil ecosystems. The impacts of microplastic pollution on soil dwelling vertebrates are not studied in much depth either, although the definition of “soil dwelling vertebrates” is context dependent, i.e. animals living on the surface, within the litter layer, or within the soil. One could consider snakes as part of the terrestrial foodweb as well, and [71] found microplastic fibres in the gastro-intestinal tract of two different snake specimens (*Natrix natrix* and *N. tessellate*) kept in a museum from 1989 – 2019. Snakes are relatively at high trophic levels in terrestrial foodwebs which may explain the presence of the plastics inside their bodies. There are other studies on vertebrates, but mainly on mice and under laboratory conditions (e.g. [72]), as conducting ecotoxicological research on soil vertebrates typically requires strict ethical consideration. Such research may be valuable for wildlife, however, ethically that may be questionable.

Lastly, there is an increase in biodegradable and/or compostable plastics manufactured of materials designed to degrade more rapidly than conventional types (e.g. polyethylene, polyethylene terephthalate or polystyrene) and several products also claim to generate no microplastics. However, (bio)degradation in the natural soil environment and the effects of those plastics when littered or incorporated in the soil especially those on soil functioning and related faunal communities will have to be better understood to assess their potential risks [73], before they are adopted as an alternative to conventional plastics, including in agriculture.

216 **Summary points**

- 217 • There is mounting evidence that (micro)plastics can affect soil animals directly and
218 indirectly via changes in soil physico-chemical properties. This manifests as lethal and
219 sub-lethal (e.g. at the molecular level) health responses.
- 220 • Recently many systematic reviews and meta-analysis have been published
221 amalgamating the scientific literature, but there is still a need for more empirical
222 research to understand the underpinning mechanisms of how (micro)plastics can
223 affect soil animals.
- 224 • Many taxa of soil fauna remain understudied. To generate a bigger picture, more
225 information on effect of microplastics on taxa which are more cryptic, e.g. micro- and
226 mesofauna.
- 227 • There is still a dearth of information on how soil animal communities, at a higher level
228 of organisation, are affected as most research has been done on one or just a few
229 animals in isolation.
- 230 • Research will have to move to studies done under field conditions, ideally long-term,
231 including focus on (bio)degradable types of plastics and understudied groups such as
232 vertebrates, although there are many (ethical) challenges to consider.

233

234 **Declaration of conflict of interest**

235 There are no conflicts of interest.

236

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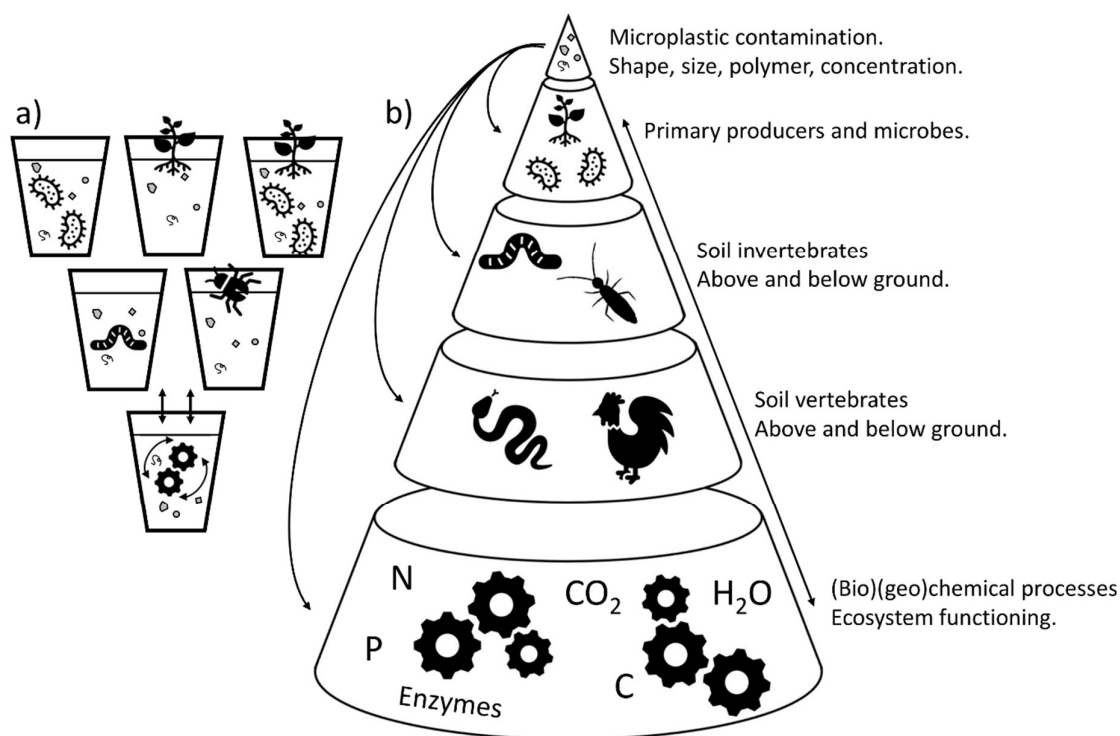
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552 **Figure 1.** Most research on the effects of microplastics have been done in isolation in
 553 laboratory studies (a), with one or several trophic groups considered such as microbes, plants,
 554 worms and invertebrates. Soil ecosystems are very complex and instead of viewing them as
 555 separate compartments, a more holistic approach needs to be adopted linking primary
 556 producers to microbes and soil fauna and associated (bio)geochemical processes (b). Symbols
 557 are obtained from MS Office 365 (Microsoft 2022), except the collembolan (credit: Shyamal
 558 L.)