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# Extent and effects of microplastic pollution in soil with focus on recycling of sewage sludge and composted household waste and experiences from the long-term field experiment CRUCIAL

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ABSTRACT

Microplastics (MP) occur in household waste products, which can be recycled as fertilizers in agricultural fields. Recycling of waste products has many benefits but concerns of the effects of MP on soil health limit recycling. MP are present in composted household waste and sewage sludge. Sewage sludge contains many small particles (primarily fibers and fragments), whereas compost mainly contains larger fragments (flakes from packaging and bags). Here, we review the extent and possible consequences of MP pollution in soil with focus on waste product recycling. We summarize the results from studies that have measured MP concentration in soil and waste products. We review the possible hazards of MP on soil invertebrates, plant growth and microbial communities based on published studies. We discuss these results in relation to MP quantities measured in agricultural fields and generally find that MP contents in fields are below the MP levels that cause negative effects in most current effects studies. Finally, we present results from the long-term field experiment CRUCIAL, which have received composted household waste and sewage sludge in dosages corresponding to more than 100 years of legal amendment. Experiments with earthworms and quantification of various soil organisms do not indicate that household waste and sewage sludge, including the inherent contaminants, affect soil health negatively. In fact, growth of earthworms and abundances of organisms were often higher in these treatments compared to NPK-fertilized or unfertilized plots, probable due to the content of organic matter in the waste product. Based on these assessments, we conclude that the potential risk of current levels of microplastics in terrestrial environments is low for agricultural soils, but more studies are needed to perform a robust risk assessment.

## 1. Introduction

Recirculation of nutrients from urban and industrial organic waste and by-products, for example composted source separated organic waste (SSO) and sewage sludge, can reduce the use of NPK-fertilizer (i.e., commercial fertilizers containing nitrogen (N), phosphorous (P) and potassium (K)), resulting in both economic and climate benefits. Because the production of nitrogen for NPK-fertilizers is associated with high energy demands [1] and phosphorus is a critical resource [2], the utilization of NPK-fertilizers raises environmental, economic and climate concerns. Transitioning from the use of NPK-fertilizers to employing nutrients derived from organic waste and by-products, notably, ensures effective recycling of phosphorous. Compost and sludge, in addition, contain significant amounts of carbon [3], and continuous supply of carbon to agricultural fields increases soil carbon content, enhancing carbon sequestration and improving soil structure, with positive effects on both climate and plant yield [4].

Organic waste products are, on the other hand, often met with skepticism, as they contain xenobiotic residues [5,6]. Contaminants of concern are heavy metals and organic compounds (pharmaceuticals, detergents, biocides, per- and polyfluorinated substances (PFAS) and pesticides). Skepticism related to heavy metals in organic waste products was justified in the past, as particularly sewage sludge previously contained significant amounts of heavy metals, which accumulated in the soil and impaired plant growth and terrestrial organisms [7]. However, heavy metal concentrations in sewage sludge in many EU

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countries have declined drastically in recent decades, and it is estimated that current levels are comparable to levels in farmyard manure [8].

Recently, there has been growing awareness and concern among scientists, policymakers, and the general public regarding the contamination of agricultural fields and terrestrial environments by plastics [9]. Often focus is on microplastics (i.e., plastic particles of 1–5000 μm), but also particles below and above this size range may be of concern. Plastics are organic polymers that, in principle, may decompose and eventually disappear through physical-chemical degradation (photooxidation in particular) [10] or biological degradation [11,12]. However, most plastics are slowly degradable, and thus likely to stay in the soil for decades given the environmental conditions [13]. Presumably, physical/mechanical fragmentation of plastic materials will increase the rate of degradation, but at the same time, the resulting smaller particles are potentially more harmful to soil organisms. When assessing pollution with microplastics, it is thus important to notice that both plastic type and size, as well as a range of additional parameters, e.g., morphology and surface chemistry, will determine the environmental fate and effects. In addition, pollution with microplastics is dynamic, as both the chemistry, size, number of particles and other relevant parameters change over time.

Sources of microplastics in terrestrial environments are many and varied [14]. In addition to the use of potentially microplastic contaminated organic resources, agricultural plastic for crop covering (i.e., plastic mulching), littering, irrigation, abrasion of car tires, paint peelings from agricultural machinery and possibly other sources contribute to terrestrial plastic pollution. Despite the relevance and increasing concern of pollution with microplastics in terrestrial environments, there are still significant knowledge gaps regarding microplastics in compost, sewage sludge and other organic waste products used as fertilizers. Among other things, we lack knowledge about the contribution of organic waste fertilizers relative to other sources of terrestrial microplastic pollution, and on how microplastics affect soil organisms and soil health at environmentally realistic concentrations and exposure conditions. Although microplastic prevalence, fate and effects are considerably more studied in aquatic environments, there is still no scientific consensus regarding the risks associated with microplastics. While many studies report negative effects on various aquatic organisms, the picture becomes more ambiguous at the lower exposure concentrations that are of environmental relevance [15–18]. Thus, even if the measured environmental concentrations are underestimated, some studies may overestimate the risk of effects. This uncertainty is even larger for the terrestrial environment, due to fewer studies on terrestrial organisms, and a limited number of studies using exposure conditions relevant to field conditions (e.g., complex mixtures of plastics and possibly other contaminants, environmentally realistic concentrations, long exposure time and natural soils).

The aim of this review is to provide an overview over extent of pollution with microplastics in agricultural soils, with a focus on potential inputs from recycled waste products, such as sewage sludge and organic household waste, and to compare this to observed effects of microplastics on soil organisms and plant growth. As a specific case, research results from the CRUCIAL experiment - a Danish field trial where plots have been fertilized with various forms of organic waste products since 2003 - are reviewed. The CRUCIAL experiment provides insight into long-term effects expected to manifest due to the total xenobiotic input from different fertilizer products.

## 2. Microplastics in terrestrial environments

Sources of microplastics in terrestrial environments are many [19], but limited focus has been on assessing the relative contribution of different sources of microplastic pollution. Although more focus has been devoted towards microplastic pollution in agricultural soils and other terrestrial environments in the very recent years, the major challenge in obtaining a substantiated assessment of microplastic prevalence in soils, is the lack of standard procedures for sampling and analysis of soil matrices as well as agreed metrics for reporting the results. Many published studies use particle number per fresh or dry weight soil or other matrix, fewer use a mass-based metric (typically plastic weight per dry weight soil), and some use particle number per area, which make comparisons among studies, organic resources, geographic areas, and different land-use difficult.

This section addresses the prevalence of microplastics in agricultural and other terrestrial environments and review the potential contribution of organic waste fertilization to microplastic prevalence in the terrestrial environment.

### 2.1. Microplastics in organic waste products

Both sewage sludge and source separated organic (SSO) resources such as composted organic household waste contain microplastics from households [20,21], but there are differences in the type, sizes and distribution of the plastic particles. Sewage sludge contain microplastics transported from households and industries via wastewater as well as microplastics from roads and other impervious surfaces in areas with combined sewage systems. Estimated >90 % of microplastics in wastewater is retained in the settling tank of the most efficient wastewater treatment plants and ends up in the sludge fraction [22]. It is often smaller particles, for example fibers from laundry and dishcloths/sponges [23], rubber residues from car tires, paint, and to a lesser extent deliberately added plastic in cosmetic products. Whereas heavy metal concentrations in sewage sludge have decreased [8], microplastic content in biosolids (i.e., sewage sludge) has increased from 1990 to 2016 corresponding to increasing plastic production since the 1950s [24].

Studies of Danish sewage sludge (Table 1) from altogether seven Danish wastewater treatment plants, showed concentrations between $1.3 \times 10^5$ and $6.8 \times 10^8$ particles $kg^{-1}$ DW [25–27]. Differences in concentrations probably primarily reflect that the studies used different analytical methods, including different lower size-limits for detection (100 μm and 10 μm, respectively), as well as actual differences in the wastewater treated at the plants and the sampling season. A median concentration of 4.5 mg plastic $g^{-1}$ wet digested sludge was estimated for 5 samples [25]. This means that approximately 0.7 % (wet weight) of the tested sludge samples were plastic particles in the size 20–500 μm. Particles observed in sludge are primarily fibers (ca. 70 %) and fragments (e.g., black rubber) (ca. 20 %) [20,26,27]. In comparison, many previous studies from other countries found considerably lower concentrations (down to ca. 500 particles $kg^{-1}$ DW; Table 1) most likely also due to different quantification methods and differences in the minimum detectable particle size.

Source separated organic waste from households (SSO-HHW) also contains microplastics, but their characteristics differ from particles found in sewage sludge. They are typically fragments of packaging and wrapping material. Fewer studies have assessed microplastics in composted SSO-HHW than in sewage sludge. Generally, SSO-HHW contains fewer particles than sewage sludge, for example 20-$2 \times 10^5$ particles $kg^{-1}$ DW were reported in compost and biopulp from HHW of various origins (Table 1). It should be noted that only particles >0.3 mm were measured in Ref. [28], while the minimum size for detection was 1 mm in Refs. [21,29]. In general, compost contains a larger proportion of bigger particles compared to sludge, and many particles are so large that they are not classified as microplastics. Due to the use of different methods for sample preparation and analysis, it is problematic in general to compare content of microplastics in sewage sludge and compost. However, the same method, primarily focused on detecting particles larger than 100 μm, was employed for the analysis of microplastics in sewage sludge [26,27] and in pre-treated and biogasified SSO-HHW [30], making it reasonable to compare these results (Fig. 1). Calculated on a dry matter basis, the particle content is higher in sewage sludge ($1.8 \times 10^5$-$2.4 \times 10^5$ particles $kg^{-1}$ dry weight) compared to SSO-HHW ($5.6 \times 10^4$-$1.8 \times$

**Table 1**
Published data on microplastic concentrations (particles/kg or g/kg) in organic resources.

| Organic ressource | Country | Analytical method | Particles detected | Particle size | Concentrations of MP particles | | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | Particles $kg^{-1}$ | g $kg^{-1}$ (‰ of DWT) | |
| Sewage sludge | USA | | Only fibres | NA | 1500–4000 | | [31] |
| Sewage sludge | USA | Microscopy + FTIR validation | Mostly blue irregular PE | NA | 1000 | | [32] |
| Sewage sludge | Canada | Microscopy | Fragments & fibres | NA | $4.4 \times 10^3$–$14.9 \times 10^3$ | | [33] |
| Sewage sludge | Sweden | Microscopy + FTIR validation | NCSP | ≥300 μm | 16,700 ± 1960 | | [20] |
| Sewage sludge | Finland | Microscopy | NCSP | >20 μm | $1.87 \times 10^5$ | | [34] |
| Sewage sludge | Finland | Microscopy + FTIR or Raman | NCSP | | $2.3 \times 10^4$–$1.7 \times 10^5$ | | [35] |
| Sewage sludge | Germany | FTIR microscopy | NCSP[a] | >10 μm | 1000-24,000 | | [36] |
| Sewage sludge | Denmark | FTIR microscopy | NCSP | 20–500 μm | $1.7 \times 10^7$–$4.1 \times 10^8$ | 0.4–7.23 | [22],[b] [25], |
| Sewage sludge | Denmark | Microscopy + FTIR validation | NCSP | >100 μm | 1.3–3.2 × $10^5$ | | [26],[c] and [27],[d] |
| Sewage sludge | Netherlands | Microscopy | NCSP | >10 μm | 510–950 | | [37] |
| Sewage sludge | Spain | Microscopy, heating method[e] + FTIR microscopy | NCSP | >11 μm | 21,840–73,010[f] | | [38] |
| Sewage sludge | China | Microscopy + FTIR validation | NCSP | 37 μm-5 mm | 1565-56,386 | | [39] |
| Sewage sludge | Sweden | FTIR microscopy | NCSP | 10–500 μm | | 0.42 | [40] |
| Biogas digestate (mainly foodwaste) | | | | | | 0.006 | |
| Digested biowaste | Germany | Microscopy + FTIR validation | NCSP | >1 mm | 10.69–16.13 | | [29],[g] |
| Digested and composted biowaste | | | | | 21.01–113.92 | | |
| Composted biowaste | Germany | Microscopy + FTIR validation | NCSP | >1 mm | 20–24 | | [21] |
| Biogas digestate (Biowaste) | | | | >1 mm | 70–146 | | |
| Composted green cuttings | Germany | Microscopy | NCSP | >0.3 mm | 12–46 | 0.05–0.63 | [28] |
| Compost household SSC (HHW) | | | | | 32 | 1.36 | |
| Biopulp from household SSO (HHW) | Denmark | Microscopy + FTIR validation | NCSP | >100 μm | 0.5–1.8 × $10^5$ | | [30] |
| Biogas digestate (HHW biopulp) | | | | >100 μm | 1.4–2.1 × $10^5$ | | |
| Biopulp (mainly HHW) from 4 pre-treatment plants. | Denmark | Visual + IR | NCSP | >2 mm | | 0.06–0.29 | [41] |

NA, not applicable or not determined; NCSP, not constrained to specific particles.
[a] Including density separation favoring polymers with densities >1.14 g/cc.
[b] Note that numbers and masses in this study is provided per kg digested sludge with a water content of 25–30 %, and therefore numbers and masses are approximately 3–4 times higher on a dry weight basis.
[c] MSc report (in Danish, supervised by A. Palmqvist).
[d] Unpublished report (in Danish).
[e] The heating method utilizes microscopy of residues from filtration before and after a heating period that changes circularity, transparency and shininess of plastic particles. See more about the method in Ref. [38] and references herein.
[f] Range in average microplastic content in sludge from 4 wastewater treatment plants.
[g] Note that numbers for 1–5 mm and >5 mm are added, and that only samples from finished compost are provided in the table.

$10^5$particles $kg^{-1}$ dry weight), but when calculated per grams of phosphorus (P), sewage sludge contains fewer particles (6200 particles $g^{-1}$ P) compared to SSO-HHW ($1.7 \times 10^4$-$4.2 \times 10^4$ $g^{-1}$ P). The comparison indicates that composted household waste will add more microplastics compared to sewage sludge when application of fertilizer is based on phosphorus content. We emphasize that this conclusion is based on few studies and could benefit from more data. Nevertheless, an annual introduction of MP pollution into agricultural soils, through the utilization of urban organic waste as fertilizers, can be roughly estimated based on the data from Fig. 1 and by making specific assumptions. In the EU, use of fertilizers is regulated at the national level through implementation of various EU regulations. According to Danish implementations, use of both composted household waste and sewage sludge as fertilizers on agricultural land is regulated based on phosphorous content assuming that all threshold values for regulated contaminants in the resources are met [42]. The maximum phosphorous application from urban organic waste per hectare and year is thus 30 kg P $hectare^{-1}$ $year^{-1}$ in Denmark [43]. Assuming a plowing zone of 20 cm depth, an average soil density of 1.5 g $cm^{-3}$ and using MP averages for biopulp and sewage sludge extracted from Fig. 1, provides estimated MP pollution to agricultural soils of approximately 250 and 60 MP particles (>100 μm) $kg^{-1}$ soil for biopulp and sewage sludge, respectively, each year the agricultural land is fertilized with these phosphorous resources alone. It should be noted, however, that only a small proportion (<10 %) of the total phosphorous supplement to Danish agricultural soils stems from urban organic waste, and that urban organic waste are only permitted for use as fertilizers on certain types of crops [42]. In comparison, van den Berg and co-authors [38] estimated an average MP input of 710 particles per sewage sludge application to 11 Spanish agricultural fields and found a clear positive correlation between soil MP content and number of sewage sludge applications. The notably higher estimated microplastic input per application in Ref. [38], compared to the estimates derived from the data in Fig. 1, is likely due to a significantly greater sludge application rate in the Spanish study, surpassing what Danish regulations would permit. The sampled fields in Ref. [38] had received 20–22 t sewage sludge per hectare and application. This application rate is 3 times higher than what is permitted by Danish regulations if calculated based on dry matter (maximum 7 t dry matter $hectare^{-1}$ $year^{-1}$ as an average over 10 years) and up to 20 times higher when application rate is based on phosphorous content, as it would be

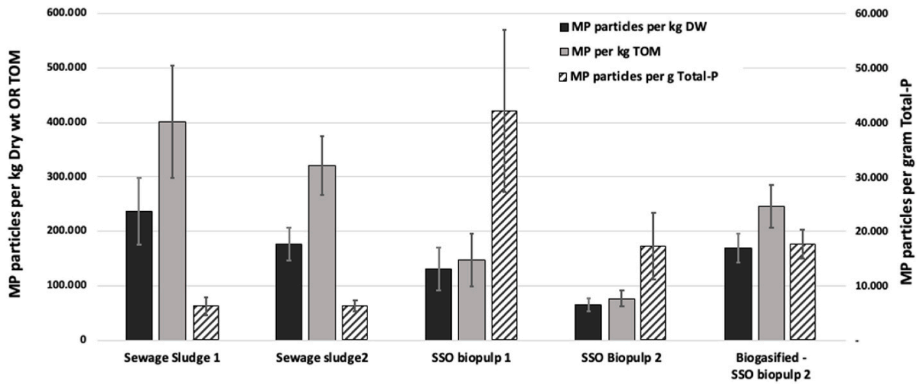


**Fig. 1.** *Comparison of the microplastic (MP) content (number of particles* per *dry matter (DW), total organic material (TOM) and total phosphorus (Total-P) in wastewater sludge from two studies* [26,27] *and biopulp of source separated organic waste (SSO) from domestic households and biogasified SSO from a third study* [30]. *The comparison is reproduced from* Ref. [30] *and indicates that microplastic content is highest in sewage sludge when compared on a dry matter basis, while it is highest in SSO from domestic households when compared on a basis of phosphorus units. The data is presented as mean values* ± *standard deviation. Note that while sample preparation and analysis method is similar for the three studies, the mode of replication is different for the different studies included in the graph: The sewage sludge 1 samples represent an average of three technical replicates. The sewage sludge 2 samples represent an average of two true replicates each divided into and analyzed as four technical replicates. The SSO samples represent an average value based on samples from two different dates (temporal replication) and three technical replicates at each date.*

due to Danish regulations, given that phosphorous content is similar in the Danish and the Spanish fertilizer products. However, irrespective of the exact microplastic input rate, it is evident that the utilization of urban waste products as fertilizers will contribute to the MP pollution in agricultural soils.

### *2.2. Microplastics content in soils*

The content of microplastics in soil depends on the amounts added with fertilizer products, via other agricultural inputs (e.g., plastic mulching, irrigation, and peeling of paint from agricultural machinery), through littering, and via deposition from the air. Therefore, location and agricultural practices partly explain variations in soil microplastic content. Published microplastic soil concentrations reviewed in Refs. [44,45] showed that the microplastic content in agricultural soils from rural areas is generally lower compared to soil from agricultural fields in urban areas, and that soils from industrial areas and other urban areas have considerably higher microplastic concentrations compared to agricultural soils (Fig. 2).

According to the 30 studies from 15 different countries, on which Fig. 2 is based, the maximum concentrations of microplastic particles in agricultural soils were 0.224 g plastic/kg dry weight soil or $5.3 \times 10^5$ particles/kg dry weight soil. In comparison, the maximum microplastic concentrations in soils from industrial sites and other urban areas were 67.5 g plastic/kg dry weight soil or $2.6 \times 10^7$ particles/kg dry weight soil. The measured soil microplastic concentrations are highly variable due to differences in sampling, sample preparation and analytical methods in addition to the differences driven by e.g., soil types, use, geographical location on both local and global scale, and different agricultural practices (e.g., fertilization with organic waste products or use of agricultural plastics). For analyses of microplastics in soil, the choice of method depends on the parameters under investigation, for example mass of particles, number of particles, type of plastic and size of the particles. Particle type and size are typically a supplement to the quantification of the particle numbers and characterize the origin of particles or the likelihood that particles are ingested. The size and type of particles are commonly considered determinants of the risk posed by plastic pollution to terrestrial organisms, underscoring the significance of understanding the sources of terrestrial microplastic pollution in addition to concentrations. Generally, it must be assumed that sewage sludge contributes to soil pollution with many small particles (especially fibers from clothes), whereas composted household waste contributes larger particles (especially flakes and foils from packaging), that may however over time disintegrate into smaller particles. Most methods for measuring mass cannot simultaneously measure e.g., number, size, shape, or color, making these measurements less biologically relevant. However, we need quantifications of mass to make better comparisons (e.g., different fertilizer products, different soils or soils over time, mass-balances) [47,48].

Quantifications based on mass are still limited, partly due to limitations in analytical methods. This is particularly problematic since most terrestrial microplastic effects studies use mass as exposure metric, and

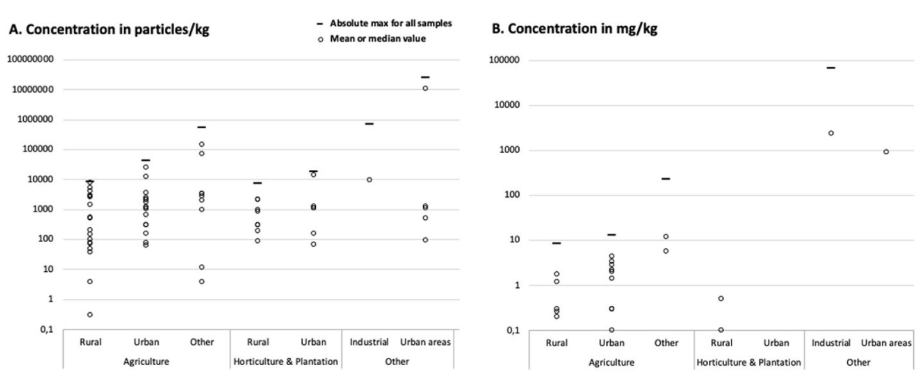


**Fig. 2.** Soil microplastic concentrations in particles per kg (A) or mg per kg (B) for different types of terrestrial environments. Datapoints represent either median or mean values based on references in Refs. [44–46]. The absolute maximum for all samples (i.e., the single highest value measured for each specific type of terrestrial environment) is provided when available.

this inconsistency in metrics between exposure and effects assessment makes risk assessments challenging or even impossible. Comparable mass measurements are furthermore necessary to assess the relative contribution of different sources of microplastic pollution in soils. In a recent study by Klemmensen et al. [46] soil samples treated with different fertilizers in the same Danish long term field trial (i.e., the CRUCIAL field study that is described in more detail in section 4), the authors found approximately 6–7 times higher microplastic concentrations in soils that had received sewage sludge compared to soils that had received either NPK-fertilizer or cattle manure. Whether this is a general pattern remains to be confirmed. In fact, in another study from Sweden [40] microplastic concentrations, estimated based on the amount of sewage sludge amended to the soils over several years, could not be confirmed in analysis of the soil content of microplastics larger than 10 μm. The study by Klemmensen et al. [46] also demonstrated that the spatial distribution of microplastic in soils is highly heterogeneous, and that the act of sampling itself may contribute to the variability observed among different studies. Using the MP inputs per fertilizing event estimated in section 2.1, an extreme worst-case scenario for the contribution of recycled organic waste to microplastic contamination in agricultural soils could range from approximately 3100 MP $kg^{-1}$ soil to 35,500 MP $kg^{-1}$ soil in the Danish and Spanish scenarios, respectively. This is calculated under the (extreme) assumption of sludge application in a specific field every year for the 50 years since the practice of recycling of phosphorous and organic matter from sewage sludge back into agricultural fields became more widespread in the 1980s. Moreover, the worst-case estimate relies on the assumption that the MP content in sewage sludge has remained consistent over the past 50 years, although this assumption is an overestimation as indicated by Ref. [24], which demonstrates an exponential increase in microplastic content in biosolids from 1980 to 2016, and a limited plastic leaching into sewers prior to 1990. In this worst-case scenario, the estimated input accounts for only a portion of the maximum MP concentrations for agricultural soils presented in Fig. 2, indicating that additional sources of MP notably contribute to soil contamination. It is important, however, to note that high variability in measured microplastic concentrations, partly stemming from inconsistencies in analytical methods used across published studies, renders the available data currently inadequate for conducting an accurate mass balance or forming a reliable estimate of the relative contribution from urban organic waste fertilizers to microplastic contamination in agricultural soils.

## 3. Effects of microplastics on terrestrial organisms

To this date it is still unclear whether environmentally relevant microplastic contents have negative effects on terrestrial animals, plants, and microbial communities. Theoretically, exposure to microplastics can cause negative effects on organisms via different mechanisms, namely 1) a physical effect resulting from the organism's direct interaction with the particle, 2) direct toxic effects of components in the microplastics (i.e., chemical residues or additives), 3) a vector- or binding effect, where adsorption of other contaminants to microplastics modifies the availability and route of exposure, or 4) indirect effects, where microplastics alter the availability or quality of food and nutrients. In this section, we focus solely on effects associated with microplastics, and therefore do not consider interactions between microplastics and other xenobiotics or effects that are clearly indirect.

### 3.1. Effects on soil invertebrates

Microplastics resemble, morphologically and chemically, common food items in soil, especially when they have been in the environment for some time. Since many terrestrial invertebrates are not particularly selective in terms of food, they may perceive and ingest microplastics as food. Microplastic ingestion is documented for several groups of organisms including nematodes [49], snails [50] and earthworms [51].

Soil invertebrates are essential for plant growth as they contribute to the conditioning of the soil, e.g., through the decomposition of organic material and soil aggregate formation. Soil invertebrates may be adversely affected by microplastics at different levels of biological organization ranging from impacts on organisms' biochemistry (e.g., enzyme activity) to impacts on the composition and function of terrestrial fauna communities (Fig. 3A). While effects on biochemical parameters indicate that individual organisms react to exposure to contaminants, in many cases these types of responses do not result in quantifiable consequences at the individual or population level. To understand if exposure is likely to result in effects at individual, population, and community level, it is thus necessary to investigate possible effects on e.g., survival, growth, and reproduction or directly on population and community dynamics at environmentally realistic concentrations. In a meta-analysis of 32 published studies, addressing microplastic effects on 18 different invertebrate species or groups of species (Fig. 3A [51–79]), it becomes evident that most of the studies have focused on effects at the sub-organism or organism level. It is also clear that a large part of the observed effects is found at exposure concentrations higher than environmentally realistic (i.e., environmentally realistic considered to be microplastic concentrations at or below the maximum concentration found in agricultural soils) (Figs. 2 and 3A), and that many studies do not observe adverse effects within the tested concentration range (marked as NOEC values (i.e., No observed effect concentration) in Fig. 3A). This indicates that the potential risk of current microplastic concentrations in agricultural soil is low, although more studies at environmentally realistic concentrations is needed to confirm this. In most cases, observed effects were negative, but in a few cases seemingly positive effects on individual endpoints were observed. For instance, 14 days exposure to 1 mg $kg^{-1}$ polystyrene particles in the size of 100 nm increased the mortality of the earthworm *Eisenia fetida*. However, no effect was observed at exposure to 1300 nm particles in the same concentration, and exposure to 0.1 mg $kg^{-1}$ polystyrene particles in both sizes reduced the mortality. In the same study, *Eisenia fetida* growth increased at all exposure concentrations and particle types compared to the control, but at the same time biochemical changes occurred, which may indicate a reaction to oxidative stress, DNA damage and cellular changes [52]. The complex outcomes of studies like this underline the importance of performing tests at environmentally realistic concentrations and exposure conditions. Out of the 32 studies included in the analysis, no studies addressed population level effects, and only one study [53] addressed effects on soil fauna communities.

### 3.2. Effects on plant growth

Although studies on the effects of microplastics on terrestrial plant growth has increased in the recent years, knowledge on effects at environmentally realistic concentrations is still limited. Rillig et al. [80] considered potential consequences of microplastics on plant growth, and particularly indirect effects are thought to have significance. Such effects could derive from changes in soil structure, water retention capacity, nutrient availability and effects on organisms that form symbiosis with plants, such as mycorrhiza and rhizobia bacteria. Some studies have assessed effects of high microplastics concentrations (>1 %) on plants grown in pots (e.g. Refs. [81–83], and found moderate effects or sometimes beneficial impacts on plant growth. For example, Meng et al. [81], found moderate effects of a micro-bioplastics but no effect of Low-density-polyethylene particles (both up to 2.5 %) on bean plant growth. Similarly, Wang et al. [82] observed an effect on maize plants at 10 % PLA (polylactic-acid) but no effect at 0.1 or 1 % PLA or at any concentrations of PE (polyethylene). The PLA effect could be partly explained by a reduced chlorophyll content in leaves of plants exposed to the two highest PLA concentrations. PLA may become increasingly relevant in an agricultural context since it is suggested and increasingly used as a more environmentally friendly substitute for PE in plastic mulching. One study [83] evaluated the effects of 6 different types of

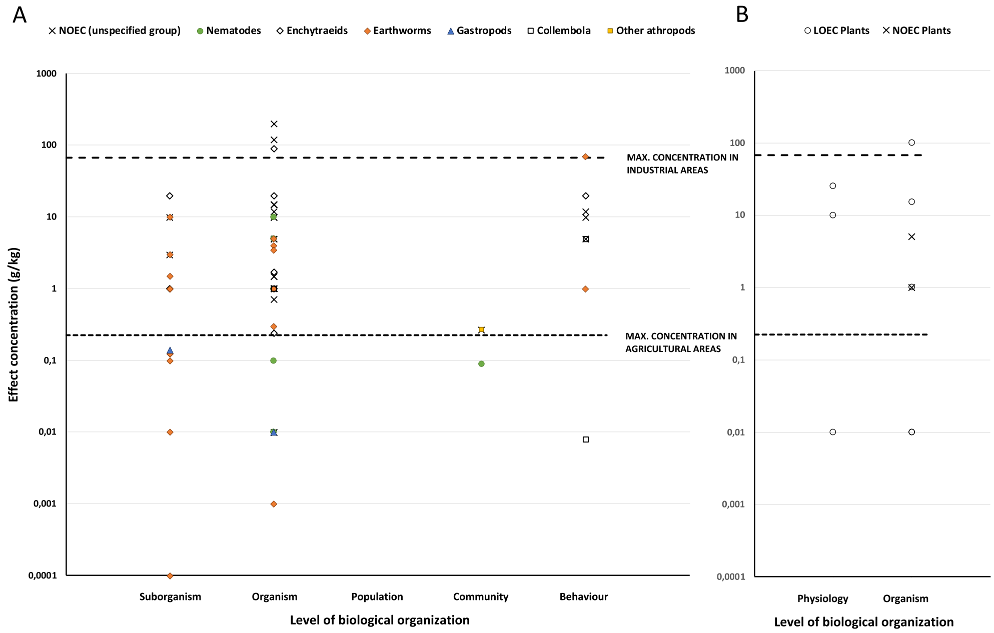


**Fig. 3.** Lowest observed effect concentrations (LOEC values in g/kg; only adverse effects are included) extracted from the published literature and divided on different levels of biological organization and different groups of soil fauna (A) or divided on physiological or organism levels effects for plants (B). For studies where the maximum tested concentration did not result in an adverse effect, a NOEC (No observed effect concentration) value has been included instead (NOEC values are not specified per group of soil fauna). The absolute maximum concentration for agricultural areas and industrial areas, respectively (found in Fig. 2) are superimposed on the graph as dotted lines. For the sorting of test endpoints to levels of biological organization, suborganism level is defined as biochemical, molecular or histological endpoints; organism level is defined as effects on the individual (e.g., survival, growth and reproduction endpoints); population level endpoints are defined as changes in population size or composition (i.e., for one species); community level endpoints are defined as changes in community composition or dynamics. For plants, physiological level typically relates to chlorophyl content or type. Data for the figure is derived from published studies [51–83].

microplastics (High-density-polyethylene, polyamide, polyester, polypropylene, polystyrene, and polyethylene terephthalate) at a 2 % exposure concentration on growth of onion bulbs and found that bulbs grew better when microplastics were added, because microplastics affected nutrient availability in the soil. Some example effect concentrations for plant growth and physiology (measured as Chlorophyl content or composition) are shown in Fig. 3B [80–83]. Like for invertebrates, test and effect concentrations for terrestrial plants are also often higher than environmentally realistic concentrations.

### 3.3. Effects on microbial communities

Several studies have observed effects on the structure and function of microbial communities in soil, when working with microplastic concentrations that are orders of magnitude higher than realistic environmental concentrations. For instance Ref. [82], found that the diversity of arbuscular mycorrhizal fungi was significantly higher at 10 % PLA compared to both control and lower PLA concentrations (0.1 and 1 %).

For bacteria, the addition of 3 % (30 g $kg^{-1}$ dry matter soil) LDPE (low density polyethylene) to a forest soil significantly reduced the diversity, whereas 0.2 % LDPE did not affect the bacterial diversity [84]. Another study [85] found no changes in bacterial diversity upon exposure to 0.1 or 1 % PVC (polyvinyl chloride) in two Chinese soils. The results of [85] corroborates another study [86], where bacterial diversity was unaffected by exposure to 1 % PVC microplastics whereas 5 % PVC and 1 % and 5 % PE microplastics reduced the bacterial diversity.

In addition to microbial diversity, microplastics may also affect microbial activity, i.e., respiration or metabolic activity, as well as nutrient turnover. In several cases, exposure to polypropylene (PP) and LDPE microplastics enhanced microbial respiration [84,87], but again, this was only seen at microplastics concentrations (0.1%–18 %) that are much higher than realistic soil concentrations (Fig. 2). The enhanced microbial respiration at 0.1–18 % LDPE was not accompanied by a corresponding effect on the microbial production of $N_2O$ [88]. The activity of the microbial enzyme urease (involved in nitrogen cycling) and acid phosphatase (involved in phosphate release) increased when the microbial communities were exposed to 1 % and 5 % PVC and to 1 % and 5 % PE [86]. For PP, acid phosphatase activity only increased significantly at 28 %, whereas PP did not affect urease activity at any of the tested concentrations [87].

The studies above show that microplastics can affect the composition and function of microbial communities. However, comparison with measured concentrations of microplastics in soil (Fig. 2) demonstrates that there is a need for experiments at environmentally realistic concentrations to get a robust understanding of how microplastics affect microbial communities and functioning in the environment.

## 4. Case: the CRUCIAL field study

The following section contains a review of studies carried out at a Danish long-term field experiment (CRUCIAL) that was established in 2003 for assessing benefits and risks of recycling organic waste products as fertilizers. The field trial is designed as a randomized block design with 39 field plots of 891 $m^2$ each, distributed into three blocks. The plots were fertilized with various urban waste products or agricultural reference treatments and managed according to conventional agricultural practices. Normal legal limitations were intentionally breached on some plots, to represent 'worst case' scenarios. Thus, some of the fertilization regimes applied in this field trial have resulted in fertilizer supply equivalent to >100 years [3]. Field plots were amended with the contaminants contained in contemporary sewage sludge and composted household waste, under conditions where contaminants interact in a realistic way representing cocktail effects. Both the concentrations of heavy metals [3,89] and organic pollutants [90] have been measured in soils from the various CRUCIAL plots. Preliminary analysis of soil samples from CRUCIAL have indicated that there are large differences in both MP content and type in soils from the different fertilizer treatments (A. Palmqvist pers. comm.). A more recent comparison of CRUCIAL soils amended with sewage soils and CRUCIAL soils amended with either NPK-fertilizer or cattle manure have confirmed that sewage sludge amended soils contain higher concentrations of MP [46].

The resilience of the soil ecosystem as studied so far has been remarkable. Briefly, it has been documented that sewage sludge and composted household waste have benefited soil structure [4], and has not impacted plant uptake of potentially toxic elements [3], soil microbial diversity [91,92], or transmission of multi-resistance [92,93]. The soil food-web consists of various species of decomposing organisms, including microorganisms, meso- and macrofauna and their predators. Their concerted activities ensure the release of plant-accessible nutrients, and as previously described, laboratory experiments have shown MP effects on some of these. The CRUCIAL field trial offers the opportunity to evaluate long-term consequences of waste-based fertilizers under realistic field conditions, where differences within groups of organisms may not only reflect direct effects of xenobiotics in the fertilizers, but instead show the full picture of the amount and type of organic material added together with its inherent contaminants. In the following, detailed results from studies of soil organisms in the CRUCIAL experiment are presented.

In 2020, we examined the influence of long-term sewage sludge and composted household waste application on populations of organisms from the soil food web in the CRUCIAL treatment plots [94]. Overall, populations of soil organisms were larger in plots that have received large amounts of organic material including composted household waste and sewage sludge. Detailed analysis showed that differences in the taxonomic composition of nematode communities did not reflect reduced representation of pollutant-sensitive taxa in sludge and compost treatments; hence, community differences probably reflected overall physicochemical differences between the different fertilizers rather than the different contents of xenobiotics.

In a mesocosm experiment, we assessed the effects of 12 weeks exposure of *Aporrectodea caliginosa,* one of the most dominant earthworm species in temperate agricultural soils, to various fertilizers, including sewage sludge and composted household waste [89]. Neither sewage sludge nor compost had negative effects on earthworm performance. Rather, sewage sludge tended to enhance earthworm body volume, and cocoon production was markedly higher in soil treated with sewage sludge. Likewise, at the field site, plots fertilized with high levels of sewage sludge had the highest abundances of earthworms [95]. To assess if high MP concentrations would affect *A. caliginosa* survival and fitness [89], we added 0.1 % of either polyethylene or acrylic MP to soil from NPK fertilized CRUCIAL plots. Even at this high MP content, no effects were measured on the performance of *A. caliginosa*.

Similarly, CRUCIAL soil fertilized with sewage sludge or composted household waste or exposure to 0.1 % polyethylene terephthalate (PET) fibers did not negatively affect the earthworm *Eisenia veneta* [95].

In avoidance experiments *E. veneta* showed a preference for soils with low content of added MP content indicating an ability to detect MP. However, *A. caliginosa* preferred soil fertilized with sewage sludge or composted household waste rather than soil fertilized with cattle manure [89].

Based on the current information, it can be concluded that the supply of sewage sludge and composted household waste in the CRUCIAL trial has improved soil health compared to unfertilized and NPK fertilized soil. Like the application of cattle manure, fertilization with sewage sludge and composted household waste has enhanced the soil organic matter content [3], densities of microorganisms and animals and plant growth. We found no indications of unwanted effects of the cocktail of xenobiotics in the waste products, including MP, heavy metals and organic contaminants. Soil has a strong capacity to adsorb organic contaminants and metals, and metals become more biologically inaccessible with time [58]. Future research should address how MP are affected over time, the extent to which further fragmentation, weathering and degradation occur in the terrestrial environment, and how this may affect the bioavailability and potential long-term effects of MP on soil organisms.

## 5. Conclusion

Based on the presented data, it may be concluded that current levels of plastic pollution of the terrestrial environment pose limited risk to agricultural ecosystems.

However, knowledge about microplastic contamination in soil and its effects is still relatively scarce. Considering the number of variables that can affect the behavior and effect of plastic particles in the environment, covering the long-term fate and consequences of microplastics in terrestrial environments will be a huge task.

A vital step is to obtain better and more comparable quantitative data on the extent of microplastic pollution. This information is important to assess whether environmental concentrations are comparable to the concentrations affecting terrestrial organisms in laboratory tests. To assess whether microplastics in organic resources, e.g., sewage sludge and organic household waste, pose a risk, it is important to determine the contribution from these organic resources to soil plastic pollution relative to other sources. We also need insights on the potential degradation of plastic materials in soils. For example, experiments quantifying plastic concentrations in both fertilizer products and the receiving soil are necessary to produce mass-balances that will help clarify the fate of microplastics in agricultural soil. Several methods for measuring the microplastic concentration in fertilizer products and soil are not comparable. It will therefore be beneficial if comparable methods are used across studies, preferably methods quantifying both the number, concentration by mass, and size of particles.

In most studies, the effects of microplastics on organisms are evaluated in controlled laboratory systems. It is essential to verify if effects also manifest in real (agro)ecosystems using field studies or model ecosystems. Here, systematic long-term experiments will have great value and give realistic predictions of the consequences of microplastics pollution for terrestrial ecosystems.

## CRediT authorship contribution statement

**Jesper Liengaard Johansen:** Conceptualization, Writing – original draft. **Jakob Magid:** Conceptualization, Funding acquisition, Writing – original draft, Project administration, Writing – review & editing. **Mette Vestergård:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. **Annemette Palmqvist:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing.

## 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.

## Data availability

No data was used for the research described in the article.

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