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Review

An overview of microplastic and nanoplastic pollution in agroecosystems

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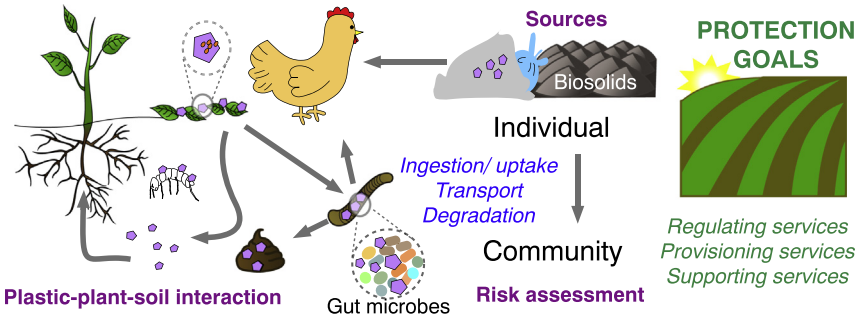
HIGHLIGHTS

- We estimate maximum loadings in agroecosystem using existing regulations.
- Lifetime loading of 2.8–63 $t \cdot ha^{-1}$ of microplastics from biosolids use alone.
- Biotic response is mediated by the organism, soil and plastic properties.
- We deduce ecosystem impact by linking organismal response to ecological role.
- Estimated loadings can be used to set up ecotoxicology experiments.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics and nanoplastics are emerging pollutants of global importance. They are small enough to be ingested by a wide range of organisms and at nano-scale, they may cross some biological barriers. However, our understanding of their ecological impact on the terrestrial environment is limited. Plastic particle loading in agroecosystems could be high due to inputs of some recycled organic waste and plastic film mulching, so it is vital that we develop a greater understanding of any potentially harmful or adverse impacts of these pollutants to agroecosystems. In this article, we discuss the sources of plastic particles in agroecosystems, the mechanisms, constraints and dynamic behaviour of plastic during aging on land, and explore the responses of soil organisms and plants at different levels of biological organisation to plastic particles of micro and nano-scale. Based on limited evidence at this point and understanding that the lack of evidence of ecological impact from microplastic and nanoplastic in agroecosystems does not equate to the evidence of absence, we propose considerations for addressing the gaps in knowledge so that we can adequately safeguard world food supply.

Contents

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1. Introduction: microplastics and nanoplastics as emerging environmental pollutants

Small plastic particles are ubiquitous throughout the environment, and cause considerable concern because micro (defined here as 100 nm to 5 mm in size) and nano (<100 nm in one dimension) sized particles are small enough to be taken up by many organisms (EFSA Panel on Contaminants in the Food Chain, 2016) and raises questions of potential bioaccumulation and biomagnification (see glossary in Box 1). There is growing evidence that microplastics are ingested by marine organisms, some evidence of translocation beyond the gut and fewer still evidence of transfer from one trophic level to the next (Galloway et al., 2017; GESAMP, 2015; Rochman et al., 2016). Nanoplastics are potentially more hazardous than microplastics because they can permeate biological membranes (Bouwmeester et al., 2015; EFSA Panel on Contaminants in the Food Chain, 2016; Nel et al., 2009). Terrestrial studies on microplastics ingestion are emerging for soil organisms (Huerta Lwanga et al., 2016; Rodriguez-Seijo et al., 2017). Recently, Horton et al. (2017) and Duis and Coors (2016) reviewed sources and fate of microplastics in terrestrial environment, and we build upon their work by exploring the extent to which plant and soil organisms in agroecosystems could be impacted, from individual level up to ecosystem level.

Leo Baekeland developed the world's first useful synthetic plastic in 1907 using formaldehyde and phenol (American Society of Chemistry National Historic Chemical Landmarks, 1993), but little was produced until around 1950s, when mass production of plastics begun and plastics found use in increasing range of applications; between 1950 and 2015, global plastic waste is estimated to be 6300 million tonnes, 79% of which has accumulated in landfills and other environmental compartments (Geyer et al., 2017). Based on the sources of microplastic pollution, agroecosystems are likely to be the most plastic-contaminated terrestrial system outside of landfills, urban spaces (Nizzetto et al., 2016) and beaches (Duis and Coors, 2016), and therefore they are excellent systems to study the implications of exposure to microplastic and nanoplastic. We will also include some findings from research on macro-plastics that we believe are relevant to understanding the overall effects of plastic pollution in agroecosystems.

In this synthesis, we present an overview of the multidisciplinary research on microplastics and nanoplastics in agroecosystems. While the relevant literature is vast, some aspects have fortunately been covered by recent reviews, which we will briefly summarise. Here, we emphasise on the following. Firstly, we identify the sources and estimate microplastics loading in agroecosystems, using reported estimates and our own calculations. Secondly, we examine the likely mechanisms and constraints underlying plastic degradation in soils and their dynamic behaviour. Thirdly, we report on the impact of these plastic

Box 1
Glossary.

Bioaccumulation	The process by which the amount of a substance, in this case, plastic particles, in an organism increases progressively because the rate of intake exceeds the rate of removal from the body.
Bioavailable	Amount of a substance that an organism absorbs (across a physiological membrane) as a result of physical, chemical and biological processes.
Biomagnification	Accumulation of a substance through a food chain by transfer of residues from diet to body tissue. The tissue concentration increases at each trophic level in the food web when uptake exceeds removal.
Cometabolism	The degradation of a substance catalyzed by an enzyme whose primary function is to react with another substrate. The other substrate is used as the primary carbon and energy source. For example, the breakdown of organopollutants, such as DDT, by white rot fungus *Phanerochaete chrysosporium* is catalyzed by enzymes responsible for breaking down lignin in plant material under normal conditions.
Home garden	Traditional, small scale agroforestry systems practiced in urban and rural areas, consisting of multipurpose trees and shrubs where livestock are often raised.
Nanoplastic	Plastic particles with one dimension between 1 and 100 nm.
Microplastic	Plastic particles in the size range between 100 nm and 5 mm.
Annual microplastic loading rate	The quantity of microplastic added per unit area per year.
Maximum or lifetime loading	This is the maximum amount of a substance per unit area given regulatory limits, e.g. contaminant limited biosolids application rate. In the case of biosolids, the limit is usually reached by a persistent contaminant such as heavy metals, thereby preventing further addition of biosolids to the land once this limit is reached.

particles on soil organisms and plants. This present work will serve as a synthesis of existing evidence as well as propose hypothetical implication at higher biological level of organisation built upon knowledge about plastic debris of all sizes and the ecological role of model organisms, such as earthworms. Finally, we propose approaches and considerations to determine the effects of microplastic and nanoplastic pollution.

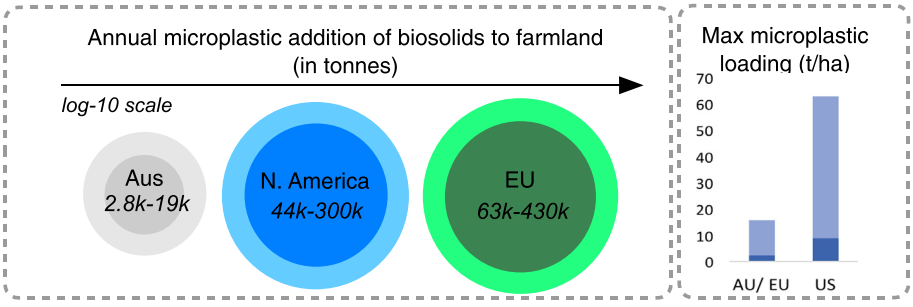
2. Major sources of microplastics and nanoplastics in agroecosytems

Microplastics and nanoplastics enter agroecosystems either as primary (manufactured) micro and nano materials (e.g. in waterborne paints, medical applications, electronics, coatings, adhesives), or indirectly as secondary microplastics and nanoplastics generated by the breakdown of larger plastic debris (Duis and Coors, 2016; Koelmans et al., 2015; Rillig, 2012). It was recently demonstrated that photodegradation of recovered marine microplastic debris (Gigault et al., 2016) and 1-cm^2-pieces of disposable polystyrene coffee cup lid (Lambert and Wagner, 2016) generated nanoplastics. Direct sources in agriculture include plastic mulch films and greenhouse materials and soil conditioners (e.g. polyurethane foam and polystyrene flakes). Indirect sources include general littering and the use of treated wastewater and biosolids (Duis and Coors, 2016; Horton et al., 2017). Microplastic and nanoplastic emissions per capita vary greatly between regions due to population size, affluence, presence and efficacy of waste management practices (Nizzetto et al., 2016; Ziajahromi et al., 2016). Here, we focus on plastics that end up in agroecosystems. Using existing data and estimates, we have derived potential annual and maximum plastic loadings in agroecosystems for Europe, USA and Australia, to illustrate the potential scale of the plastic problem.

Globally, between 0.8 and 2.5 million tonnes of microplastics – two-thirds of which are due to synthetic fibres released during washing and erosion of tyres while driving – are estimated to end up in oceans every year (Boucher and Friot, 2017). Of the microplastics that pass through wastewater treatment plants, some 95% of the microplastics are estimated to be retained in biosolids (Ziajahromi et al., 2016). As both treated wastewater and biosolids are used in agriculture for irrigation and as fertiliser (Mohapatra et al., 2016; Nizzetto et al., 2016), the microplastic loading on agricultural land is likely to be high. In Europe, Nizzetto et al. (2016) estimated that some 63,000 to 430,000 tonnes of microplastic enter agroecosystems annually through biosolids alone, while estimates for North America ranged from 44,000 to 300,000 tonnes of microplastics annually. We use Australia as a case study to further evaluate plastic particle loading rates per unit area per year and maximum (lifetime) loading given our in-depth knowledge of Australia's regulations on biosolids use. We estimate that between 2800 and 19,000 tonnes of microplastics are applied to Australian agroecosystems each year through biosolids (Box 2 and supplementary information, SI).

Besides biosolids, composts derived from non-source-separated residential waste or mixed municipal solid waste, and source-separated garden organic waste (to a lesser extent) are also sources of plastic pollution in agroecosystems. The physical degradation of plastics from these sources, abrasion and fragmentation due to mixing and transport,

Box 2
An estimation of loading rates and total loadings for microplastic from biosolids in agricultural soils in Australia, EU and USA.



In Australia, 1.5 million tonnes of wet biosolids were produced in 2015 (Australian and New Zealand Biosolids Partnership, 2016), with 64% of the biosolids used in agriculture and predicted to further increase in the future (Australian and New Zealand Biosolids Partnership, 2016). Using 125 to 850 tonnes of microplastics per million inhabitant (Nizzetto et al., 2016) as a basis, we estimate that between 2800 and 19,000 tonnes of microplastic could be applied to Australian agroecosystems each year through biosolids alone; Nizzetto et al. (2016) estimated 44,000–300,000 and 63,000–430,000 tonnes of microplastics could be applied to North American and European agroecosystems respectively. This estimate of between 2800 and 19,000 tonnes of microplastics in the 1.5 million wet tonnes of biosolids in Australia, equates to a likely presence of between 9 and 63 kg of microplastics per tonne of dry biosolids, assuming a total solids content of 20% (Eldridge et al., 2008) for the biosolids.

The application of biosolids in Australia is tightly regulated by state regulations which are largely derived from the New South Wales guidelines (EPA-NSW, 1997). Loading rates are limited by plant available N supply and contaminant loading to ensure that biosolids applications do not raise the level of contaminants in the soil above the accepted maximum allowable contaminant level for agricultural soils. There are also limits on the time interval between applications. Combined, these results in the theoretical maximum ceiling (lifetime loading) for biosolid application rate to agricultural land in Australia to be around 250 dry t·ha^{-1}. At 250 dry t·ha^{-1}, this would represent a maximum (lifetime) microplastic loading of between 2.3 and 15.8 t·ha^{-1} incorporated into the top 75 to 100 mm of soil.

A comparison of estimates of microplastic loadings through biosolid application

The US regulations pertaining to biosolid application to agricultural land (i.e. USEPA 40 CFR 503, 1993) are less stringent than EU regulations (EU Directive 86/278/EEC, 1986). These limits on biosolid application rates would suggest maximum potential rate of microplastic inputs from biosolid in the order of 0.5 to 3.2 t·ha^{-1}·yr^{-1} in the US and from 0.045 to 0.63 t·ha^{-1}·yr^{-1} in Europe. Based on copper and zinc contaminants limited biosolid application rate for the respective regions, we would expect similar maximum microplastic loadings for agricultural land between Australia and Europe, while maximum (lifetime) microplastic loadings for US farmland may be as high as 9 to 63 t·ha^{-1}. Details for the regulations, calculations and uncertainties of the estimates for Box 2 are elaborated in SI.

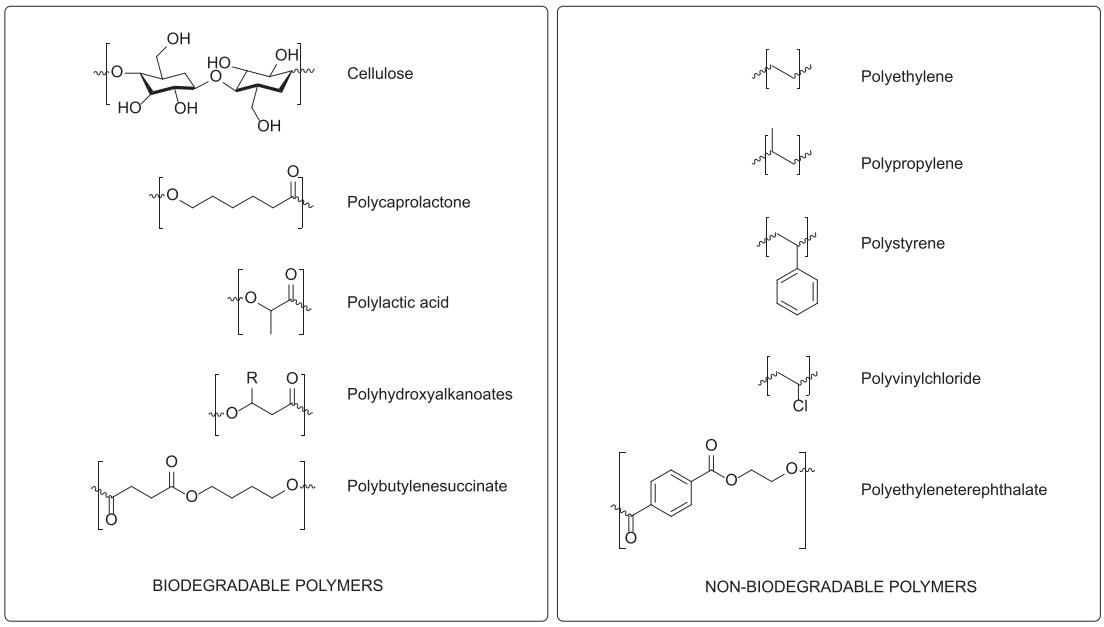


Fig. 1. Chemical structures of some important biodegradable polymers and examples of non-biodegradable polymers commonly contaminating soil.

is also likely to produce secondary microplastics. Brinton (2005) found that polyethylene, plastic fibres, and polystyrene foam occupied up to 5% w/w in compost from mixed municipal solid waste for all size fractions between 420 μm and 25 mm; with around 0.5 to 0.6% having sizes <2 mm. The quality and use of composts are regulated to varying degrees across the globe. For example, Australian standard (AS4454, 2012) for compost, soil conditioners and mulches retailed to backyard gardeners and farmers in Australia allows up to 0.5% dry matter w/w rigid plastic and 0.05% dry matter w/w of light, flexible or film plastics. This is equivalent to having up to 5 $t \cdot ha^{-1}$ of rigid plastic and 0.5 $t \cdot ha^{-1}$ of light plastic to a depth of 10 cm for a lifetime compost loading of 1000 $t \cdot ha^{-1}$. Hence, the potential contamination of agroecosystems by secondary microplastics and nanoplastics, could be significant.

In the early 2000s, 0.7 million tonnes of mulch film was used annually worldwide in agriculture, with China being the largest user (~80%; Espí et al., 2006). Plastic mulch film covers some 20 million hectares of farmland in China (Liu et al., 2014). Plastic mulch films with thicknesses between 6 μm and 20 μm are widely used in intensive production systems because of four perceived benefits: modification to soil temperatures, reduced evapotranspiration, better weed control, and reduced soil blemish of the product. As plastic mulch is applied with each crop cycle, soils become enriched with plastic residues that have been intentionally or unintentionally left behind on the field by farmers (Steinmetz et al., 2016). In the Xinjiang region of China, where plastic mulch is extensively used, the film residue content in soils ranged from 0 to 502 $kg \cdot ha^{-1}$ (mean 121.5 $kg \cdot ha^{-1}$), with the quantity being positively correlated with the number of years under mulching (Zhang et al., 2016).

3. Polymer degradation and dynamic behaviour of plastic particles on land

Polymer degradation refers to a chemical change in the molecular structure of the polymer that alters its properties. There exists an enormous number of polymers that, depending on their chemical structure, are rendered more or less susceptible to different types of degradation processes.

The biodegradable polymers possess heteroatoms (O, N, S) distributed along the polymer backbone that act as sites for hydrolytic or enzymatic reactions, leading to significant decreases in the molecular weight of the polymer in a relatively short timeframe (days to several years). These processes cause the structure of the polymer to break down into lower molecular weight molecular fragments that microbial cells can assimilate and subsequently mineralise to produce CO_2, H_2O and biomass in aerobic environments (or CO_2 and CH_4 in anaerobic environments). Examples of biodegradable polymers include aliphatic polyesters such as polylactic acid, polycaprolactone and polybutylenesuccinate, and natural biopolymers such as cellulose and polyhydroxyalkanoates (Fig. 1).

Many of the commonly used polymers contaminating our environment, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), possess a carbon backbone that is resistant to hydrolytic and enzymatic degradation (Fig. 1). As such, microbes are generally unable to assimilate and mineralise the polymers, resulting in the environmental accumulation of these materials. Some projections indicate that the lifetime of polyolefins on land is in the vicinity of hundreds of years (Kyrikou and Briassoulis, 2007). The ultimate degradation of these types of polymers in soil will involve several mechanisms including (i) photo- and thermo-oxidative degradation and (ii) some degree of biodegradation by microorganisms after a prolonged period of environmental exposure and oxidation. Nguyen (2008), and Singh and Sharma (2008) have authored thorough reviews on the general topic of plastic degradation. The present article will therefore provide an overview of the degradation processes on land.

3.1. Photo- and thermally-initiated oxidative degradation

Common polymer contaminants in or on soil are susceptible to some degree of photo- or thermo-oxidative degradation. The general mechanism for abiotic oxidative degradation of polymers with a carbon backbone is given in Box 3. Oxidative degradation is triggered by free radicals generated when the materials are exposed to ultraviolet or thermal energy under aerobic condition. As these degradation processes rely on the combination of radicals with oxygen, they will only occur when plastic is at, or very near to, the soil surface. In the field, radicals are most likely to form by (i) direct photolysis of C—C and C—H bonds in the polymer, (ii) residual catalyst or chromophoric chain defects present from synthesis, or (iii) as a result of other additives such as photosensitisers (e.g. TiO_2), pro-oxidants (usually salts of transition metals including iron, nickel, cobalt and manganese), fillers, dyes and pigments (Carlsson and Wiles, 1976; Gardette et al., 2013), which in

Box 3
General mechanisms for oxidative degradation of carbon-based polymers.

Initiation	$PH + X\bullet \rightarrow P\bullet + XH$	[1]
Propagation	$P\bullet + O_2 \rightarrow PO_2\bullet$	[2]
	$PO_2\bullet + PH \rightarrow POOH + P\bullet$	[3]
Chain branching (autocatalytic)	$POOH \rightarrow PO\bullet + \bullet OH$	[4]
	$PO\bullet + PH \rightarrow POH + P\bullet$	[5]
	$HO\bullet + PH \rightarrow HOH + P\bullet$	[6]
	$PO\bullet \rightarrow$ various chain scission reactions	[7]
Termination	$P\bullet + P\bullet \rightarrow P—P$ or $P—H + P(—H)$	[8]
	$PO_2\bullet + PO_2\bullet \rightarrow$ inactive products	[9]

PH designates the polymer, P• is a macroradical and X• is an unspecified radical (Hawkins, 1964; Nguyen, 2008). This is the general mechanism for the oxidative degradation of polyolefins such as PE and PP and it is also applicable to other types of polymers with a carbon backbone.
The first step of the oxidation pathway begins by abstraction of hydrogen from the polymer to produce the macroradical species, P•, regardless of how the radical is generated (Eq. (1); Hawkins, 1964). A chain reaction ensues in the propagation stage, involving combination of the macro-radical with oxygen (Eq. (2)) to produce a peroxy polymer radical. The peroxy radical then abstracts hydrogen from another polymer molecule to produce a molecule of hydroperoxide and a new macroradical (which subsequently undergoes reactions [2] and [3], and so on). Eqs. (4–7) show the autocatalytic chain branching phase which increases the oxidation rate further. Here, the hydroperoxides formed in the previous step decompose into radicals, which in turn abstract hydrogen from polymer molecules to generate more macroradicals. Termination eventually occurs when radicals couple together or undergo disproportionation. The interested reader is referred to the following articles for more specific details on the mechanisms of photo- and thermally-triggered oxidative degradation in PE (David et al., 1992; Gardette et al., 2013), PP (Carlsson and Wiles, 1976), PS (Grassie and Weir, 1965), PVC (Palma and Carenza, 1970) and PET (Jabarin and Lofgren, 1984).

some cases may involve the production of singlet oxygen as a reactive intermediate (Rabek and Rånby, 1975). The degree to which these oxidative processes can occur is highly dependent on the environmental conditions (e.g. UV exposure, temperature, soil composition, moisture, oxygen); as well as the chemical structure and crystallinity of the plastic (with oxygen diffusion and degradation occurring more readily in amorphous regions of the materials) (Nguyen, 2008).

At the macroscale, photo and thermally triggered oxidative degradation leads to the embrittlement, cracking and weakening of plastics with time. Thus the materials become more susceptible to fragmentation when they are exposed to abrasive or mechanical forces, e.g. from farm equipment, generating micro and nanoplastics. At the molecular level, the polymer chemical structure changes due to a combination of events including chain scission (decrease in polymer molecular weight), crosslinking (increase in molecular weight), branching (increase in molecular weight) and incorporation of oxygen containing functional groups at the surface of the plastic particle, e.g. esters, ketones and alcohols, which also reduces the surface hydrophobicity of the plastic (Singh and Sharma, 2008).

As plastic particles age in the environment, their movement through the soil profile is expected. Earthworms (Huerta Lwanga et al., 2017; Rillig et al., 2017) and collembola (Maaß et al., 2017) have been observed to transport microplastics, and agricultural practices such as ploughing, would also contribute to their vertical transport. This new subsurface location would negate photo and thermal degradation, which are crucial for reducing the size of polymers with carbon backbones like PE, PP, PS and PVC (as described above) before any substantial biodegradation can occur. Furthermore, anaerobic conditions may develop in deeper layers of the soil and inhibit oxidative degradation processes (Thomas et al., 2012).

3.2. Biodegradation

After extensive initial photo- or thermo-oxidative degradation, biodegradation plays an important role in the ultimate fate of plastics in soil. Biodegradation is the process of mineralisation of an organic material by microorganisms to generate CO_2 and H_2O under aerobic conditions, or CO_2 and CH_4 under anaerobic conditions (Mohan, 2011). The molecular weight, chemical structure and morphology, hydrophobicity, water absorption, and surface roughness of plastic materials all have an impact on their susceptibility to biodegradation (Table 1). Even low molecular weight components of PE subjected to extensive pre-oxidation in accelerated conditions (i.e. artificial weathering where UV light and/or heat between 50 and 70 °C is applied) not reflected in the field, can only be partly biodegraded (Thomas et al., 2012). The accelerated weathering conditions certainly decrease the molecular weight of the PE, a critical step towards achieving microbial degradation; however the majority of the oxidised sample is still too high in molecular weight to be mineralised (Table 1).

Although numerous organisms are recognised to biodegrade or partially biodegrade even some of the most persistant types of plastics – e.g.

Table 1
General rules of thumb indicating the likely impact of certain polymer properties on susceptibility to biodegradation.

Property	Impact on biodegradation	Sample format
Molecular weight	Only low molecular weight compounds can be assimilated by microbial cells and enzymatically degraded. Carbon-chain backbones do not biodegrade until the molecular weight is <1000 g/mol (Potts et al., 1973)	Molecular
Chemical structure and morphology	Certain functional groups provide sites for enzymatic cleavage (ester, ether, amide, urethane) (Kawai, 2010)	Molecular
	Branched structures are more difficult for microbes to assimilate (Potts et al., 1973)	Molecular
	Amorphous materials biodegrade faster than crystalline ones (Reed and Gilding, 1981; Yoo and Im, 1999)	Macro
Surface hydrophobicity	Hydrophobic surfaces inhibit biofilm formation, hydrophilic surfaces (water contact angle 40–70°) promote it (Lee et al., 1998)	Surfaces of thin films
Water absorption	Bulk hydrophilicity and water absorption give microbes access throughout the bulk material (Göpferich, 1997)	Macro
	Water absorption softens polymers, and softer materials biodegrade faster than harder ones (Foruzanmehr et al., 2015)	Macro
Surface roughness	Microbes adhere to rougher surfaces more easily than smooth ones (Wan et al., 2005)	Surfaces of thin films

certain bacteria (Huerta Lwanga et al., 2018; Yang et al., 2014; Yoshida et al., 2016) and insect larvae (Bombelli et al., 2017), these specific organisms (such as the bacteria *Ideonella sakaiensis* isolated from recycling site) and/or their hosts (such as the caterpillar of the moth *Galleria mellonella* or larvae of Indian mealmoth *Plodia interpunctella*) may not be naturally present in agroecosystems. Even if plastic-degrading organisms are present in soils, such as the plastic-degrading bacteria (*Microbacterium awajiense*, *Rhodococcus jostii*, *Mycobacterium vanbaalenii*, *Streptomyces fulvissimus*, *Bacillus simplex* and *Bacillus* sp.,) identified from earthworm's gut (Huerta Lwanga et al., 2018), less energetically expensive carbon resources would be present in soils, therefore biodegradation of such plastic particles would be less likely to become a relevant process, with cometabolism being a more likely scenario. Cometabolism, which is the degradation of a compound in the presence of another compound that is used as carbon source, has been extensively studied for bioremediation of organic pollutant such polyaromatic hydrocarbon, but its effectiveness in field is thus far limited and requires extensive and costly intervention (Ghosal et al., 2016).

3.3. Particle changes through biophysicochemical interactions at particle-soil interface

During oxidative degradation, anionic or polar surface groups are likely to be introduced on plastic particles, providing surfaces for further interaction with soil components. The interaction between plastic particles and soil components is a dynamic process involving a series of interconnected physical-biological-chemical changes. As a result, the physicochemical state of plastics in soils is likely to be highly dynamic. Moreover, plastics are typically a complex mixture of polymers, residual monomers, catalysts and additives (Teuten et al., 2009), which affect the plastic particle characteristics and behaviour, and therefore the interactions of different plastics with soil organic and inorganic matter.

Consider the apparently simple two-way relationship between plastic and an agrochemical (e.g. pesticide) present in the soil. Studies so far indicate that there is a subtle interplay between environmental factors and plastic composition that can affect the stability of both agrochemicals and plastics commonly used in agriculture. Various pesticides' accumulate and/or become stabilised on the surface of plastic mulch film (Ramos et al., 2015), while other plastics treated with agrochemicals actually become more susceptible to photodegradation and embrittlement than the corresponding clean plastics (Schettini et al., 2014).

Additionally, as degradation proceeds, smaller sized plastic particles are generated. Studies on nanomaterials indicate that the smaller the particle, the larger its surface-to-volume ratio and its reactivity, thus, the more dynamic the behaviour of nanoparticles (P. Wang et al., 2016; Wiesner et al., 2011). The fragmented microplastics released in the casts of *L. terrestris* (Huerta Lwanga et al., 2016) are surrounded by ecocoronas or biofilms comprising soil biota, and soil-derived organic and inorganic macromolecules. These ecocoronas change the density, surface charge, size and shape of micro or nanoplastic particles, and may therefore also alter the mobility, degradation, bioavailability and toxicity of the encapsulated plastic particles (Artham et al., 2009; Galloway et al., 2017; Nel et al., 2009).

When considering nano-sized particles in soils, it is argued that the prevalence of black carbon and natural carbonaceous nanoparticles in soils would exceed that of manufactured nanomaterials (Koelmans

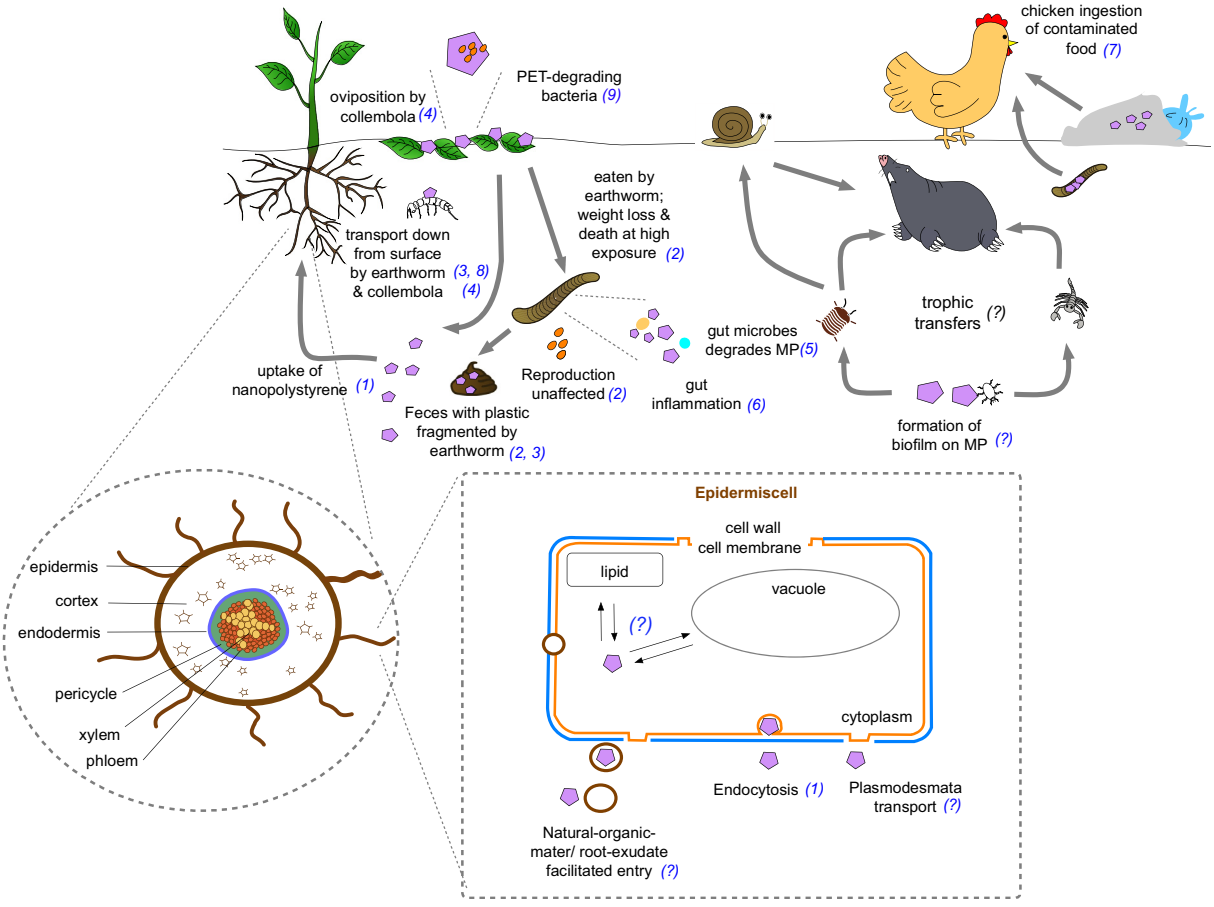


Fig. 2. Microplastic and nanoplastic uptake and interaction with soil biota and plants. (1) Bandmann et al. (2012); (2) Huerta Lwanga et al. (2016); (3) Huerta Lwanga et al. (2017); (4) Maaß et al. (2017); (5) Huerta Lwanga et al. (2018); (6) Rodriguez-Seijo et al. (2017); (7) Huerta Lwanga et al. (2017); (8) Rillig et al. (2017); (9) Yoshida et al. (2016); (?) refers to unknowns. MP refers to microplastic or nanoplastic.

et al., 2009). Using examples from Simpson and Hatcher (2004), there would be between 520 and 2010 $t \cdot ha^{-1}$ of black carbon to 7.5 cm depth for a Canadian Chernozem (5.2% total C) and German Mollisol (1.9% total C) respectively. In comparison, our estimates of maximum microplastic loading from biosolid use in agroecosystems in Box 2, assuming eventual 100% conversion into nano-sized particles, would correspond to between 2.3 and 63 $t \cdot ha^{-1}$ of nanoplastics in soils. This finding raises three questions: (1) what is the relative importance of plastic as a host or carrier of organic and inorganic matter relative to other carriers in the soil, such as mineral particles and natural soil carbonaceous polymers, (2) how will plastic interact with these soil carbonaceous materials, and (3) if soil biota and plants have historically evolved within this environment, do these materials pose no threat or has the biota developed mechanisms to live amidst these materials? These also highlight the crucial task to quantify actual plastic loading and their sizes in agroecosystems.

4. Response of soil biota to microplastic and nanoplastic pollution

4.1. Organismal-level response

It is reported by studies in marine environment that microplastic ingestion is rarely lethal at environmentally relevant concentrations (Galloway et al., 2017; Rochman et al., 2016). In earthworms, two outcomes have been observed so far in controlled experiments: i) the organism survives, the microplastics may be fragmented further internally, and plastic particles are transported in soil via defecation and when the organism moves or, ii) the organism suffers weight loss, and then dies at high exposure concentration (Fig. 2). In one study, earthworms *Lumbricus terrestris* exposed to concentration of 28% PE microplastics (w/w in dry plant litter) and above, experienced growth inhibition (<1.4 mg weight gain compared to 10.3 mg weight gain in control with no exposure to microplastic) and subsequently died (8–25% compared to 0% in control with no exposure to microplastic) even though their reproduction was unaffected (Huerta Lwanga et al., 2016). These are high exposure concentrations that could occur under contaminated land scenario. Another study using *Eisenia fetida* exposed to 0.25 and 0.5% of PS microplastic (w/w in dry soil) showed no growth inhibition, with growth inhibition only occurring at exposure concentrations >1% (Cao et al., 2017). In another study using *Eisenia fetida*, inflammation in the guts was observed when the earthworm is exposed to concentration of 0.0125% PE microplastic (w/w in dry soil) and above but this does not translate to any significant effects on survival, reproduction and biomass at concentration up to 0.1% of PE microplastic (w/w in dry soil) (Rodriguez-Seijo et al., 2017).

Studies on algae in the aquatic environment showed that nanoplastics are adsorbed onto the cell wall of microalgae such as *Scenedesmus*, *Chlorella* and *Pseudokirchneriella subcapitata* (Bhattacharya et al., 2010; Nolte et al., 2017a), with binding mediated by cell morphology (Bhattacharya et al., 2010), the particle's charge and ionic strength of the medium (Nolte et al., 2017a; Nolte et al., 2017b). These experiments, lasting for hours to a few days, indicated these nanopolystyrenes were not lethal to the algae at concentrations up to 100 $mg \cdot L^{-1}$. However, they did reveal that these nanoplastics can lead to the physical inhibition of algal photosynthesis due to increased water turbidity and light scattering, coverage of the algal cell surface with microplastics, or immobilisation of algae at concentration of around 1.5 $mg \cdot L^{-1}$ and above (Bhattacharya et al., 2010; Nolte et al., 2017a). It remains to be explored if interferences from nanoplastics in photosynthesis and induction of physiological stress responses can occur in soil-dwelling algae.

Despite their ecological importance, the exposure of soil filter feeders such as some nematodes, rotifers and ciliates to microplastics and nanoplastics have not yet been determined to our knowledge. Filter feeders in marine ecosystems have been shown to ingest microparticles (Van Cauwenberghe and Janssen, 2014; Wright et al., 2013) while filter feeders in freshwater ecosystems, *Daphnia magma* and *Thamnocephalus platyurus*, have been shown to be sensitive to nanoplastics (Besseling et al., 2014; Casado et al., 2013). Uptake by such organisms is determined by their ability to discriminate food and non-food, which depends on a mixture of chemical (taste and olfaction) and physical (size) mechanisms (Kiyama et al., 2012). Kiyama et al. (2012) demonstrated that the nematode *Caenorhabditis elegans* on buffer solution and agar plates take up PS microspheres of 0.5 and 1 μm, particularly in the absence of food bacteria. Organisms with other feeding modes are also susceptible to microplastic ingestion. Recently, Taylor et al. (Taylor et al., 2016) found synthetic microfibers on and inside six out of nine deep sea organisms from the phyla Cnidaria, Echinodermata and Arthropoda with predatory and detritivorous feeding mechanisms. As such, woodlice, snails, caecilians and other soil organisms with similar feeding mechanisms would be subjects of interest in agroecosystems.

Information about the bioavailability and bioaccumulation of microplastics in soil organisms is generally lacking. Early investigations indicate that mussels take up particles <10 μm and these particles were translocated from gut to the circulatory system and retained there for the duration of the testing period (48 days) (Browne et al., 2008). We know that nanoplastics can enter cells, as fluorescent nanoplastic polymers have been used as molecular probes for a wide range of biological studies with mammalian cells, for example to measure blood flow in tissue and as tracers for phagocytic processes (see e.g. carboxylate-modified microsphere F8888 by Invitrogen; Katz and Iarovici, 1990; Rembaum and Dreyer, 1980). The translocation of a range of microparticles by mammalian gut into the lymphatic system have been demonstrated in human (particle sizes 160 nm – 150 μm), rabbits (100 nm – 10 μm), dogs (3–100 μm) and rodents (10 nm – 40 μm) (see details in review by Hussain et al., 2001). There is no experimental evidence of nanoplastics being transferred from invertebrates to vertebrates in soils; there is evidence of the transfer of microplastics from contaminated land to vertebrates, and potentially from earthworm to chicken. In one study developed for homegardens, it was observed that chickens' digestive tract became polluted with plastic particles (62.6 ± 49.5 particles per gizzard, 16.45% of which were <5 mm and 83.55% were >5 mm; 11 ± 15.3 particles per crop, all of them macroplastics, no microplastics were found in crops; Huerta Lwanga et al., 2017).

Understanding organismal-level response is the basis for toxicological studies and risk assessments, and translating this response to population and ecosystem-level consequences upon which policy and decisions are often based, is a challenge. For the purpose of decision-making, complementing the above individual endpoint measurements with models could allow prediction of the pollutant burden over time and translate the individual-level response to a population model (Jager, 2016). For example, toxicokinetic and toxicodynamic models examine internal concentrations of a contaminant as function of uptake, transformation, distribution and elimination, and the subsequent response of the test organism (Rohr et al., 2016) could be applied to well-studied earthworm, where there is a strong understanding of the organism's biology.

4.2. Response of soil microbiome

The time and space scale for microbes, given their relatively short life history and small size, give us a chance to study processes that would be difficult at field scale (Jessup et al., 2004) and allow us to capture the emergent properties of a system which would be impossible with individual-level trait studies. While no studies have specifically examined micro- and nano-sized plastic particle effects on soil microbiome, an experimental study on plastic mulch residues provides some preliminary indication of potentially useful measures. In a pot trial experiment with 67.5 and 337.5 $kg \cdot ha^{-1}$ plastic mulch residues (20 mm × 20 mm) maintained at constant moisture content, soil microbial biomass, enzyme activities (dehydrogenase and fluorescein diacetate hydrolysis)

and functional diversity (community level physiological profile) tended to decrease with increasing concentrations of plastic mulch residue (J. Wang et al., 2016). The concentration (67.5 kg·ha^{-1}) used in this experiment is environmentally relevant for soils with over 5 years of plastic mulch film use (Zhang et al., 2016). Given the long-term use and misuse of plastic mulch in some agroecosystems, studying their soil microbiome may provide insights into the long-term implications of plastic pollution on land.

5. Response of plants to microplastic and nanoplastic pollution

5.1. Uptake of nanoplastic by plants

Uptake of microplastics by plants is not expected. The high molecular weight or large size of the plastic particles (Teuten et al., 2009) prevents their penetration through the cellulose-rich plant cell wall. In contrast to microplastics, nanoplastics indeed have been shown to enter plant cells (Fig. 2). Bandmann et al. (2012) have demonstrated uptake of 20 and 40 nm nanopolystyrene beads by tobacco BY-2 cells in cell culture via endocytosis, while 100 nm beads were excluded. However, no studies have investigated whole plant, instead of plant cell culture, uptake of nanoplastics to the best of our knowledge.

Plant species vary in their uptake, translocation and accumulation of contaminants due to a range of anatomical and physiological differences. Plant properties that are known to affect the uptake of organic compounds include root properties (volume, density, surface area), xylem properties (volume, surface area), transpiration, growth rate, water and lipid fractions, plasma membrane potential, tonoplast potential, cytoplasm pH and pH of vacuoles (Trapp, 2000). Characteristics and permeability of the plant cell wall varies, but as a rule-of-thumb, particles <6 nm in one dimension may be able to permeate the cell wall (Carpita et al., 1979).

Studies on plant uptake of engineered carbonaceous nanoparticles – structurally dissimilar to plastics but they can be produced with similar particle size, shape, surface functional groups to microplastics – may shed light on the possible modes of nanoplastic interaction with plants and bioavailability (see reviews by Ma et al., 2010; Rico et al., 2011; J. Wang et al., 2016). In plants, such engineered carbon-based nanoparticles are being targeted as molecular transporters to study plant cell biology, or deliver agrochemicals and biomolecules (Morales-Díaz et al., 2017; J. Wang et al., 2016). Uptake of these carbon-based nanoparticles has been documented in whole plants such as rice (*Oryza sativa*), maize (*Zea mays*), soybean (*Glycine* max) and arabidopsis (*Arabidopsis thaliana*) (Lin et al., 2009; Zhao et al., 2017). Based on the above studies, the proposed pathways for entry of carbonaceous nanoparticles into plants, depending on plant species and nanoparticle properties, include endocytosis through the plasmodesmata; passage via ion transport channels, carrier proteins or aquaporins; and also soil carbon or root exudate mediated entry (Fig. 2).

There are no studies on translocation and storage of nanoplastics in plants. However, the translocation of engineered carbon nanoparticles in the size range of 40 to 70 nm to stem and/or leaf have been demonstrated in rice using fullerene C_{70} (Lin et al., 2009) and soybean, maize, rice and Arabidopsis using carbon nanotubes (Zhao et al., 2017). There have not been any studies evaluating the transgenerational transmission of nanoplastics. Transmission, has been reported in rice using fullerene C_{70} mixed with natural organic matter obtained from natural waters, which contains a mixture of hydrophobic and hydrophilic acids and other soluble organic compounds (Lin et al., 2009). The transport and fate of the engineered carbon nanoparticle is strongly influenced by interaction with natural organic matter (Hyung and Kim, 2008), and therefore, the effect of soil organic matter adsorption to nanoplastics should be explored if we are to understand its effects on the fate of nanoplastics in soil and plant.

5.2. Toxicity, stress and response of plants to nanoplastic

Similarly, there is no data on the toxicity of nanoplastics on plants. Since the first studies reported plant cell uptake of engineered carbon-based nanotubes and fullerenes (Lin et al., 2009; Liu et al., 2009), studies on nanotubes and fullerenes indicate a range of positive, neutral and negative effects in a range of edible crops (see reviews by Husen and Siddiqi, 2014; Ma et al., 2010; Rico et al., 2011; J. Wang et al., 2016). All four reviews presented a sum of six studies (with numerous overlaps) on nanotubes and two studies on fullerene C_{60}, with all crops being grown under hydroponic and broth culture, except the studies by Kole et al. (2013) who used sphagnum moss and Torre-Roche et al. (2013) who used a mixed vermiculite-soil medium.

General observations on toxicity of carbon nanoparticles that may have relevance to future studies using nanoplastics, are: (1) phytotoxicity tests such as germination, root elongation and growth measures across studies indicate that sensitivity depends on plant species and the physicochemical properties of the engineered carbon nanoparticle; (2) cell damage occurs through genotoxicity and cytotoxicity (Shen et al., 2010; Tan et al., 2009); and (3) interactions between different types of engineered carbon nanoparticle with pesticides can increase or decrease the uptake of pesticides by different crops (Torre-Roche et al., 2013).

Plant can metabolize a range of pollutants, including polychlorinated and polycyclic hydrocarbons (Sandermann Jr., 1992). During pollutant metabolism, oxidative stress can result from a combination of (1) reactive oxygen species generated during cytochrome P450 mediated oxidation, and (2) glutathione depletion through gluthatione-S-transferases catalyzed conjugation with pollutant (Scandalios, 2001). Zhao et al. (2017) measured the uptake of ^{14}C-labelled carbon nanotubes (2.25 ppm) in rice, maize, soybean and arabidopsis; and found that biochemical parameters, such as antioxidant enzyme activities, were more sensitive than physiological measures, such as pigment and total protein contents. Biochemical parameters, therefore, may be a good indicator of plant response to nanoplastics. Pollutants are often stored as soluble and insoluble conjugates in various parts of the plants rather than degraded (Sandermann Jr., 1992). As such, it is also necessary to determine whether detoxification processes produce harmless metabolites, or whether new toxins might be introduced into the food chain. Estimates made using plant uptake models and quantities of micropollutants in irrigation water, indicate that human exposure to 27 emerging micropollutants (including pharmaceuticals, fragrances, flame retardants and plasticizers) range from <1 to >461 ng per person per day through vegetable and fruit consumption (Calderón-Preciado et al., 2011). Several of these chemicals are additives in plastic production. We have, to maintain brevity in this overview, refrained from discussing the residual monomers, catalysts and additives that are part of plastic and need to be considered in future studies. Readers interested in this matter are referred to reviews on biological effects of plastic additives (Meeker et al., 2009; Oehlmann et al., 2009), or phthalate esters occurrence and degradation in the environment (Gao and Wen, 2016; Staples et al., 1997).

6. From organismal to ecosystem-level responses

So far, studies on the ecological impact of plastic in soils are mostly at organismal level, or on the soil microbiome. This approach is not unique to soils, since a majority of impact studies in marine ecosystems have demonstrated impacts only at suborganismal and organismal levels (Browne et al., 2015). Clearly, more work needs to be done at higher biological organisation levels (Browne et al., 2015; Galloway et al., 2017; Rochman et al., 2016). But impact studies at higher biological organisation are difficult. Browne et al. (2015) suggests that existing knowledge of ecological linkages, where known, and population models, where the linkages are unknown, can be used to deduce such impact.

There is evidence that water infiltration is correlated to earthworm biomass and burrow length (Blouin et al., 2013), that is soil porosity is linked to earthworm presence. Using the approach suggested by Browne et al. (2015), one can then hypothesise that when earthworm mortality is high as a result of high microplastic contamination, as per reported by Huerta Lwanga et al. (2016) and discussed in Section 4.1, soil porosity would be impacted. Currently, only one laboratory study explored such ecological linkage. The study showed that *L. terrestris* had lower biomass under the exposure of microplastics at 7% microplastics (w/w in dry plant litter) while the burrows occurred in significantly higher numbers and the burrow walls were denser compared to the control without exposure to microplastics, however the burrow length was similar across all treatments during the 14 day experiment (Huerta Lwanga et al., 2017). These results indicate soil porosity may increase as a result of earthworm-microplastic interaction but further work is still necessary to validate their longer-term implications for soil porosity. Additionally, microplastics may also have direct effect on soil porosity, as both synthetic water-soluble and gel-forming polymers are used as soil conditioners to improve water infiltration, water retention and soil stabilisation (Bouranis et al., 1995).

In aquatic systems, microplastics have become a floating mobile habitat for algae, bryozoans, dinoflagellates, isopods, marine worms and microbes (Reisser et al., 2014). There is evidence that the ingestion of plastic debris by seabirds is linked to dimethyl sulfide, a chemical cue released by phytoplankton in response to foraging activity (Savoca et al., 2016). Savoca and colleagues demonstrated experimentally that PE and PP microplastics exposed in the ocean for three weeks produce dimethyl sulfide. The migration facilitated by microplastics can affect population and ecosystem dynamics. In soils, there is no lack of substrate compared to open water but plastic particles could serve similar habitat functions. Collembola has been observed to use microplastics as a site for oviposition (Maaß et al., 2017). Earthworm casts are naturally rich ecosystems of microbes (Gómez-Brandón et al., 2011; Toyota and Kimura, 2000) and casts enriched with plastic particles (Huerta Lwanga et al., 2016) would be hosting microbiomes. These findings suggest that biofilms on plastic in soils could promote uptake by other organisms higher up in the food chain.

7. Considerations for assessing risks of microplastics and nanoplastics in agroecosystems

7.1. Challenges and lessons from studies on plastic particles, engineered nanoparticles and other persistent contaminants

Current methodologies used to extract, quantify and characterise microplastics from water or sediment samples, would require adjustment to enable equivalent information from soil samples; not to mention the entire lack of nanoplastic isolation methods (Duis and Coors, 2016; EFSA Panel on Contaminants in the Food Chain, 2016; Syberg et al., 2015). Soil is a heterogeneous medium which makes the isolation or enrichment of plastic particles from it extremely challenging. The presence of soil organic matter, sometimes stabilised by interaction with soil minerals, complicates the removal of soil organic matter that distort spectroscopic techniques for identification of plastic particles. Recently, a method for extracting and quantifying the number, size and mass of micro-sized low-density polyethylene and polypropylene from soil using flotation and heating was published by Zhang et al. (2018). Some similarities can be drawn from sediment studies, and adaptation of recent procedures for the isolation of microplastics from fine sediments could potentially be used in the future to quantify micro and nanoplastic loads in agricultural soils (Coppock et al., 2017). These allows the identification of microplastics in soils, but more efficient and faster techniques are required. In addition, standardisation of the units of measurement in terms of weight, number and/or volume should be prioritised to allow comparison of results from different experiments.

The representativeness of synthetic plastic particles used in many experiments is questioned, since the aging of plastics in the environment alters their surface chemistry and behaviour. Rapid aging could be simulated by subjecting plastics containing pro-oxidant additives to artificial, accelerated weathering and abrasion, but we would still need to relate the structure and chemical properties of the artificially generated microplastic or nanoplastics with those isolated from field samples. An alternative approach is the preparation of a range of standard testing materials aged in a set of selected soils with different characteristics.

The adoption of high doses is often used in assessing effects of a pollutant in laboratory studies to elicit toxicological endpoint and determine dose-response curves. However, studies on pesticides and other endocrine-disrupting chemicals have shown that nonlinear or non-sigmoidal dose-response relationships are common, such as the U-shape or inverted U-shaped responses, (Clotfelter et al., 2004; Imfeld and Vuilleumier, 2012), and studies on nanoparticles indicated that particle surface area or particle number concentration may be more relevant than mass-based dose metric for determining biological effects for nanoparticles (Petersen et al., 2015). The range of doses could be narrowed down through spatiotemporal data detailing the occurrence of microplastic and nanoplastic debris in agroecosystems. This can be achieved by prioritising data collection in agroecosystems that receive recycled organic inputs or use plastic mulch.

Choosing the right subjects, variables and controls in the studies is also challenging (Horton et al., 2017; Syberg et al., 2015). If the test organisms are already exposed to high background levels of the pollutant, its lack of response compared to the treatment can be merely an artifact of the organism's prior exposure. Certain organisms are also more sensitive; such as root crops (Eggen et al., 2013), or juvenile organisms in early developmental stages (Clotfelter et al., 2004; Talsness et al., 2009), and these should be prioritised in initial screenings. Interspecific variation in susceptibility within a taxonomic group, intraspecific variations between age class, sexes and populations and ability to recover must all be carefully considered. Beyond individual species, choosing the right measures at the population and ecosystem level is also necessary as other abiotic or biotic stressors may enhance the sensitivity of the test organism or system to plastic pollution (Rohr et al., 2016).

Classical soil ecotoxicological approaches use isolated organisms and standard substrates, with measures taken for survival, growth, reproduction and avoidance behaviour over a period of days and weeks. Such approaches may not capture the full impact of chemical additives in plastics that act as endocrine disruptors in addition to those which bioaccumulate, where long-term exposure at low doses may alter cell functions or cause DNA damage. Such damage manifests later in life or across generations as the damage accumulates (Clotfelter et al., 2004). The use of short-lived organisms is the norm and provides an opportunity to study multigenerational effects of plastic pollution. However, great care must be taken in any attempt to extrapolate laboratory studies to spatially and temporally relevant scales for processes, ecological interactions and ecosystems.

7.2. Knowledge gaps and key research questions

This review highlights several major gaps in our understanding of what happens to microplastics and nanoplastics in soils and their ecological consequences. Some broad issues and key questions are briefly summarised below.

1. Shortcomings in defining and standardising parameters for determining magnitude of microplastic and nanoplastic contamination on land; e.g.

- What cost-efficient techniques can we use to detect, isolate, measure, and identify microplastics and nanoplastics in soils, and in organisms?
- Can artificially generated micro and nanoplastics be used as suitable models to gain an understanding of their potential ecotoxicity?

2. Improve understanding of the dynamics and fate of microplastics and nanoplastics in soils; e.g.

- What is the concentration of microplastics and nanoplastics in soils from each major pollution source?
- How do the products of fragmented plastic particles behave in the soil profile?
- How does the interaction of plastic particles with agrochemicals affect their behaviour?

3. Determine the bioavailability of plastic particles to plants and soil organisms; e.g.

- What are the features of plants and soil organisms that determine uptake, the capacity to exclude, or the capacity to isolate or sequester plastic particles internally?

4. Insufficient understanding of the consequences on plants and soil biota; e.g.

- What are the physical, physiological, and biochemical impacts of plastic residues – polymer, additives and their degradation products – within plants or soil organisms?
- How do nanoplastics affect the microbiome in phyllosphere, endosphere, spermosphere and rhizosphere of the plant?
- Do plastic pollution alter plant and soil biota response to other existing agrochemical or environmental stressors?
- What is the impacts of plastic pollution on the capacity of the agroecosystem to produce biomass?

8. Concluding remarks

Currently, considerable uncertainty exists because of the limited number of studies that have been published regarding the impact of microplastic and nanoplastic on most trophic levels in agroecosystems – demonstrated evidence of effects and demonstrated evidence of no-effects are equally few at this point in time. The existing regulations based on heavy metal contaminants and available nitrogen for land application of biosolids provide us with the possibility to estimate plastic loading in some agroecosystems. We could then use these loading rates to set up ecotoxicology experiments to determine if these loadings would pose acceptable ecological risk and if not, at what loading concentration would there be a problem.

Based on the mechanisms and constraints described above, the degradation of plastics applied to land is expected to be limited within the timescale of a human lifetime. As such, precautionary measures, even if the cause and effect relationships are not established for agroecosystems, may be warranted. Ultimately, many actions to mitigate microplastic and nanoplastic emissions on land will also benefit the wider environment. They are also likely to be less costly in the long-term and allow us to reap the benefits of plastics with much lower plastic pollution on land and in water.

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Appendix A. Supplementary data

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