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Nanoplastics in the soil environment: Analytical methods, occurrence, fate and ecological implications☆

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A B S T R A C T

Soils play a very important role in ecosystems sustainability, either natural or agricultural ones, serving as an essential support for living organisms of different kinds. However, in the current context of extremely high plastic pollution, soils are highly threatened. Plastics can change the chemical and physical properties of the soils and may also affect the biota. Of particular importance is the fact that plastics can be fragmented into microplastics and, to a final extent into nanoplastics. Due to their extremely low size and high surface area, nanoplastics may even have a higher impact in soil ecosystems. Their transport through the edaphic environment is regulated by the physicochemical properties of the soil and plastic particles themselves, anthropic activities and biota interactions. Their degradation in soils is associated with a series of mechanical, photo-, thermo-, and bio-mediated transformations eventually conducive to their mineralisation. Their tiny size is precisely the main setback when it comes to sampling soils and subsequent processes for their identification and quantification, albeit pyrolysis coupled with gas chromatography-mass spectrometry and other spectroscopic techniques have proven to be useful for their analysis. Another issue as a consequence of their minuscule size lies in their uptake by plants roots and their ingestion by soil dwelling fauna, producing morphological deformations, damage to organs and physiological malfunctions, as well as the risks associated to their entrance in the food chain, although current conclusions are not always consistent and show the same pattern of effects. Thus, given the omnipresence and seriousness of the plastic menace, this review article pretends to provide a general overview of the most recent data available regarding nanoplastics determination, occurrence, fate and effects in soils, with special emphasis on their ecological implications.

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1. Introduction

Nowadays, it is clear that plastic pollution, which is one of the most important environmental problems that humans have to face, is ubiquitous in the environment: from deep sea sediments (Zhang et al., 2020) to the highest mountains (Napper et al., 2020), passing through the coasts and rivers of all over the world (Elizalde-Velázquez and Gómez-Oliván, 2021). Once in the environment, plastics may be fragmented into tiny pieces (microplastics, MPs, or nanoplastics, NPs) as a result of abiotic (termo-oxidation, photo-oxidation, atmospheric oxidation and mechanical degradation) or biotic processes (Crawford and Quinn, 2016). In particular, the presence of MPs, which have a length, in their largest dimension, between 5 mm and 1 μm (the most accepted definition), is constantly being reported in all thinkable matrices (Padha et al., 2022). Of particular importance is the fact that they have even been found in human faeces (Zhang et al., 2021), lungs (Amato-Lourenço et al., 2021), placenta (Ragusa et al., 2021) or blood (Leslie et al., 2022), though the scientific community still needs to continue research in this field to try to definitely reveal if they constitute a real threat to human health.

Despite the fact that the presence, behaviour and properties of MPs and their potential negative effects is currently a key and relevant issue that still needs to be understood, their further fragmentation into NPs -size below 1 μm-could be even more important. In mammalians, for example, NPs can accumulate in the ovaries and testes, trigger inflammatory and oxidative gonadal damage and impair germ cells (Marcelino et al., 2022). They can also disrupt the ecological function of biofilms, causing adverse effects in aquatic organisms, and bioaccumulate (Kihara et al., 2021), among other issues. In general, the lower the size, the more widespread presence in the environment and the more possible harms in it.

In terrestrial environments like soils, the presence of plastic is also of special concern. In fact, it is estimated that the amount of mismanaged plastic waste can be up to 4–23 times greater than those reported for marine environments (Horton et al., 2017). In the particular case of agricultural soils, for example, plastics may appear as a result of soil management techniques and, to a lesser extent, due to atmospheric deposition. Conventional farming applies an extremely high amount of plastic products which include mulching, packaging, greenhouse shedding, seedbeds, and water pipes, among others. Concerning the presence of MPs and NPs, irrigation (i.e. treated wastewater) or the application of sewage sludge or compost are particularly important (Yang et al., 2021; You et al., 2022).

The great majority of laboratory evidence indicates that NPs may generate a wide range of harmful effects on the chemical, physical and biological properties of the soils. Chemical, in the sense that they can leach plastic additives or adsorb/desorb chemicals; physical like the density, porosity, water-bearing capacity or the stability of soil aggregates, among others; and biological, by influencing the living organisms, including microbial communities (Yang et al., 2021; You et al., 2022). However, the lack of consensus on the research methodologies leads to mixed and inconsistent conclusions (Wang et al., 2021). Therefore, it becomes necessary the development of new standardized analytical methods that allow accurate NPs determination in soil samples. So far, there are very few works in this sense and most of them make use of previously existing protocols for the determination of MPs (Cai et al., 2021).

As a result of such important issues derived from the presence of NPs in soils, this review article has been intended for addressing the following aspects: i) how NPs arrive to the soil environment, and their behaviour, migration, transformation mechanisms and fate; ii) the methodologies utilised for NPs analyses, including soil sampling tips, procedures for NPs removal from complex matrixes, and techniques employed for quantification, size distribution and composition establishment; iii) the influence of NPs pollution on soil microorganisms, directly by affecting their population and biodiversity, or indirectly by disrupting the soils physicochemical parameters and nutrients cycles; iv) the consequences of the ingestion of NPs by soil dwelling fauna; v) the uptake of NPs by plants and their impact in vegetable growth, morphology, nutritional status and biochemistry; and vi) the global ecological implications, current gaps and research needs.

2. Nanoplastics definition, sources, transport and transformation

2.1. Definition and sources

It is important to mention that the definition of NPs is still under discussion. If particle size (largest dimension) is considered, studies generally set the upper size limit at 100 or 1000 nm, which places them after MPs in the rating by size of plastic debris (Mitrano et al., 2021; Wang et al., 2022a; Wu et al., 2020). Similar to MPs, NPs have a wide range of shapes (e.g. sphere, granule, fibres and films) and composition (e.g. polyethylene -PE-, polypropylene -PP-, polystyrene -PS- and polyvinyl chloride -PVC-). They may be directly manufactured for electronic, pharmaceutical, cosmetic and personal care product industries, and even for science purposes (primary NPs). Additionally, they may result from the fragmentation and degradation of bigger plastic pieces exposed to abiotic and biotic environmental processes, such as thermo-oxidation, photo-oxidation, atmospheric oxidation, hydrolysis, mechanical and microbial activities (secondary NPs) (Hayes, 2019; Pinto da Costa et al., 2019; Yu et al., 2021b; Yu and Flury, 2021). Thus, the presence of NPs in soils is closely linked to MPs inputs from different natural processes and human activities. For example, atmospheric deposition, runoff, and abandoned debris plastic are pathways of entry for MPs, and consequently NPs, in non-agricultural soils (Bläsing and Amelung, 2018; He et al., 2018; Rezaei et al., 2022). Agricultural soils have an additional contribution of MPs and NPs through the degradation of conventional and biodegradable plastic mulching, irrigation water or fertilisation with sewage sludge, biosolids, compost and polymer coated slow-release fertilisers and pesticides (Crossman et al., 2020; Katsumi et al., 2021; Yu et al., 2021b; Zhou et al., 2022). Therefore, in this type of soils the abundance of MPs-NPs has been associated with both agricultural management and waste treatment, specially from wastewater treatment plants. Finally, it has even been hypothesised that the abundance of NPs could be much higher than MPs in the natural environment, although research remains limited due to the technical difficulties and challenges involved in analysing NPs in complex matrices such as soil (Maity et al., 2022).

2.2. Transport

The main difference between MPs and NPs particles lies in their environmental behaviour. In this sense, NPs constitute the category of plastic debris that exhibits the highest surface-to-volume ratio and they can have colloidal properties (Reynaud et al., 2022). Due to this peculiarity, NPs present in soils a higher mobility and different fate than MPs particles (Mitrano et al., 2021). In short, the vertical and horizontal transport of NPs in soils is regulated by: i) physicochemical properties of NP particles (e.g. size, shape and surface charge), ii) physicochemical soil properties (e.g. structure, porosity, organic matter content, pH, infiltration capacity and water retention), iii) human activities (e.g. digging, pronging, tilling, etc.), and iv) interactions with biota (e.g. ingestion/egestion, push and adhesion on their surface) (Huerta Lwanga et al., 2017; Liu et al., 2021b; Pinto da Costa et al., 2019; Wu et al., 2020).

Those plastic nanoparticles that are free in the soil solution will be more mobile than those that are attached to natural soil particles (such as organic matter, iron oxides and clays), which may present potential risks to the groundwater and increase the bioavailability of NPs by facilitating their uptake by plants roots (Castan et al., 2021; Maity et al., 2022) (Fig. 1a). It has also been experimentally shown that the cracks

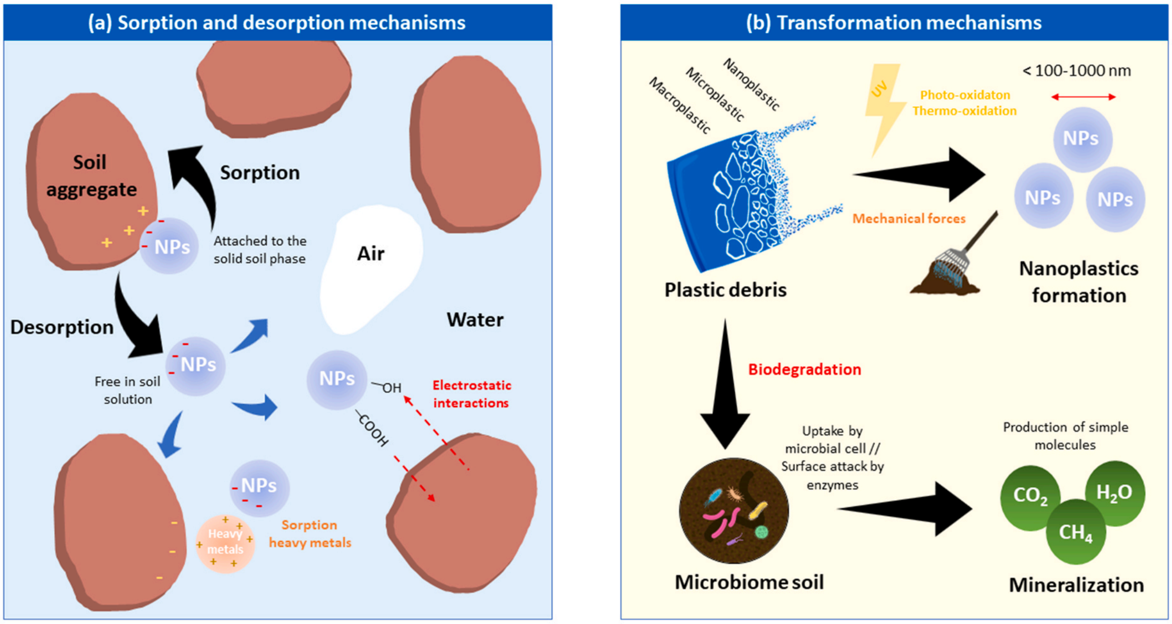

Fig. 1. Main mechanisms influencing the transport and transformation of NPs in soils.

and pores formed by roots in soil promote the upward movement of plastic particles by floatability effect while soil infiltration processes cause downward transport (Li et al., 2021a). In fact, some studies have observed how wetting and drying cycles influence the advance of these particles into deeper soils layers (O'Connor et al., 2019). Soil porosity also contributes to the ability of NPs to adhere at the air-water interface by electrostatic or hydrophobic interactions (Liu et al., 2021b; Yu and Flury, 2021). Due to its effect on surface charges, soil pH plays a crucial role in these mechanisms. Thus, at elevated pH values the long-distance migration of NPs increases in the soil (Wu et al., 2020). In addition, functional groups present in NPs may contribute to adsorption mechanisms with soil organic matter by changing its stability and thus reducing their transportability (Shen et al., 2019; Wang et al., 2021). As shown in Fig. 1a, NPs can interact with other coexisting contaminants, such as heavy metals and organic contaminants (Liu et al., 2021b). The distribution of NPs within the soil profile may also be regulated by soil biota, particularly earthworms are able to move these plastic particles via bioturbation (Heinze et al., 2021). Recently, a study showed that soil amoebae, specifically *Dictyostelium discoideum*, can excrete NPs during slug migration, which can influence both their distribution and biodegradation (Zhang et al., 2022c).

2.3. Transformation

Regarding the transformation of NPs in soils, anthropogenic pressure, high temperatures and ultraviolet (UV) radiation intensify the environmental degradation of plastic particles, especially at the soil surface. By contrast, in deeper soil layers, where there is no photoaging process associated to solar exposure and anaerobic conditions may prevail, the transformation of NPs mainly depend on biodegradation since the photo-oxidation process is inhibited (Fig. 1b) (Chai et al., 2020; Khanna et al., 2021; Liu et al., 2021b).

The photoaging consists of a light-mediated oxidative degradation process which causes molecular changes in plastic chemical structures via ruptures and rearrangements of their constituent polymers, as well as the introduction of oxygenated functional groups (Liu et al., 2021b). As a consequence, a physical enfeeblement of the plastic occurs, yielding to macroscopic cracking and to the formation of particles with a higher degree of crystallinity and a tinier size which can facilitate the accumulation of toxic chemicals. Furthermore, the presence of oxygen-rich functional groups increases the NPs polarity and hydrophilicity, disrupting their transport processes and also increasing both the affinity for soil contaminants as its releasing ability (Liu et al., 2021b; Menzel et al., 2022; Pathan et al., 2020; Yang et al., 2021).

Biodegradation of NPs is a cascade process which starts with the biodeterioration and bio-fragmentation of the plastics, i.e. the microbial-based disruption of their physicochemical properties to trigger the breakdown of the polymeric chains via plastic degrading enzymes into less complex monomers. These can be eventually assimilated as a carbon source by the microorganisms, which mineralise them releasing simple inorganic by-products such as CO_2 or H_2O (Tiwari et al., 2021). Thus, plastic biodegradation has a promising impact in the development of new strategies and technologies to remediate plastics from the environment (Zhou et al., 2022). Indeed, fungal and bacterial organisms present in soils and capable of degrading plastic particles are increasingly being discovered. In this sense, the chemical nature of NPs (e.g. density, types of functional groups and plasticisers or other additives added during plastic processing), environmental conditions (e.g. substrate, pH, temperature and oxidative stress) and type of soil microorganisms are factors that determine the rate of biodegradation (Zhang et al., 2022c; Zhou et al., 2022). However, these processes are not exclusive to soil dwelling microorganisms because bigger fauna can also ingest plastic particles (Beriot et al., 2021; Huerta Lwanga et al., 2017) and accelerate their degradation by means of the gut bacterial community (Pathan et al., 2020). Plant-microbe interactions are also able to transform NPs, since exposure to plastic contamination has induced changes in root exudation in response to the abiotic stress, driving the degradation of NPs in the rhizosphere (Yoon et al., 2021). As a consequence of these plastic biodegradation processes, it may occur the release of adsorbed toxins, albeit the same microbial enzymes involved in the plastic degradation may also act as degraders of environmental pollutants (Zhou et al., 2022).

3. Analytical methods

In recent decades, the design, development and application of analytical methodologies for the determination of contaminants in samples of environmental origin have been carried out under the premise that these contaminants were dissolved or adsorbed in the matrix of the sample (Mitra, 2003; Ribeiro et al., 2014). This has led to an important development of analytical methodologies, based mostly on the use of solvents and solid materials that allow the selective separation of contaminants for their subsequent determination using an appropriate technique (López-Lorente et al., 2022). However, the emergence of new contaminants in the form of solid particles of different sizes and chemical composition, like MPs and NPs, has posed a new challenge in terms of analytical methodologies, since, despite the important advances mentioned above, these methodologies cannot be extrapolated for such purpose.

In recent years, great efforts have been made developing new procedures and techniques for the extraction/separation, quantification and identification of MPs in environmental samples, although no unified standard methods have been established yet (Li et al., 2020c; Ye et al., 2022). These procedures generally involve a previous digestion step followed by floatation in a high-density solution and its subsequent filtration. However, and despite some attempts have been made, some of these procedures cannot be directly applied to the determination of NPs, since when the size of the particles falls on the nano-scale, the physicochemical properties of the material change, even if it is composed of the same type of plastic. This is the case of the separation by flotation, as the buoyant force is not strong enough for such small particles (Cai et al., 2021; Li et al., 2020d). Thus, the determination of NPs constitutes nowadays a real challenge for the scientific community, and it is clearly reflected in the limited number of publications in which the determination of NPs have been addressed, especially if the analysis of real samples is considered (Cerasa et al., 2021). Regarding the specific case of soils, the complexity of the sample has resulted, to the best of our knowledge, in an extremely low number of works regarding the presence of NPs in real samples and in very little information about their occurrence into the environment (Möller et al., 2020).

Despite the additional difficulties involved in the determination of NPs in environmental samples, most of the steps of analytical methods are the same considered for the determination of MPs, such as an adequate design of the zones and the sampling procedure taking into account the characteristics of the sample, sample treatment (removal of organic matter, separation and removal of other particles, etc.), and quantification and characterisation of the isolated plastic particles (Cerasa et al., 2021). As any other analytical method, the final goal in this field is the development and validation of analytical methodologies that can be applied to real samples providing reliable results, guaranteeing a good accuracy, precision, selectivity, sensitivity and robustness. In this context, the emergent nature of NPs means that there are no reference materials, making it necessary to spike the matrices with a known amount of NPs and thus be able to evaluate the aforementioned parameters (Cerasa et al., 2021).

3.1. Sampling of plastic polluted soils

As it is well known, sampling is an essential step of any analytical method to ensure the reliability, significance and representativeness of the results. Thus, any error made in the sampling stage will render useless any care and rigor in the subsequent stages of the sample treatment or the determination of the analytes (Zhang and Zhang, 2012). This stage is specially relevant when it comes to environmental samples, since, in general, they are dynamic systems, where the design and sampling varies greatly depending on the objective of the analysis and the type of sample, being necessary some specific devices in some cases (Lai et al., 2021; Silva et al., 2018). Regarding soils or sediments, the sampling protocols are very similar in terms of the devices used. In this sense, when plastic particles can be distinguished by naked eyes, a selective sampling can be carried out by sieving or by picking them out using tweezers (Hidalgo-Ruz et al., 2012). However, these methods cannot be applied to NPs, so bulk sampling is necessary, in which all particles are collected, including both MPs and NPs. This type of sample collection is normally carried out using metallic spoons, shovels or spatulas (Lai et al., 2021), although in some cases stainless steel core samplers have been used (Pérez-Reverón et al., 2022). This last option is very interesting to study the vertical distribution of plastic particles, at the same time that prevents the use of additional containers to preserve and transport the sample to the laboratory for its analysis (Lai et al., 2021).

3.2. Separation of nanoplastics from the matrix

An important issue that has to be considered when determining NPs in soils, is the possible presence of aggregates formed between non-plastic particles and/or natural organic materials and NPs, as well as the similar density among plastic types typically found in soils (Cerasa et al., 2021). In consequence, digestion steps to remove the organic matter and separation techniques have to be applied to isolate NPs efficiently, at the same time that no alterations of their physicochemical properties are produced (Cerasa et al., 2021; Li et al., 2020d; Schwaferts et al., 2019). Due to the fact that NPs found in environmental samples (not only in soils) are always coated with biofilms or organic matter in general, two common approaches can be distinguished to remove that coating: chemical and enzymatic digestion (Li et al., 2020d). The former is based on the exposition of samples to an oxidizing agent solution (H_2O_2 30%), Fenton's reagent (H_2O_2 and Fe^{2+}), alkali solution (NaOH, KOH), strong acid solutions (HCl, HNO_3), or even a surfactant (sodium dodecylsulphate, SDS), sometimes increasing the temperature to accelerate and favour the process, but not too much to avoid NPs elimination (Li et al., 2020d). The last method has demonstrated a high efficiency for organic matter removal when complex environmental samples are analysed. However, the main drawback that this alternative presents is the high cost of the enzymes used, such as Proteinase-K, although other cheaper enzymes have been applied for plastic particles clean-up including protease, cellulase, lipase or trypsin (Hurley et al., 2018; Li et al., 2020d; Schwaferts et al., 2019; Zarfl, 2019).

In addition to the different digestion processes mentioned above, there are other methods that have been applied for the separation of MPs and NPs from other particles present in the sample matrix (not always of environmental concern). Some of them consist of filtration processes (Hernandez et al., 2019), sometimes using membranes of different pore sizes placed in series, which allows discrimination by particle size, or ultracentrifugation processes (Hernandez et al., 2017; Junhao et al., 2021). These types of systems are mainly used for aqueous samples and are expensive. In addition, the application of some techniques used for the separation of nanomaterials to the analysis of NPs has also been proposed, such as field flow fractionation (Fuller and Gautam, 2016) or cloud point extraction (Zhou et al., 2019). However, to the best of our knowledge, none of these approaches have been applied to the determination of NPs in soils, though, it is possible to find different methods developed for the determination of MPs in soils and sediments, including the use of pressurized fluid extraction (Fuller and Gautam, 2016), oil extraction taking advantage of the lipophilic surface of plastic particles (Crichton et al., 2017), as well as the classical density separation using inorganic salts solutions (i.e. NaCl, NaI, Zn_2Cl, NaBr, etc.) (Möller et al., 2020), among others.

3.3. Characterisation and quantification of nanoplastics

In order to accurately evaluate the potential risks derived from the presence of NPs in the environment, NPs separation and cleaning-up is not enough, being necessary more information about their chemical composition, size distribution and the concentration of the particles found in the sample. At this point, and after having carried out the treatment of the sample, the techniques used are generally the same regardless of the matrix analysed (Li et al., 2020d). Regarding particle size distribution and/or morphology determination, some techniques used for nanomaterials characterisation have been adapted to NPs, including light scattering (Correia and Loeschner, 2018; Wahl et al., 2021), electron microscopy (Gigault et al., 2016), or nanoparticle tracking analysis (Lambert and Wagner, 2016). Nevertheless, these techniques do not allow the determination of the composition of plastic particles. In this sense, smallest MPs have been typically characterised using Fourier transform infrared (FTIR) microscopy and Raman microscopy (Li et al., 2020d; Nguyen et al., 2019). However, these techniques are normally limited by the detection of single particles below 20 μm for FTIR and 1 μm for Raman spectroscopy (Cerasa et al., 2021), being necessary to resort to other techniques that allow carrying out this type of analysis, such as X-ray photoelectron spectroscopy or pyrolysis coupled with gas chromatography-mass spectrometry (Pyr-GC-MS) (Schwaferts et al., 2019). In this sense, Pyr-GC-MS has emerged as one of the most powerful tools in this field, thanks to its selectivity and sensitivity, although it presents the drawback of being a destructive technique, since it requires the pyrolysis of the NPs (Li et al., 2020d). Other techniques based on the use of MS can be found in the literature for the detection of plastic particles in environmental samples, for example thermoextraction and desorption coupled with GC-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, or thermogravimetry-mass spectrometry (Li et al., 2020d).

3.4. Determination of nanoplastics in soils

Several studies regarding the effect of NPs in soils (Zou et al., 2022) or their role on the transport and accumulation of different contaminants in porous media (Jiang et al., 2022; Xi et al., 2022) have been carried out. However, to the best of our knowledge, up to date only two works have dealt with the determination of NPs in soils (Wahl et al., 2021; Wang et al., 2018). In this sense, Wahl et al. (2021), evaluated the occurrence of NPs in contaminated agricultural soils. All samples were dried at room temperature, sieved at 2 mm and stored in the dark, controlling the contamination of the sample during this process by using a soil control. NPs were extracted using ultrapure water in a soil:water ratio of 1:4 under stirring for 72 h. After this time, samples were filtered through 0.8 μm filters. Water-extract filtrates were fractionated by asymmetric flow-field flow fractionation coupled to UV spectroscopy and static light scattering, establishing three different populations in the ranges <5 nm, 20–150 nm and 150–500 nm. The two biggest groups of NPs were analysed by Pyr-GC-MS to determine their composition, obtaining a series of multiple peaks at constant time characteristics of PE (Fig. 2). Besides, specific plastic markers were also identified, such as naphthalene and styrene monomers. Wang et al. (2018) studied the extraction efficiency of MPs and NPs from biosolids and soils using spherical PS beads of 0.05, 1.0, 2.6, 4.8 and 100 μm as model MPs and NPs. Regarding soil, it was firstly spiked with the plastic particles and deionized water was added at soil:water ratio of 1:2, manually shaken the mixture and letting it settle for 48 h. Then, organic matter was oxidized with H_2O_2 at 60 °C during 7 days. A separation by floatation was also evaluated using a $ZnCl_2$ solution, and it was seen that the floatation time for 0.05 μm beads was exceedingly long (86–186 h), since these particles diameter were close to the lower critical particle size of 0.024 μm. In general, the extraction efficiency for NPs was low compared to the one obtained for MPs. The diameters of NPs (0.05 μm) after sample treatment was determined by laser light scattering, and scanning electron microscopy (SEM) was used to determine if the oxidation process caused changes in the surface and shape of the beads. For quantification, UV–Vis spectroscopy (216 nm for 0.05 μm beads) was used.

3.5. Analytical methods for the evaluation of the phytotoxicity induced by nanoplastics

Apart from the previous mentioned techniques, it should also be highlighted that other techniques have been employed to study NPs phytotoxic effects. Spanò et al. (2022) stained fixed root and shoot tissues with uranyl acetate and lead citrate to perform transmission electron microscopy (TEM) observations. Luo et al. (2022) doped PS

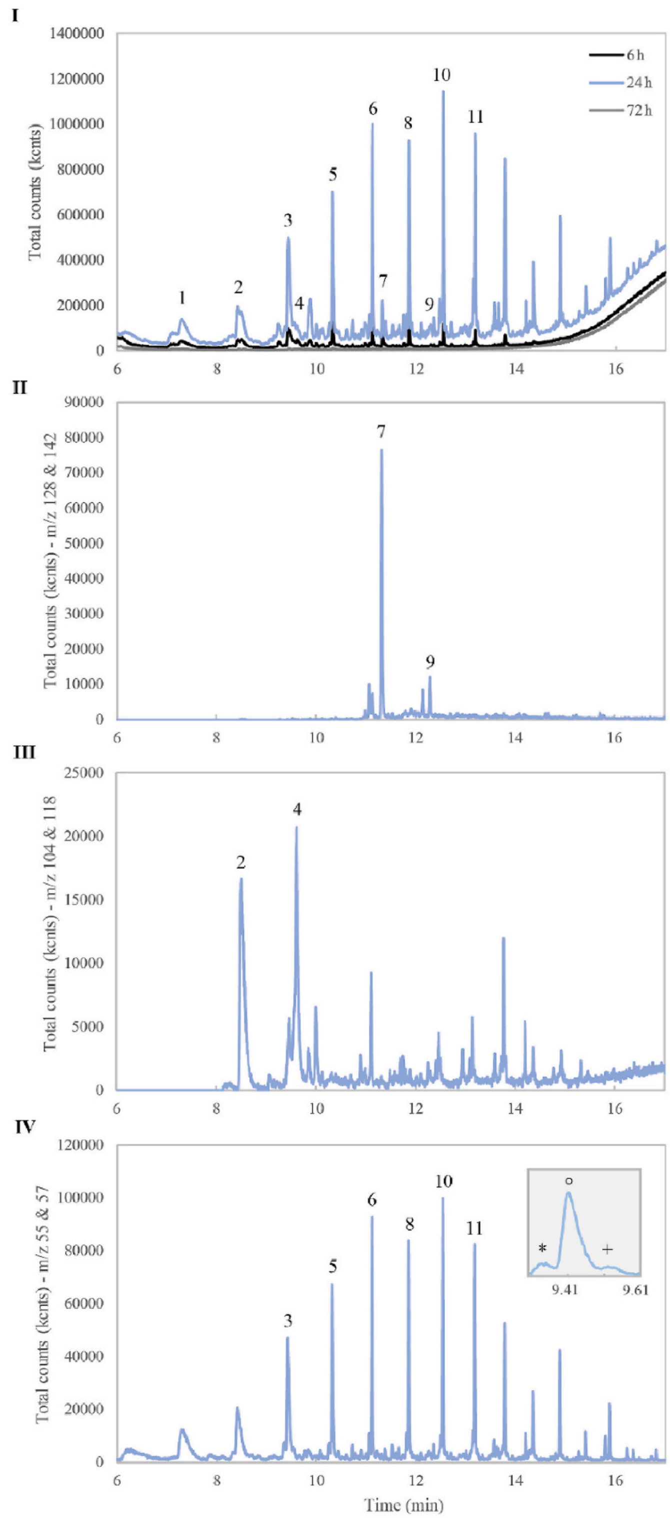

Fig. 2. I: Pyrograms of the 3 soil:water extracts at 6, 24 and 72 h. Numbers correspond to specific markers of plastic: 1: toluene, 2: styrene, 3: 1-decene, 4: α-methyl styrene, 5: 1-undecene, 6: 1-dodecene, 7: naphthalene, 8: 1-tridecene, 9: naphthalene 1-methyl, 10: 1-tetradecene, 11: 1-pentadecene. II, III and IV: Ion chromatograms at 24 h for m/z = 128 and 142, m/z = 104 and 118, and m/z = 55 and 57, respectively. Finally, the box in the top right corner in IV details the triplet n-alkadiene *, n-alkene ° and n-alkane +. Reprinted from (Wahl et al., 2021) with permission of Elsevier.

particles with the europium chelate Eu-β-diketonate and used inductively coupled mass spectrometry (ICP-MS) to quantify them. del Real et al. (2022) employed Pd-doped NPs and then visualized the particles via micro X-ray fluorescence (μ-XRF). Fluorescently labelled NPs have also been widely used to detect their absorption and distribution in plants via fluorescence microscopy (Wang et al., 2022b; Wang et al., 2022e) and confocal laser scanning microscopy (CLSM) (Liu et al., 2022a; Wang et al., 2022b; Wang et al., 2022e; Zhu et al., 2022). These analyses agreed about NPs traversed the root cell wall and accumulated in vascular systems of plant tissues after the absorption, becoming a potential risk to food safety.

Finally, it is important to remember that these small plastic particles are ubiquitous, so any effort in the development of sample treatment methodologies or the use of advanced instrumentation is useless if sample contamination is not controlled during treatment. In this sense, the use of control blanks, suitable cleaning protocols, air purification systems in the laboratory, the use of glovebox, or even laboratory coats of a specific colour, as well as the use of non-plastic laboratory materials, are some of the most extended practices to avoid an overestimation of the NPs content (Möller et al., 2020).

4. Effects of nanoplastics on soil microbiota

Microorganisms, which can inhabit the bulk soil and/or the rhizosphere, are the main participants in many processes and functions developed by soils (Philippot et al., 2013). The activities of such microbial communities are also indicators of contamination effects (Delgado-Baquerizo et al., 2016). In this sense, nowadays, it is well-known that MPs and NPs can make changes on the soil properties and microbial communities, and these changes indirectly affects plant growth (Chen et al., 2022). However, the real impact of the organic and inorganic pollutants associated to these particles, as well as their potential ecological risks in soil microbial activities, are still unknown (Boots et al., 2019; Hu et al., 2019).

NPs are expected to be more hazardous to microbial health than MPs because of their higher surface to volume-ratio (Wiesner et al., 2011). Most studies about microbial effects in soils have been carried out with MPs alone or combining MPs and NPs (Allouzi et al., 2021; Guo et al., 2020; Iqbal et al., 2020; Joos and de Tender, 2022; Ren et al., 2022; Sun et al., 2021b; Yoon et al., 2021), and many of them have been conducted on agricultural soils due to the application of sewage sludges (Allouzi et al., 2021; Hurley and Nizzetto, 2018; Iqbal et al., 2020; Joos and de Tender, 2022; Khalid et al., 2020; Ren et al., 2022; Sun et al., 2021b; Zhu et al., 2018)

Soil fertility can be reduced with MPs and NPs particles in soils as a result of the decrease of microbial communities in the rhizosphere that have a significant role in plants growth (Allouzi et al., 2021; Chen et al., 2022). As an example, Ren et al. (2022) studied the combined effects of MPs and NPs (5 μm and 70 nm PS beads) on the growth of wheat seedling and rhizosphere microbes, showing effects in the dissolved organic matter in soils, survival and growth of seedlings and their associated rhizosphere microbes. PS-beads MPs and NPs resulted in lesser diversity of soil microbes and selective effects on specific microbes.

Awet et al. (2018) demonstrated for the first time that PS NPs (Fig. 3) affect negatively microbial biomass in soils, thus suggesting antimicrobial activity of PS in the soil environment. Several studies later indicated a decrease in soil microbial biodiversity or biomass when NPs were present in soils (Joos and de Tender, 2022; Ren et al., 2020; Wang et al., 2016). However, it should also be indicated that, after serial dilution, plastic mulching can significantly increase the soil microbial population, increasing the so-called plastisphere (Wang et al., 2011).

Nutrients cycling is also affected by the presence of micro and nanoparticles. Iqbal et al. (2020) studied their implication for nitrogen cycling and soil microbial activity. Concerning NPs pollution on the soil-plant system, they identified different potential ecological risks associated with nitrogen cycling and also physical and chemical mechanisms and direct toxicities than can generate shifts in soil microbial activity. The oxygen flow in soils can also be affected by the presence of NPs, changing the soil moisture and porosity, and altering the habitat of aerobic and anaerobic microorganisms (Allouzi et al., 2021). Studies about the behaviour of pH under the influence of NPs are still lacking, albeit it is known that pH changes when MPs are present in soils. On the one hand, it has been reported that low-density PE (LDPE) and polylactic acid (PLA) increased pH in soils; on the other hand, high-density PE (HDPE) reduced pH values, affecting soil microbial community (Guo et al., 2020; Khalid et al., 2020).

5. Effects of nanoplastics on soil fauna

NPs may be ingested by organisms and accumulated in their bodies, thereby entering the food chain. This fact, together with their high surface-to-volume ratio, make relevant to investigate their harmful effects on environmental species (Kim et al., 2019; Pathan et al., 2020). Soils might constitute a larger reservoir for NPs than marine ecosystems and, therefore, determination of nano-sized particles in soil fauna would provide an assessment both of the NPs exposure effects in terrestrial organisms and monitoring soil environment pollution (He et al., 2018; Masseroni et al., 2022). Nematodes, earthworms, collembola, snails and isopods as well as other larger animals like rodents, are the most commonly studied living species of soil fauna (Channarayappa and Biradar, 2018; Görres and Amador, 2021; Kiran et al., 2022). Despite the wide variety of soil organisms, to the best of our knowledge, only nematodes, earthworms and mice have been used to carry out the majority of studies on NPs. By contrast, research in collembola and snails has been very limited (only one study each), and studies in terrestrial isopods have been performed only for MPs (Helmberger et al., 2022; Jemec Kokalj et al., 2021, 2018; Selonen et al., 2021, 2020). Effects of NPs exposure in these organisms have been reviewed and summarized in Table 1.

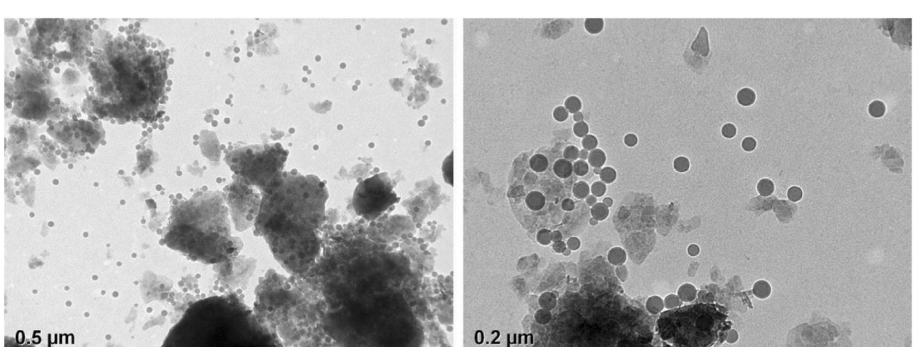

Fig. 3. Transmission electron microscope (TEM) image of PS-NPs in soil. Reproduced from Awet et al. (2018) with permission from Springer.

Table 1
Effects of NPs in soil fauna.

Organism	Plastic particles		Exposure conditions		Effects	References
	Size	Type	Concentrations tested	Exposure time (days)		
Caenorhabditis elegans	100 nm	PS	1–1000 μg·L^{-1}	4.5	Transgenerational toxicity, intestinal permeability enhancement, defecation cycle prolonged	Zhao et al. (2017)
	100, 500 nm 1, 2, 5 μm	PS	1.0 mg·L^{-1}	3	Survival rates, body length and lifespan decrease, neuronal toxicity, oxidative stress, locomotor behaviour disruption	Lei et al. (2018a)
	0.1, 1.0, 5.0 μm	PS	0.5–10 mg·m^{-2}	2	Size-dependent toxicity on lethality, intestinal calcium decreases and intestinal accumulation	Lei et al. (2018b)
	50, 200 nm	PS	17.3, 86.8 mg·L^{-1}	1	Energy metabolism disruption, locomotion and reproduction decrease, induced oxidative stress	Kim et al. (2019)
	102 nm	PS	1–100 μg·L^{-1}	NA	Neurodegeneration, locomotion behaviour disruption, dynamic autophagy induction	Qu et al. (2019a)
	35 nm	PS-NH_2	1–1000 μg·L^{-1}	NA	Reproductive capacity reduction, gonad development disruption, germline apoptosis and germline DNA damage	Qu et al. (2019b)
	42, 530 nm	PS	0.1–100 mg·L^{-1} (in liquid media) 0.01–100 mg·kg^{-1} (in soil media)	1	Offspring number reduction at low concentrations and larger NPs size	Kim et al. (2020)
	30 nm	PS	0.1–100 μg·L^{-1}	1	Fungal infection co-exposure lifespan and locomotion behaviour decrease at high concentrations	Li et al. (2020a)
	30 nm	PS	0.1–100 μg·L^{-1}	NA	Brood size reduction, germline apoptosis induction, locomotion behaviour decrease, ROS induction. Less toxic than Al_2O_3 or TiO_2 nanoparticles	Li et al. (2020b)
	100 nm	PS	0.1–100 μg·L^{-1}	6.5	Fat metabolism alteration, ROS production, locomotion behaviour decrease	Liu et al. (2020a)
	100 nm	PS	1–1000 μg·L^{-1}	NA	ROS production, locomotion behaviour decrease	Liu et al. (2020b)
	25, 50, 100 nm	PS	1–1000 μg·L^{-1}	3	ROS increase, mitochondrial damage, neurotoxicity, lipofuscin accumulation, apoptosis	Liu et al. (2020c)
	0.1–10 μm	PS	0.04–12.5 mg·L^{-1}	4	Toxicity determined by total surface area of NPs, food availability alteration	Mueller et al. (2020)
	30 nm	PS	1–1000 μg·L^{-1}	8	Lifespan reduction, locomotion behaviour decrease, oxidative stress, autophagy induction	Qiu et al. (2020)
	1 μm	PS	0–100 μg·L^{-1}	3	Adverse physiological effects, intestinal damage, lipofuscin accumulation, oxidative stress	Yu et al. (2020)
	20, 100 nm	PS	0.1–100 μg·L^{-1}	6.5	Transgenerational toxicity, locomotion behaviour decrease, brood size reduction, oxidative stress	Liu et al. (2021a)
	35 nm	PS-NH_2	1–100 μg·L^{-1}	4	Transgenerational toxicity, reproductive capacity and gonad development decrease, germline apoptosis	Sun et al. (2021a)
	100 nm	PS	1–100 mg·L^{-1}	3	Transgenerational toxicity, brood size reduction, intestine accumulation in first generation, oocytes aberrations, germline apoptosis	Yu et al. (2021a)
	30 nm	PS	1–100 μg·L^{-1}	6	Transgenerational toxicity, ROS production, brood size and locomotion behaviour inhibition	Zhang et al. (2022b)
Enchytraeus crypticus	0.05–0.1 μm	PS	0, 0.025, 0.05, 10% d.w.	7	Body weight reduction, reproduction increase, gut microbiome disruption and bacteria abundance decrease	Zhu et al. (2018)
Eisenia fetida	100, 1300 nm	PS	100, 1000 μg·kg^{-1} soil	14	Intestinal accumulation and cell damage, oxidative stress, DNA damage	Jiang et al. (2020)
Eisenia andrei	180, 250 μm	PE	1000 mg·kg^{-1} soil	21	Spermatogenesis and male reproductive organs damage, cytotoxicity	Kwak and An (2021)
Lumbricus terrestris	256 nm	PS	0.56 g·kg^{-1} (0.06%)	2	PS ingested and excreted but no visible adverse effects	Heinze et al. (2021)
Eisenia fetida	10, 100 μm 100 nm	PS	10, 100 mg·kg^{-1}	21	MPs co-exposure caused higher toxicity (oxidative stress, immune balance disturbed, metals and semimetals accumulation) than NPs co-exposure	Xu et al. (2021b)
Lumbricus terrestris	500 μm MF 187 nm PS	PET MFs PS	50–500 μg MF·g^{-1} d.w., 22–2206 μg NP·g^{-1} d.w.	7 MFs 42 PS	Body burden increase, food intake reduction, faeces reduction, PS retention and therefore accumulation	Lahive et al. (2022)
Eisenia fetida	10 μm 100 nm	PS	10, 100 mg·kg^{-1}	21	Concentrations of MPs higher than NPs, pyrene accumulation and toxicity high in MPs, co-exposure pyrene and NPs co-exposure caused severe effect in the intestinal microflora	Liu et al. (2022b)
Lobella sokamensis	470, 530 nm 27, 32, 250, 300 μm	PS PE	4–8 mg·kg^{-1} 1000 mg·kg^{-1}	NA	Reduction of mobility. Plastic particles fate associated with springtail behaviours.	Kim and An (2019)
Achatina fulica	28 nm	PS	0–100 mg·kg^{-1} (mung bean direct exposure)	14 (indirectly exposure)	Growth rate, feeding and foraging speed and gut microbiota viability decrease, histological damage	Chae and An (2020)
Mice	100 nm	PS-NH_2, PS-COOH	10 mg·mL^{-1}	28	Liver, spleen, lung, kidney, intestines, testis, brain accumulation, alveolar walls thickened, pulmonary interstitial fibrosis, renal tubular atrophy, immune cells enrichment, malformed neurons, cell apoptosis, intestinal barrier disruption	Xu et al. (2021a)
	50 nm, 5 μm	PS	10^2–10^6 ng·L^{-1}	18.5	Growth-restricted, cords length decrease, potential neurodevelopment and chronic diseases	Aghaei et al. (2022)
	100 nm	PS	0.1–10 mg·L^{-1}	21	Birth and postnatal body weight reduction, liver weight decreases, oxidative stress induction, testicular disruptions	Huang et al. (2022)
	50, 500 nm	PS	0.5–1000 μg·day^{-1}	14	Neurodevelopment abnormal, cognitive deficits, transgenerational toxicity through breast milk	Jeong et al. (2022)
	42 nm	PS	0.5–50 mg·kg^{-1} b.w.	7	Brain accumulation, blood-brain barrier permeability increase, neuronal damage, ROS production	Shan et al. (2022)

b.w.: body weight; d.w.: dry weight; DNA: deoxyribonucleic acid; MFs: microfibres; PE: polyethylene; NA: not available; PET: polyethylene terephthalate; PS: polystyrene; PS-COOH: carboxy-modified PS; PS-NH_2: amino-modified PS; ROS: reactive oxygen species.

Despite the recent interest in NPs and increasing research in soil organism effects, many points are still to be investigated. NPs media might impact on the exposure and toxicity, so further research focusing on the media dependence are needed. On the other hand, the main polymer used to assess NPs toxicity is PS, and most studies employ pristine PS. However, more realistic studies are required to estimate the real exposure in the environment where NPs are constituted by various polymers (i.e. PE, PP, polyester, PA, etc.) and particles are mostly degraded. Additionally, concentrations used in exposure studies would have to be further explored with the aim of being able to evaluate the effects on soil fauna realistically.

5.1. Nematodes

Caenorhabditis elegans, a specie of nematode, is widely used as model for toxicological assessment thanks to its small size, short life cycle, ease cultivation and manipulation and well-described genetic background (Lei et al., 2018a ; Zhao et al., 2017). A number of articles have shown transgenerational reproductive capacity, gonad development damage (Qu et al., 2019b; Sun et al., 2021a; Zhao et al., 2017) and chromosomal oocytes alterations (Yu et al., 2021a) caused by nano-particles translocation into reproductive organs (Zhao et al., 2017) and germline apoptosis (Qu et al., 2019b) with a brood size reduction as a consequence (Li et al., 2020b).

Liu et al. (2020c) found that PS exposure causes *C. elegans* lipofuscin accumulation and apoptosis which might involve, according to Qu et al. (2019a), neurotoxic effects like neurodegeneration and locomotion behaviours. The increase of mitochondrial damage and reactive oxygen species (ROS) could be slightly responsible, but not limited to, of the neurodevelopmental perturbation (Liu et al., 2020c). However, ROS production induced in adult *C. elegans* (Qiu et al., 2020), as well as in their offspring (Zhang et al., 2022b), an evident oxidative stress which might be mitigated with natural antioxidants (Lei et al., 2018a). Locomotion behaviours and ROS production were also disturbed after exposure to 100 nm PS particles (Liu et al., 2020b).

On the other hand, Kim et al. (2019) revealed that several metabolites related with energy metabolism, TCA cycle, amino acids or neurotransmitter precursors were disrupted by exposure, while Liu et al. (2020a) reported that observed NHR-8 mutation might suggest a fat metabolism alteration. Additionally, intestinal damage and NPs accumulation, probably due to intestinal development alterations and barrier hyperpermeable state, were observed (Yu et al., 2020; Yu et al., 2021a; Zhao et al., 2017).

Regarding size-dependent, media-dependent and surface modification-dependent effects, Liu et al. (2021a) exposed nematodes to 20 and 100 nm and observed that lower size particles were more toxic. Conversely, Lei et al. (2018a) and Lei et al. (2018b) exposed *C. elegans* to five and three, respectively, different PS sizes (from 100 nm to 5.0 μm), being 1.0 μm the most toxic in both cases. Liu et al. (2020c) used three particle sizes (25, 50 and 100 nm) being the weaker impacts detected at 25 nm particle size, and Kim et al. (2020) described more toxicity when 530 nm particle size was used. Furthermore, Mueller et al. (2020) reported that NPs toxicity depend on the total surface area and when the surface of NPs was modified with amino groups, the toxicity was increased compared with that of the nematodes exposed to pristine particles (Qu et al., 2019b; Sun et al., 2021a). Likewise, Li et al. (2020a) observed that the toxicity caused by *Candida albicans* might increase by PS exposure. Thus, even if there are some inconsistencies that need to be further explored, it is clear that particle size, media in which NPs are found and their surface modifications might sway the adverse effects caused by NPs.

5.2. Collembola

Kim and An (2019) exposed *Lobella sokamensis* springtail to different size of NPs and MPs. They observed that the movement of collembola was diminished in contaminated soils. In a later study, the same authors exposed *Folsomia collembola* to 1–5, 27–32 and 53–63 μm PE and they reported that 2 μm of size was the most ingested particle while fluorescence in gut was not observed to >66 μm size of PE, concluding that the edible particle size for this specie is less than 66 μm. Also, as in their previous study, a significant reduction in velocity movement was detected (Kim and An, 2020).

5.3. Earthworms

Earthworms are representative organisms in soil ecosystems and can be easily identified; consequently, they are also an important model for NPs effects evaluation (He et al., 2018). Jiang et al. (2020) exposed earthworms *Eisenia fetida* to 100 nm and 1300 nm PS particles. They observed that exposure induced an increase in growth rate, accumulation in the intestines and gut damage. In accordance with this, Zhu et al. (2018) reported that *Enchytraeus crypticus* exposed to elevated concentrations of PS (with diameter size from 50 to 100 nm) underwent changes in gut microbiome and produced a variability in relative abundances in some bacterial groups; however, they described a reduction in earthworm weight. Lahive et al. (2022) used polyethylene terephthalate (PET) microfibres and PS with 500 μm of length and 187 nm, respectively, to assess the exposure of *Lumbricus terrestris*, and no substantial changes were observed in weight terms while the amount of faeces decreased according to the increasing concentration of microfibres. Additionally, a retention of microfibres in the gut took place at higher concentrations in soil. Concerning NPs, a significant body burdens and nano-sized particles retention in tissues were found at the highest exposure. Reproductive disorders such as male reproductive organs damage and spermatogenesis harm in *Eisenia andrei* (Kwak and An, 2021), as well as raise of cocoon production in *E. crypticus* (Zhu et al., 2018), were also reported. Likewise, oxidative stress was observed (Jiang et al., 2020; Kwak and An, 2021) and due to accumulation of ROS in tissues, DNA damage might occur at high concentrations exposure regardless of particle size studied (Kwak and An, 2021). Additionally, Xu et al. (2021b) and Liu et al. (2022b) exposed *E. fetida* to micro- (both at 10 μm size, and Xu et al. (2021b) also at 100 μm size) and nano-PS (100 nm particle size). They observed that (semi)metals (Xu et al., 2021b) and pyrene (Liu et al., 2022b) were accumulated more easily when co-exposed with micro-sized particles than with nano-sized.

On the other hand, Heinze et al. (2021) found no detrimental impacts on earthworms after exposure to 256 nm PS particles, but they observed that *L. terrestris* plays an important role in fate and transport of NPs via bioturbation because of the deep-burrowing activities. Heinze et al. (2021) and Kwak and An (2021) also described that earthworm fed with MPs might generate nano-sized plastics in their digestive tract and introduce them into soil through cast excretion.

5.4. Snails

Chae and An (2020) fed African giant snails with mung bean previously exposed to 28 nm PS microbeads for 14 days. They reported that snails exposed had a decrease growth rate and a reduction in feeding speed was observed in organisms exposed to high NPs concentrations. Song et al. (2019) found a significant excretion disruption after PET microfibres (with 76.3 and 1257.8 μm of size) exposure. In addition, they observed an increase of ROS and inhibition of antioxidant enzymes which may cause oxidative stress. A decline in the viability of snail gut microorganisms (Chae and An, 2020) as well as damage in gastrointestinal tissues have also been described (Chae and An, 2020; Song et al., 2019). Furthermore, snail movement was significantly slower in exposed groups compared with the control group (Chae and An, 2020).

5.5. Mice

Recently, NPs have been found in placenta of women (Ragusa et al., 2021), lungs (Amato-Lourenço et al., 2021; Jenner et al., 2022) and even in human blood (Leslie et al., 2022) increasing the concern about the unclear effects of plastics in human health (Yong et al., 2020). For this reason, studies of MPs and NPs in mammal models, in particular in mice, have been carried out. Recently, mice maternal exposure and trans-generational impact have been evaluated. Huang et al. (2022) exposed mice to PS (plastic size of 100 nm) during pregnancy and lactation and reported a decrease in body weight of brood. Aghaei et al. (2022) also found that offspring with exposed mothers had a growth decline and cords reduction that might be caused by deficient nutrition through the placenta. Likewise, Jeong et al. (2022) observed that neuronal damage and cognitive deficits may occur in broods after high concentrations of maternal exposure due to an anomalous development of brain, while Xu et al. (2021a) found that PS, with size of 100 nm, might accumulate in the brain and in cells which cause ROS increasing.

Testicular disruptions like spermatogenesis alteration, testis weight reduction and testicular oxidative injury were found in offspring by Huang et al. (2022), which suggests that there is evident testicular toxicity. Regarding the surface modification-dependence, Xu et al. (2021a) exposed mice for 28 days with PS, PS-NH_2 and PS-COOH. An accumulation, inflammation and disorders were observed but while PS were detected in stomach, intestine, testis and kidney, PS-NH_2 and PS-COOH were also spread in the lungs and even PS-COOH was found in brain. Nevertheless, the bodyweight, intestine weight as well as testis weight were declined in mice exposed to PS-NH_2, whereas alterations were not observed in mice exposed to PS and PS-COOH. On the other hand, testicular tissue exposed to all three types of PS as well as brain exposed to PS-NH_2 showed a cell apoptosis and disruptions in lung and kidney.

6. Phytotoxic effects of nanoplastics

Some reviews have been published in 2022 covering the literature about the impact of MPs and NPs in plant species (Azeem et al., 2022; Campanale et al., 2022; Chen et al., 2022; Hartmann et al., 2022; Maity et al., 2022; Wang et al., 2022c, 2022d). All these reports agree that MPs and NPs pollution is a complex environmental problem and their impact on terrestrial plants is one of the most concerning aspects. The uptake and translocation of plastic pollutants, as well as their effects on the morphological and biochemical plant parameters, are frequently discussed. However, as new research about the phytotoxic effects of NPs is steadily appearing in the scientific literature, these authors also agree with the necessity of frequent overviews. Therefore, to help with this goal, herein we selected all the research articles published in the first semester of 2022 about phytotoxic effects of NPs and summarized and discussed the significant variations of several plant growth and biochemical parameters as a consequence of NPs exposure. Table S1 of the Supplementary Material shows the studied plants (mainly edible crops), the kind of NPs (regular or functionalized PS in 91% of the cases), its size (from 50 to 1000 nm), its concentration (covering 6 orders of magnitude from 0.01 to 10 000 mg·L^{-1}), the measurement date (MD) and 48 measured parameters (comprising seeds, aerial organs and roots) which appeared in at least 2 of the selected publications. The table also includes the plant scientific name, phenological stage at the beginning of the experiment, kind of experimental design, evaluated co-factors and 111 measured parameters.

Some general highlights can be easily deduced from Table S1 of the Supplementary Material. For instance, the presence of NPs is detrimental to seeds germination (Guo et al., 2022) and also leads to higher values of H_2O_2 (Dong et al., 2022b; Guo et al., 2022). This excessive accumulation of H_2O_2 and other ROS such as superoxide radical (O_2·⁻) also occurs in above and underground tissues (Gao et al., 2022; Giri and Mukherjee, 2022; Yildiztugay et al., 2022; Zhang et al., 2022d). The increase of substances like malondialdehyde (MDA) are indicators of this oxidative stress (Gong et al., 2022; Zhang et al., 2022d). As a consequence, the plant defence system releases several compounds to alleviate this oxidative stress, such as antioxidant defence enzymes superoxide dismutase (SOD, which catalyses the dismutation of O_2·⁻ into O_2 and H_2O_2) and catalase (CAT, which decomposes H_2O_2 into H_2O). Thus, an increase in SOD and CAT activities in shoots and roots was generally observed (Gao et al., 2022; Giri and Mukherjee, 2022; Gong et al., 2022; Guo et al., 2022; Spanò et al., 2022; Wang et al., 2022b; Wang et al., 2022e; Yildiztugay et al., 2022; Yu et al., 2022; Zhang et al., 2022d). Moreover, plant-produced non-enzymatic antioxidants (e.g. glutathione (GSH) and ascorbate (AsA) which react quickly with ROS, and also act as enzymatic substrate for glutathione reductase (GR) and ascorbate peroxidase (APX), among others. An increase in the contents and activities of these substances is frequently observed (Spanò et al., 2022; Yildiztugay et al., 2022; Yu et al., 2022). The harmful effect of this oxidative stress lead to morphological changes, normally producing decreases in aerial and subterranean length and weight (Dong et al., 2022a; Dong et al., 2022b; Giri and Mukherjee, 2022; Guo et al., 2022; Spanò et al., 2022; Sun et al., 2022; Wang et al., 2022b; Wang et al., 2022e). Finally, NPs affect photosynthesis via stomatal and non-stomatal factors. On the one hand, the exposure to NPs usually produces a partial closure of the stomata by epidermal guard cells, thus decreasing transpiration rate (Tr) and stomatal conductance (Wang et al., 2022b; Wang et al., 2022e; Yildiztugay et al., 2022). On the other hand, the biochemical control of photosynthesis is also damaged by NPs, as can be deduced from the usual reduction of photosynthetic pigments chlorophyll-a and b and carotenoids, which play a photoprotective role during photosynthesis (Sun et al., 2022; Zhang et al., 2022a; Zhang et al., 2022d). Fig. S1 of the Supplementary Material summarizes those parameters in seeds, underground and aboveground parts of plants exposed to NPs which have always shown in the references discussed herein equivalent and/or increasing (green upwards arrows) or equivalent and/or decreasing (reddish downward arrows) values regarding control plants.

Most of the evaluated parameters depend on the studied conditions, i.e. the composition, size and concentration of the NPs, as well as the MD. For instance, Zhang et al. (2022a) studied the response of Chinese cabbage (*Brassica rapa* L.) seeds when exposed to 3 concentration levels (1, 10 and 100 mg·L^{-1}) of 2 kinds of NPs (PS and PS-NH_2). They determined the photosynthetic pigments (carotenoids and chlorophyll-a and b) contents in cotyledons at photomorphogenesis and whole stages, i.e. 18 and 42 h after seeds treatments under the studied conditions, respectively. At 18 h, exposure to PS only yielded a pigment significant decrease in the case of chlorophyll-a at 1 mg·L^{-1} concentration (dose dependence). By contrast, chlorophyll-a always decreased significantly when seeds were exposed to PS-NH_2 (composition dependence). Interestingly, a generalized decrease in the three pigments contents was found at 42 h for both NPs independently of the concentration (MD dependence). It can be concluded that the relevance of the MD gains importance in assays focused on the evaluation of the short-term phytotoxic effects. Whereas NPs composition is relevant, its surface morphologies does not seems so, as observed by del Real et al. (2022) when submitted wheat (*Triticum aestivum* L.) to smooth or rough PS of 160 nm. The applied dose has even shown influence in the number of differentially expressed genes in wheat (*T. aestivum* L.) roots and leaves (Lian et al., 2022). Regarding the NPs size, Zhang et al. (2022d) laid bare its importance in their hydroponic study of corn (*Zea mays* L.) treated with 50 mg·L^{-1} of PS with sizes of 100, 300 and 500 nm. They observed a size independent increase in some parameters such as roots dry weight and ROS content. By contrast, SOD activity and Tr increased with 100 and 300 nm PS, but are comparable to control with 500 nm PS. Interestingly, dry stem biomass decreased with 100 and 300 nm PS, but increased with 500 nm PS.

Besides the study of the NPs effects on the above discussed parameters, many works also focused on their uptake and translocation pathways in plant organs (del Real et al., 2022; Liu et al., 2022a; Luo et al., 2022; Spanò et al., 2022; Wang et al., 2022b; Wang et al., 2022e; Zhu et al., 2022). In this regard, it is important to establish relationships between NPs exposure and the nutritional status of plants. Yu et al. (2022) studied conifer Chinese nutmeg yew (*Torreya grandis*) after 7 days of being sprayed with a 10 g·L^{-1} PS (100 nm) solution. They found significant changes for Zn and Fe (decrease) and Mg (increase) content, whereas the rest of studied elements (Ca, K, P, Na, S, Cu and Mn) remained unchanged regarding control. Lettuce (*Lactuca sativa* L.) was exposed for 21 days to four doses (10, 20, 50 and 100 mg·L^{-1}) of polymethyl methacrylate (PMMA) of 131 nm, obtaining a generalized decrease for K, Fe, Ca, Mn, Mo, Zn, P and Cu. Boron content also decreased at all the doses except at 20 mg·L^{-1}, for which remained unchanged regarding control plants, as occurred with Mg content for all the doses (Yildiztugay et al., 2022). Moreover, in that study, authors reported that the total saturated fatty acids amount decreased at lower concentrations (10, 20 and 50 mg·L^{-1}) of PMMA, whereas the unsaturated fatty acids increased at those concentrations. Gong et al. (2022) found a slightly higher accumulation of iron in roots but not in leaves of lettuce (*L. sativa* L.) exposed to 50 mg·L^{-1} of 100 nm PS.

In addition to their inherent toxicity discussed above, the accumulation of NPs in plants generally worsen the negative effects of other factors such as organic pollutants (Sun et al., 2022), metal oxide nanoparticles (Gong et al., 2022), heavy metals (Dong et al., 2022a; Gao et al., 2022), and low temperature (Wang et al., 2022b; Wang et al., 2022e). Sun et al. (2022) found that PS-NH_2 aggravated the phytotoxicity caused by the phthalate esters (PAEs) di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP), increasing the PAEs accumulation in corn (*Z. mays* L.) leaves and decreasing the plant growth. Both PS and Fe_2O_3 nanoparticles provoked phytotoxicity on lettuce (*L. sativa* L.), and the oxidative stress, root deformation, translocation of damaged cells and iron accumulation in tissues aggravated under co-exposure (Gong et al., 2022). The concurrence of NPs and the toxic heavy metals As and Pb has been evaluated. Different doses of As and PMMA were exogenously and independently added to rapeseed (*Brassia campestris* L.) seeds yielding phytotoxic effects during germination. More interestingly, germination index, fresh biomass, and root and shoot lengths were even lower under co-exposure to both pollutants, thus showing a joint toxicity (Dong et al., 2022a; Dong et al., 2022b). However, according to Gao et al. (2022), the chemical nature of the NPs seems to play an important role in the interaction with heavy metals. They found that the addition of Pb increased the negative effects (higher levels of ROS and enzymes activity) on dandelion (*Taraxacum asiaticum* Dahlst) treated with PS and PS-COOH, albeit not with PS-NH_2, thus revealing that NPs surface charge should not be underestimated. The role of the temperature was evaluated with an experiment in which barley (*Hordeum vulgare* L.) was exposed to PS (66 nm, 2000 mg·L^{-1}) for 7 days at 26/18 °C and an extra day at 2/0 °C. For several parameters (net photosynthetic rate, initial and total Rubisco activities) no differences were found between PS-treated and control plants at room temperature, albeit decreases were found as a consequence of the low temperature. Moreover, these decreases were significantly more pronounced when NPs were present (Wang et al., 2022b; Wang et al., 2022e).

Nevertheless, some positive effects associated to the presence of NPs as alleviating of other pollutants has also been reported (Dong et al., 2022b; Ren et al., 2022; Wang et al., 2022b; Wang et al., 2022e). For instance, the addition of PS (50 nm, 50 mg·L^{-1}) to rice (*Oryza sativa* L.) in the presence of the organic pollutant phenanthrene (PHE) reduced its accumulation both in roots and shoots, as well as the inflicted stress. Moreover, both compounds are individually detrimental to the photosynthetic system, albeit this effect is alleviated when combined (Wang et al., 2022b; Wang et al., 2022e). Soils polluted with As were evaluated for rice (*O. sativa* L.) crop in the presence of PS and polytetrafluoroethylene (PTFE), and both NPs inhibited the As uptake. Consequently, higher root, stem, leaf and grain biomass were achieved by adding NPs to the As-contaminated soil (Dong et al., 2022b). Ren et al. (2022) studied how PS (70 nm, 10 and 100 mg per kg of soil) affected wheat (*T. aestivum* L.) growth parameters with and without degradable mulching film (PLA and PBAT, 4.5 mm, 1% w/w) as co-factor. When comparing with control soil, only a significant decrease of diameter was found. However, starting from soils with degradable mulching film pieces, the presence of PS at higher concentration yielded an increase of the aerial biomass and height, and no differences in base diameter.

Very little is still known about the mitigation of the negative impact of NPs on crops (Giri and Mukherjee, 2022; Gong et al., 2022; Li et al., 2021b). Giri and Mukherjee (2022) studied short-term biochemical effects caused by regular, aminated and carboxylated PS at three concentrations in onion (*Allium cepa* L.). They observed a mitigation of cell death and oxidative stress when NPs were previously treated with extracellular polymeric substances (EPS) obtained from rhizosphere secretions. TEM images of treated NPs showed the formation of an eco-corona layer which forms micron size aggregates, probably reducing the NPs uptake by the roots. Similarly, Gong et al. (2022) found that humic acid was able to disperse the harmful aggregates of NPs and Fe_2O_3 nanoparticles, therefore alleviating their joint phytotoxicity in lettuce (*L. sativa* L.). Recently, Li et al. (2021b) also reported that PS caused in wheat (*T. aestivum* L.) seedlings an increase of the H_2O_2 content and a decrease in the maximal photochemical efficiency (F_V/F_M). However, the exogenous application of melatonin counteracted these effects and also diminished the NPs uptake, their translocation and their negative effects on carbohydrate metabolism. All these encouraging findings provide new insights to mitigate the phytotoxic effects caused by NPs and the consequent risk for human nutrition and health.

7. Conclusions and potential ecological implications

Soil health can be considered as the cornerstone on which the functioning of terrestrial ecosystems pivots, affecting key processes such as biogeochemical cycles, biomass production and maintenance of air and water quality. Any external element, for example NPs, that generates changes in the structure of the soil system, will trigger a series of interrelated processes that could ultimately affect global mechanisms such as the production of greenhouse gases or the biodiversity conservation. The question that must be asked is whether there is enough information to establish that contamination by NPs is already causing changes in soil quality that can represent a threat to the sustainability of terrestrial environments.

In various sections developed earlier in this review it has been shown that a significant number of studies report that the presence of NPs can potentially affect the microbiota, micro-, meso- and macrofauna of the soil, and the growth and development of vegetation. These effects can be produced by direct or indirect toxicity through the reactivity of NPs with other extrinsic organic or inorganic chemicals present in the soil. Plant performance depends on soil diversity and particularly on rhizosphere colonizing microorganisms so if NPs, in addition to direct phytotoxicity, have the potential to affect soil microbiome it could have unknown consequences in plant diversity and community composition. Due to the crucial role that vegetation plays in modulating climate, even small effects at the local level on plant productivity could be amplified at the ecosystem level, which would have important repercussions on climatic conditions (Rillig et al., 2019).

However, although knowledge about the impact of NPs is rapidly increasing due to the large number of investigations carried out in recent years, there are several limitations that still prevent us from determining whether these pollutants are causing significant damage at an ecosystem scale. While an ecological approach has already been applied to the study of other pollutants, for example pesticides, the study of NPs in soils is beginning to take its first steps in this direction (Rillig and Lehmann, 2020). Main limitations from reaching that perspective would be: i) concentration of NPs in soil environments remains poorly unknown (Azeem et al., 2021). Many studies assess high exposure concentrations that could be non-realistic for natural environment. Moreover, research needs to approach the diversity of these particles mainly in terms of chemistry and aging; ii) most research has focused on agricultural systems but to a much lesser extent on other natural ecosystems such as forest, grassland or drylands where NPs dynamics could be different. Also, more studies should be done in different soil types (e.g. different textures) since so far most works have been using sandy loam soil with simplified representations (Azeem et al., 2021; de Souza Machado et al., 2020); iii) new studies are needed to assess the interactions between multiple types of NPs instead of focusing on the effects of single types of NPs on soil biota and plants. Similarly, relatively few studies have tried to understand NPs effects in combination with other abiotic stresses (Yoon et al., 2021); iv) NPs can affect soil biota and plant performance in several ways therefore within a soil ecosystem different species can respond differently to that abiotic stress (Rillig et al., 2021). Most of the studies carried out to date just focus on a single species (Wang et al., 2022c), so a new experimental approach is required to test the effects of NPs at the community level; v) attending to the soil food web high complexity, it is also necessary to inquire the bioaccumulation and biomagnification of NPs polymers, associated additives, and adsorbed substances along the food web (de Souza Machado et al., 2020); finally vi) many studies address the very short-term effects of NPs on soil organism, but these contaminants degrade very slowly into the soil, so they can be considered a long-lasting environmental stressor (Yoon et al., 2021). For this reason, studies are needed to assess the impact of NPs long-term exposure on soil organisms.

In summary, it is necessary to deepen the knowledge of the environmental responses to the addition of large amounts of NPs in the soils. To achieve this objective, field and mesocosm-level experiences must be developed to analyse the medium-long-term effect on the soil organism community, including plants. In essence, it is about knowing the ecological interactions to understand the global effects of pollution by NPs on terrestrial ecosystems, also allowing researchers to get closer to knowing the threshold levels of pollution that trigger harmful effects on these environments.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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