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Review

Soil Pollution from Micro- and Nanoplastic Debris: A Hidden and Unknown Biohazard

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Abstract: The fate, properties and determination of microplastics (MPs) and nanoplastics (NPs) in soil are poorly known. In fact, most of the 300 million tons of plastics produced each year ends up in the environment and the soil acts as a log-term sink for these plastic debris. Therefore, the aim of this review is to discuss MP and NP pollution in soil as well as highlighting the knowledge gaps that are mainly related to the complexity of the soil ecosystem. The fate of MPs and NPs in soil is strongly determined by physical properties of plastics, whereas negligible effect is exerted by their chemical structures. The degradative processes of plastic, termed ageing, besides generating micro-and nano-size debris, can induce marked changes in their chemical and physical properties with relevant effects on their reactivity. Further, these processes could cause the release of toxic oligomeric and monomeric constituents from plastics, as well as toxic additives, which may enter in the food chain, representing a possible hazard to human health and potentially affecting the fauna and flora in the environment. In relation to their persistence in soil, the list of soil-inhabiting, plastic-eating bacteria, fungi and insect is increasing daily. One of the main ecological functions attributable to MPs is related to their function as vectors for microorganisms through the soil. However, the main ecological effect of NPs (limited to the fraction size < than 50 nm) is their capacity to pass through the membrane of both prokaryotic and eukaryotic cells. Soil biota, particularly earthworms and collembola, can be both MPs and NPs carriers through soil profile. The use of molecular techniques, especially omics approaches, can gain insights into the effects of MPs and NPs on composition and activity of microbial communities inhabiting the soil and into those living on MPs surface and in the gut of the soil plastic-ingesting fauna.

Keywords: microplastics (MPs); nanoplastics (NPs); soil; reactivity; toxicity; human health

1. Introduction

Despite the fact that annual plastic release into soil is approximately 4 to 23 times higher than that into the sea [1], the study of plastic pollution in the oceans preceded that of soil contamination [2,3]. Interest and concern regarding plastic pollution has been increasing, since it is reported that plastics can accumulate and persist in the environment for several hundreds of years under low-light and low-oxygen conditions [1]. In addition, their constituent monomers and oligomers, such as bisphenol A and styrene, are toxic; this is because, as monomers of polyvinyl chloride (PVC), they are endocrine system disruptive and carcinogenic [4]. Some plastic additives and plasticizers are toxic substances such as phthalates (diesters of 1,2–benzenedicarboxylic acid) and brominated flame retardants [5].

Both toxic monomers and additives are released during the slow plastics degradation into the soil and they can reach to the aquatic environments by leaching [1]. Furthermore, plastic debris can act as carriers that pick up hydrophobic organic and inorganic contaminants as well as pathogens, which are present in soil and water, thus increasing their environmental toxicity. However, their adsorption and mobility properties depend on the surface/volume ratio, which is high in MPs (Microplastics) and NPs (Nanoplastics). The methods for determining plastics and their inputs and fate in the soil have been reviewed by Bläsing and Amelung [4], whereas the presence, fate and behavior of MPs in freshwater and terrestrial environment was reviewed by Horton et al. [1]. Due to their high surface/volume ratio, both MPs and NPs are particularly toxic, as they can easily enter into the food web, being ingested by animals because of their small size [6].

The European Food Safety Authority (EFSA) defines MPs as plastics with a diameter ranging from 0.1 to 5000 micrometres (μm) and NPs as those whose diameter ranges from 0.001 to 0.1 μm (http://www.efsa.europa.eu/en/press/news/160623). MPs are present in the environment as microbeads or spheres, fragments, fibers, and granules, whereas the shapes of NPs in the environment are relatively unknown due to the methodological problems related to detecting and characterizing them.

The aim of the present review is to discuss the state of soil pollution from MPs and NPs, the relative methodological complications to detect and characterize them, and the knowledge gaps about their behavior in soil to promote the future researches. Compared to the review by Horton et al. [1], we have given more attention to the role of soil biological properties, soil-reactive particles and important biological molecules, such as DNA and proteins. We have also focused on future environmental challenges that will require solutions for environment and human safety. We further discuss the role of biodegradable plastics and bioplastics that would be partially or fully degradable by microorganisms. The latter are polymeric substances similar to the synthetic plastics produced by bacteria under nutrient-deficient conditions, such as the lack of macro elements (phosphorus, nitrogen, trace elements, oxygen) combined with an excess supply of carbon sources [7]. PHAs have also been produced by genetically modified plants [8]. Frequently, we refer to methods for determining and characterizing properties of MPs and NPs and information on their behavior in aquatic systems, since the relative information in soil is not available yet. Of course, the application of these methods and the occurrence of the relative behaviors in soil should be tested.

2. Preliminary Aspects

MPs are directly released (Primary MPs) in the environment from pellets and resins that are used in the plastic industry or from plastic exfoliators such as face and body scrubs, or produced (Secondary MPs) by the fragmentation and weathering of larger plastic materials [9,10]. The surface ablation and fragmentation of plastics, caused by weather (UV radiation, oxidative reactions, wetting and drying cycles) and human (mechanical forces due to tillage) degradative actions, not only produce MPs but also NPs and the size of produced plastic debris depends on layer thickness and surface heterogeneity [11]. The integrity of the layer between the surface and the underlying virgin polymer, leads to fragmentation by delamination [11]. The exposed chemical groups by mechanical alteration can bind exogenous chemicals, with effects on the plastic degradation rate [12,13]. Finally, abiotic hydrolysis with the formation of potentially harmful MPs and NPs can occur during the slow mineralization (it can last several months) of biodegradable plastics (https://www.isse.utk.edu/projects/seedprojects/2016-17/FateofTerrestrialMicroplastics.html).

Despite the fact that plastics differ for their chemical composition (acrylonitrile butadiene styrene, acrylonitrile/methyl methacrylate, acrylonitrile-styrene-acrylate, high-density polyethylene, low-density polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyvinyl butyral), their behavior in the soil mainly depends on their physical properties. Indeed, the reactivity of plastics depends on their crystalline structure; for example, plastics with low values of crystalline/amorphous ratio are more reactive compared to those with high ratio values. This could be due to the higher pore surface and higher adsorption of chemicals which may enhance their degradation and the formation

of additional secondary MPs and NPs [11]. As mentioned above, MPs and NPs differ for their size and thus for their surface/volume ratio, with nanoplastics having a higher chemical reactivity and mobility than MPs and a different colloidal behavior [14,15]. The colloidal property influences the stable or unstable hetero-aggregation of NPs, which also depends on the ionic strength and pH value of the solution and thus it should depend on mineral composition and organic matter content of the soil [15] (Figure 1).

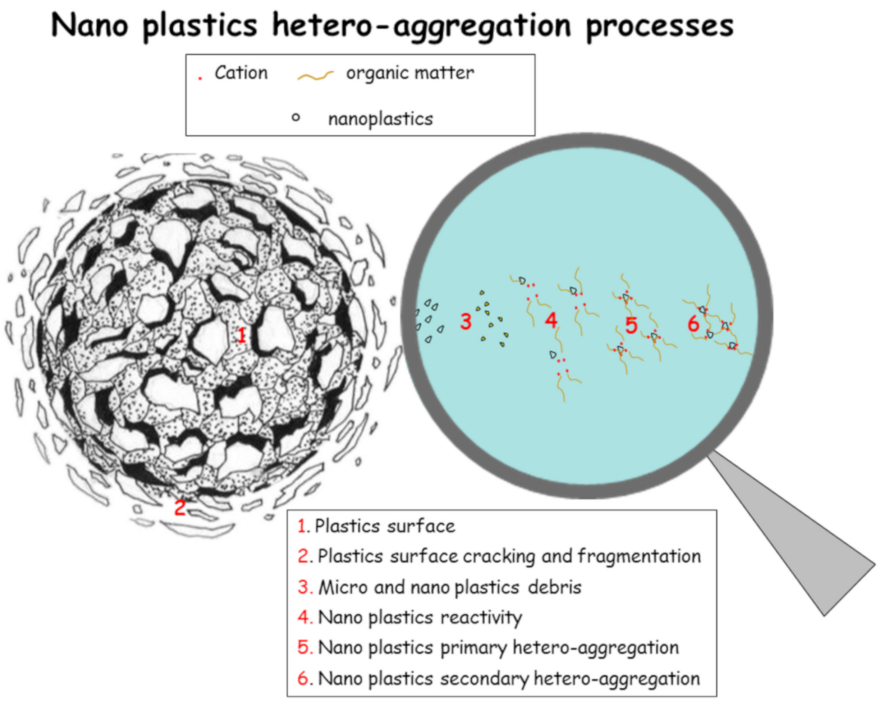

Figure 1. Plastics hetero-aggregation process (modified from Andrady A.L. 2017).

As previously mentioned, plastic materials consist of a wide range of additives, such as chemicals added for enhancing their elasticity, rigidity, UV stability, flame retardation and color [4]. These additives are often weakly bound or unbound to the polymer molecules and thus they will leach out from the plastic overtime. The leached additives are mainly lipophilic and may penetrate cell membranes and possibly causing toxic effects [16], following leaching depending upon the type and amount of additives present in the plastic [17]. Besides additives, auxiliary substances (catalysts of polymerization, initiators and accelerators) may also be present in plastics [3]. Organic compounds, nutrients, hydrophobic contaminants, metals and microorganisms (bacteria) can be rapidly and selectively adsorbed by the plastic surface from the environment. Additionally, bacteria can be attracted by the nutrients present on the plastic surface [3,11]. Adsorbed pollutants can penetrate into the plastic structure and reach higher concentrations in the microplastics than those of the surrounding environment [18]. However, the mechanisms, guiding the penetration of these chemicals inside the plastic are poorly known and future research should fill these knowledge gaps [13]. Surface area is more important than chemical properties [19]. For example, NPs can adsorb toxic chemicals up to 100 times more than MPs (Wageningen University Research Center 2014, Wageningen, The Netherlands, https://www.wur.nl/en/Dossiers/file/Microplastics-and-Nanoplastics.htm).

The microenvironment of plastics' surface area, known as ecological corona (eco-corona), is characterized by the complex and dynamic adsorption of extracellular organic molecules, leading to a surface organic layer (corona), which affects the plastics' behavior and their interactions with organisms [20] and soil constituents. In addition, components in the plastic eco-corona layer can be bioavailable to organisms. The eco-corona can be soft or hard depending on their adsorption affinity toward the target molecule. The hard-eco-corona layer has high binding affinity, long residence time,

slow exchange time, and probably causes extensive conformational changes to the present contaminant molecules. On the other hand, the soft eco-corona consists of a more loosely associated and rapidly exchangeable layer of exogenous molecules with a low degree of conformational changes [21]. Furthermore, it could also influence the pollutants transport into the soil through their different binding affinity to plastic surfaces.

Plastics function as habitats, and are being rapidly colonized by microorganisms that form dense biofilms on the plastic surface, known as the "Plastisphere". Further, the plastisphere defines the microbial community that is inhabiting in the eco-corona [22]. It is mainly the bacterial biofilm that markedly affects the eco-corona reactivity, for example, by increasing the chemicals adsorption by microplastics [13]. Interaction between the eco-corona and the plastisphere is shown in Figure 2. The biofilm matrix contains not only the bacterial cells but also proteins, extracellular DNA, lipids and polysaccharides [23,24]. However, the presence and the influence of bacterial biofilms on the ecological properties of MPs are poorly understood [13,25]. The NPs' size (i.e., smaller than bacteria and most viruses) precludes the presence of adsorbed microbial cells and thus the presence of biofilms, but NPs can be associated with pathogens. Indeed, NPs below a size of 50 nm pass through eukaryotic and prokaryotic cell membranes with absorbance of pathogenic microbes, thus increasing the biohazard potential of pathogens [26]. Therefore, both MPs and NPs show the so-called "Trojan horse effect" since they can passively transport chemicals, [27] and pathogens [22,28], whereas the spread of antibiotic resistance is only associated to MPs' diffusion [29]. The Trojan horse effect can impact MPs and NPs, which take a toll on the environment, with potential and neglected hazard for human health [29].

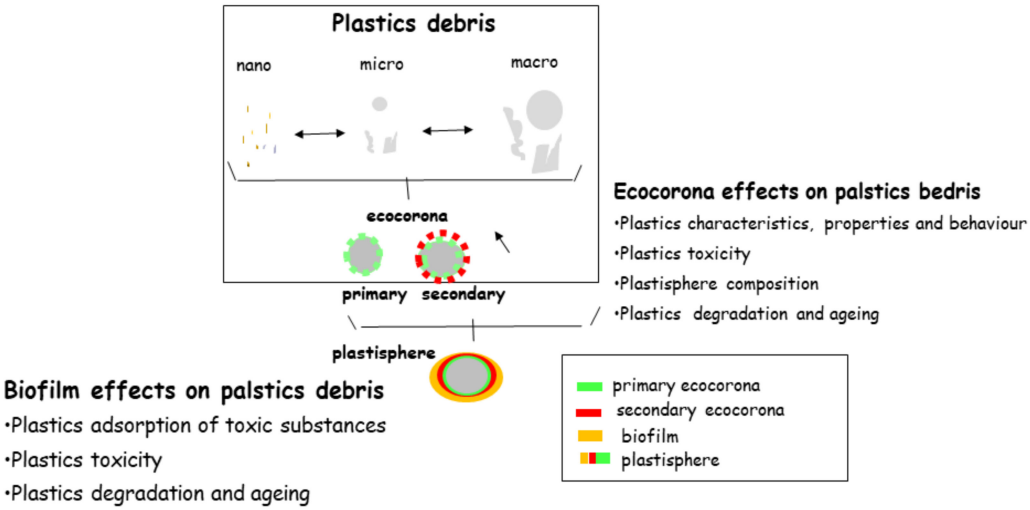

Figure 2. Eco-corona and Plastisphere.

3. Sources of Soil Contamination

Soils may receive MPs and NPs from different human activities and natural processes. The main human sources are represented by agricultural practices, such as plastic mulching, application of soil amendments containing plastics, and irrigations with plastic-polluted wastewaters. Additional anthropogenic sources comprise landfills, littering along roads and trails, illegal waste dumping and road runoff. Natural sources are represented by atmospheric inputs [30] and flooding with lake water or river water [1]. Bläsing and Amelung [4] showed that compost and sewages sludge could contain 2.38–1200 and 1000–24,000 mg of MPs kg^{-1}, respectively, whereas plastic polluted with untreated and treated wastewaters used in irrigation accounted for 1000–627,000 and 0–125,000 fragments m^{-3}, respectively. Maris et al. [31] reported that in Europe almost 30% of plastic waste was deposited in landfills in 2014. The natural soil pollution caused by flooding with lake or river waters accounted for 0.82–4.42 plastic fragment m^{-3} [4]. Finally, there are no estimates of soil pollution by NPs because there

are no methods to detect them in soil due to their size. Therefore, nowadays this pollution represents a hidden and unknown environmental biohazard.

3.1. Landfills

Wind, storms and water losses can cause MP diffusion from landfill to the surrounding soil [32]. The mechanisms underlying the degradative processes that producing secondary MPs in landfill depend on the plastics location. Degradation of those particles located on the surface is caused by the high adsorption and scatters of UV rays, whereas those located in deeper landfill layers are degraded by the leached acidity and by the chemical activity of compounds present in the layers of interest [33]. The chemicals released by the plastic degradation can diffuse into the environment as a landfill leachate and the diffusion depends on the pore size of the plastic and the molecular size of the additive [34]. The European Commission has proposed to eliminate plastics in landfills by 2025 (https://ec.europa.eu/environment/waste/plastic_waste.htm; https://ec.europa.eu/environment/circular-economy/pdf/plastics-strategy-brochure.pdf).

3.2. Flooded Areas, Rise up of Saline Waters in Coastal Soils and Eolian Transport

About 80% of plastic waste in the sea derives from land-based sources, generally through leaching and soil erosion [32,35]. The coastal lands are extremely vulnerable to plastics pollution, due to both human activities and pollution from the sea environment. Agricultural soils alongside the coasts may be irrigated with saline waters if the groundwater is contaminated by seawater, which is a relevant source of MPs and NPs. For example, the concentration of MPs in different samples of commercial salt ranged from 50 to 280 MPs/kg salt [36]. In addition, wind can spread salts and MPs from the sea to the coastal soils. It is important to underline that plastics degradation is faster at the water surface due to UV action than in the deeper layers with the slowest degradation rates observed in the seabed, where there is no UV radiation and temperatures are colder than in the upper layers [37]. Aeolian transport represents an important diffusion of plastics as demonstrated by the presence of MPs in Swiss floodplain soils located far from the urban areas [38].

3.3. Soils Irrigated with Waste Waters or Fertilized with Sewage Sludge or Composts

The annual estimates of MPs added to farmlands in Europe and North America are 63,000–430,000 and 44,000–300,000 tons, respectively, either through direct application of sewage sludge or application of waste derived from processed bio-solids [39]. The wastewaters from treatment plants represent the main vectors for MPs derived from industry, landfill, domestic wastewater and storm-water [39]. Thus, these wastewaters should be purified before their use for irrigating agricultural soils. The efficiency of these processes depends on the applied technology and the threshold concentration of plastics is established by the legislation governing the application of wastewaters and bio-solids to agricultural lands. It is relevant to underline that, at present, there is no specific policy designed to prevent environmental pollution by MPs [40] and NPs in Europe. The efficient removal of MPs from waters depends on their size, because removal efficiency decreases with the particles size and it is low for small particles (20–300 μm) [9,17,41]. Most of the removed MPs are deposited in the sewage sludge after the wastewater treatment and, thus, the use of sewage sludge as fertilizer can potentially introduce MPs into soil and then into aquatic systems via runoff (http://www.waterborne-env.com/headlines/MPs-in-wastewater-treatment-plants-and-receiving-waters/). In the last few years, there has been an increasing interest to consider sewage sludge as an important source of nutrients but their use as fertilizers requires a careful control of pollutants. Recently, Mahon et al. [42] have monitored MPs in sludge samples from wastewaters subjected to anaerobic digestion, thermal drying, or lime stabilization. The anaerobic digestion significantly reduced the MPs concentration in the treated water. New technologies like supercritical oxidation during gasification can keep the nutrients in sewage sludge but reduce the plastic concentration [43].

According to the bio-based economy, the sewage sludge may be used to produce bioplastics (polyhydroxyalkanoates, PHAs) (www.aiforo.com/development) [44]. Composts used as fertilizers can be a source of MPs pollution in agricultural soils [4]. Weithmann et al. [45] found that composts from supermarket waste contained the highest amount of plastic particles (895 pieces > 1 mm per kg dry weight), but the concentration of MPs was also high in household compost (20–24 pieces per kg of dry weight). Styrene-based polymers, polyethylene, and polyester were the most frequent plastics, being commonly used in food packaging such as labels directly put on fruits and vegetables. In contrast, digestates from agricultural biogas production contained a low amount or even no plastic particles [45].

The EcCycle (www.ecocycle.org/MPsincompost) reports some actions to reduce the risks of plastic pollution by applying compost as a fertilizer, including education about not using misleading packaging and promotion of practices using compostable products. However, despite the problems of plastic pollution, some countries still accept the presence of exogenous organic matter, such as plastics, in fertilizers; for example, the German regulation on fertilizer quality allows up to 0.1 weight % (wt %) of plastics, without considering their particle size [45].

3.4. Soil under Plastic Mulching

Plastic mulching is a widespread agricultural practice to improve crop quality and yield by regulating soil temperature, reducing soil erosion, increasing water use efficiency [46–48] and exerting the pathogen control [49–51]. Worldwide, the surface covered by plastic mulching is expected to grow annually by 5.7% [52] and the most used polymers are the low-density polystyrene and PVC because of their low costs. The mulching practices produce plastics debris and release harmful additives likes phthalates; both MPs and additives may be incorporated into soil [53] with the risk of being introduced in the food chain and thus with potential risks for human health [54–56]. The use of biodegradable plastics and bioplastics can reduce the environmental risks caused by plastic mulching, but it is limited due to their high cost. To overcome this problem, researchers at ETH Zurich and at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) have suggested using the polymer poly (butylene adipate-co-terephthalate) as an alternative to polyethylene films, because it is more biodegradable in soil [57].

3.5. Techno-Soil with Remediated Sediments

In last few years, the use of sediments as substrates to recover degraded land or in horticulture has rapidly increased, due to the use of remediation techniques eliminating or reducing the pollutants. Indeed, Melgarejo et al. [58] have shown that dredged and remediated sediments can be used as alternatives to peatlands, whose sources are limited, as substrates in horticulture. Civil engineers suggest reinforcing unstable soils for building by using plastics with an addition rate of 2–4% of the total weight of the soil [59]. However, even if this technique represents a useful method of recycling plastics, the fate of MPs and NPs in reinforced soils should be monitored.

4. Microplastics and Nanoplastics Fate in Soil

The plastic degradation in soil mainly depends on physico-chemical properties of plastics, type of soil, presence of an active microbial community and environmental conditions [4]. For example, exposure to UV radiation and weathering forces increased plastics degradation, which was higher in clay soils than in sandy soils [60]. This could be due to the differences in microbial activity between the two soils [3,61]. In soil, the plastic biodegradation occurs in two phases: Surface degradation of the polymer is followed by the degradation of the fragments originating from the first phase [61]. The initial plastic biodegradation rate depends on the plastics' available surface area and it is characterized by an intense mycelial growth on the plastic surface [62] as well as by the formation of bacterial biofilms [61]. Several soil inhabiting micro-organisms (see below) can partially or totally degrade synthetic plastics [57,63,64] with co-metabolism being the main degradative pathways [65]. Nutrients availability is not a relevant limiting factor in plastics degradation in soil, whereas the degradation

of MPs and NPs depend on the auto- and hetero-aggregation, and thus on surface hydrophobicity, as reported in aquatic environments [66,67].

As already mentioned, the degradation of the additive organic components can also occur during plastic degradation [68,69], causing production of small particles that can spread in the environment [70]. There is evidence that the eco-corona could select the bacteria that colonize the plastics' external surfaces. In fact, in the case of pollutants adsorbed onto plastics, metagenomic studies of the surface colonizers could lead to novel pollutant-degrading microbes [22]. Future research using metagenome and amplicon sequencing may provide insights on the presence of plastics degraders in soil [71,72]. The fate and effects of MPs and NPs in soil depends on the eco-corona properties, which, for example, can affect plastics interactions with clay minerals and organic matter, and also plastics ingestion and toxicity by soil feeders, such as earthworms [73,74]. In addition, it is important to get insights on: (i) how important biomolecules in soil, such as DNA, enzymes, root exudates, etc., are adsorbed onto the MPs surfaces and what effects they have on the eco-corona properties; (ii) how different eco-corona properties affect the ecological behavior of MPs and NPs, which is their interactions with soil components and thus on their persistence, mobility, bioavailability and toxicity [20].

As it concerns the interactions of MPs and NPs with surface-reactive soil particles, such as clays and soil organic matter (SOM), soil pH can affect the surface charge of these plastics, except those characterized by a hydrophobic surface, which are uncharged. In undisturbed natural soils, the downward movement of MPs should be favored by the presence of macro-pores and preferential path flows, such as bio-pores and cracks, and limited by micro-porosity with MPs accumulation in the surface layer of the soil [4]. Of course, plastic mobility in soil also depends on dissolved organic matter (DOM) and clay minerals because plastic particles can interact with these soil constituents [75–77], as discussed below. In addition, leaching or movement of MPs and NPs by capillarity may occur through the soil profile, as it occurs for high molecular weight compounds, such as extracellular DNA in soil [78], but this should be experimentally proven. Nizzetto el al. [39] and Siegfried et al. [79] created model systems for MPs transport by leaching and soil erosion to quantify the MPs distribution in aquatic and terrestrial environments. However, the absence of experimental data meant that the accuracy of the proposed models could not be verified.

Tillage practices have positive effects on the topsoil porosity and aggregation, thus improving leaching. Zhang and Liu [80] have observed the high amount of plastics debris associated with aggregates (72%) and the prevalence of MPs fibers into micro-aggregates of a cropped soil. The plastics debris inclusion into aggregates can promote their accumulation in soil. This could affect not only the aggregate turnover but also interactions of biota inhabiting the aggregates and soil constituents. The effects depend on the type of MPs because, for example, polyethylene and polypropylene increased aggregate formation [81]. Therefore, MPs can affect soil structure and thus soil functioning. However, tillage may also limit MPs and NPs mobility in soil due to the plough pan formation and this can increase concentration of plastics in the soil's top layers. The study of plastics debris in sediments of river and in temporary or permanent flooded soils along the coast may provide insights on plastics debris transportation and fate in the soil [82,83]. The deposition of MPs from water columns onto sediments is slow due to the low MPs density, but the deposition rate increases with the hetero-aggregation of MPs by particulate organic and inorganic material due to the increased density of these hetero-particles compared to the separate particles [82,84].

5. Effects of Microplastics and Nanoplastics on Soil Properties

5.1. Soil Chemical-Physical Properties

The presence of MPs and NPs in soil aggregates can alter biological, chemical and physical properties of soil [85–88], and affect the estimation of soil carbon sequestration [89]. The effect of plastics on soil aggregate formation and humic acids properties are shown in Figure 3. Indeed, Atuanya et al. [87] observed that the addition of plastic granules to soil increased the total organic

carbon content of soil because the current methods used to quantify the soil organic carbon also determine the invisible MPs fraction of soil aggregates (e.g., polystyrene or polyethylene contain almost 90% carbon) [90]. Therefore, Rillig [90] recommended re-evaluating what is the "true" soil carbon storage in plastic polluted soils. De Souza Machado et al. [91] studied the effects of MPs with different shape, density and chemical composition, on the bulk density, water holding capacity, water stable aggregates and microbial activities of the soil. The authors concluded that micro-plastic could potentially trigger functional changes in soil that are difficult to be predicted due to the complexity of the soil system. However, the effects of MPs properties were not taken into consideration.

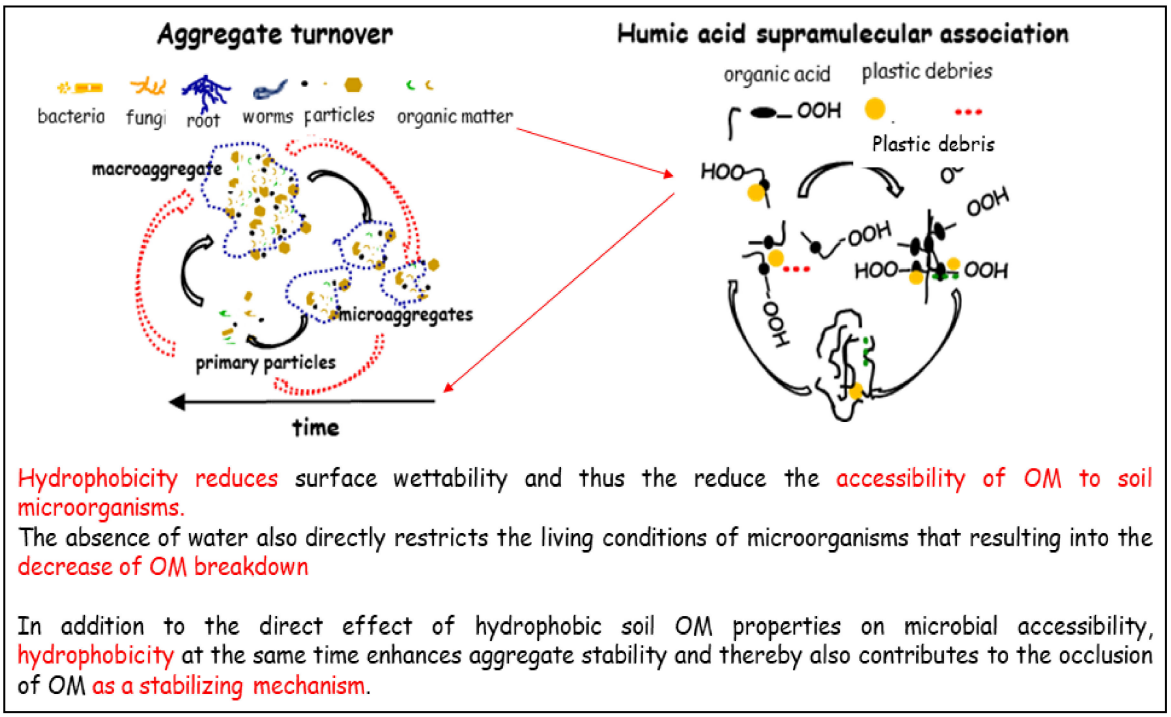

Figure 3. Plastics effects on Humic acids properties and soil aggregate formation (OM-Organic Matter).

As previously mentioned, the interactions of micro and nanoplastics with soil reactive components, and with the main extracellular biological molecules may affect soil functionality, which could directly affect the soil fertility and consequently yield and quality of crops. However, only a few reports confirm the environmental relevance of MPs in soil. MPs and NPs can interact with various functional groups of the dissolved fraction (dissolved organic matter, DOM), which may be associated with inorganic pollutants [92–94]. The result would be the formation of organic complexes being potentially very toxic and moving through the soil profile [95]. Indeed, humic substances interacted with MPs (phenanthrene) and heavy metals (Pd and Cd), when adsorbed on a clay mineral (bentonite) [96]. MPs can interact with the aromatic structure of DOM via π-π conjugation and then with carboxyl groups and C=O bonds [95]. Microplastics seem to act as electron donors to humic substances leading to highly conjugated co-polymers with an increased electron density [95] (Figure 3). These effects depend on the plastics size and the pH of the soil solution. On the other hand, NPs can accelerate the kinetic assembly rate of DOM by weak electrostatic interactions and hydrophobic interactions thus forming the particulate organic matter [97]. The interactions of MPs with organic compounds also depends on the plastic's age, but contradictory differences in reactivity between the aged and the relative original plastics have been observed [14,98]. Interactions of MPs with organic matter can also affect the nutrient availability to biota in soil, for example, by decreasing the dissolved organic N and dissolved organic P forms [99].

5.2. Soil Active Extracellular Molecules

Extracellular enzyme activities play a key role in soil functioning, since they are involved in the degradation of high molecular weight organic polymers [100]. Adsorption of extracellular enzymes by hydrophobic MPs and NPs may increase the half-life of the enzyme due to the protection against proteolysis and a decrease in thermal denaturation [101]. According to Awet et al. [102], polystyrene nanoparticles decreased the extracellular enzymes activity of soil after 28 days of incubation. However, the origin of the enzyme activities is not clear. The measurement done by Awet et al. [102] could be from both intracellular and extracellular enzymes [100]. In addition, Awet et al. [102] observed a negative effect of polystyrene nanoparticles on microbial biomass. However, the negative effect of nanoparticles may not persist when they form aggregates. Pure glyphosate increased MPs degradation and phosphatase activities but not urease and β-glucosidase activities in soil, whereas the presence of MPs had no effects on glyphosate degradation [103].

Both intracellular and extracellular DNA can have ecological importance in soil [104,105]. The role of intracellular DNA in cell division (DNA replication) and protein synthesis (transcription and translation) is well known and established. However, involvement of extracellular DNA is less considered, despite the fact that it can act as a plant bio-stimulant or bio-repressor [106–108] and it is involved in the bacterial biofilm formation [109] and gene transfer by bacterial transformation [110]. No information is available about the contributing percentages of both forms, extra- and intra-cellular DNA to total DNA associated with plastics.

It is reasonable to hypothesize that hydrophobic MPs and NPs can interact with the N bases of the extracellular DNA molecule, especially with those of double-stranded DNA which is more hydrophobic than single-stranded DNA, NPs may also be entrapped within major grooves of the DNA molecule. In addition, the surface of charged MPs and NPs can interact with the phosphate backbone and N bases of the extracellular DNA molecules depending on the sign of the charges. As already mentioned, bacteria can form biofilms on plastics surfaces and the role of extracellular DNA in this formation is well established. The extracellular DNA allows the bacterial adhesion to the solid surfaces through acid–base interactions onto hydrophilic surfaces and through Van der Waals forces onto hydrophobic surfaces [111]. Low-density MPs surfaces are characterized by high porosity, and extracellular DNA molecules can not only be adsorbed or entrapped on these surfaces, but also be intercalated as single strand between sheets as it occurs with clay mineral sheets [112]. However, extracellular DNA is mainly present in the soil environment in a dirty state represented by DNA molecules surrounded by proteins and lipids [104]. Therefore, the interaction between the extracellular DNA molecule and MPs or NPs depends not only on plastics eco-corona properties but also on extracellular proteins and other cellular components released by cell lysis and inorganic compounds surrounding the DNA molecule. The adsorption of extracellular DNA by MPs and nanoplastics may make the adsorbed molecule more resistant to DNases compared to the free extracellular molecule, as observed when DNA is adsorbed by clays [104]. However, future research should fill the knowledge gaps concerning the survival of extracellular DNA in soil and its interaction with plastic debris.

Furthermore, plastics act as vectors for heavy metal ions in marine environments [82,113–115], while in terrestrial ecosystem, MPs could also act as vectors to increase metal exposure in earthworms [116]. However, the related risk is unlikely to be significant for essentials metals such as Zn that are regulated by metabolic processes [116]. However, future research should investigate the mobility of other heavy metals than zinc (particularly non-essential metals) and other MPs than poly-ethylene in soil since physical-chemical properties differ among heavy metals and MPs and these properties affect plastic mobility [113].

5.3. Soil Microbial Community

The transport of microbial invasive species via plastic waste, especially the role of MPs in transporting microorganisms, is poorly identified due to methodological problems. Pesticides can move through the soil profile with MPs, as hypothesized by Horton et al. [1]. The concern on these

issues should endorse future research with the aim to understand the role of MPs not only as vectors of microorganisms but also as vector of pollutants. MPs can affect some microbial properties such as, MPs-associated bacteria, which showed higher plasmid transfer rates than free-living bacteria [29]. Since, bacterial community inhabiting biofilms can acquire a wide antibiotic resistance capacity, it is reasonable to hypothesize that MPs may act as antibiotic resistance vectors [29]. Both conjugation and transformation may be involved in DNA transfer in the biofilm [117].

NPs can easily penetrate into cells lipid membranes affecting the cell's functionality [118]. However, microorganisms can prevent the entrance of nanoparticles into cells by adopting different self-protecting mechanisms, such as changes in the structure of cellular membrane, the secretion of molecules neutralizing the contaminants [119,120], and the barriers imposed by the bacterial cell walls and any biofilm matrix [121,122]. Perhaps the movement of cells from a planktonic state to a biofilm state will impede contact with NPs [121]. The protective action of biofilms against NPs toxicity is exerted by passive and active mechanisms. The passive protection is due to physical-chemical properties of the biofilm matrix, such as the high density and the presence of functional groups on the extracellular polymeric substances that are capable of entrapping and binding NPs into the biofilm surface layer, respectively [123]. In addition, NPs detach from biofilms being released when they saturate the biofilm surface [121], which is probably due to NPs tendency to form hetero-aggregates in the presence of inorganic colloids and natural organic matter regardless of the bacterial activity [121]. However, the presence of NPs may induce resilience and functional redundancy properties in the bacterial community inhabiting the biofilm [122]. All these mechanisms are difficult to be proven in soil due to methodological problems. Unfortunately, no data are available on the effects of MPs on the composition of soil microbial community and future research should fill these gaps by monitoring the relative effects by determining microbial diversity by metagenomics or amplicon sequencing [71,72].

5.4. Soil Fauna

Soil fauna, particularly earthworms and collembola, can incorporate MPs and NPs and thus move them through the soil profile [73,85,124], with the former being particularly effective because of their high capacity to filter soil [116]. By considering the presence of 2000 earthworms per m^2, multiplying this number by the single worm gut volume of 450 mm^3 (100 mm × 1.44 mm^2 × π), we predict that 1 m^3 of soil can contain nearly 1 L of earthworm gut material [125]. The collembola effect on the mobility of MPs and NPs in soil can be important, considering their high abundance, ranging from 10,000 to 100,000 individuals per m^2 of soil within the first 10 cm of the soil profile [126]. However, the effect of other ecologically important soil biota, such as protists, the main consumers of soil bacteria, is poorly known [127]. These organisms may be potentially important vehicles for the delivery of MPs into the soil food chain [90]. Protists can discriminate between different types of bacteria [128] but not between latex microplastics spheres and bacterial cells [129]. The uptake and incorporation rate of MPs by protists depend on species, their nutritional status and age and MPs concentration [90].

Plastic feeders seem to prefer the aged MPs because of the presence of microorganisms [130]. The feeders ingest regularly shaped MPs faster than irregular MPs [131]. The ingestion of MPs and NPs by soil fauna can change the composition of gut microflora as shown for collembola and the oligochaete *Enchytraeus crypticus*. The NPs ingestion by the latter decreased the body weight and affected the composition of gut microorganisms involved in organic matter decomposition and N reactions [6,132]. Indeed, MPs and NPs can interact not only with soil-dwelling invertebrates but also with other organisms, such as terrestrial fungi and plant pollinators, which are involved in essential ecosystem services and functions [81]. However, the negative effects of NPs on the annelid worm, *Enchytraeus crypticus*, were lower than those due to other nanomaterials [89,133–135]. Not only microorganisms but also molecules adsorbed by plastics affect the interactions between plastics and soil biota. For example, the earthworm *Eisenia fetida*'s preferentially recognized nanoparticles when coated by its coelomic proteins compared to those coated by fetal bovine serum [136]. MPs with

glyphosate negatively affected the earthworm burrowing activity [137]. An exhaustive description of the MPs interaction with soil biota was recently published by He et al. [138].

5.5. Soil Pedogenesis

An intriguing aspect arising from the abovementioned effect of MPs and NPs on soil properties, due to a prolonged time of residence and high reactivity, is related to their possible effects on soil pedological processes. It is possible to hypothesize the MPs and NPs presence as a characterizing factor to classify the surface and subsurface soil horizons. Moreover, how could this debris modify the pedological processes is not yet discussed in the literature to our knowledge. This possibility is preeminent and harbinger of interesting developments. In this context, it is important to consider the recently discovered pyroplastics, originating from the frequently adopted practice of burning waste debris [139,140]. Due to their recalcitrance to degradation, these forms of plastics may become part of the soil's geological cycle.

5.6. Plants

The soil plastics contamination could exert a direct and indirect affect cultivated plants as a consequence of their root uptake or effects on soil chemical-physical and biological characteristics, respectively. Concerning the direct effects in the last two years, there was an increase number of evidence of MP and NP contamination in plants [74]; plant metabolism of pollutants or their storage of recalcitrant contaminants could be the main issue [141]. The plant uptake of MPs and NPs depends on the anatomical and physiological properties of the plant (plant species), on plastics properties, especially those of the eco-corona, and on environmental ageing affecting surface chemistry and behavior [65]. Li et al. [142] have determined that plastic debris with size up to 0.2 μm can be taken up by lettuce roots and provided evidence of intercellular presence of plastics in the form of a "grape-like" cluster. Recently, Sun et al. [143] reported that uptake of NPs is significantly depends upon their surface charge. The authors showed that accumulation and uptake of negatively charged NPs was much higher in roots compared to positively charged NPs, which could be due to due to their high binding affinity with radical mucilage and their size increasing through the hetero-aggregation induction by root's exudates. However, these results not exclude the risk of the MPs entry into the human food chain through vegetable food as a consequence of their adhesion to the surfaces of salad and root vegetables [143]. Further, Smith et al. [144] showed that MPs coming from industrial produced compost could accumulate in plants that are grown in those compost.

The excessive accumulation of plastic debris in the root can induce several risks for the plants such as the interruption of the nutrient transport system by the obstruction of the cell connection and/or the pores in the cell wall [145], the excessive production of reactive oxygen species (ROS) [145] and the reduction of the plant disease resistance by inducing downregulation of disease resistance genes [143]. After accumulation of plastic debris into roots, can further transferred to stem and leaves via the vascular system following the transpiration stream, where they can be detected at intercellular level as "string-like" cluster and dispersed forms, respectively [142].

The intracellular NPs uptake is expected but the MPs cannot pass through the cell membrane due to their higher size (from 0.1μm to 5 mm) than NPs (<0.1 μm) [33,146]. Plant cells take up NPs by endocytosis, through ion transport channels, by carrier proteins or by aquaporins. NPs size is important for plant uptake because nano-polystyrene beads with a diameter of 20 to 40 nm were taken up by tobacco cells, but not those of 100 nm [146]. Kettler et al. [26] suggested that the threshold value is 50 nm. The plant uptake of NPs also depends on anatomical and physiological properties of the plant, and thus on plant species, on the part of the plant, on nanoparticle properties, especially those of the eco-corona, and on environmental ageing, affecting the surface chemistry and behavior of NPs [65]. Further research is needed to better understand NPs' translocation, storage, and toxicity on plants and the defense mechanisms of plants against NPs [55].

In relation to MPs' and NPs' indirect effects on cultivated plants, the main aspects to be considered are their effects on soil structure, nutrient immobilization, contaminant adsorption and diffusion, soil microbial community root-associated microbiome and root symbionts. Most of these topics have been considered in the present review. However we can focus on the possible plastic waste interference in the chemical communication in rhizosphere that may affects the rhizosphere microbial community [147,148] but also the plant's interaction with symbionts and pathogens [149]. Finally, in relation to the toxic substances release from plastics waste degradation in soil, it is intriguing to evidence the plants' high enantio-selectivity, as recently evidenced for hexabromocyclododecanes (HBCDs) and pentabromocyclododecenes (PBCDEs) monomers [150].

Biological Indicators of Microplastics and Nanoplastics in Soil

Seed germination and plant growth [151,152], intensification of the toxicity to earthworms [153], and inhibition of nitrification [146] were used as indicators of plastic toxicity. As already mentioned, soil-filtering earthworms may accumulate both MPs and NPs and thus they can be used to extract plastics from the soil [132]. The microbial degradation of MPs can produce volatile compounds (VOCs), such as methane and ethylene, indicators of the presence of MPs in the analyzed sample [154–156]. However, the use of this method is challenged by the poor knowledge of the mechanisms (such as those due to microbe-microbe and plant–microbe interactions) producing VOCs during plastic degradation and by different factors, such as pH, moisture, organic carbon content and clay minerals, and different microbial diversity affecting this production in soil [157,158].

6. Analyses of Soil Microplastics and Nanoplastics

6.1. MPS and NPs Sources and Relevance of Ageing Processes

In relation to study the MPs and NPs, the reactivity and toxicity of plastics in soil has to consider the response to their chemical and physical characteristics [10]. Among them, the most relevant are, respectively, the type of polymer, considering also the eventually added chemicals (plasticizers), the shape and size. Commercially produced particles may be purchased with designed properties or directly synthetize the requested plastic polymer [102] but often this is not possible and PS chemical-physical adaptive treatments are required. These approaches mainly consist of physical reduction in size by chopping, milling and grinding of the plastics into micro- and nano-sized debris [2,81,159–161]. However, these treatments do not provide plastics debris with chemical and physical characteristics similar to those determined by the environmental degradative processes [113]. In fact, the plastics degradative pathways under environmental conditions is termed as ageing and markedly influence the plastics debris reactivity [14,98]. Further, each degradative pathway differently affects the plastics surface characteristics even on the same polymer, thus leading to a large variety of aged plastics differing in surface properties [162,163].

To solve this problem, methods that mimic the ageing process under laboratory conditions have been developed. These methods are mainly based on exposing plastics to natural abiotic degradative factors such as light (UV), temperature, oxidative conditions (H_2O_2) and moisture at defined levels [14,69]. However, it is relevant to underline the existence of other abiotic ageing factors, such as fouling or mechanical abrasion, that could induce different surface properties [14]. Recently, the Laser Ablation technique has been successfully tested to simulate plastic ageing to obtain nano size debris [164]. Further, up to now the ageing methods have not considered the biotic processes due to the polystyrene resistance toward biodegradation [165]. However, the current research on polystyrene biotic degradation [65,154], it is reasonable to expect an increased utilization of plastics ageing methods based on biotic degradative processes.

The chemical-physical characteristics of the utilized micro- and nanoplastic debris have to be determined by appropriate techniques [2]. For example, photoxidation has been recently proposed to utilize the carboxylic index that is determined by attenuated total reflection Fourier-transform

infrared spectroscopy (ATR-FTIR) [166]. Additional methods to complete the aged plastics surface characterization are the Scanning Electron Microscopy (SEM) coupled with N_2 BET specific surface areas and Differential Scanning Calorimetry (DSC) [14]. We stress that the aged plastics surfaces may react differently than the native forms such as in their adsorptive capacity [14,99].

To avoid the lack of reliable techniques to study these exchange processes, it has been recently proposed that modelling by poly-parameter linear free-energy relationship (ppLFER) may provide information on multiple adsorption by different molecules [14].

6.2. Microplastics Characterization

MPs' extraction from complex environments such as soils, especially those rich with organic matter, is a challenge; it is not a coincidence that, until now, no standard method has been adopted. As discussed by Fuller and Gautam [167], the current methods to extract MPs fragments using the floatation or density separation step with dense salt solutions (NaI and $ZnCl_2$) do not extract plastics from organic samples. The intrinsic difficulties have been clearly highlighted by Wang et al. [82], discussing the poor extractive results obtained by coupling organic matter degradative pretreatment with flotation method. In fact, the current methods to extract MPs fragments using the floatation or density separation step with dense salt solutions (NaI and $ZnCl_2$) do not efficiently extract plastics from organic samples [167], mainly because SOM has densities similar to that of several plastics [4]. In addition, methods based on chemical digestion (acid or alkaline solutions) and H_2O_2 [82] could also degrade plastics debris, whereas those based on enzymes seems to be more plastics friendly [168].

To avoid this problem, Hurley et al. [169] have set up an efficient and plastics friendly organic matter degradative approach that is compatible with the existing methods to analyze MPs from soil. After comparative tests with H_2O_2, Fenton's reagent and alkaline solutions (NaOH and KOH) with different polymers, Fenton's reagent have shown the best performances evidencing also a high compatibility with MP density-based extractive techniques. Further, the method has shown similar extractive efficiency regardless of whether an organic matter degradation step occurs before or after treatment. The only request reported by the authors is to monitor the pH values during the Fenton's treatment to avoid the risk of plastics obscuration by iron sulfide precipitation at higher pH value such as pH 5–6. However, we have to consider that the common soil sample processing of grinding and sieving techniques destroy the aggregates organization and do not allow linking plastics extraction data with plastics debris allocation in soil. Further, floatation or density separation methods determine the number of plastic fragments but cannot give the relative concentration [4].

Recently, Ng et al. [65] has reviewed different methods used for the identification of MPs that are extracted from soil, such as the Fourier Transformed Infrared (FTIR) [170,171] and Raman [172,173] spectroscopy and reported that these methods were not destructive and identified both the type and size of particles. Plastics can also further be discriminated by size exclusion chromatography (SEC) and be characterized by two vibrational spectroscopies detection methods (Raman and FTIR). Fuller and Gautam [167] have developed a new method for MPs detection in soil, based on the pressurized fluid extraction, which uses solvents at subcritical temperatures and pressure conditions with recovery of semi-volatile organics from solid materials; once solvents were removed, MPs were determined by FITR. The method efficiently extracted the plastic particles less than 30 μm, which are difficult to be extracted by floatation and other physical separation procedures but it is ineffective in extracting plastic particles higher than 30 μm [174]. Zhang et al. [175] used flotation to extract low-density MPs (polyethylene and polypropylene) from soil and identified the different size and shape of extracted MPs by microscope after heating. The microplastics' mass was calculated using an empirical model. However, heating-based methods are not suitable for analysis of MPs eco-corona due to its degradation. The electrostatic method, discriminating charged MPs from uncharged particles set up by Felsing et al. [176], probably cannot work in soil due to the presence of charged soil particles. Oil extraction of microplastics from sediment was effective due to the hydrophobic properties of microplastics and the successive FITR spectroscopy characterization was possible but requires to

eliminate the oil residues by ethyl alcohol washing [177]. The positive and negative aspects of micro- and nano-plastic separation methods based on external field forces have recently been well reviewed by Fu et al. [178]. Among them, the authors reported the best performances of asymmetrical flows via a semi-permeable membrane (AF4) and gravitational/centrifugal sedimentation (FFF). However, drawbacks of these methods are high sample dilution, membrane pore size and the low density of plastics. For those methods based on electrical and magnetic fields force application, the main drawback is related to the presence in the soil of mineral and organic charged components.

The use of spectroscopic techniques, such as FTIR and Raman scattering, gives spectra of MPs and dissolved organic matter that often overlap due to similar spectral peaks and, thus, they fail to reveal the small spectral changes caused by their interaction [179–181]. To solve this problem almost simultaneously, Chen et al. [97] and Watteau et al. [182] have proposed two different methodological approaches. Chen et al. [97] have set up a chemometric analysis, which may solve these problems. The method is based on the excitation-emission matrix coupled with the parallel factor analysis (EEM-PARAFAC) and two-dimensional correlation spectra (2DCOS). The EEM-PARAFAC provided information on the MPs hetero-aggregation and MPs role in the transport of contaminant whereas the integration of 2DCOS with FTIR (FTIR-2DCOS) gave insights on the plastics fragmentation processes producing secondary MPs under various natural conditions [97]. The combination of FTIR and focal plane array detection eliminates the problems of the previous visualization of MPs but it is time consuming [183].

Watteau et al. [182] have developed a method combining soil fractionation, microscopic observation and chemical characterization to follow the fate of plastics in soils and apply this approach to soil amended with municipals solid waste compost. The presence of plastics was detected and analyzed using morphological and analytical characterization by transmission electronic microscopy (TEM-EDX) and pyrolysis coupled to gas chromatography and mass spectrometry (Py/GC/MS). The presence of specific plastic tracers in the plastics polymers have allowed identification of MPs polymers also in presence of organic matter even in the <200 μm soil fractions. These plastics tracers could be intentionally added to polymers or inserted between the polymer constitutive molecules. These tracers could also be detected by pyrolysis as volatile organic compounds (VOCs). An example is represented by the toluene/styrene ratio in the pyrolysis products. This ratio result ranging between 4–5 in natural soil is rich with organic matter, whereas the presence of plastics could markedly decrease toluene/styrene ratio, indicating the presence of plastics [184]. Further, the detection of styrene dimer and trimer conformation is indicative of plastics presence in soil [185].

In the case of no interest to preserve the plastics polymer debris integrity, it has been recently proposed that an automated thermal extraction-desorption (TED) coupled with gas chromatography mass spectrometry (GC-MS) [186] can be used to characterize MPs. This technique (TED-GC-MS) is a two-step method in which the gasified sample by a thermo-gravimetric furnace (TGA) is concentrated and purified onto a solid phase absorber (TPA) and then analyzed by thermal desorption gas chromatography mass spectrometry (TDU-GC-MS). TPA also allows to overcome the risk of GC column clogging as a result of the extremely lower GC operating temperature (200 °C) compared to those of the TGA (1000 °C). The method can be easily optimized by regulating the GC purge gas flow and heating rate. This technique (TED-GC-MS) allows a comprehensive characterization of MPs polymers and their degradation processes even in soil identifying the different polymers and determining the relative weight concentrations. Moreover, Dynamic light scattering (DLS) is a non-invasive technique suitable to determine the micro- and nanoplastics' hydrodynamic size and zeta potential measurements in a wide range of liquid samples. The DLS also permit the detection of the microplastics' aggregation. The main limits of the DLS is the sample purification to avoid the presence of an elevated light-scattering background [187].

6.3. Nanoplastic Characterization

Unfortunately, there are no ad hoc methodologies for extracting NPs from soil for quantification and characterization [188,189]. In relation to the present technological gap, it is possible to adapt the existing methodologies utilized for MPs [174]. The main technological gap is represented by the difficulties with sample preparation and subsequent analysis. In fact, due to the NPs' low concentration and higher reactivity, which induce hetero-aggregation with other soil constituents, it has been necessary to improve the extraction, purification and concentration methods. Further, in relation to their extremely small size, it has also been necessary to adapt the present analytical technologies until the development of new and more performants ones. In relation to NPs purification, the same considerations of MPs, with the additional hetero-aggregation induction exerted by acids, alkaline, oxidative (H_2O_2) or enzymatic treatments, are valid [168].

Concerning the concentration step, several different methods are suitable and are determined by the subsequent adopted identification and characterization approaches. The more suitable methodologies consist of ultrafiltration and ultracentrifugation by asymmetric flow field fractionation (A4F) [190] and analytical ultracentrifugation (AUC) [191]. Among the suitable separation techniques reported in the literature, the most performed is represented by flow field fractionation (FFF) in the AF4 variant [192], chromatography by the hydrodynamic chromatography (HDC) version [193]. Further, the AF4 technique coupled with multi-angle light scattering (MALS) for detection of nanoplastics may require adjustment of the separation according to characteristics and properties of different NPs [194].

NP characterization in terms of size and morphology firstly requires the conservative pre-treatment, and the main techniques are those based on Light Scanning (LS), Electron Microscopy (EM) and Scanning Probe Microscopy (SPM) to obtain information related to particle size and their distribution (Particle Size Distribution—PSD), and to shape and surface conformation, respectively. The more suitable LS reported in the literature are the Dynamic Light Scattering (DLS) and Nanoparticles Tracking Analysis (NTA); those of EM techniques are the Scanning (SEM) and Transmission (TEM) EM. The SPM are mainly represented by Atomic Force Microscopy (AFM), Scanning Probe Microscopy (SPM) [195] and Scanning Transmission X-ray Microscopy (STXM) [196]. With the Brunauer, Emmett and Teller technique, it is also possible to determine the surface area and porosity of MPs and NPs [61]. The more suitable NPs chemical characterization technologies are limited to Raman Microscopy (RM) and X-ray photoelectron spectroscopy (XPS) [186].

An exhaustive review on the particle size distribution was carried out by Schwaferts et al. [174]. Both authors have critically evaluated the different analytical techniques such as dynamic light scattering (DLS), Raman spectroscopy (RM), nanoparticle tracking analysis (NTA) and asymmetric flow field-flow fractionation (AF4). The results provided by these techniques have been compared. NTA and AF4 gave higher resolution than DLS. Further, AF4 has proven to be a precise analytical technique for the separation of nano sizing particles of different sizes. Schwaferts et al. [174] have also evaluated RM supported by SEM and multi angle light scattering (MALS) to enhance the sub-nano and nanoplastic debris detection coupling the chemical information that Raman spectroscopy provides with those related to detection, separation and the inline quantification obtained by SEM and MALS, respectively.

6.4. Eco-Corona and Plastisphere Characterization

The determination of microbial species in the eco-corona and plastisphere of MPs and NPs requires the use of molecular techniques, such as metagenomics [22,197], to overcome the problem of non-culturable microorganisms and to understand functions, gene expression, proteomics and metabolomics must be use. The approach is termed mehaphenomics according to Jansson and Hofmockel [198]. For the DNA-based techniques, it is important to consider that the efficiency of the direct extraction of DNA from plastic debris decreases by decreasing the size of the plastic particles and it is lower with irregular than regular shapes of plastic particles [199]. DNA has also been extracted through the indirect method from plastics debris [200]. However, this method shows a lower DNA recovery than the direct DNA extraction methods [72].

Future and imaginative research should fill the knowledge gaps regarding the formation of eco-corona, plasti-sphere and biofilms in MPs and their evolution in soil. Indeed, the only papers dealing with these issues, concern the marine environment mainly utilizing a metagenomics approach. Nasser and Lynch [20] distinguished between hard and soft eco-corona by using the culture-dependent method followed by SDS-PAGE, whereas other studies determined microbial properties of plasti-sphere and biofilm, even at network analysis level [22], distinguishing the microbial community in the biofilm formation and maturation phase [201–204] and also determining the MPs-dependent and independent biofilm assemblage factors [205]. These data should be finally matched with that of metabolic activities, such as toxins, bacteriolytic substances, extracellular polymeric substances (EPS) and enzymes, to obtain a putative signature of biofilm formation steps. Finally, future research should be done to distinguish the molecules that are present in the matrix of biofilms from those are result of biological activities of soil inhabiting biota and further distinction is required to differentiate between extracellular and intracellular locations [24].

7. The Reduction of Microplastic and Nanoplastic Inputs to Agricultural Soils

It is important to reduce or eliminate the inputs of MPs and NPs to soils, especially to agricultural soils, to avoid the contamination of the food chain. Procedures should reduce the plastics contents in composts, waters and wastewater sludge. In addition, it is important to promote the use of degradable plastics, for example, the use of bioplastics or biodegradable plastics, such as poly (butylene adipate-co-terephthalate) (PBAT) for mulching [57]. The importance of this issue is also shown by the recent H2020 call for studying microbial communities involved in plastics bio-degradation (RIA) (H2020-NMBP-TR-IND-2018-2020). However, the policy has not yet formulated the laws for reducing the risk of environmental plastic pollution, despite up to 12.7 million metric tons of plastic litter entering the oceans from land-based sources each year [1]. G7 meeting (G7 leaders, Leaders' Declaration 2015) on plastic declared, "Plastic litter poses a global challenge".

7.1. Biodegradable and Bio-Based Plastics

According to Wackett [206], bioplastics are those partially or totally biodegradable and could be divided in synthetic, partially biological and totally biological (bio based). The Poly (butyl adipate-co-terephthalate) (PBAT) [57] and Polycaprolactone (PCL) [207] are the main biodegradable synthetic plastics (fossil base) in which the aliphatic molecular part is responsible for its biodegradability. On the other hand, the bio-based plastics (i.e., plastics synthesized from biomass or renewable resources) are mainly composed by poly hydroxyalkanoic acids (PHAs), poly-lactic acid (PLA), poly-butylene succinate (PBS) and PBS-co-adipate (PBSA) [63,208]. These plastics are used for replacing conventional plastics in agriculture, medicine and in the dairy industry. Bacteria, fungi and actinomycetes can degrade both natural and synthetic plastics by causing both physical and chemical changes in these materials. Usually the biodegradation occurs aerobically, but in sediments and landfills, it can occur under anaerobic conditions and in compost and soil under partial aerobic conditions [209,210]. The bioplastics degradation rate in soil depends on chemical composition of bio-plastics and on bacterial biomass, but not on bacterial diversity [208]. The microbial degradation rates increase with greater surface to volume ratio, and thus the intake of oxygen and water, with stimulation of oxidative and hydrolytic processes [69,211].

According to the "OK Biodegradable soil" Certification by the Belgian Institute Vinçotte, the French standard NF U 52-001 [212], the Italian standards UNI 11462 [213], and the UNI 11495 [214], biodegradable plastics should degrade by 90% in 2 years and their eco-toxicity on microorganisms, plants [151,152] and animals [152] should be determined prior to their use [159]. Chenon et al. [215] suggested to use the biodegradation test [216], as an indicator of biodegradation, and the determination of nitrification as indicator of eco-toxicity [217], before using biodegradable plastics. In Europe, biodegradable and compostable plastics are subjected to independent certificates by DIN Certco and Vincotte. Certificates for compostable plastics are also issued by the Biodegradable Products Institute

(BPI, New York, US), the Japan BioPlastics Association (JBPA, Tokyo, Japan) and by other less known organizations. In fact, compostable plastics are the plastics being degraded during the composting processes but direct degradation in soil biosphere is difficult due to their specific requirement of different environmental parameters such as temperature, moisture, nutrient concentrations and often the addition of chemicals in specific concentrations and ratio [218]. An example of a non-biodegradable compostable plastic is polylactic acid (PLA) which achieved only 4% degradation under soil burial condition [219].

It is well known that biodegradable materials are indeed more expensive than non-biodegradable materials; however, the long-term ramifications of not using them, including environmental pollution and the massive landfill utilization, put the cost into the perspective. Further, the most suitable end-of-life solution depends not only on plastics characteristics but also on their volume on the market application, available collection and processing infrastructure. However, both the production of degradable plastics and the management of their wastes by defining the optimal end of life will require the existing industries to be revamped. This adaptive process is also required by the recently published EU Roadmap for a Strategy on Plastics in a Circular Economy—one of the main blocks of the European Green Deal, Europe's new agenda for sustainable growth (https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1583933814386&uri=COM:2020:98:FIN). The main emerging challenges for the sustainability of bio-based plastics utilization identified by the commission are to evaluate where the use of bio-based raw materials leads to real environmental benefits and clearly define the biodegradable or compostable plastics application criteria. These objectives aim at the correct utilization of "biodegradable" or "compostable" plastics to ensure that their use can be beneficial for the environment.

7.2. Development of Clean Up and Bioremediation Technologies

An interesting and promising approach to reduce the MPs and NPs pollution in soil involves the enhancement of the in situ degradation by increasing the activity of fungi, bacteria and other soil organisms inhabiting the soil. Sehkar et al. [220] isolated *Enterobacter* sp., *Citrobacter sedlakii, Alcaligenes* sp. and *Brevundimonas diminuta, capable of degrading* polystyrene, and electronic plastic (e-plastics) containing decabromodiphenyl oxide and antimony trioxide. Muenmee et al. [221] investigated the biodegradation of waste plastics by a bacterial consortium oxidizing methane by setting a lysimeter experiment. They mixed UV light pre-aged plastic polymers with unsterilized stabilized organic waste sampled from an open dump. The detected plastics waste-colonizing bacteria consortium consisted of heterotrophs, autotrophs and methanotrophs, with the latter being the main decomposers. Screening of plastic degrading microbes from various dumped soils at different time highlighted that *Bacillus sp*., *Pseudomonas sp*., *Streptococcus sp*., and fungal *Aspergillus sp*. and two *Fusarium sp*., completely degraded the plastics polymers after 120 and 75 days, respectively [63]. Considering that plastics biodegradation occurs on the surface, it is reasonable to hypothesize that the biodegradation efficiency may depend on the surface/volume ratio of plastics debris. However, the biodegradation rate of polymers is also affected by other physical properties, such as melting point, glass transition temperature, crystallinity and storage modulus [209], and by the chemical nature of the polymer, environmental conditions and activity of the microbial degraders [61]. In addition, the degradation of the MPs and NPs occurs in hot spots, such as the worms' guts, where the activity and abundance of microbial degraders are higher than in other sites [74]. The bacterial community of mealworms guts (the larvae of *Plodia interpunctella*) degrades plastics [222] and the bacterium *Ideonella sakaiensis* 201-F6 and larvae of the wax moth *Galleria mellonella* can degrade polyethylene terephthalate and *polyethylene,* respectively [223,224]. Further, termites are also capable of chewing and eating plastics indirectly by gut bacterium such *Lysinibacillus* and *Bacillus* sp. T2 [225]. Organic compounds can increase the biodegradation rate of plastics [159]. For example, EcoPure®, a blend of organic ingredients produced by Bio-Tec Environmental, is used to increase the rate of plastic biodegradation in landfill and the stimulation depends on different factors such as plastic type, the product configuration, solid content,

temperature and moisture levels of the landfill (http://www.goecopure.com). Plastics are transformed in oxo-plastics (that contain a pro-degradant catalyst such as salt of Magnesium or Iron) in the presence of small amounts of metal salts; however, heavy metals salts cannot be used since the EU Packaging Waste Directive 94/62 Art 11 restricts their use. The abiotic degradation of oxo-plastics in the presence of oxygen will be faster than that of ordinary plastics. Generally, the final products of plastic degradation produced via carboxylation and/or hydroxylation are low molecular weight organic compounds, which are easily biodegraded as the result of eliminating plastic fragments in the environment. (Source:https://ipfs.io/ipfs/QmXoypizjW3WknFiJnKLwHCnL72vedxjQkDDP1mXWo6uco/wiki/Oxo_Biodegradable.html).

A better understanding of properties and microbial habitat of the eco-corona is essential to increase the plastic degradation rate, specifically by stimulating the adsorption of microbial plastic degraders by the eco-corona in soil. The discovery of microorganisms which are capable of using plastics as a source of carbon and energy and thus capable of synthesizing enzymes involved in the plastic degradation, suggests the possibility to increase the microbial performance through enzyme engineering [226]. The environmental risk due to contamination of NPs contained in waste water or in compost produced by waste water sludge, is reduced by microbial promotion of NPs aggregation [122], leading to their flocculation, a process triggered by the EPS and proteins interaction with NPs, as shown by FTIR [123].

8. Socio-Economic Impacts of MPs and NPs Soil Pollution

As described in the above part of the review, the socio-economic impact caused by MPs and NPs pollution in soil could exert their effects in a wide range of environments at a different scale level. In fact, as reported by several authors, the soil is the main sink of MPs and NPs that act as reserve for the other ecosystems (water and air) [1,227]. The plastics pollution affects all the soil compartments such as the rhizosphere, bulk, soil crust, etc., markedly influencing the functionality and thus potentially affecting the arising ecosystem services mainly represented by food production, water purification, and air quality with consequence on human health. All these aspects have in common a high economic cost which, at present, is not possible to quantify due to our lack of knowledge on this kind of pollution at all the ecological level. An example is represented by the bioaccumulation and bio-magnification effects of MPs and NPs pollution on the food web and the finally on humans.

As far as we know until now, there are few published studies related to their effects on human health, probably because plastics are considered inert [228]. However, their risk also depends on the content of chemical additives [5,229]. Polyvinylchloride, polystyrene, polycarbonate, polyethylene, polyester, and polyurethane can contain and release toxic carcinogenic monomers, harmful for humans, rodents, and invertebrates [230,231]. As already mentioned, the high surface reactivity makes both MPs and NPs vectors of environmental toxic contaminants, whereas only MPs can be vectors of potential pathogens [28]. As reviewed by Galloway [232] and Revel et al. [228], the ingestion of MPs can cause localized gut inflammation, immune system alteration, intestinal blockage or tissue abrasion, alteration of oxidative stress, energy and lipid metabolism, but also neurotoxic effects. The plastics debris can change their surface chemical and eco-corona properties after their transit trough the digestive trait and this may stimulate the adsorption of toxins or antigens [228,232].

NPs can penetrate cells more easily than MPs and their entrance in the gut epithelium can increase their potential toxicity (http://www.efsa.europa.eu/en/press/news/160623) due to their interaction with biological macromolecules, such as lipids, protein and DNA [233]. However, only NPs with diameter lower than 50 nm can reach to the blood circulation [228]. NPs can cause lung carcinoma cells (A549) [234], affect inflammatory gene expression and cell morphology, induce gastric pathologies, and cross the blood–brain barrier [235] in humans. Finally, it is important to underline the possible different effects exerted on human health by NPs and engineered nanoparticles due to their different physical-chemical properties [236], as reviewed by Galloway [232] and Ravel et al. [228].

9. Conclusions

Despite the fact that contamination of soils by MPs and NPs is an important issue nowadays, there are several knowledge gaps about their fate and effects that should be filled by future research. In this context, a fundamental precondition is to improve the present applied methodologies that are characterized by a good quality level for MPs but become a real technological challenge for NPs. The ambitious goal is to combine efficiency, low cost, time request and standardization despite the extremely complexity and variability of soil environment. The relevance of this target is demonstrated by the progressive numbers of published articles on the topic in the last few years. The future research activities will have to be able to cover the MPs and NPs wide diffusion in soil from nano to macro scale and the arising huge number of complex interactions. These interactions involve all the biotic and abiotic soil components and often cause marked effects on their properties and reactivity. For these reasons, the research strategy will request a holistic approach that is able to combine data from specific aspects in a general framework that contextualize their effects at the ecosystem level. This approach will further allow to better evaluate the actions and activities of MPs and NPs and provide a clear picture of their relevance at ecosystem scale. This knowledge will represent the fundamental prerequisite to deeper study on the soil MPs and NPs pollution and counteract their hazardous effects on the soil's ecosystem functionality. The combined use of these approaches will allow the studies of the fundamental problem related to MPs and NPs in soil.

A representative example is the persistence of the NPs, the more active and potentially higher hazardous form. The persistence of this form seems to be regulated by the hetero-aggregation process that describe their complex and reversible interaction with organic and inorganic soil particles. The holistic approach has permitted evaluation of the hetero-aggregation process at a higher-scale level, with focus on effects on the carbon cycle and the aggregate turnover. Furthermore, it has been possible to extend the relevance of this data by considering their effects at soil physcal-chemical level and to its biota. The knowledge resulting from research on the MPs and NPS interactions with important biological molecules, such as humic substances, extracellular DNA, EPS and enzymes will integrate those on the composition and activity of microbial communities inhabiting soil and gut of soil fauna as well as on the microbial plastics degraders, often just present on the plastics debris surfaces. The successive holistic approach will allow to evaluate the MPs and NPs effects at level of soil inhabiting biota and the relevance at ecosystem level. Strategic will be our ability to apply the metagenome, amplicon sequencing, metatrascriptomic and proteomics to give insights into ecosystem functioning. Finally, it is important to detect and characterize the fate of additives of plastic products (plasticizers, retardant, antioxidants, and photostabilizers) and the adsorbed chemicals by plastics in soil. The ecological relevance of the MPs and NPs fate and behavior in soil will allow better definition of the risk to the human food chain and the effects on human health. The final considerations are the relevance of MPs and NPs as universal antropogenic stressors with ignored environmental consequences and the infancy of the current research on this topic has been highlighted.

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