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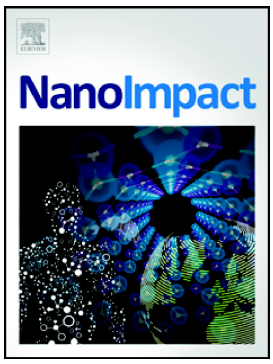

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PII: S2452-0748(23)00024-1

DOI: https://doi.org/10.1016/j.impact.2023.100473

Reference: IMPACT 100473

To appear in: *NANOIMPACT*

Received date: 11 March 2023

Revised date: 20 June 2023

Accepted date: 28 June 2023

Please cite this article as: D. Masson, M. Pédrot, M. Davranche, et al., Are nanoplastics potentially toxic for plants and rhizobiota? Current knowledge and recommendations, *NANOIMPACT* (2023), https://doi.org/10.1016/j.impact.2023.100473

Are nanoplastics potentially toxic for plants and rhizobiota? Current knowledge and recommendations

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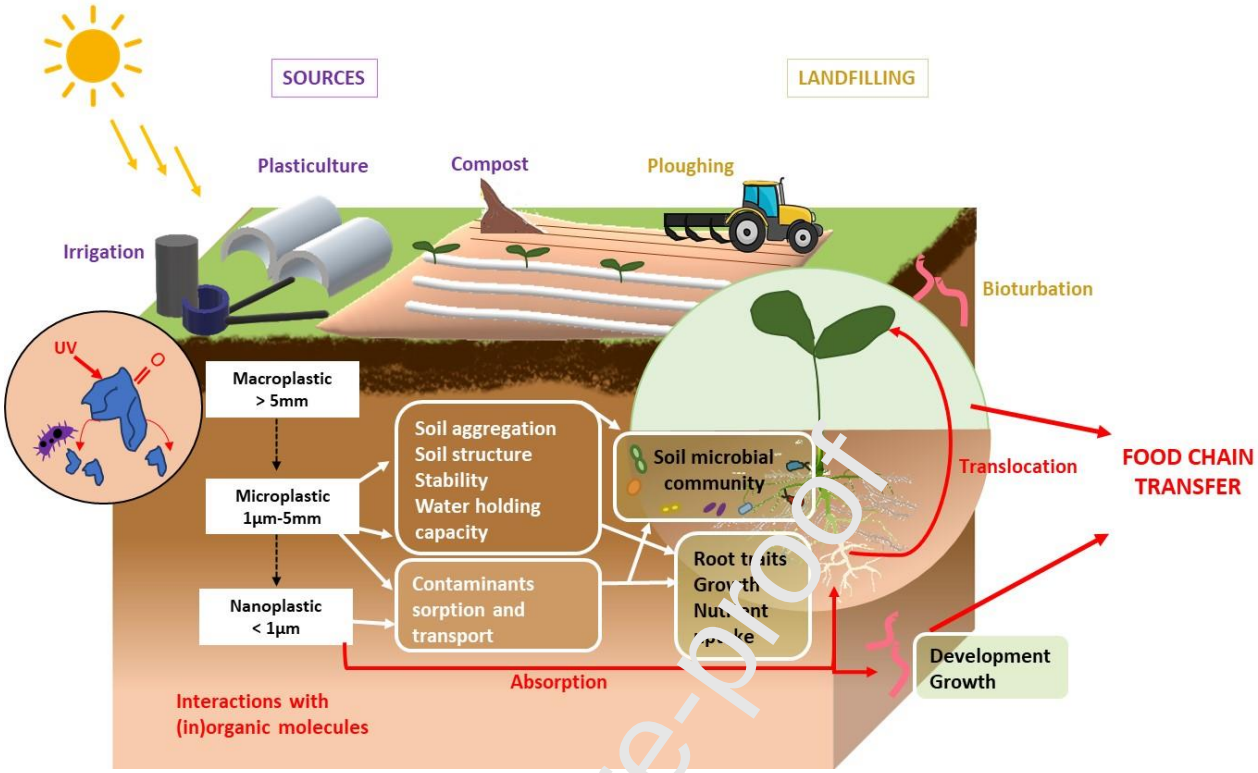
Abstract

Soil is now becoming a reservoir of plastics in response to global production, use/disposal patterns and low recovery rates. Their degradation is caused by numerous processes, and this degradation leads to the formation and release of plastic nanoparticles, i.e., nanoplastics. The occurrence of nanoplastics in the soil is expected to both directly and indirectly impact its properties and functioning. Nanoplastics may directly impact the physiology and development of living organisms, especially plants, e.g., by modifying their production yield. Nanoplastics can also indirectly modify the physicochemical properties of the soil and, as a result, favour the release of related contaminants (organic or inorganic) and have an impact on soil biota, and therefore have a negative effect on the functioning of rhizospheres. However all these results have to be taken carefully since performed with polymer nano-bead not representative of the nanoplastics observed in the environment. This review highlight thus the current knowledge on the interactions between plants , rhizosphere and nanoplastics, their consequences on plant physiology and development in order to identify gaps and propose scientific recommendations.

Keywords : nanoplastic, ecotoxicity, plants, soils, rhizomicrobiota, physico-chemical conditions

Graphical Abstract



Expected plastic cycle in the soil system

1. Introduction

Most scientific studies dedicated to the environmental dissemination of plastics are focused on their source, occurrence, and impact on living organisms in the marine environment. However, less studies were interested on the arrival, fate and degradation, of plastics debris in terrestrial environments as compared to marine environment, even though their presence were attested in rivers and soils (Dris et al., 2018; Rillig, 2012; Wahl et al., 2021) . Moreover, although microplastics have received much attention (de Souza Machado et al., 2019), there is a lack of knowledge regarding the nano-sized fraction of plastics debris, i.e., nanoplastics from 1 to 1000 nm. However, they were recently detected in plastic-contaminated soil (Wahl et al., 2021). Nanoplastics are released into the environment as engineered nanoplastics (from paints, medical applications, electronics, coatings, adhesives), so-called primary nanoplastics either as secondary nanoplastics generated unintentionally by the degradation of larger plastic debris (Gigault et al., 2018, 2016). As engineered particles, engineered nanoplastics are produced as nano-beads although they are largely asymmetrical-shaped when produced under environmental conditions (**Figure 1**).

a) b)

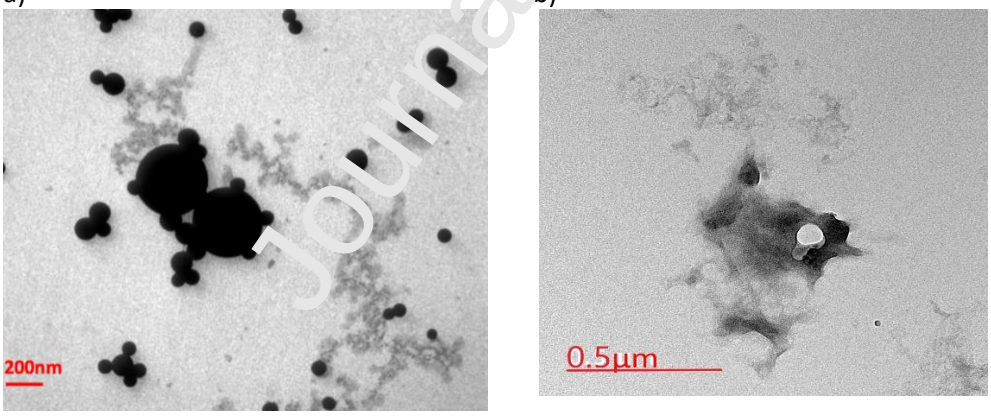


Fig. 1 Transmission electron microscopy observation of a) Engineered nanoplastics (nano-beads of polystyrene generally used as standard/model for nanoplastics) b) a nanoplastic extracted from a soil contaminated with a household compost enriched in plastic debris (Wahl, 2022) showing the asymmetrical shape

The size of the nanoplastic is the major parameter controlling their properties (i.e., surface reactivity), mobility and bioavailability. Although nanoplastics are considered to be mainly retained in porous media (Pradel et al., 2021) and the risk of vertical transfer is minimal (Bläsing and Amelung, 2018), nanoplastics can be formed at depth through agricultural practices such as plowing or by bioturbation

(Huerta Lwanga et al., 2017; Maaß et al., 2017). This potential occurrence of nanoplastics in the uppermost layer of soil and in the rhizosphere, could have a significant impact on the development and production of plants. Due to their nanometric size, they are likely to be absorbed by plants. This absorption is the first step for their transfer into the food chain. However, some studies have been dedicated to the interaction between nanoplastics and plants as well as their potential toxicity for plants. But, the majority of these studies has focused on marine and fresh water species and on the micro to nanoplastics size continuum (Azeem et al., 2021; Horton et al., 2017; Kalčíková, 2020; Li et al., 2023; Lian et al., 2020a; Mateos-Cárdenas et al., 2021; F. Wang et al., 2022; Yin et al., 2021). The few studies that are available have shown reduction in the germination rate and growth abnormalities (Azeem et al., 2022; Giorgetti et al., 2020; Pflugmacher et al., 2020a). One way to circumvent this lack of knowledge is to assume that nanoplastics behave similarly to nanoparticles as already performed in a recent review dedicated to the impact of nanoplastics-plants interactions on soil (Zhou et al., 2023). The approach that consist to learn from the similarity and difference with engineered nanoparticles was also already used for microplastics (Hüffer et al., 2017). Due to their nanometric size, nanoplastics are also likely to be translocated and accumulated in plants (Azeem et al., 2021; Li et al., 2015; Sun et al., 2020). As ionized nanoparticles like metallic nanoparticles, nanoplastics can bind many other pollutants (organic or inorganic) on their surface and can therefore act as a Trojan horse by releasing their pollutant loading within the plant (Dang et al., 2022; Sun et al., 2023; Zhang and Xu, 2022) which is expected to produce reactive oxygen species (ROS) (Domingo et al., 2019). Nanoplastics may therefore impact the health, development, and productivity of plants. Their presence in soils is therefore becoming a global environmental concern.

Although this context, all the reviews interested into plastic impacts on plants have considered micro and nanoplastic as a whole group with a size continuum (**Figure 2** and Azeem et al., 2021; F. Wang et al., 2022; Yin et al., 2021). However micro and nanoplastics have different properties such as their size, shape, specific surface aera, charge and functional surface group density, stability in solution and what is often forgotten, their additives amount (Gigault et al., 2021). The smaller size of nanoplastics allow

to penetrate different biological barriers while microplastics do not as demonstrated from different polymer for fauna (Sorensen and Jovanović, 2021). Fadare et al., (2019) observed that polystyrene nanoparticles (PS-NPs) are more toxic to *Daphnia magna* than PS microplastics in aquatic systems. Their toxicity to plants also differs, Azeem et al. (2022) demonstrated, for example, that nanoplastic modify plants root morphology although microplastics affect germination but not root. Gaylarde et al., (2021) explained that nanoplastics are more hazardous and with different effects than microplastics It is therefore no pertinent to study micro and nanoplastic as a whole group. Another reason explaining why micro and nanoplastics were considered as a size continuum group with quite similar properties is that the proof of nanoplastic occurrence in soil is very recent (Wahl et al., 2021). The consequence is a strong lack of data for their toxicological impact on plants and information can only be extrapolated/deduced from studies using polymer nanoparticles such as polymer nanobeads (ex. Polystyrene nanoparticles, PS-NPs) (**figure 2 and 1a**). Some papers were devoted to the impact and plants uptaking of PS-NPs (Li et al., 2021; Liu et al., 2022; Sun et al., 2021; Zhu et al., 2022). However, the comprehensive impact of different types, shapes and sizes of environmental nanoplastic on terrestrial plants has not been yet sufficiently considered (Zhu et al., 2022). Internal bioavailability of nanoplastics for terrestrial plants and interaction mechanisms in rhizosphere are a key goal for future investigations (Hua et al., 2023). Understanding the features of the bioavailability of nanoplastics in plants from the soil will make it possible to find effective plant species for soil phytoremediation. In addition, the Trojan horse effect as a phenomenon of possible enhancing the bioavailability of organic and inorganic compounds adsorbed onto nanoplastics remains little studied (Dang et al., 2022; Sun et al., 2023; Zhang and Xu, 2022). Thus, the novelty of research on the impact of nanoplastics on plants, especially terrestrial plants, is beyond doubt.

All these statements are partially illustrated in the figure 2 corresponding to the bibliometric network of the co-occurrence keyworks made with a publications database (353 from 2015 to 2023) collecting from the web of Science using plants and nanoplastics entry keywords. Figure 2 confirms that 1) nanoplastics and microplastics are often associated in research papers and reviews, 2) polystyrene is

the most considered polymer for nanoplastics, 3) few studied are dedicated not only to plants but also to phytotoxicity, oxidative stress and plant growing. Finally, figure 2 shows that studies interested to quantification are recent and scarce.

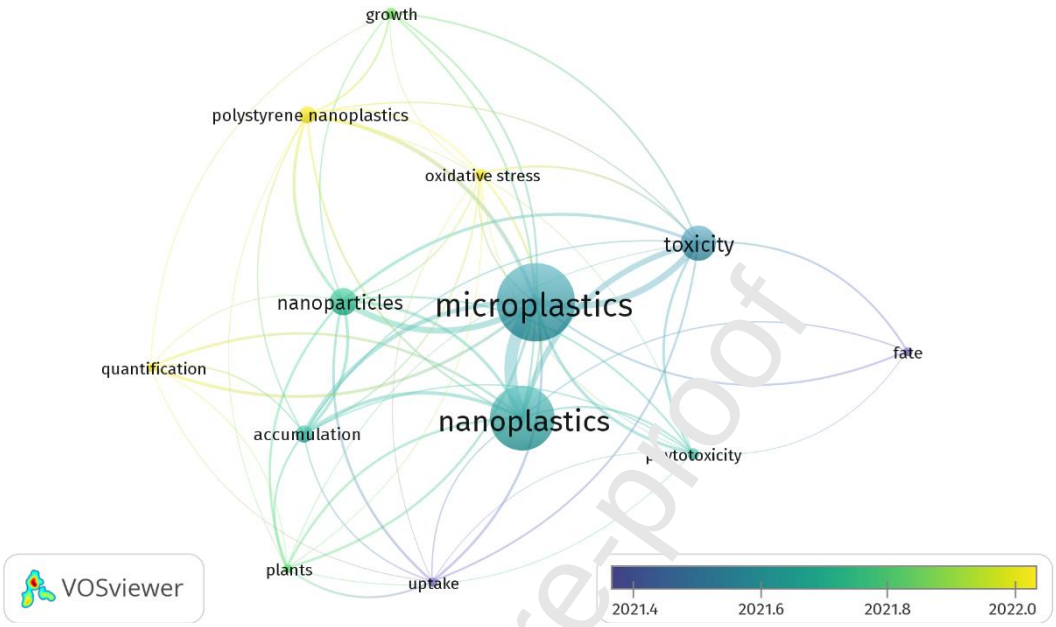


Figure 1 : Bibliometric network visualization for the co-occurrence of the relevant keywords performed with VOSwiever

Hence, the present review addresses the impacts of nanoplastics on plants and highlights knowledge gaps and prospects for future studies in the field. This review focuses more particularly on secondary nanoplastics produced under environmental conditions. For simplicity, these secondary nanoplastics are just called nanoplastics. We are here more specifically interested in these nanoplastics because they are expected to be more numerous than engineered nanoparticles, notably in soils. The use of primary nanoplastics is indeed very limited as manufacturing is difficult and expensive. Considering that under environmental conditions, nanoplastics come from the degradation of plastics debris, Besseling et al., (2019) estimated that NP concentrations is 10^{14} times higher than those currently measured for microplastics (MPs).

2. Methodology for review.

In a first step, potential available literature dedicated to plants and nanoplastics interactions was compilated through a screening of peer-reviewed researches and reviews, collected from Web of

Science from 2015 to 2023 (**Figure 2**) with plants and nanoplastics as entry keywords. The final database comprising 353 papers was analyzed using the bibliometric mapping tool, VOSviewer. The co-occurrence of the keywords allowed to create a bibliometric network map (**Figure. 2**). The map show that nanoplastics and microplastic generally co-occurred together and with all the terms but more peculiarly with "toxicity, "nanoparticles ," and 'accumulation". The term plants and phytotoxicity co-occurred in a lesser extend with nano and microplastics. We finally selected 107 papers from this database that were completed, when necessary, with papers found in other well-recognized databases (i.e. Scopus and ScienceDirect). The selected studies were relevant reviews or experimental studies, 1) allowing to precisely describe the nanoplastics properties, 2) dedicated to the impact of nanoplastics models (mainly PS-nanobeads) on terrestrial plants (under hydroponic or soil conditions) and 3) since this literature was scarce, we also used literature focusing on the impact of polymer and metallic (in a small order) nanoparticles/nanomaterials on terrestrial plants as already performed in previous review (Zhou et al., 2023). Sometimes, because of the lack of information on the metabolic and physiological impact on terrestrial plants, articles relating to microplastics were also used.

In the following text, NPs referred to nanoparticles and the full name was used for nanoplastics and microplastics.

3. What are nanoplastics? Source, properties, and role as a carrier of contaminants.

3.1. Definition

Two categories of nanoplastics can be distinguished: 1) 'Engineered' nanoplastics which correspond to spherical nano-sized particles released directly into the environment from industrial processes and products (ex. **Figure 1a)** and 2) 'Environmental' nanoplastic which enters the environment with plastic debris degradation under environmental conditions (ex. **Figure 1b**). According to the International Standard Organization, the manufactured nanomaterial is 'intentionally produced for commercial purposes to have specific properties or specific composition' (ISO/TS 80004e1:2015). By contrast, 'Environmental' nanoplastics are unintentionally produced, directly under environmental conditions from the degradation of plastic objects and breakdown of plastic particles (Gigault et al., 2018; Jahnke

et al., 2017) (Figure 1b). In this present work, we are interested by this second category which is expected to be produced from all the plastics debris released in the environment and notably in the soil (ex. plasti-culture, landfills).

Nanoplastics have only recently been defined (Gigault et al., 2018) and observed in the environment (in the ocean: Ter Halle et al., 2017; in soil: Wahl et al., 2021). This definition includes the morphological and colloidal properties of nanoplastics observed in the environment. Nanoplastics are unintentionally produced poly-dispersed nano-sized particles (size continuum), varying in size from 1000 to 1 nm. They have a heterogenous and asymmetrical shape with an open structure (Blancho et al., 2021; Gigault et al., 2018) (**Figure 1b**). They exhibit a colloidal behaviour that controls their stability in solution relative to the physiochemical condition that may involve homo- or hetero-aggregation. This plastic (polymer + additives) nanoparticles have thus a large specific surface area and a negative surface charge controlled mainly by the density of the -COOH site resulting from the oxidation of the pristine polymer as demonstrated by several authors via FTIR analysis, potentiometric titration and surface binding site tracers (Blancho et al., 2022, 2021; Prunier et al., 2019). Thus, 'environmental' nanoplastics are able to bind numerous organic and inorganic contaminants.

Note that nanoplastics differ from 1) microplastics in terms of size (<1000 µm against 5mm to 1000 µm), shape (strongly heterogeneous against small piece), reactivity (higher specific surface aera and site density for nanoplastic, and behaviour in solution (colloidal stability against floating) and from 2) polymer nanobeads/nanoparticle

es (mainly PS-NP in literature) in term of composition (with additives against no additives), shape (strongly heterogeneous against round), reactivity (carboxylic sites against with or without chosen grafted chemical groups). Therefore, nanoplastics behaviour and impact can only be partially deduced from microplastic and polymer-NPs.

3.2. **Mobility in soil**

In soil, the mobility of nanoplastic depends on the soil pH and solution pH, soil mineralogy, organic matter, NPs surface functional groups and nanoparticle size(Velzeboer et al., 2014; Wang et al., 2021).

Wu et al. (2020) showed that the mobility of nanoplastics increased with the increasing soil pH in response to the increasing electrostatic repulsion between PS-NP and soil particles (recovery of PS-NPs ranging from 0 to 96% and pH increase from 5 to 10). They also showed that the transport of PS-NPs was significantly influenced by both the ionic strength and the cation present in the soil solution. The transport of PS-NPs significantly decreased with the increase in ionic strength due to the compression of the electrical double layer, leading to a decrease in the negative charge of both soil particles and PS-NPs, thereby reducing the repulsive forces between PS-NPs and soils (Wu et al., 2020).

Nanoplastic transport can be limited by the attachment of particles to the mineral surfaces in the immobile substrate. These attachment mechanisms between PS-NPs and the substrate are often similar to the interactions between NPs and colloidal or aqueous components (Brewer et al., 2021; Song et al., 2019). Due to the greater stability of the particles, larger PS-NPs particles are more mobile. The presence of humic acid, which is part of the soil humus, can increase the migration of PS-NPs in response to the formation of organic matter coating the PS-NPs surfaces, which prevents the aggregation of nanoparticles (Brewer et al., 2021; Dong et al., 2020; Song et al., 2019; Wang et al., 2021).

The mobility of nanoparticles in porous media is also affected by the aggregation and resulting size increase that controls the absorption of nanoplastics by plants (Lowry et al., 2012). Aggregation can be a homo-aggregation between the nanoparticles themselves or a hetero-aggregation with colloids. Due to the lower expected concentration of nanoplastics compared to natural colloids, there is negligible homo-aggregation (Labille et al., 2015). Besseling et al. (2017) showed that nanoplastics can hetero-aggregate with clay colloids. The aggregation efficiency increases with the decreasing plastic particle size. Soil clays are considered as important sinks of NPs (Usman et al., 2020). The hetero-aggregation of nanoplastics also increases with biofilm formation (through the sticky action pf polysaccharides) (Besseling et al., 2017). By contrast, the coating of organic molecules tends to decrease aggregation through the effects of electrostatic repulsion and steric hindrance thereby ensuring the stability of the

NPs and nanoplastics (Lowry et al., 2012; Pradel et al., 2021). By modifying the surface properties of the NPs, the coating can potentially mitigate the impact of the NPs (Lin et al., 2008).

More recently, Gao et al. (2022) have demonstrated that the transfer of metallic elements (i.e., As and Cd) may be indirectly controlled by the occurrence of PS-NPs. They demonstrated an increasing mobility of As with PS-NPs. Through the development of a displacement model, they demonstrated that the increased As mobility resulted from competition between the negatively charged NPs and As for the soil binding sites.

3.3. **Carrier of potential plant contaminants**

Plastic debris include many other organic or inorganic contaminants potentially adsorbed on their surface, or trapped in their structure as additives used in the formulation of plastics (Hermabessiere et al., 2017; Rochman, 2015). The toxicity of nanoplastics may therefore be related to the release of these associated contaminants in response to the physicochemical conditions inside and outside the plants.

Because of their nanometric size and their surface properties, nanoplastics potentially have a high sorption capacity for many organic and/or inorganic contaminants. Among those contaminants, the interaction of metallic elements with nanoplastics has recently been taken into account (Blancho et al., 2022; Davranche et al., 2019). Although metallic elements are widely used as additives in the formulation of plastics, it is not expected that they can persist in nanoplastics because of their nano size and high degree of alteration. But, they can be bound to them (Blancho et al., 2022; Davranche et al., 2019). Catrouillet et al. (2021) demonstrated that metals adsorbed on the altered layer of microplastics, which is responsible for the release of nanoplastics, mainly originated from the metallic additive solubilized by the degradation of the plastic. Many metallic trace elements are classified as harmful to organisms (ex. Pb , Ni, Cr,…) even at low concentrations due to their high toxicity and their carcinogenic effects. They are generally used as catalysts for polymerization (compounds based on Zn, Sb, Sn, Ti or Al), as a pigment (which may contain Cd) or as a stabilizer (e.g. Pb) (Becker et al., 2010; Nakashima et al., 2012). Despite these benefits, metallic trace elements are known to be potentially

toxic to fungi, flora and fauna. Their release into the environment in response to plastic degradation (Wang et al., 2017) or their association with nanoplastics are of concern. The adsorption of Pb and rare earth elements on standard and environmental nanoplastics has already been demonstrated (Blancho et al., 2022; Davranche et al., 2019). This high affinity of nanoplastics for metals is explained by their large specific surface area and their functional surface sites, mainly -COOH) resulting from the photooxidation of polymers (Blancho et al., 2022, 2021; Wang et al., 2017, 2020). Electrostatic attraction, surface complexation and intra-particle diffusion are the main adsorption mechanisms evoked to explain this (Blancho et al., 2022; Davranche et al., 2019). Lian et al. (2020b) showed that nanoplastics reduced Cd toxicity for wheat plants in response to a decrease in the Cd concentration resulting from Cd adsorption by nanoplastics. This sorption capacity also allows nanoplastics to act as a Trojan horse when absorbed by plants in response to the physicochemical variations (pH, ionic strength, competitive ligands, or ions, etc.) inside the plant and plant organs (Dang et al., 2022; Zhang and Xu, 2022). Although no data are available to demonstrate this process, Hodson et al. (2017) observed a high desorption of Zn from microplastics (40-60%) in the intestine of earthworms (*Lumbricus terrestris*) and concluded that plastics may increase the bioavailability of associated ETMs. Moreover, when nanoplastics and ETMs are simultaneously present, it is expected that a cocktail effect will be produced. The synergistic effect potentially increases the ecological risks. Although no data are available for the ETM-nanoplastics combination, chronic toxic impacts and the increased bioaccumulation of ETMs in marine organisms have been demonstrated for a co-exposure to microplastics and ETMs (Kim et al., 2017; Zhu et al., 2020).

Nanoplastics are also expected to affect the fate of persistent hydrophobic organic pollutants (POPs), such as PCBs and PAHs (Liu et al., 2018), in the environment. The difference in the polarity of organic pollutants also results in different sorption capacities (Brewer et al., 2021; Wang et al., 2021) Polar compounds bind to nanoplastic parts via surface adsorption, although non-polar or weakly polar compounds tend to be adsorbed in the internal matrices of the nanoplastics (physical capture) (Liu et al., 2018; Wang et al., 2021). Consequently, the bioavailability of organic pollutants associated with

nanoplastics is varied. Studies of PCB sorption by nanoplastics have shown that sorption by nanoplastics is 1-2 orders of magnitude higher than that of microplastics. The sorption parameters differed depending on the PCB congener. Velzeboer et al. (2014) obtained the highest Freundlich constant (*log Kf*) for $PCB_{180,\ 126}$, and the lowest for PCB_{44}. This is especially important for dioxin-like PCBs, which are extremely toxic to humans with toxicity equivalence factors TEF for PCB 126 equal to 0.1 (*Recommended Toxicity Equivalency Factor for Human Health Risk, EPA, USA, 2010*). Because PCBs are persistent organic contaminants, their detoxification in the environment resulting from sorption onto nanoplastics can be further slowed down. In addition, the probability of bioaccumulation of lipophilic PCBs in adipose tissue with nanoplastics, which penetrates the biota, increases.

4. Direct impacts on the health, development, and production of plants : potential toxicokinetic and toxicodynamic

Currently, as far as we are aware, very few studies have been carried out on the impact of nanoplastics on terrestrial plants. However, the first observations are worrying and show, for example, an accumulation of nanoplastics in plants and toxicity effects (Azeem et al., 2021; Li et al., 2023; Lian et al., 2020a; Mateos-Cárdenas et al., 2021; Sun et al., 2020).

4.1. Toxicokinetic, absorption and translocation

The processes of toxicokinetic, absorption, translocation and accumulation of contaminants vary according to plant species due to anatomical and physiological differences (W. Wang et al., 2022). Particles with a diameter <6 nm can generally penetrate the cell wall (Carpita et al., 1979). However, nanoplastics can penetrate plant cells, even when their size is greater than 6 nm. The presence of nanoplastics can damage the cell wall and modify the size of pores (Maity and Pramanick, 2020). While NPs affect the porosity of the cell wall, their uptake by plants can also be affected leading to the absorption of larger nanoplastics, as listed in Table 1. Bandmann et al. (2012) showed that the 20 and 40 nm Polystyrene nano-beads (PS-NPs)penetrate tobacco BY-2 cells by endocytosis, whereas 100 nm beads are excluded. Using confocal laser scanning microscopy, Jiang et al., 2019 demonstrated that 100 nm PS-nanoparticles can penetrate bean roots (*Vicia faba*), probably through the root epidermis,

and accumulate in the root tissues. Polystyrene-NPs (100 nm) were detected in the root xylem and stems of wheat (*Triticum aestivum*) (Lian et al., 2020b). Li et al. (2020a) highlighted the entry of larger NPs (200 nm) into wheat roots through Casparian band defects. The Casparian strip in the root endodermis is known to play an apoplastic barrier role during radial nutrient transport (Barberon and Geldner, 2014; Peterson, 1987). However, the effectiveness of this barrier may be reduced in the young parts of the roots where the Casparian strip is not fully developed or at sites where the Casparian barrier is not present, e.g. at sites of lateral root emergence (Barberon and Geldner, 2014). It therefore becomes possible for these 200 nm plastic beads to enter into the apical meristem through these cracks (Li et al., 2020a). Fluorescent labelling also showed the presence of PS-NPs in the stem and leaves, suggesting that PS-NPs diffuse through the apoplastic spaces of the apical zone into the vessels of the root xylem, which makes their rapid transport into the wheat roots possible. Li et al. (2020a, 2020b) confirmed these observations in two hydroponic culture studies. In lettuce (*Lactuca sativa*) and wheat they showed that toxicokinetic consisted in absorption followed by the translocation of PS-NPs (200 nm) in the vascular system and the cell walls of the root tissues. Homo-aggregates cluster or chain-like aggregates of PS-NP were observed in the intercellular spaces of the vascular tissues in the root and stem of lettuce plants (Li et al., 2019). For wheat plants, PS-NPs were observed in the root, stem, and leaf cells. After penetrating the central cylinder of the root (the stele), PS-NPs are likely to move to the aerial parts of the plant through the vascular system (Li et al., 2020b). The extent of NPs translocation may depend on the transpiration flow of the plant: high transpiration leads to increased accumulation of NPs in the roots and upper parts of the plant, transpiration could represent a major driving force for an upward movement (Z. Li et al., 2020). A recent study highlighted the presence of PS-NPs (300 nm) in cucumbers (*Cucumis sativus*) (Z. Li et al., 2020). For crop plants, the absorption, toxicokinetic, and translocation of nanoplastics could constitute a health hazard that would add to the ecological risk (Bouwmeester et al., 2015). The absorption of plastic by plants might have implications for other trophic levels, which could pose a potential risk to food yield, quality, and safety (**Figure 2b**). Even though this more likely concerns root crops, the transport of nanoplastics from the roots to the

leaves via the xylem allows them to reach all parts of the crops that are of agronomic interest and to accumulate there, as observed in mung beans (*Vigna radiata*) where PS-NPs accumulated in the leaves. The contaminated leaves were then ingested by giant African snails (*Achatina fulica*) which caused growth and behavioural problems in animals (Chae and An, 2020)

4.2. ***Factors influencing absorption***

The absorption and translocation of NPs within plants do not only depend on the size of the NPs but also on their physicochemical properties, particularly their surface charge. Spielman-Sun et al. (2017) found that CeO_2(+) NPs adhered most easily to the roots of wheat plants due to their higher affinity with negatively charged cell walls (presence of surface carboxyl groups). After 34 h of exposure, the NPs concentrations of CeO_2(-) and CeO_2(0) in the leaves are higher than those of the NPs of CeO_2 (+). Therefore, the translocation of NPs within the plant seems to favour neutral or negatively charged particles, probably due to electrostatic repulsions between NPs and electronegative cell walls (Spielman-Sun et al., 2017) (**Figure 3**). The same process was observed for nanoplastics (Bhattacharya et al., 2010), their adsorption on a cellulose film being promoted for positively charged particles. Kibbey and Strevett (2019) studied the interaction of sulphate-modified (negative) and amine-modified (positive) PS-NPs with root surfaces. It was found that only positively-charged PS-NPs attached to the root surfaces, confirming these observations. However, (Sun et al., 2020) observed a stronger uptake and internalization of the negative PS-NPs into the roots of *Arabidopsis thaliana* by studying the absorption of PS-NPs (sulphate-modified (negative) and amine-modified (positive) - 55-70 nm). This result could be explained by a greater exudation of oxalate in the presence of positive PS-NPs than by treatment with negative PS-NPs. The absorption probably occurred via the root hairs and the PS-NPs were then directed to the stele in an apoplastic manner. The size of the positively charged PS-NPs aggregates increased with increasing oxalic acid concentrations, whereas the size of the negatively charged PS-NPs remained unchanged. Therefore, the aggregation of nanoplastics onto the root surfaces could affect their mobility and absorption. The large size of the positive PS-NP aggregates

may make it difficult for them to penetrate into the root tissue. The positive PS-NPs preferentially remained adsorbed on the root hairs which caused greater epidermal damage (Sun et al., 2020).

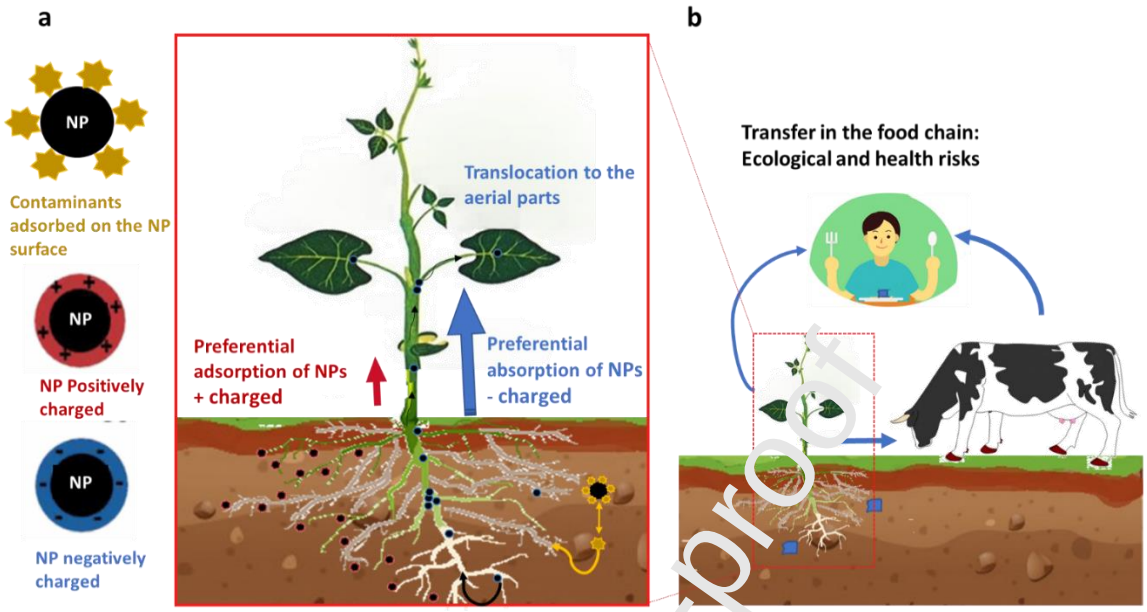


Fig. 3 a) Absorption and translocation of nanoplastics (NP) within the plant: influence of surface charge and the potential release of contaminants. b) Risk of transfer into the food chain via soil microbiota and rhizomicrobiota.

4.3. ***Effects on plant development***

An increasing number of studies are dedicated to the effects and toxicodynamic of microplastics on the development of terrestrial plants. Pflugmacher et al. (2020b) noted a decrease in the germination rate of watercress (*L. sativum)* in the presence of microplastics (3 mm). They highlighted the significant toxicity of plastic leachates, whose presence caused a 77% decrease in the germination rate. The toxicodynamic of microplastic leachates was also observed by Balestri et al. (2019) who showed developmental abnormalities (deformed seedlings) and reduced growth (shorter radicle than the control plants). A significant decrease in above-ground and root biomass was observed in the presence of PE, PET, PP, PS; PE microplastics (de Souza Machado et al., 2019) and biodegradable PE microplastic (Qi et al., 2018). Studies that specifically focus on the effects of nanoplastics are scarce and, to our knowledge, only concerned PS-NPs. Nevertheless, the available studies showed that PS-NPs (≤ 300 nm) have similar effects on biomass, i.e. a decrease in the dry and fresh weight of the underground parts (Jiang et al., 2019; Sun et al., 2020) and aerial parts (Chae and An, 2018; Z. Li et al., 2020; Sun et al.,

2020). Several authors have pointed out anomalies in root development such as altered growth. This alteration is reflected either by the decrease in mean diameter observed in mung beans (by about 82.5%) and cucumbers in the presence of 300 nm (50 mg L^{-1}) and 28 nm (10-100 mg kg^{-1} soil) PS-NPs, respectively, or by a decrease in root length. This was also observed for onion plants (*Allium cepa*), for which the root length decreased by 41.5% in the presence of 50 nm (1 g L^{-1}) PS-NPs compared to the controls (Giorgetti et al., 2020). These observations may be due to a blockage of cell connections or pores in the cell wall triggered by the nanoplastics (Jiang et al., 2019). This physical blockage could cause a disturbance in water absorption needed for normal imbibition, germination, and primary root growth (Bosker et al., 2019) or it could affect the absorption and transport of nutrients by the plant (Jiang et al., 2019; Sun et al., 2020). Note however that germination was only delayed in this case (Bosker et al., 2019). Similar effects were observed on other plants exposed to nanoplastics (Table 1). However, the nanoplastics-related toxicity to plants is expected to be variable with the species, the cultivation practices (hydroponic or soil) or the plastic properties. For example, positively charged nanoplastics promotes the plant development (Sun et al., 2020). Lozano et al. (2022) observed an increasing seed germination synchrony of *Daucus carota* L., probably related to the occurrence of mild stress on seeds. Lian et al. (2020) showed that the incorporation of PS-NPs (100 nm, 10 mg L^{-1}) in a hydroponic solution did not significantly impact the plant growth. These variations in the plant response may also be partly related to the various concentrations of nanoplastics used (van Weert et al., 2019). The phytotoxicity induced by nanoplastics remains thus to be explored in coherence with the environmental concentrations and conditions.

Table 1
Physiological impacts due to the presence of plastic nano- and microparticles

Species	Plastic/Diameter	Experiment	Observations	References
*Scenedesmus (*multicellular green alga*)*	PS 20nm	Hydroponics	Decreased photosynthetic activity	(Bhattacharya et al., 2010)
	PS 70 nm	Hydroponics	Decreased growth rate *Decrease in chlorophyll concentration	(Besseling et al., 2014)
Vicia faba (bean)	PS 100 mg L^{-1} 100 nm	Hydroponics 10, 50, 100 mg L^{-1}	Reduction in the weight of the roots Decreased roots elongation	(Jiang et al., 2019)
Triticum aestivum L. (wheat)	PS 100 nm	Hydroponics 0.01-10 mg L^{-1}	No significant difference in germination rate Root elongation	(Lian et al., 2020b)
	PE (bioPE) 50 µm-1mm	Microcosm soil 1% p/p	Decrease in aboveground and root biomass	(Qi et al., 2018)
Allium fistulosum (spring onion)	PE-PET-PP-PS	Microcosm soil 2% p/p	Decrease in aboveground and root biomass Change in the basic composition of tissues	(de Souza Machado et al., 2019)
Lepidium sativum (watercress)	PC 3 mm and leachate	Hydroponics Microcosm soil	Decreased germination rate Higher leachate toxicity compared to microplastics	(Pflugmacher et al., 2020)
	Leachate HDPE	Solution	Developmental anomalies Reduced seedling growth	(Balestri et al., 2019)
Vigna Radiata (mung bean)	PS 28 nm	Microcosm soil 10 – 100 mg Kg-1 of soil	Reduction in the weight of the aerial parts Altered root growth: decrease in average root diameter	(Chae and An, 2020)
Arabidopsis thaliana (Ladies cress)	PS-SO3H 55 nm PS-NH2 71 nm	Microcosm soil 0.3 – 1 g Kg^{-1} of soil	Reduction in the weight of the aerial and root part	(Sun et al., 2020)

			Decrease in chlorophyll content Generation of ROS in the roots Variation according to the load of the PS	
Cucumis sativus (Cucumber)	PS 100-700 nm	50 mg L^{-1}	With 300 nm: Decrease in the average root diameter. Reduction in the weight of the aerial parts Reduction in the stem length	(Z. Li et al., 2020)
Allium cepa (onion)	PS 50 nm	0,01 – 0,1 – 1 g L^{-1}	With 1g L^{-1}: Reduction in the root length	(Giorgetti et al., 2020)
Zea mays (maize)	PS-COOH 24 nm PS-NH_2 22 nm	Foliar exposure 1 mg L^{-1}	Inhibitory effect on photosynthesis and a stimulation to the activity of antioxidant systems (with a higher effect for PS-NH_2)	(Sun et al., 2021)
Zea mays (maize)	PS 100-500 nm	50 mg L^{-1}	No effect on photosynthetic characteristics Damaged on the root microstructure Stimulation to the activity of antioxidant systems	(Zhang et al., 2022)
Oryza sativa (rice)	PS 50 nm	0.1 – 1 g L^{-1}	Affected root cell ultrastructure, the germination process, seedling growth and root mitotic activity	(Spanò et al., 2022)

4.4. ***Cellular and molecular modifications***

The profound impact of nanoplastics at the cellular and molecular levels is well documented for animals and other organisms (Rai et al., 2021). However, the first insights into the cellular and molecular impacts of nanoplastics on terrestrial plants have only recently been observed. To the best of our knowledge, (Jiang et al., 2019) carried out the first study on these aspects; these researchers evaluated the oxidative potential, cytotoxicity, and genotoxicity of PS-NPs of different sizes (100 nm and 5 µm) and concentrations (10, 50 and 100 mg ml^{-1}) on faba bean plants (*Vicia faba*). They reported that NPs accumulate in the root tips and that they pose a greater threat to *V. faba* than micro-sized NPs at a 100 mg L^{-1} concentration. Thus, 100 nm PS-NPs produced more ROS, resulting in higher oxidative stress, cytotoxicity, and genotoxicity as indicated by increased lipid peroxidation, reduced mitotic index, and increased micronucleus (MN) frequency, notwithstanding the increased activity of superoxide dismutase and catalase antioxidant enzymes. It is interesting to note that lower concentrations of 100 nm PS-NPs led to a higher increase in the antioxidant defence mechanism and helped maintain the redox balance while avoiding oxidative stress and genotoxicity. The importance of oxidative stress driving the phytotoxicity of nanoplastics was later highlighted by (Z. Li et al., 2020)who were working with cucumber plants in hydroponics exposed to 50 mg L^{-1} of PS-NPs of different sizes (100 to 700 nm). Thus, PS-NPs increased oxidative stress (lipid peroxidation, H_2O_2 and proline) and certain enzymatic antioxidants (peroxidase and ascorbate peroxidase activities and gene expression). In addition, they reduced other enzymatic antioxidants (catalase and superoxide dismutase activities and gene expression), photosynthetic pigments, and the soluble sugar contents of cucumber leaves, with an overall increasing adverse impact with increasing PS-NPs particle size (Z. Li et al., 2020)

Recently, Giorgetti et al. (2020) and Maity and Pramanick (2020) confirmed the cytotoxic and genotoxic potential of PS-NPs. They investigated the potential toxicity of 50 nm (25-400 mg L^{-1}) and 100 nm (10-1000 mg L^{-1}) PS particles on onion plants (*Allium cepa* L.), respectively. Both articles reported the induction of ROS production and lipid peroxidation, and ROS-induced cytotoxic (decrease in mitotic

index) and genotoxic (augmentation of chromosomal and nuclear abnormality indexes, and MN) effects. Some of the cytogenotoxic effects (or even oxidative stress as reported (Maity and Pramanick, 2020) started from the lowest doses. In agreement with their cytogenotoxicity, (Maity and Pramanick, 2020) showed that PS-NPs down-regulated the expression of the *cdc2* gene which encodes a plant cyclin-dependent kinase A, an important cell cycle regulator through interaction with D-type cyclins in the G1 phase (Qi and Zhang, 2020). It is worth noting that only (Giorgetti et al., 2020) who used the smallest PS-NPs, reported the internalization of PS-NPs into root tissues, but both studies provided evidence for the deposition of PS-NPs on the root surface. Other studies have also reported a toxic impact without the integration of plastic NPs in plant tissues, suggesting that these particles can be toxic to the plant without getting inside the plant tissue by blocking the pores on the surface of cell wall and seed coat (Bosker et al., 2019) or the pores on the root surface (Giorgetti et al., 2020; Maity et al., 2020; Sun et al., 2020).

In addition, both processes, penetration into plant tissues and cellular/molecular effects, were shown to be affected by the particle charge and chemical modification, as illustrated in Sun et al. (2020). They exposed *Arabidopsis thaliana* L. plants to different concentrations (0.3 g kg^{-1} and 1.0 g kg^{-1} in soil or 10, 50 and 100 mg L^{-1} in agar-medium) or two functionalized PS-NPs, sulfonic-acid- (PS-SO_3H, 55 nm) and amino-modified (PS-NH_2, 71 nm) PS-NPs. Both PS-NPs resulted in the accumulation of ROS (H_2O_2 and O_2^-) in the roots, but the positively charged PS-NH_2, which aggregated in the external medium and had limited accumulation in roots, induced a higher accumulation of H_2O_2 than the negatively charged PS-SO_3H NPs.

It is extremely complex to evaluate the toxicity of nanomaterials as multiple factors are involved. Given the cytogenotoxic potential of nanoplastics, it is necessary to supplement physiological dose-response studies and the targeted characterization of important biological functions, with comprehensive approaches such as omics techniques- the only approaches that can immediately identify the mechanisms of toxicity and those of detoxification and adaptation at once. To date, few metabolomic and one transcriptomic analysis have been carried out to determine the impacts of nanoplastics on

terrestrial plants. Lian et al. (2020b) studied the impact of PS-NPs (88 nm, 10 mg L^{-1}) on the metabolome in wheat leaves and reported that changes in the leaf metabolic profiles under PS-NPs stress were mainly linked to carbon (galactose, starch, and sucrose; and glyoxylate and dicarboxylate metabolisms), energy (TCA cycle) and amino acid (alanine, aspartate, and glutamate metabolism) metabolism regulation. This metabolic analysis highlighted the significant potential of PS-NPs to modulate wheat growth or development. In this regard, the metabolomic profiling of leaves from wheat plants grown in hydroponics with a PS-NPs and/or Cd treatment indicated that the positive effects of PS-NPs in terms of alleviating cadmium-induced oxidative damage were due to the concomitant stimulation of carbohydrate and amino acid metabolisms (Lian et al., 2020b).

Three recent transcriptome analyses have provided even more detailed information about the mechanisms involved in the response of plants to nanoplastics. Sun et al. (2020) confirmed that positively charged PS-NPs have a greater impact than $PS\text{-}SO_3H$. These authors also related the exposure to PS-NPs and the enhanced antioxidant chemical activities and tolerance/defense responses in A. thaliana roots as well as processes related to growth, photosynthesis, survival and response to abiotic stresses in A. thaliana shoots. (Zhou et al., 2021) also showed that PS-NPs improve certain antioxidant pathways and carbon metabolism in rice roots. Quite recently, (Lian et al., 2022) also showed that the exposure of wheat plants to PS-NPs leads to the higher expression of genes involved in photosynthesis pathways for carbon fixation (i.e., photosynthesis antenna, carbon metabolism, and pentose phosphate pathway) as well as several energy metabolism pathways sustaining plant growth. In contrast, different genes involved in responses to pathogen defences were down-regulated by PS-NPs in *A. thaliana* (Sun et al., 2020), rice (Zhou et al., 2021) and wheat (Lian et al., 2022). Moreover, transcriptomics analyses also determined that PS-NPs altered phytohormone homeostasis pathways such as jamonic acid (JA) in rice roots (Zhou et al., 2021) and wheat (Lian et al., 2022), as well as auxin and ethylene in wheat (Lian et al., 2022). Lastly, Lian et al. (2022) also showed that PS-NPs down-regulated genes involved in the metabolism and biosynthesis of amino acids in leaves, and in nutrient ion transport, which could ultimately impact the quality and yield of wheat seeds.

5. Impacts of nanoplastics and associated contaminants on soil biota

To assess the possible toxicological effects of nanoplastics (around 40 nm) in the soil and subsequently on plants, Awet et al. (2018) worked on the abundance of microbial biomass and the functional diversity of enzymes. Both aspects were frequently mentioned as suitable indicators of soil health. They showed that the presence of PS-NPs reduces microbial biomass and enzymatic activities in soils. They also suggested that PS-NPs exhibit antimicrobial activity in the soil environment. Extracellular enzymes play a key role in soil ecosystem functions, particularly in nutrient cycles and microbial metabolism. Although they seem to be less efficient in soil aggregation than bacteria and fungi (Lehmann et al., 2017), soil animals have a positive effect on the dynamics of organic matter and nutrients recycling. However, the presence of PS-NP (around 50 nm) was reported to have an impact on the bacterial diversity in the white worm (*Enchytraeus crypticus*) microbiome. A significant decrease in the abundance of proteobacteria was observed (Zhu et al., 2018). These proteobacteria families contain key microorganisms that contribute to the nitrogen cycle and to the breakdown of organic matter. The same authors also showed that the ingestion of PS-NPs resulted in significant weight loss. Kim et al. (2019) used the nematode *Caenorhabditis elegans*, which lives in the solid to liquid phase of rotting plant material, as a real-life model organism to show metabolic disruptions and toxic effects. Therefore, via a cascade effect, nanoplastics could strongly affect soil fertility and the resulting plant production (Van Groenigen et al., 2015). However, Kibbey and Strevett (2019) recently highlighted that the physicochemical surface properties of PS-NPs could control their impact on soil bacteria and plants. These authors demonstrated that when the PS-NPs surface was negatively charged (sulphate-modified 100 nm PS-NPs), the rhizosphere bacterial counts increased. In contrast, positively charged PS-NPs (amine-PS-NPS of 1000 nm), involve significant decreases in both rhizosphere bacterial counts, and plant root and stem growth were observed (Kibbey and Strevett, 2019).

PS-NPs could influence the root exudation of low-molecular-weight organic acids (LMWOA) which partly control the microbial activity of rhizospheres. As a result, Sun et al. (2020) observed an increase

in oxalate exudation by *A. thaliana*, notably with positively-charged PS-NPs thereby indicating that root exudation was influenced by the nanoplastic surface charge.

6. Conclusion and Prospects for further research

By compiling data from the literature, this work provides a review of the current knowledge on the potential toxicological (toxicokinetic and toxicodynamic) impacts of nanoplastics on plants and associated rhizomicrobiota as well as their controlling mechanisms. The occurrence and production of nanoplastics in soils was now proven. Most studies present nanoplastics as emerging pollutants able of exerting strong pressure on terrestrial ecosystems. They could modify the chemical properties of soil and soil biota and act as trojan horses for metallic pollutants coming from the soil or coming from the pristine plastic additives released by the plastic degradation. However, uncertainties still exist with regards to their positive and/or negative impact and the underlying processes. As described above for terrestrial plants, studies on the toxicity of nanoplastics were mainly focused on PS (82% of published studies; Shen et al., 2019), thus likely underestimating the toxicological impact of other polymers. Even though no studies are available on the cellular and molecular impacts of other nanoplastic polymers on terrestrial plants. The studies carried out with polymer nanoparticles, did not reach the same conclusions. Therefore, not enough data are available in the literature to back up the real toxicological impacts of nanoplastics alone, that is then still debated. Currently the only established consensus is that nanoplastics can act as a source and carrier of metal pollutants for plants. Moreover, there is a major lack of data on the nanoplastics behaviour when introduced in soils and, more importantly, on their impacts on plants/rhizomicrobiota interaction. The few existing field studies addressed the difficulties to collect and quantify nanoplastics under environmental conditions which have avoided to perform experimental studies under realistic environmental conditions. Based on this detailed review of the literature, we identified which further studies are crucial for determining the toxicity of nanoplastics from the soil to the plant.

- If the size continuum between micro and nanoplastics is an important issue to understand the overall environmental plastic contamination, it is important to consider that micro and

nanoplastic, in response to nanoplastic smaller size, behave differently in terms of physico-chemical properties and also toxicity. Therefore, further ecotoxicological studies have to be dedicated specifically to micro or to nanoplastics and not for the whole group.

- All the studies performed on the ecotoxicity of nanoplastics were forecasted from engineered nanoparticles or standard polymer nanoparticles .However their size, shape and surface properties are very different from those observed under natural conditions (Ter Halle et al., 2017; Wahl et al., 2021) (**Figure 1**). Therefore, all the conclusions drawn from previous studies must be validated with environmentally relevant nanoplastics models. In this perspective, new protocols to produce such models have recently been proposed (Blancho et al., 2021, El Hadri et al., 2020).
- Field monitoring of nanoplastics in soil-plant system has to be performed to highlight the real environmental surroundings of multifaceted processes of mutual influence of nanoplastics on soil and plants. To address this issue, various kind of soils, plastic contamination sources and time of plastics exposure in soils have to be identified.
- No information is available on the concentration of nanoplastics in soils and more generally in environmental samples. Therefore, a major effort must be made to develop quantitative analytical methodologies. Only the development of a multi-approach strategy combining new analytical methodologies could provide such information in such complex and heterogeneous media.
- No comprehensive tools for direct quantitative determining the nanoplastic in plant exist. This information is however absolutely necessary to determine accumulation factor or translocated amount. Most of polymer NPs quantitative methods in plants are indirect, for example by TOC method or metal tracer monitoring by ICP-MS. Direct quantification method based on efficient nanoplastics extraction using, for example, organic solvents (toluene, dichloromethane) and efficient detection/quantification methods (ex Py-GCMS) has to be developed.
- It is crucial to assess the plant exposure pathways under environmentally relevant physicochemical exposure conditions (in qualitative and quantitative terms) in order to determine the

toxicodynamics and toxicokinetics of nanoplastics and associated elements in plants. Such experiments must be combined with field experiments.

- Metrics have to be harmonized between the different studies since to allow comparison among studies and with environmental measurement whether they are measured or extrapolated.
- Knowledge of the interactions between nanoplastics and contaminants is needed to evaluate their potential cocktail and Trojan horse effects on plants. Adsorption models must be developed to identify and quantify the mechanisms involved. The potential of nanoplastics to promote bioaccumulation and the trophic transfer of the associated contaminants must also be clarified.
- The toxicological impact (toxicodynamic and toxicokinetic) of nanoplastics on plants depends on the biophysical parameters of soils, on fauna and microbial diversity and activity has therefore to be investigated. For example, the ability of nanoplastics to modify the soil pH, cation exchange capacity, aggregate structure, and their consequences on the alteration of soil microbiota (Boots et al., 2019).
- Lastly, little is known about the factors that influence the absorption, translocation, and fate of nanoplastics in plants/rhizomicrobiota, namely the toxicokinetic, apart from the surface charge. To date, no studies have investigated whether rhizospheric processes control the bioavailability of plastics in plants. Studies using several plant species including crops and wild are therefore required in order to assess the risk of transfer into the food chain.

Acknowledgments

This work was supported by the ANR (Agence Nationale de la Recherche) PRC program through the PLASTISCARE (ANR-19-CE04-0007), the CNRS EC2CO-HYBIGE program via the project NAME and the ADEME from the CINAPE project and finally the PAUSE project allocated to Prof. N. Ryzhenko by the Collège de France. We also acknowledge Ludivine Ruault from the THEMIS platform from ScanMAT, UAR 2025 UR1-CNRS, for the SEM images, and Sara Mullin for the English editing (https://www.proz.com/profile/677614)

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Declaration of interests

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.

Highlights

•No information on the impact of nanoplastics produced under environmental conditions.
• Extrapolation from model polymer nanobeads allow expecting direct metabolic and indirect, through soil properties modifications, impacts on plants
• The inexistence of quantitative methods limits toxicodynamic and toxicokinetics identifications.
•Main future challenges lie in using relevant nanoplastics models in size polydispersivity, shape and surface properties.
•Plant exposure pathways have to be assessed under environmentally relevant physicochemical exposure conditions