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Review:

Interactions between engineered nanomaterials and agricultural crops: implications for food safety*

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Abstract: Engineered nanomaterials (ENMs) are being discharged into the environment and to agricultural fields, with unknown impacts on crop species. In this paper, we review the literature on ENMs uptake, translocation/distribution, and generational transmission in various crop species, as well as potential material trophic transfer. Previous studies reveal that ENM-exposed crops exhibit adaptive processes in response to stress, including endocytosis/endosome activities, production of antioxidant enzymes, regulation of genes related to cell division/extension and membrane transport. Some agronomic traits of crops are compromised during the adaption response, including photosynthesis, fruit yields, nutritional quality and nitrogen fixation. Cultivation of crops in ENMs-contaminated environments has unknown implications for food safety and quality. Notably, mechanisms underlying ENMs phytotoxicity and bioavailability are unclear. Additional investigations focused on developing novel techniques for *in vivo* identification/characterization of ENMs are critically needed. Given the abundance of uncertainty in the literature, it is clear that more research is urgently needed in the area of ENMs-crop interactions; only then can one accurately assess exposure, risk, and overall implications for food safety and also enable guidance development for the sustainable implementation of nanotechnology in agriculture and food production/manufacturing.

Key words: Engineered nanomaterials (ENMs), Uptake, Trophic transfer, Food safety, Toxicity and impact
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1 Introduction

Nanotechnology has revolutionized many facets of modern society through extensive application in the fields of material science, energy, environmental remediation, agriculture, and medicine. As this technology continues to expand, nano-scale materials will inevitably being discharged into the environment and have become emerging contaminants of concern. Importantly, the implications of nanotechnology for the environment and agriculture remain unclear; without this fundamental knowledge, development of regulations and guidelines for safe use of engineered nanomaterials (ENMs) will not be possible.

The dramatic increase in worldwide production and application of ENMs is due to novel and useful material properties that become evident at the nanoscale. On the scale of nanometers, the relatively large surface area of ENMs results in enhanced chemical/biological activity. In addition, quantum effects become significant with size reduction, subsequently changing particle optical, electrical, and magnetic behaviors. However, great variation exists among different ENMs, including size, shape, physical conformation, specific surface area, surface charge, and the presence of coatings/functionality (Hassellov *et al.*, 2008; Parsons *et al.*, 2010; Pan and

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Xing, 2012). From the perspective of nano-biological interactions, the most attractive ENMs traits include a high degree of surface reactivity and a size-dependent ability to cross biological membranes. Because ENMs will be on the same scale as the key components of cells, including proteins, nucleic acids, lipids, and cellular organelles, significant particle-cellular interactions (both positive and negative) can be anticipated (Fadeel *et al.*, 2007; Auffan *et al.*, 2009).

The widespread presence of ENMs in the environment will bring significant and unique challenges to food safety and security. The global production and application of ENMs make particle accumulation in soil and groundwater inevitable. Plant species exposed to ENMs over time may undergo morphological, physiological, genetic, and epigenetic changes that may subsequently affect crop growth, yield, or nutritional status. Furthermore, after ENM transfer from soil to the edible/reproductive organs of crops, particles may accumulate in the food chain with unknown consequences to humans and other sensitive receptors. As such, consumption of food products from contaminated soil presents an unknown risk to public and environmental health.

There are many studies reporting the results of ENM toxicity tests conducted on model organisms and aquatic species such as *Arabidopsis thaliana* (Liu *et al.*, 2010; Wang H. *et al.*, 2011) and algae (He *et al.*, 2012). These studies and others provide evidence of both beneficial and detrimental effects on plants upon ENMs exposure. However, the literature is far too anemic to shed light on the responses of edible terrestrial plants with regard to food safety and overall nanotechnology sustainability. In this review we summarize and interpret the literature on ENM-crop interactions so as to further efforts to achieve a comprehensive understanding of (1) the exposure conditions and scenarios of agricultural crops to ENMs in the environment; (2) the uptake pattern of ENMs internalization and translocation *in vivo*; (3) potential trophic transfer; and (4) the impact of ENMs on agricultural crops at morphological, physiological, and genetic/epigenetic levels. Based on this review, we will identify critical knowledge gaps and highlight future research priorities.

2 Exposure scenarios

2.1 ENMs in real environments

Although ENMs are ubiquitous in the environment, actual data measuring ENMs concentrations in various media is scarce (Klaine *et al.*, 2008). Much of this is due to limited techniques for separation/extraction, characterization, and quantitation of ENMs environmental samples. One group has predicted environmental ENMs concentrations through probabilistic material flow analysis (Mueller and Nowack, 2008; Gottschalk *et al.*, 2009; 2013). As described in their work, the annual increase of ENMs in sludge amended EU or US soil was predicted to range from 1 ng/kg for fullerenes to 89 μg/kg for nano-TiO_2 (Gottschalk *et al.*, 2009). However, the predicted data are highly variable due to the poorly defined model inputs such as production volume and flow coefficients. Although ENMs concentrations in soils were reported or predicted to be low, these materials will accumulate over time in soils and rates may vary in response to unknown parameters (Boxall *et al.*, 2007; Gottschalk *et al.*, 2009). Research about potential risks of these particles should be completed before contamination or detrimental effects are observed. To achieve this goal, a solid data set of ENMs concentrations in environmental media is of critical importance. When ENMs are discharged into the environment, they can persist in air, water, and soil similar to naturally occurring nanoparticles. Crop roots could be exposed to ENMs in soils and irrigation water while the leaves and stems will be in direct contact with atmospheric ENMs. Crop growth in contaminated soils is an obvious major avenue of exposure, and soils could be contaminated through various intentional or accidental ENMs releases.

The main routes of entry for ENMs into agricultural fields include intentional application in agrichemicals to enhance crop protection, as well as through soil remediation efforts. Specific uses of ENMs in agrichemicals include incorporation into nanodevices as delivery system to specific target tissues, as additives in pesticides to increase solubility of active ingredients or to protect against premature active ingredient degradation (Gonzalez-Melendi *et al.*, 2008; Baruah and Dutta, 2009; Kah *et al.*, 2013; Kumari and Yadav, 2014). For example, farmers may use pesticides containing silver nanoparticles because of their capability to suppress the growth of harmful organisms (Bergeson, 2010). ENMs are also used to remediate contaminated soils; the most common example is nano-zero-valent iron (nZVI). The large surface area and high surface reactivity of nano Fe particles has proven effective at transforming and detoxifing a wide variety of common contaminants, including chlorinated organic solvents, organochlorine pesticides, and polychlorinated biphenyls (PCBs) (Zhang, 2003).

Accidental release or transport of ENMs from other media (water, air, soil amendments) into soil is also likely to occur. For instance, nano-CeO_2 is added into commercial diesel fuel as combustion catalyst. A case study showed that cerium oxide concentrations were estimated to vary between 0.32 and 1.12 μg/g at a distance of 26 m from the edge of highway, and between 0.28 and 0.98 μg/g for a distance of 96 m away (Park *et al.*, 2008). In addition, ENMs could be transported from water to soil. It was reported that the conventional drinking water treatment can only remove 2%–20%, 3%–8%, and 48%–99% of Ag, TiO_2 and ZnO nanoparticles, respectively, and that nano-sized metals were still detectable in finished water (Chalew *et al.*, 2013). Besides atmospheric deposition and ENMs persistence through water treatment, another important exposure pathway is through the application of biosolids on agricultural soils. Within the US, more than 60% of biosolids produced each year are added to agricultural fields (National Research Council Committee, 2002). ENMs released from biosolids may enter soils, subsequently interacting with crops and potentially affecting the quality, yield, and safety of food products.

2.2 Laboratory designed exposure conditions

The long-term growth of crops in ENMs-contaminated soils is obviously the most environmentally relevant approach, but less complex systems (hydroponic, model media) under shorter exposure scenarios will be necessary to thoroughly characterize particle toxicity and accumulation mechanisms. For seed germination and seedling growth assays less than 7 d, ENMs exposure solutions are typically prepared in water (Lin and Xing, 2007; Cifuentes *et al.*, 2010; Lopez-Moreno *et al.*, 2010a; Klancnik *et al.*, 2011; Wang S. *et al.*, 2011; Larue *et al.*, 2012a). For hydroponic seedling growth assays with exposure intervals exceeding 7 d, ENMs were more commonly suspended in nutrient media such as Hoagland's solution (Stampoulis *et al.*, 2009; Castiglione *et al.*, 2011; Musante and White, 2012; Wang *et al.*, 2012). Homogeneous dispersions can be achieved by amendment with external surfactants or through the use of surface functionalized ENMs. For example, multi-wall carbon nanotubes (MWCNTs) and C_{70} fullerene were stabilized in natural organic matter (NOM, including humic acid) and gum Arabic solutions in the media (Lin *et al.*, 2009; Larue *et al.*, 2012b); metal-based ENMs such as Fe_3O_4, Au, Ag, and $Ni(OH)_2$ were coated with citrate, tannate, or polyvinylpyrrolidone (PVP) (Parsons *et al.*, 2010; Wang H. *et al.*, 2011; Judy *et al.*, 2012a; Lee *et al.*, 2012). There are also reports using agar or semi-solid media such as Murashige and Skoog (MS) medium that may be amended with ENMs prior to solidification (Lee *et al.*, 2008; 2012; Miralles *et al.*, 2012b; Yang *et al.*, 2012; Yan *et al.*, 2013). In either agar or aqueous systems, biocompatible agents such as humic acid are preferable due to greater environmental relevance and less potential toxicity than synthetic surfactants such as sodium dodecyl sulfate (SDS) or PVP.

Semisolid media in petri dishes and hydroponics are simple systems, facilitating homogeneous mixing and immediate contact of ENMs with root surfaces; this experimental approach allows a focus on intrinsic particle properties. But from a practical and realistic perspective, soil-based studies are more relevant and important. Soil or similar porous materials can physically and chemically alter the stability and availability of ENMs to terrestrial biota, including plants. To date, the knowledge of ENMs-crop interactions in soil-based systems is very limited. In some published trials, sand or soil was either amended with nanomaterial powders or with ENMs suspensions (Du *et al.*, 2011; Dimkpa *et al.*, 2012; El-Temsah and Joner, 2012; Priester *et al.*, 2012; Zhao *et al.*, 2012a; 2012b; Khodakovskaya *et al.*, 2013). A design with outdoor lysimeters under field condition was first introduced to investigate the impact of ENMs on *Triticum aestivum* (wheat) growth and soil enzyme activities; here, the topsoil was *ex-situ* amended with TiO_2 and ZnO nanoparticles (Du *et al.*, 2011). This type of research, although still limited in scope, provides important environmentally relevant information on the fate and bioavailability of ENMs in agricultural systems. Additional similar yet expanded studies will be needed to fully characterize the impact of ENMs on crop growth, uptake and potential particle trophic transfer.

It is also worth noting that some less intuitive routes of ENMs entry into aerial plant tissues have also been considered, including injection, leaf spray and atmospheric exposures (Corredor *et al.*, 2009; Birbaum *et al.*, 2010). Although leaves may possess resistant barriers, these studies will help to clarify all possible ENM exposure routes.

3 Uptake of ENMs into agricultural crops

Among terrestrial plant species, major agricultural crops raise obvious concerns due to their direct consumption as food. The crops that have been evaluated for ENMs accumulation and toxicity are numerous and include *Triticum aestivum* (wheat), *Cucumis sativus* (cucumber), *Zea mays* (corn), *Allium cepa* (onion), *Cucurbita pepo/mixta* (zucchini/pumpkin), *Lycopersicon esculentum* (tomato), *Oryza sativa* (rice), *Glycine max* (soybean), *Lactuca sativa* (lettuce), and *Nicotiana xanthi* (tobacco). Among these species, *Cucurbita pepo/mixta* and *Cucumis sativus* are commonly used in uptake and translocation experiments due to their large-size vascular bundles and significant water uptake capacity. In terms of ENM detection *in planta*, magnetic and carbon-based nanoparticles can be detected *in vivo* by magnetic resonance imaging and radiolabeling, respectively. Additional complementary techniques to provide evidence for root or shoot uptake of ENMs, include (1) microscopic techniques, e.g., transmission electron microscopy (TEM), scanning electron microscopy (SEM), scanning X-ray fluorescence microscopy (XFM) and confocal laser scanning microscopy (CLSM), coupled with (2) other qualitative/quantitative techniques, such as energy-dispersive spectroscopy (EDS), inductively coupled plasma-mass spectrometry (ICP-MS), and Raman spectroscopy, and occasionally assisted with (3) isotope tracer or labeling techniques. Although viable techniques for ENM *in planta* detection, information is still far too limited to mechanistically characterize uptake processes conclusively. However, based on knowledge from the existing literature, some similarities and patterns in uptake process are evident and summarized below.

3.1 Surface contamination and metal speciation

When root surfaces are exposed in ENMs-containing media, these materials tend to accumulate on the epidermis or adhere onto surficial tissues as individual particles and/or aggregates (Lin and Xing, 2008; Wild and Jones, 2009; Zhao *et al.*, 2012b). The initial contact or interaction may occur via electrostatic adsorption, mechanical adhesion or hydrophobic affinity of certain ENMs (Zhang H.F. *et al.*, 2011). During accumulation experiments, it is critical to distinguish adsorption and actual ENMs uptake. In most uptake studies of metal-based ENMs, tap or deionized water is used to rinse the root surface, and then the whole tissue is digested for metal content determination. However, uptake data based on this type of procedure is really a semi-quantitative evaluation since a fraction of strongly adsorbed ENMs will be retained and digested with the tissues. Agents such as NaOAc and Na_4EDTA may be used to remove adsorbed CuO nanoparticles from *Triticum aestivum* (wheat) root surface (Zhou *et al.*, 2011). This amendment significantly facilitated CuO desorption from root surfaces without introducing new stress, presenting clear advantages over metal competing ions and surfactants. But the efficiency of removal was not widely tested among other ENMs and crop species, and it is not a standard approach when quantifying root uptake. When evaluating accumulation, data derived from aboveground tissues not in direct contact with exposure media is far more relevant. Moreover, it is necessary to include ion and bulk particle controls in experiments. Even so, multidisciplinary and orthogonal techniques should be applied to provide more accurate information, such as visualization via electron microscopy or metal speciation. Metal speciation is necessary not only because crops may accumulate dissolved ions instead of particles, but also it provides potential information on metal biotransformation *in vivo*. In addition, plant root exudates or biomass could potentially affect the dissolution and accumulation of metal-based ENMs. Some metal oxide nanoparticles undergo dissolution and their metal ions are subsequently accumulated by plants. It was also reported that *Medicago sativa* alfalfa shoot biomass was capable of reducing gold(III) to form gold(0) colloids in aqueous solutions and living alfalfa plants could uptake silver(I) and undergo nucleation to form silver nanoparticles (Gardea-Torresdey *et al.*, 2000; 2003). As such, it becomes difficult to determine whether the metal species detected in plant tissues result from direct ion uptake or biotransformation within plant cells. While all Au remained as particles in plant shoots and 79% of Ce maintained the original CeO_2 coordination, CuO ENMs accumulation by *Triticum aestivum* (wheat) shoot were in particulate ((64±10)%) and complexed forms (Cu(I)-sulfur complexes ((36±10)%)). ZnO ENMs-exposed plants (*Triticum aestivum*, *Glycine max*) have Zn as Zn(II)-phosphate or Zn-citrate complexes; no elemental particles were observed (Lopez-Moreno *et al.*, 2010a; Judy *et al.*, 2011; Dimkpa *et al.*, 2012; 2013; Hernandez-Viezcas *et al.*, 2013). This cursory review of the literature clearly suggests that observed phytotoxicity likely result from a combination of ion-driven and size-dependent effects.

3.2 Processes of root uptake

3.2.1 Size-based selection by plant cell wall

Plant cell walls are a complex matrix containing a network of cellulose microfibrils cross-linked with hemicellulose and lignin, and further impregnated by pectin (Serag *et al.*, 2013). With this characteristic structure, root epidermal cell walls restrict the passage of large ENMs aggregates or agglomerates. Agglomerates are particles assemblages that could be fractured by considerable forces, while aggregates are a more definite pattern of prenucleation structures (Nichols *et al.*, 2002). Cell wall pores are estimated to have sizes in a range of 5 to 20 nm (Carpita *et al.*, 1979; Tepfer and Taylor, 1981); smaller clusters or individual particles could diffuse through pores and enter apoplastic and/or symplastic flow. In some instances, extensive ENMs sorption onto root surfaces could cause structural damage and compromise cell integrity. Also, carbon nanotubes may physically pierce epidermal and root hair cell walls (Wild and Jones, 2009), physically altering tissues in ways that may subsequently facilitate ENMs entry into the cellular cytoplasm.

3.2.2 Apoplastic route

When ENMs traverse porous cell walls, particles may diffuse in the space between the cell wall and plasma membrane: a route known as the apoplastic pathway and which is subject to osmotic pressure or capillary forces (Lin *et al.*, 2009). Through the apoplast, particles may bypass epidermal and cortical cells to reach the endodermis. However, aggregates often accumulate in the endodermis as a result of the significant barrier imposed by the waxy Casparian strip (Larue *et al.*, 2012a; Zhao *et al.*, 2012b). For effective translocation to the shoot system, ENMs in apoplastic flow must eventually merge into the symplast so as to penetrate into vascular system, as presented in Fig. 1b.

3.2.3 Symplastic route

The symplastic route is hypothesized to be the more important and highly regulated pathway for transporting ENMs into crops. It has been proposed that cell internalization of ENMs could occur by binding to carrier proteins, through aquaporins, ion channels, endocytosis, or by creating new pores (carbon nanotubes) (Rico *et al.*, 2011). While experimental data are scarce and many proposed mechanisms are under intense debate, endocytosis has been demonstrated definitively through the use of temperature control and the addition of endocytosis-inhibiting agents such as wortmannin (Onelli *et al.*, 2008; Liu *et al.*, 2009; Iversen *et al.*, 2012; Miralles *et al.*, 2012b). Enhanced expression of aquaporin proteins and up-regulation of water channel genes were found to support possible passive uptake mechanisms (Khodakovskaya *et al.*, 2012). Hydrophobic/hydrophilic effect could also alter the interaction of ENMs with plant cell membranes. Hydrophobic nanomaterials tend to embeds into the hydrophobic core of the membrane without results in membrane leakage; while hydrophilic nanomaterials favor the adsorption on the surface of the bilayer, and they are more likely to bind to intracellular vesicles (Li *et al.*, 2008; Stark, 2011). ENMs in the cytoplasm may be surrounded by protein or other biomolecules that form a corona (Nel *et al.*, 2009). Once within cells, the ENMs-containing endosomes or ENMs-protein complex could undergo efficient transport to neighboring cells via plasmodesmata, which typically have a diameter of 20–50 nm. Notably, the structural

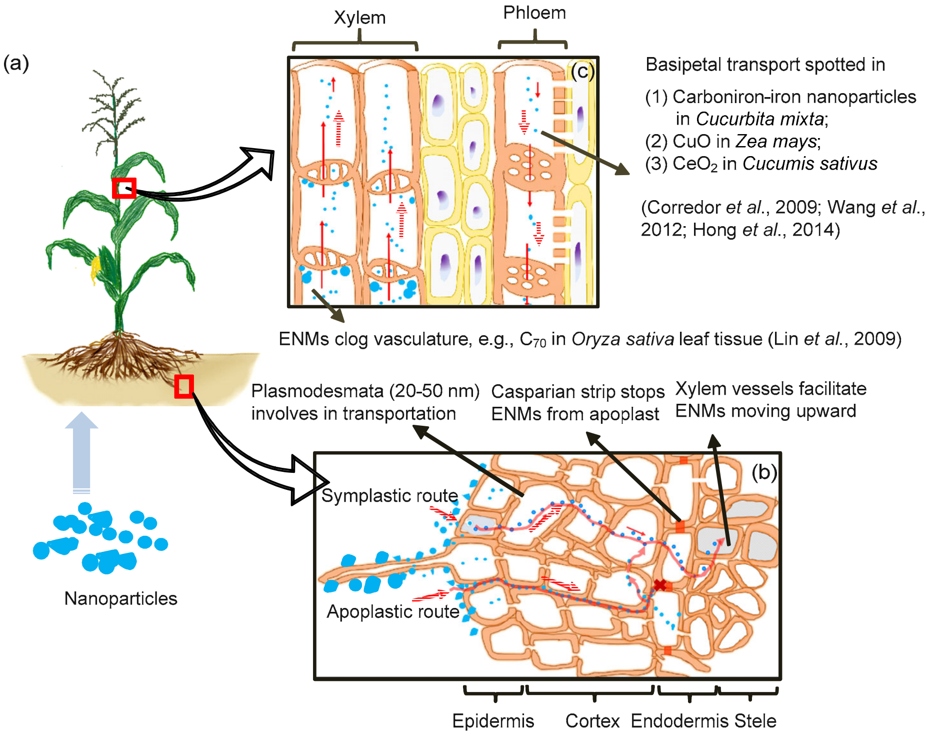

Fig. 1 Schematic diagram of uptake process and routes

(a) A model crop plant; (b) Root uptake processes and translocation into vasculature starting from root surface accumulation, symplastic/apoplastic flows into cortex and then traverses endodermis into xylem vessels; (c) Upward movement in xylem and downward transportation in phloem in both root and foliar uptake scenarios

integrity of plasmodesmata is maintained by cytoskeleton microfilaments and certain ENMs (ultrasmall TiO_2 nanoparticles) have been reported to disrupt such microtubular networks in *Arabidopsis thaliana* (Wang S. *et al.*, 2011; Larue *et al.*, 2012a). Additionally, Rab proteins were hypothesized to have a role directing "cargo" to specific areas near plasmodesmata locations (Cifuentes *et al.*, 2010). As a result, the transportation of ENMs may be facilitated through the normal and coordinated activity of organelles, transport proteins and trans-wall channels. Because of the high volume of material involved in symplastic flow, this pathway may prove to be highly effective at transporting ENMs through endodermis and into stele and subsequent vascular tissues.

3.3 Foliar uptake

As previously mentioned, foliar uptake was investigated in several studies that sought to characterize the possibility of phloem-based ENMs transport. ENMs have the potential to penetrate leaf surfaces through stomatal pores (Eichert *et al.*, 2008; Larue *et al.*, 2014). Basipetal translocation of carbon-coated iron nanoparticles was evident from the epidermis of exposed *Cucurbita mixta* petioles; similarly, Ce was found in *Cucumis sativus* roots after leaf exposure to nanoparticulate CeO_2 (Corredor *et al.*, 2009; Hong *et al.*, 2014). Split-root experiments performed on *Zea mays* roots also indicated the movement of CuO from shoots to roots via phloem (Wang *et al.*, 2012). Interestingly, in the above-mentioned study with *Cucurbita mixta*, ENMs aggregates were found to be present in "chains" of several adjacent parenchymatic cells oriented radially to the stem surface. Conversely, in root uptake studies, ENMs were found randomly distributed, suggesting that translocation through phloem may be more tightly regulated and organized than through xylem. Although experiments using atmospheric exposure are difficult, foliar uptake studies provide useful mechanistic information about ENMs distribution and translocation within crop plants.

3.4 Translocation and distribution

The *in planta* translocation and uptake of ENMs is a dynamic process that will depend on exposure conditions, ENMs properties, and crop species. Key characteristics of ENMs that will impact bioavailability/bioactivity include particle size, surface charge, affinity to water (hydrophilic/hydrophobic), and protein/biomolecule adsorption. Upon accumulation, particle distribution via the vasculature (Fig. 1c) could occur rapidly; ENMs were detected in shoots as short as 24 h after carbon-coated magentic nanoparticle exposure to *Helianthus annuus* (sunflower), *Lycopersicum sculentum* (tomato), *Pisum sativum* (pea) and *Triticum aestivum* (wheat) (Cifuentes *et al.*, 2010). An *in vivo* plant flow cytometry assessment showed that the average velocity of linear flow in *Lycopersicum sculentum* stems exposed to quantum dot-carbon nanotube conjugates was approximately 0.2 mm/s (Nedosekin *et al.*, 2011).

Various ENMs have been shown to translocate within stems, leaves, petioles, and fruits of different crops. Table 1 outlines a portion of the relevant literature, with a focus on transport to edible tissues. It is worth noting that all the listed studies present rather definitive evidence of ENMs transport, supported by direct visualization of ENMs or whole-plant mapping of ENMs signals. Based on these studies, some trends are evident: (1) Observable ENMs in shoots tend to be concentrated or restricted to locations near or within vascular tissues. This is likely a function of the transpiration flow/pattern through the leaf structure (Ghafariyan *et al.*, 2013); (2) Small aggregates or individual particles are obviously more capable of long-range movement from root to subapical tissues, as compared to large aggregates from the same type of ENMs; (3) It is commonly found that leaf concentrations of ENMs are higher than that stems when expressed in amount per dry weight tissue; and (4) Separate from vascular transport, specific locations for ENMs distribution, such as the leaf periphery and trichomes, may be implicated in detoxifying pathways (Cifuentes *et al.*, 2010). However, it is clear that large-scale patterns of *in planta* distribution are poorly understood. Unknown detoxification pathways may also confound assessment of ENM distribution within exposed plants.

Although multiple routes of ENMs translocation are possible, many ENMs will not transport to subapical tissues, with particle size being the primary reason for negligible accumulation. While 20 nm Fe_3O_4 was found to penetrate and move into *Cucurbita mixta* (pumpkin), 25 nm did not translocate within the plants (Zhu *et al.*, 2008; Wang H. *et al.*, 2011). A study involving $nTiO_2$ of different size (Anatase 14, 25, 140 nm and Rutile 22, 36, 655 nm) concluded that ENMs with primary size under 36 nm could reach the stele in *Triticum aestivum* (wheat) roots whereas ENMs with sizes between 36–140 nm could only move to the root cortex. Another typical example is MWCNTs, which usually have lengths in the micrometer range. MWCNTs tended to adsorb onto root surfaces and pierce root epidermal tissue. The tubes induce physical damage and therefore stress crop plants without typically being internalized (Miralles *et al.*, 2012a). Surface coating is a second important role in determining the possibility of ENMs uptake. As depicted above, ENMs may be surface-modified with biocompatible agents. These agents or surface functional groups have affinity with plasma membranes and significantly enhance ENMs uptake and translocation.

4 Trophic transfer and potential risks to food safety

Of particular concern is the internalization of ENMs into edible and reproductive tissues of plants; this has been shown for fullerol in fruits of *Momordica charantia* (Kole *et al.*, 2013), fullerene (C_{70}) in *Oryza sativa* grains and second-generation seedlings (Lin *et al.*, 2009), and MWCNTs in flowers of *Lycopersicum sculentum* (Khodakovskaya *et al.*, 2013). In spite of these demonstrated phenomena, relevant research on ENM bioaccumulation and trophic transfer is poorly understood; a discussion of the limited published literature follows below.

Notably, the majority of existing data related to ENM trophic transfer come from studies in freshwater plants and aquatic invertebrates, e.g., transfer of quantum dots from ciliated protozoans to rotifers, $nTiO_2$ from daphnia to zebrafish and quantum dots from dosed algae to *C. dubia* (Bouldin *et al.*, 2008; Holbrook *et al.*, 2008; Zhu *et al.*, 2010). No biomagnification was observed in the above aquatic studies, with bioaccumulation factors (BAFs) ranging from 0.004–0.04 (Hou *et al.*, 2013). However, research more related to agricultural systems points to the possibility of trophic transfer and biomagnification

Table 1 Translocation and distribution of engineered nanomaterials in aerial tissues of agricultural crops through root uptake

Crop name	ENMs type	ENM size	Exposure concentration ($\times10^{-6}$)	Growth matrix	Location in aboveground parts	Accumulated concentration	Reference
Bitter melon	$C_{60}(OH)_{20}$	1.5±0.2 nm, 5.0±0.7 nm	1, 5, 10, 11.5, 50	Germination in aqueous media for 48 h and moved into soil	Petioles, leaves, flowers, and fruits	NA (not available)	(Kole *et al.*, 2013)
Cucumber	CeO_2	7 nm	20	Aqueous media for 10–14 d	Accumulated along leaf margins in young leaves; spread all over the older leaves	162–550 ng/g (leaves); 25.1–231.0 ng/g (stems)	(Zhang Z.H. *et al.*, 2011)
		25 nm	20	Aqueous media for 10–14 d		33.7–383 ng/g (leaves); 10.6–253 ng/g (stems)	
Pea	Carbon-Fe	10 nm		Aqueous media for 24–48 h	Observed in cortex, leaf petioles, internodes; within and outside vascular tissues	NA	(Cifuentes *et al.*, 2010)
Pumpkin	Fe_3O_4	20 nm	500	Aqueous media for 20 d	Strong magnetic signals detected in all leaf specimens regardless of their distances from the roots; much weaker from the stem tissue samples except those close to the roots	0.6% of initial Fe in leaf tissues, 45.4% in root tissues	(Zhu *et al.*, 2008)
Rapeseed	MWCNTs	41.2 nm	1000	Aqueous media for 7 d	Higher accumulation at peripheral areas of leaves; more accumulated in newly developed leaves	Transfer from suspension to leaves below 0.005‰	(Larue *et al.*, 2012)
Soybean	SPIONs (FeO_x)	9 nn	60	Aqueous media for 28 d	Diffused toward interior of the stem parenchyma; detected in stem and leaves, vascular and parenchyma tissues	TF (root to leaf) 1.0% for SPIONs, 1.4% for NH_2-SPIONs and 1.6% for COOH-SPIONs	(Ghafariyan *et al.*, 2013)
Rice	C_{70}	1.19 (major), 17.99, 722.10 nm	20	Germination in aqueous media for 14 d and moved in soil for 6 mol	Predominantly present in and near the stem's vascular system; in leaves; spotted in leaf tissues of the second-generation plants	NA	(Lin *et al.*, 2009)
Sunflower	Carbon-Fe	10 nm		Aqueous media for 24–48 h	Observed in cortex, leaf petioles, internodes; within and outside vascular tissues	NA	(Cifuentes *et al.*, 2010)
Tobacco	Au	10, 30, 50 nm	30	Aqueous media for 7 d	Observed within leaf mid rib near petiole	Between 2.2 and 53.5 mg/kg in aerial tissues	(Judy *et al.*, 2012)
Tomato	Carbon-Fe	10 nm		Aqueous media for 24–48 h	Observed in cortex, leaf petioles, internodes; within and outside vascular tissues	NA	(Cifuentes *et al.*, 2010)
	MWCNTs	25 nm	50, 200	Soil for 6–9 weeks	Clustered CNTs observed in the flower structures	NA	(Khodakovskaya *et al.*, 2013)
	Carbon	10–35 nm (MWCNTs), 0.86–2.22 nm (SWCNTs), 2–5 nm (graphene)	50	Agar MS medium for 10 d	Observed outside the leaves' vascular system; randomly among individual cells; only a few were found in close proximity to the leaf vasculatures	NA	(Khodakovskaya *et al.*, 2011)
Wheat	MWCNTs	41.2 nm	1000	Aqueous media for 7 d	Higher accumulation at peripheral areas of leaves; more accumulated in newly developed leaves	NA	(Larue *et al.*, 2012)
	Carbon-Fe	10 nm		Aqueous media for 24–48 h	Observed in cortex, leaf petiotles, internodes; within and outside vascular tissues; strongly accumulated in leaf trichomes	NA	(Cifuentes *et al.*, 2010)

through the food chain. One group working demonstrated BAFs of 6.2, 11.6, and 9.6 for tobacco hornworm consuming leaves of *Nicotiana xanthi* (tobacco) grown in 5, 10, and 15 nm Au nanoparticles suspensions (Judy *et al.*, 2012a). The authors later reported a much smaller BAF (0.16) for caterpillars consuming *Lycopersicum sculentum* leaves that were surface contaminated with Au nanoparticles (Judy *et al.*, 2012b). Meanwhile, nano Au (20 or 55 nm) can be taken up by earthworms (*Eisenia fetida*) from soil, and nano Au were found more bioavailable through trophic exposure (earthworms to bullfrogs (*Rana catesbeina*)) than direct exposure (bullfrogs to nano Au contaminated soils) (Unrine *et al.*, 2010; 2012). These studies suggest that ENMs trophic exposure and biomagnification is possible and of concern in agriculture. The limited information and large knowledge gaps make accurate assessment of ENMs exposure and risk during trophic transfer impossible (Gardea-Torresdey *et al.*, 2014); clearly, considerable work in this critical area is needed to enable food safety guidance and policy development.

5 Impact on plants

In addition to food safety concerns related to ENM accumulation, the quality and yield of agricultural crops may also be compromised by ENM exposure. The hypothesis that ENMs can affect crop growth has been tested repeatedly under a number of exposure scenarios. The effects of ENMs exposure can vary greatly, ranging from subtle changes in the soil environment to direct and overt phytotoxicity involving alternations in morphology, physiology, and gene expression. The final effect will also be impacted by species-specific defense systems in response to ENMs induced stress. In spite of some studies reporting ENMs beneficial effects on crop species, the negative effects on crops may well exceed possible advantages of ENMs application in agriculture, and more importantly, concerns on safety and quality of food products arise from the evidence of negative effects, which include growth inhibition in seeds/seedling stages, oxidative stress, altered photosynthesis, genetic damage, compromised agronomic and yield characteristics, and ENMs facilitated uptake with other soil contaminants.

5.1 General toxicity tests on seeds/seedlings

Although there are no established specific testing standard protocols for ENMs, US Environmental Protection Agency (EPA) and Organization for Economic Cooperation and Development (OECD) phytotoxicity guidelines are frequently used. Measured biological endpoints include germination index (time and rate), root elongation, shoot/root biomass, and root tip morphology. ENMs exposure concentrations are typically quite high, often approaching 1000–4000 mg/L. At these high levels, overt toxicity is frequently observed. For example, reduced germinated was noted with nanoparticle ZVI on *Hordeum vulgare* and *Linum usitatissimum* seeds at 250 mg/L, NP-ZnO on *Zea mays* seeds at 2000 mg/L, and CeO_2 on *Medicago sativa*, *Zea mays* and *Cucumis sativus* seeds at 2000 mg/L (Lin and Xing, 2007; Lopez-Moreno *et al.*, 2010b; El-Temsah and Joner, 2012; Feizi *et al.*, 2012; Ghafariyan *et al.*, 2013). Compared to metal-based ENMs, CNTs were shown to not affect seed germination even at 2000 mg/L (Lin and Xing, 2007; Miralles *et al.*, 2012a). Positive effects on seed germination were reported mainly in TiO_2 and industrial-grade CNTs, particularly on seeds with inherently low germination (Zheng *et al.*, 2005; Miralles *et al.*, 2012a; Feizi *et al.*, 2013). Thus, reactive oxygen species (ROS) induced by nano-TiO_2 may have enhanced seed stress resistance and facilitated capsule penetration for water and oxygen intake that enabled more rapid germination (Khot *et al.*, 2012). For industrial-grade CNTs, catalyst impurities, including Fe and Al_2O_3, can approach and exceed percent levels and as such, these materials rather than CNTs could enhance seed vigor by similar hormesis.

Compared to seed germination, root and shoot growth of seedlings is generally regarded as a more sensitive indicator of toxicity. Although exposure dose and duration vary significantly in the literature, negative effects on seedlings root/shoot elongation and biomass were noted for metal-based ENMs such as ZnO, CuO, Ag, and Al_2O_3, largely due to toxicity from the enhanced release of ions from ENMs. However, positive effects were noticed upon NP Au, Fe_2O_3, CeO_2 exposure over ion or bulk particle controls, including size-specific effects on root elongation (Barrena *et al.*, 2009; Lopez-Moreno *et al.*, 2010a; Ghodake *et al.*, 2011; Dimkpa *et al.*, 2012; Alidoust

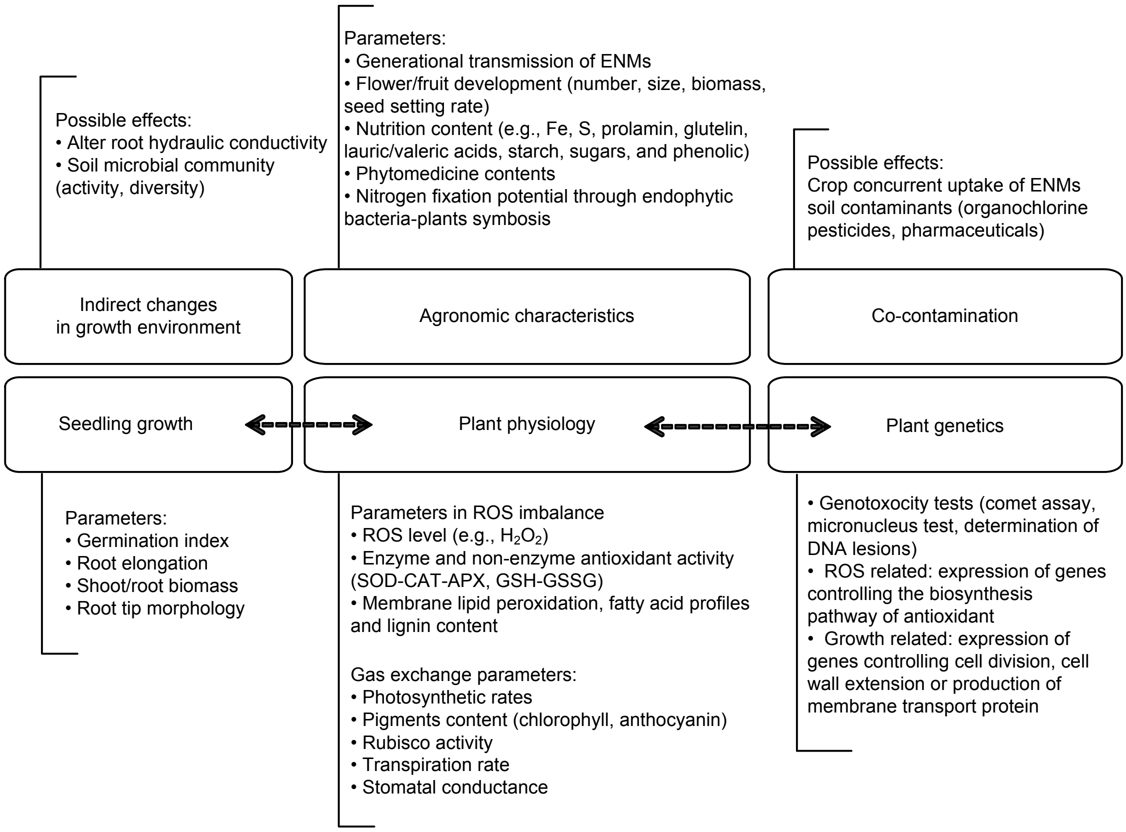

Fig. 2 An overview of ENMs impact on agricultural crops, including toxicity, growth performance, and physiological changes observed from seeds/seedlings stage to mature crop plants

and Isoda, 2013; Ghafariyan *et al.*, 2013). Conflicting data in the literature are common for carbon-based ENMs such as graphene, SWCNTs, and MWCNTs (Canas *et al.*, 2008; Begum *et al.*, 2011; Miralles *et al.*, 2012a). More disconcerting than the contradictory findings in the literature is the almost complete lack of mechanistic understanding for the observed plant responses.

5.2 Physiological changes

When compared to more generic growth parameters, physiological endpoints are potentially more informative with regard to the interactions between ENMs and crop species. The two most common physiological parameters involve the generation of (or effects from) ROS with related antioxidant defenses and various aspects of photosynthetic system function. ROS generation and oxidative stress were hypothesized to be the most likely paradigm for ENM phytotoxicity, largely due to the direct relationship between high reactive surface area and oxidative capability of these materials (Nel *et al.*, 2006). Photosynthesis has been a focus due to its critical importance as an ecosystem service and its direct relationship to crop yield and quality; however, a mechanistic understanding here is generally lacking (Long *et al.*, 2006).

5.2.1 Oxidative stress and antioxidant enzymes

ROS are by-products of aerobic metabolism, usually in reduced forms of O_2 such as superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO^-). These products are actively generated as signaling molecules during cellular homeostasis, and rapidly scavenged by antioxidant defense machinery prior to significant toxic effects (Mittler, 2002; Apel and Hirt, 2004). The antioxidant defense system includes enzymatic (superoxide dismutase (SOD); catalase (CAT); ascorbate peroxidase (APX); glutathione reductase (GR); monodehydroascorbate reductase (MDHAR); dehydroascorbate reductase (DHAR); glutathione peroxidase (GPX); guaiacol peroxidase (GOPX), and glutathione-S-transferase (GST)) and non-enzymatic antioxidants (ascorbic acid (ASH); glutathione (GSH); oxidized glutathione (GSSG); phenolic compounds, alkaloids, non-protein amino acids and a-tocopherols) (Gill and Tuteja, 2010). Among the antioxidant defenses, enzymatic group of SOD-CAT-APX and non-enzymatic group of GSH-GSSG are typically most commonly evaluated.

An imbalance between production and scavenging can lead to an oxidative burst, a phenomenon commonly noted in stressed plants. Oxidative stress and over-accumulation of ROS will induce cell damage, e.g., membrane lipid peroxidation, protein oxidation, enzyme inhibition, DNA and RNA damage, or activate the programed cell death pathway (PCD). An early report on oxidative stress involved the interaction between NP Fe_3O_4 and *Cucurbita mixta*, with the authors noting significantly higher levels of SOD and CAT along with enhanced lipid peroxidation in the root tissue (Wang H. *et al.*, 2011). In another study focusing on graphene-exposed *Brassica campestris* (cabbage), *Lycopersicon esculentum* (tomato) and *Amaranthus tricolor*/*lividus* (red spinach), ROS production was found to be dose-dependent (0, 500, 1000, 2000 mg/L) (Begurn *et al.*, 2011). Conversely, nano TiO_2 at 5, 25, and 50 mg/L did not alter any oxidative stress biomarkers in *Vicia faba* shoot (Foltete *et al.*, 2011). In such cases, NP exposure may either not induce ROS production or may induce ROS levels that are well managed by antioxidant defenses. Dimkpa *et al.* (2012) investigated the oxidative stress of *Triticum aestivum* grown in NP CuO or ZnO amended sand. Although lipid peroxidation, increased GSSG level in shoots, and higher peroxidase (POD)/CAT activity were noted, the magnitude ROS over-production and stress was not great. The increase of certain antioxidant enzymes or lipid peroxidation is consequence of ROS imbalance, which is does indirectly suggest initial ROS over-production. Direct evidence such as quantitative measurement of ROS should be presented where possible, e.g., data using oxidatively sensitive probes 2’,7’-dichlorofluorescein diacetate (DCFH-DA) and 3,3’-diaminobenzidine (DAB). Direct evidence of ROS overproduction can also be determined through measurement of antioxidant related gene expression levels (Begurn *et al.*, 2011)). Dimkpa *et al.* (2012) also highlighted another important issue, which involves being able to distinguish between ion-induced and NP-dependent stresses. In this study, Cu detected in the shoots was in both NP and Cu(I)-sulfide forms. It was noticed that the increased production of CAT/POD was also found in *Triticum aestivum* treated with Cu ion solution, which might support the view that oxidative stress was a function of Cu ions released from nanoparticles rather than the presence of nanoparticles or their aggregates themselves (Gajewska and Sklodowska, 2010).

Alternatively, Zhao *et al.* (2012b) investigated the stress response of CeO_2 nanoparticles on soil-grown *Zea mays*, and provided a comprehensive assessment of stress-related parameters including H_2O_2, CAT and APX activity, heat shock protein 70 (HSP70), lipid peroxidation, and cell death. Importantly, timing was noted as a critical factor in detecting plant stress response. Over-production of H_2O_2 in shoots was observed at day 10 but levels then gradually decreased until day 20, when over-accumulation was no longer evident. This time-dependent response suggests an adaptive process for crops grown in ENMs-contaminated environments. Also, the timing of biological endpoint determination should be taken into consideration when comparing ENMs-crop interactions across particles and species.

ROS over-production is also reflected in membrane fatty acid profiles and lignin content. For example, fatty acid content in *Oryza sativa* roots was significantly altered by nano CeO_2 treatments (Rico *et al.*, 2013b). Plant lignin content was also reduced with increasing nano CeO_2 dosage, likely due to the fact that the balance between peroxidase activity and H_2O_2 content has a regulatory role in lignin synthesis (Rico *et al.*, 2013a).

5.2.2 Photosynthesis and gas exchanges parameters

The production and scavenging of ROS are closely related to photorespiration pathways, photosynthetic apparatus, and mitochondrial respiration. As a consequence of ENMs stress, it is possible that photosynthetic processes may be suppressed or adversely affected.

Initial studies on the impact of NP TiO_2 on *Spinacia oleracea* (spinach) photosynthesis (Zheng *et al.*, 2005; Su *et al.*, 2007; Yang *et al.*, 2007) showed increased photosynthetic rates, greater chlorophyll formation, and higher Rubisco activity with exposure, although a mechanistic explanation was not offered. Other studies with different ENMs and crops revealed no differences in photosynthesis and gas exchange (net photosynthetic rate), transpiration, and stomatal conductance for CeO_2-*Zea mays*, TiO_2-*Triticum aestivum*, TiO_2-*Vicia faba* or Fe_2O_3 nanoparticles-*Glycine max* (Foltete *et al.*, 2011; Larue *et al.*, 2012a; Zhao *et al.*, 2012a; Ghafariyan *et al.*, 2013). However, unaltered gas exchange parameters did not mean that plants were unaffected; in fact, photosynthetic pigments and enzymatic structures at different stages of the photosynthesis reaction were found to be more sensitive endpoints than overall photosynthetic rates. For example, chlorophyll a and b contents in *Oryza sativa* seedlings were significantly diminished with CeO_2 NP treatment, as well as with *Triticum aestivum* treated with CuO and ZnO NPs (Dimkpa *et al.*, 2012; Rico *et al.*, 2013c). Similar suppression was also observed in some microalgae, and it was speculated to be a result of shading effect of nanomaterials, which cannot explain the inhibitory in higher plants (Sadiq *et al.*, 2011; Oukarroum *et al.*, 2012).

5.3 Genetic alternations and damage

Perhaps the most accurate and informative means to assess plant response to ENMs exposure involves molecular analysis. In addition, genetic studies would also provide important information to support physiological or phenotypic observations. For instance, *Zea mays* treated with SWCNTs exhibited enhanced root growth, and accordingly, the expression of seminal root associated genes was increased (Yan *et al.*, 2013). Similarly, it is of great interest to study the up- and down-regulation of antioxidant-related genes in conjunction with the production of antioxidant parameters, but unfortunately, there are no sufficient studies with terrestrial crop species. In the model plant *Arabidopsis thaliana* L., the expression of genes controlling the glutathione (GSH) biosynthesis pathway was significantly altered, confirming the oxidative stress. Specifically, NP In_2O_3 exposure increased glutathione synthase (GS) transcript production by 3.8–4.6-fold whereas nano CeO_2 yielded only a 2-fold increase (Ma *et al.*, 2013). In addition, in *Nicotiana xanthi* cells treated with MWCNTs, the expression of aquaporin (*NtPIP1*) gene, as well as marker genes for cell division (*CycB*) and cell wall extension (*NtLRX1*), were significantly up-regulated upon exposure. This data suggests that the enhanced growth of cells was directly related to water uptake and cell division (Khodakovskaya *et al.*, 2012).

Additional genotoxicity tests include the comet assay and micronucleus test, both of which have been used extensively on plant protoplast and mammalian cell lines. Among crop species, the micronucleus test has been more commonly employed, such as on the root tips of *Allium cepa*. Chromosomal aberrations, sticky chromosomes, disturbed metaphase, and multiple chromosomal breaks of varying degrees were noted as soon as 3 to 24 h after ENMs exposure; a relationship of these effects to overall increased lipid peroxidation was proposed (Kumari *et al.*, 2009; 2011; Ghosh *et al.*, 2010; Castiglione *et al.*, 2011; Foltete *et al.*, 2011; Klancnik *et al.*, 2011). Also, as a result of overproduced ROS and lipid peroxidation, genotoxicity of CuO exposure as measured by significant accumulation of oxidatively modified, mutagenic DNA lesions was described in *Raphanus sativus* (radish), *Lolium perenne* (perennial ryegrass), and *Lolium rigidum* (annual ryegrass), e.g., 7,8-dihydro-8-oxoguanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 4,6-diamino-5-formamidopyrimidine (Atha *et al.*, 2012). Additional studies focused on genetic and epigenetic endpoints as a function of ENMs exposure are clearly needed for terrestrial crop species so as to adequately guide food safety considerations.

5.4 Impacts through subtle changes in growth media

In addition to direct stress from ENMs, plant growth may also be affected by particle-induced changes in growth media. Although these changes are generally more subtle than direct stress effects, they cannot be ignored as a supplementary factor in plant response and as an input parameter for food safety risk assessment efforts.

Certain ENMs have been shown to extensively cover plant root surfaces; it is possible the reduced hydraulic movement between soil and roots would therefore induce symptoms of water stress. A study on *Zea mays* seedlings found that root hydraulic conductivity was reduced, likely resulting inhibition of leaf growth and transpiration (Asli and Neumann, 2009). Importantly, this effect was time-dependent and after 6 weeks, and no difference was evident between control and ENMs treatment. In this case, a more in-depth investigation into gene or protein expression would be informative. Another potentially important indirect impact may result from changes in the soil microbial community as a function of ENM exposure. Arbuscular mycorrhizal fungi (AMF) exposed to iron oxide nanoparticles had significantly reduced glomalin content and clover biomass due to compromised nutrient acquisition (Feng *et al.*, 2013). However, in many cases, linking soil microbial activity or diversity to plant responses may be difficult. For example, different groups of microorganisms were found to increase or decrease in soil amended with MWCNTs, but the overall bacterial diversity did not change and the changes in soil microorganisms that did occur were not correlated with *Lycopersicon esculentum* growth (Khodakovskaya *et al.*, 2013).

5.5 Impact on agronomic and yield characteristics

Much of the ENM phytotoxicity literature has focused on short-term exposure (seedlings/vegetative phase), but to fully characterize particle fate and effects, as well as implications for food safety, research must include studies conducted until full maturity/ harvest. Only under those conditions can the impacts of ENM exposure on fruit/grain production quantity and quality (nutrition) be adequately assessed. Specific endpoints should include growth parameters (number of flowers/fruit, fruit mass/size, seed mass/number) and nutritional traits (protein and amino acid content, oils and fatty acids, carotenoids and minerals). As mentioned above, molecular analysis tracking the expression of critical genes may also be highly instructive.

Lin *et al.* (2009) conducted on *Oryza sativa* exposed to C_{70}, and showed aggregated ENMs in second-generation seedlings when the first generation was exposed only during germination. Transmission of nanomaterials to the progeny through seeds suggests the potential that ENMs may present a longer-term chronic exposure hazard to human and non-human receptors. Khodakovskaya *et al.* (2013) illustrated that *Lycopersicon esculentum* grown in soil amended with MWCNTs produced two times more flowers and therefore, induced significantly greater fruit development as compared to control plants and plants receiving activated carbon. The promotion in flowering and reproductive system performance was likely attributed to active expression of water channel protein (aquaporin) and up-regulation of aquaporin and related genes. Similar promotion in fruit yield was reported in *Momordica charantia* (bitter melon) treated with fullerol [$C_{60}(OH)_{20}$] (Kole *et al.*, 2013). Furthermore, the contents of two anticancer and two antidiabetic phytomedicinals were significantly increased with fullerol treatment (Kole *et al.*, 2013). Another study on *Oryza sativa* treated with MWCNTs and C_{70} presented contrary results. The flowering of rice plants was delayed at least one month and the seed setting rate was reduced by 4.6% and 10.5%, with C_{70} and MWCNTs treatments, respectively (Lin *et al.*, 2009). However, these studies are highly limited and the impact of ENM exposure on flowering and fruit development remains an important unanswered question.

The nutrition level of crops grown in ENMs-contaminated soil may also be compromised. It was reported that *Oryza sativa* grains harvested from nano CeO_2 exposed plants had lower content of Fe, S, prolamin, glutelin, lauric and valeric acids, and starch (Rico *et al.*, 2013b). In a similar exposure setting, nano CeO_2 decreased the Mo concentration in *Cucumis sativus* fruit and also altered nonreducing sugars, phenolic content and fractionation of proteins (Zhao *et al.*, 2014). These tests on nutritional content of ENMs-treated edible tissues suggest that ENMs would affect fruit flavor, antioxidant content, and nutrition levels, as well as growth performance. However, it is still not fully understood whether these changes in nutrition content are indicators of stress induced by ENMs. Given the potential widespread application of nanotechnology in agrichemicals and agriculture, resolution of this question remains a critical issue of concern.

Another noteworthy aspect of ENMs impact on crops regarding food production is through influence on endophytic bacteria-plants symbosis. In *Glycine max* (soybean), nitrogen fixation potential per nodule was also severely diminished at medium and high nano CeO_2 treatments (50, 100 g/kg soil), which are correlated to absent bacteroids in nodules (Priester *et al.*, 2012). Similarly, nano TiO_2 disrupted *Rhizobium*-

legume symbiosis between *Pisum sativum* (peas) and *R. leguminosarum bv. viciae* 3841 by altering the wall structures of the infection thread in nitrogen-fixing nodules, and subsequently delayed nitrogen fixation (Fan *et al.*, 2014). These two studies suggest that Legume nitrogen fixation is very sensitive to ENMs exposure and that yield may be compromised. Clearly, more research is required to understand the potential ENMs impacts on crops and their important symbiosis with endophytic bacteria.

5.6 Impact on co-existing contaminants

ENMs interaction with co-existing contaminants may have implications for the quality and safety of crops. ENMs in agricultural soils can possibly affect the bioavailability of other soil pollutants (e.g., heavy metals, organochlorine pesticides, pharmaceuticals). For instance, C_{60} significantly increased weathered chlordane uptake from soil by *Lycopersicon esculentum* or *Glycine max* whereas MWCNTs decreased chlordane and DDx accumulation in *Cucurbita pepo*, *Zea mays*, *Lycopersicum sculentum*, and *Glycine max* (De La Torre-Roche *et al.*, 2012a; 2012b; 2013; Kelsey and White, 2013). Nanoparticle Ag was found to decrease the p,p′-DDE content of *Glycine max* tissues more than bulk Ag (De La Torre-Roche *et al.*, 2012b). Although pharmaceutical contaminants and heavy metals in soils are currently not extensively tested in co-contamination studies, it is likely the co-existence of ENMs would affect their bioavailability, posing an important food safety concern. The interactions of ENMs with other soil contaminants could be complex, including disruption in membrane integrity, increased/decreased expression in membrane transport proteins, interference with dissolved organic matter, and competition between ENMs and other nutrients/contaminants. The resulting implications for food safety could be far reaching, but the current level of knowledge is still insufficient.

6 Perspectives and research priorities

Because ENMs are transported and may accumulate in agricultural soils through multiple pathways (e.g., biosolid application, atmospheric fallouts, irrigation with recycled water), exposure to crops is likely, potentially resulting in accumulation with largely unknown impacts on growth and productivity. In terms of food safety, trophic transfer of ENMs from crops to humans remains a major concern. Transmission of ENMs through the food chain is possible, but will be dependent on particle ability to move across the soil-plant barrier and accumulate in edible tissues. Existing evidence of ENMs transfer in food chains is largely restricted to invertebrates in aquatic systems, although a small number of studies with select terrestrial invertebrates have been published, but the risk posed to humans consuming these food products is completely unknown. Meaningful and accurate health risk assessment of ENMs is dependent on a thorough understanding of material fate and effects, including ENMs concentrations in agricultural soil/water, crops and subsequent trophic levels, as well as potential transformation *in vivo*.

Two additional research focus areas with regard to ENMs-crop interactions are: (1) elucidation of underlying molecular mechanisms of interaction and (2) illustration of various environmental factors that affecting ENMs-crop interactions under realistic conditions. First, a mechanistic understanding of ENMs-crop interactions is elusive but will be critical to accurate ENM risk assessment. There are many questions to be resolved. As mentioned above, metal-based ENMs must be compared with appropriate ion and bulk material controls, to reveal actual instances of size-dependent toxicity. Another open question is how surface functionalization of ENMs would affect their fate in particle uptake and accumulation. It is possible that *in vivo*, ENMs surface functional groups could covalently bind to protein/DNA or lipophilic molecules within the membrane or cytoplasm (Stark, 2011; Maurer-Jones *et al.*, 2013). Additional emphasis should be placed on molecular/genetic level investigations to discover factors mediating transport and stress response to ENMs *in vivo*. In addition, metabolomics and proteomics could be introduced as an approach to probe stress response, including monitoring changes in secondary-metabolite profiles under ENMs exposure. Second, considering the complexity of agroecosystems, the influence of various environmental factors on ENMs-crop interactions needs to be addressed. Agricultural soils involve complex abiotic and biotic components, including mineral/nutrient, water, NOM, microorganisms/endophytic bacteria, soil invertebrates and co-existing contaminant. Among these environmental factors, NOM is of primary interest, because of its ability to dramatically stabilize ENMs in aqueous media and subsequently affect aggregation/mobility (Ghosh *et al.*, 2008; Johnson *et al.*, 2009). However, the impact of NOM on the bioavailability/phytotoxicity of ENMs has not been thoroughly evaluated. Similarly, the role of other environmental factors (root exudation, soil pH, cations/ionic strength, microbial diversity) in ENMs-crop interactions is poorly understood. It is also of great importance to investigate how ENMs would affect crop plants uptake of other co-existing soil contaminants. Hence, investigations of ENMs-crop interactions should be conducted under conditions that approximate environmental factors in rhizosphere.

Currently, measurement techniques remain a major limitation for assessing ENM fate and transport, including interactions with agricultural species. Without appropriate instrumental analysis, efficient tracking of ENMs *in vivo* or *in situ* will be difficult and prone to error. Synchrotron-based microfocused X-rayfluorescence (μ-SXRF) with microX-ray absorption near-edge structure (μ-XANES) or microX-ray diffraction (μ-XRD) are currently the most powerful set of techniques used for a complete characterization of biological samples. Among the set of techniques, μ-SXRF is very useful, allowing *in situ* mapping of nanoparticles with high sensitivity, negligible sample damage, and enable tuning of the incident energy as desired (Ma *et al.*, 2011; Majumdar *et al.*, 2012; Hernandez-Viezcas *et al.*, 2013; Hummer and Rompel, 2013). Other promising advances include single particle inductively coupled plasma-mass spectrometry (spICP-MS), solid sampling high-resolution-continuum source atomic absorption spectrometry (HR-CS AAS) and two-photon excitation microscopy (TPEM) (Wild and Jones, 2009; Feichtmeier and Leopold, 2013; Gray *et al.*, 2013). However, the most advanced instrumentation platforms used for these types of studies are costly and of limited accessibility (Szakal *et al.*, 2014). As such, a multidisciplinary and orthogonal approach is needed, including the development of new or hybridized measurement techniques, in order to promote the understanding of ENMs-crop interactions (Gardea-Torresdey *et al.*, 2014; Petersen *et al.*, 2014).

Many details of ENMs-crop interactions remain poorly understood, including the possibility of ENMs transmission from crops to human beings, co-contamination effects of ENMs with other soil pollutants, the mechanisms in plant uptake and stress response, and environmental factors mediating these interaction processes. With such a limited knowledge in ENMs-crop interactions, the quality, quantity, and safety of food products from ENMs-containing environments are impossible to assess. An awareness of potential risks from growing crops in ENMs-contaminated soils and with the development of novel measurement techniques will help to overcome these knowledge gaps.

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中文概要：

本文题目：纳米材料与农作物之间的相互作用：食品安全与启示

Interactions between engineered nanomaterials and agricultural crops: implications for food safety

研究目的：通过综述作物对纳米材料的吸收途径和积累，以及纳米材料对农作物生长和营养的影响，为纳米污染在农业中的风险提供理论分析和启示。

创新要点：归纳了纳米材料被作物吸收的路径和对作物生理、遗传、营养各水平上产生的胁迫。

重要结论：当前纳米与作物的研究应集中在食品安全相关的问题上，考虑农业实际情况和环境因素，分析纳米材料通过食物链富集和传递的可能性，探讨纳米材料与其他土壤有机污染物可能产生的复合污染。

关键词组：纳米材料；植物吸收；食物链传递；食品安全