

Critical Review: Role of Inorganic Nanoparticle Properties on Their Foliar Uptake and *in Planta* Translocation

Astrid Avellan,* Jie Yun, Bruno P. Morais, Emma T. Clement, Sonia M. Rodrigues, and Gregory V. Lowry*



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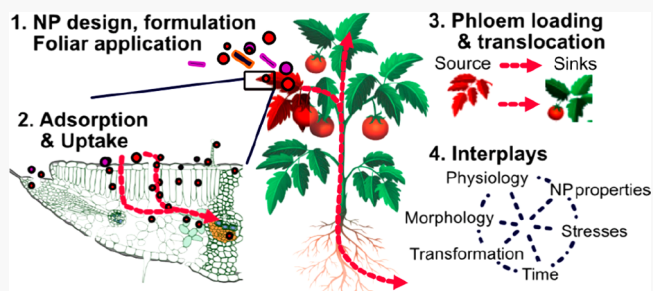
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ABSTRACT: There is increasing pressure on global agricultural systems due to higher food demand, climate change, and environmental concerns. The design of nanostructures is proposed as one of the economically viable technological solutions that can make agrochemical use (fertilizers and pesticides) more efficient through reduced runoff, increased foliar uptake and bioavailability, and decreased environmental impacts. However, gaps in knowledge about the transport of nanoparticles across the leaf surface and their behavior *in planta* limit the rational design of nanoparticles for foliar delivery with controlled fate and limited risk. Here, the current literature on nano-objects deposited on leaves is reviewed. The different possible foliar routes of uptake (stomata, cuticle, trichomes, hydathodes, necrotic spots) are discussed, along with the paths of translocation, via the phloem, from the leaf to the end sinks (mature and developing tissues, roots, rhizosphere). This review details the interplays between morphological constraints, environmental stimuli, and physical-chemical properties of nanoparticles influencing their fate, transformation, and transport after foliar deposition. A metadata analysis from the existing literature highlighted that plant used for testing nanoparticle fate are most often dicotyledon plants (75%), while monocotyledons (as cereals) are less considered. Correlations on parameters calculated from the literature indicated that nanoparticle dose, size, zeta potential, and affinity to organic phases correlated with leaf-to-sink translocation, demonstrating that targeting nanoparticles to specific plant compartments by design should be achievable. Correlations also showed that time and plant growth seemed to be drivers for *in planta* mobility, parameters that are largely overlooked in the literature. This review thus highlights the material design opportunities and the knowledge gaps for targeted, stimuli driven deliveries of safe nanomaterials for agriculture.

KEYWORDS: nano-bio interface, nanofertilizers, plant barriers, sustainable agriculture



1. MOTIVATION TO BETTER UNDERSTAND NANOPARTICLE-LEAF INTERACTIONS, UPTAKE, AND LEAF-TO-SINK TRANSLOCATION

Global food demand is predicted to increase by 50% by 2060, requiring rapid agricultural adaptation. Further, global changes and environmental increased pressure on the agro-ecosystems raise fears of a weakening of crops in the face of biotic aggressions and abiotic stresses.¹ Fertilizer application rates are highly variable depending on countries, ranging from 60 kg/ha in south African countries to 137 kg/ha in Northern America, 160 kg/ha in Europe, and 344 kg/ha in East Asia and the Pacific.² In 2017, pesticide use followed similar trends, with a yearly use of 27 000 tons in South Africa, 480 000 tons in Europe and Northern America, and 1.85 million tons in Eastern Asia.³ Following our current practices, it is predicted that agrochemical (defined in this manuscript as fertilizers, plant-protection chemicals, or pesticides) demand will undergo more than a 2-fold increase in the use of conventional

fertilizers (N, P, K), and 1.9 to 4.8-fold increase in pesticide use by 2050.^{4,5}

Yet, current use of agrochemicals is highly inefficient. It has been estimated that up to 50%⁶ of the nutrients and >95% of pesticides⁷ that are applied on crops never reach their target and are wasted, causing soil pollution,⁸ antibiotic resistance,⁹ and runoff that degrades the ecosystems.¹⁰ These wastes and contaminations are partly due to the poor design of agrochemicals along with their application strategies. In this context, legislative decisions are taken. For instance, the European Commission released a “farm to fork” strategy that

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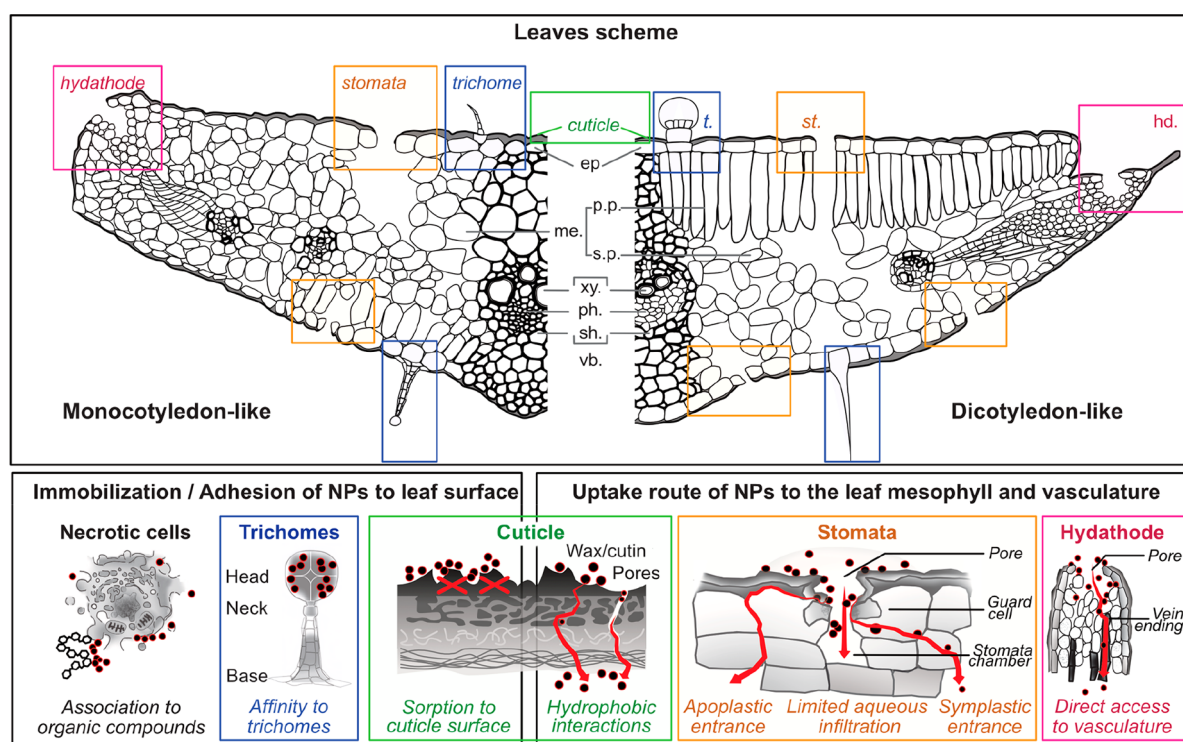


Figure 1. Leaf morphologies and structures that control NP immobilization on a leaf surface and their uptake into the mesophyll. NPs are represented as black spheres with a red outline. The different leaf structures are highlighted using colored boxes. *ep.*, epidermis; *me.*, mesophyll; *p.p.*, palisade parenchyma; *s.p.*, spongy parenchyma; *xy.*, xylem; *ph.*, phloem; *sh.*, sheath cells; *vb.*, vascular bundle. NP properties that have been demonstrated to impact these interactions include their specific surface area, core speciation, surface functionality, and hydrophobicity

aims at reducing fertilizer use by 20% and nutrient losses by 50% by 2030. Agro-ecosystems and agricultural practices have thus entered a major transition period, resulting from environmental stressors, systems weakening, and legislation orientations that are pushing the sector to evolve toward better practices. In this context, scientific research has the responsibility to propose solutions for the design of agrochemicals that fertilize and protect crops, but also their agro-ecosystems. There are several strategies to develop a safer, more sustainable agricultural management.¹¹ Foliar application of nanoenabled agrochemicals offers an opportunity to decrease our inputs, lowering the waste of resources, along with environmental contaminations.

Existing crop fertilization and protection strategies include the addition of solid fertilizers as soil amendments or fertigation, and by foliar applications. While the relative amounts of soil-applied and foliar-applied agrochemicals could not be accurately determined, agrochemicals are routinely sprayed onto crop leaves to treat infections and/or meet (micro)nutrient needs. First reported in the 19th century, foliar applications have since become a widely adopted standard practice for crop management.¹² For example, foliar application of N, P, K, and micronutrients for fertilization are common on cereals and fruit crops.^{13,14} This is a standard practice in Asia, the USA, and Europe.¹³ The foliar application of pesticides is also commonly used to manage weeds and to protect for diseases¹⁵ all types of crops: viticulture, arboriculture, market gardening, horticulture, and arable field crops.¹⁶ Foliar protection and fertilization is typically done when soil conditions (e.g., pH conditions) prevent nutrient bioavailability,¹⁷ for an improved plant priming,¹³ or when leaves need to be protected from pathogens.¹⁸ Therefore,

improvements in the foliar delivery of nutrients and pesticides will likely have a large impact on the sustainability of agriculture.

However, the formulation of foliarly applied agrochemicals mainly consists of molecules, metal salts, or chelated metal ions. The bioavailability of these compounds on leaves is relatively low, and their adhesion to the leaves is poor, leading to rain wash-off (leaf drip) and contamination of the surrounding soil and coupled ecosystems. Among them, inorganics are of particular risk due to their persistent nature. Improving their formulation is a promising strategy to optimize foliar agrochemical practices. Taking advantage of the increasing knowledge regarding nanostructure tuning for targeting reactivity and fate, through the use of nanoenabled agrochemicals, is a great opportunity to design such formulations.

Nanoparticulate forms of many key plant nutrients show promise for improving foliar delivery because of their small size, tunable surface properties, and inherent slow release of active compounds, which could allow application of the right amount and form of *what* the plant needs, delivery of it *where* it will be used most efficiently, and release of the active compounds *when* it is needed based on plant-induced stimuli.¹⁹ Energy balance calculations and expert consensus have indicated that these approaches are most beneficial for targeted applications, e.g., foliar applications of pesticide and nutrients or seed coatings to promote germination and early stage growth of plants.²⁰ Moreover, the most promising near term applications are for effective delivery of micronutrients for nutrition and pest management, new approaches to pest management (e.g., RNAi delivery), or improving the effectiveness of existing active ingredients.²¹

Several challenges arise prior to successfully designing efficient and safe nanoenabled agrochemicals. First, one should ensure that the designed structures are significantly safer than their conventional counterparts for the entire agro-ecosystem, biota, and biodiversity by assessing their environmental implications. While progress has been made to understand the structures of the nanoparticles and the conditions leading to nanorelated impacts and risks for human and environmental health, assessing the safety of these materials will have to continue in parallel with their development following a safe and sustainable by-design approach.^{22,23} This is also necessary to build stakeholder trust for these new materials and approaches. Second, although agriculture has, for decades, taken advantage of the ability of plant leaves to take up required compounds, many unknowns remain as to what parameters (biological, chemical, and environmental) will influence the fate of a nanoparticle after being foliarly deposited. This lack of knowledge impairs our capacity to design nanostructures with tunable properties regarding foliar adhesion, uptake, *in planta* mobility, accumulation, and transformations.

This review aims at analyzing the current state of knowledge regarding (i) NP adhesion to specific leaf feature and surfaces, and the different identified routes of uptake through biological barriers, (ii) NP mobility *in planta* and delivery to and/or accumulation in specific plant reservoirs (leaves, stem, fruits, seeds, roots, rhizosphere), and (iii) NP transformations that release nutrients, either through thermodynamic equilibrium, or via stimuli-driven reactions. Finally, the NP physical-chemical characteristics, environmental parameters, and/or biological properties that modulate these processes are assessed using multivariate analyses. Research needs to advance the state of knowledge about NP–plant interactions to promote more sustainable and environmentally friendly nanoenabled approaches to delivering nutrients to crop plants are also discussed.

2. NANOPARTICLE INTERACTION WITH LEAF SURFACE FEATURES

The plant leaf is an organ whose structure allows the right balance between protecting the plant against water loss, pathogens, and pests while allowing gas exchanges for photosynthesis reactions. Although plant leaf morphology is diverse across plant species, the leaf surface is generally composed of the following features (Figure 1): trichome hairs, stomata, and hydathode pores (whose functions are described below). The leaf is covered with cuticle on most of the surface. The cuticle is a lipophilic waxy layer (thickness of 100–250 nm^{24,25}) that can be thinner or absent at the surface of trichomes and necrotized cells. Stomata²⁶ and hydathode²⁵ openings are free of cuticle. After being deposited on the plant surface, inorganic nanomaterials could interact with and adhere to any of these structures, affecting adhesion and uptake. NP adhesion (i.e., the ability of an NP to remain on the plant surface after a rain event), sometimes called rainfastness, is regulated by the affinity between the functional groups of the NP surface^{27,28} and specific compounds of the foliage surface including glucosides and proteins (hydroxyls, aldehydes, carboxylic acid, and amine groups)²⁹ and wax (containing polysaccharides, fatty acids, fatty alcohols, and fatty aldehydes).³⁰

The benefits of using nanoforms of agrochemicals are clear when comparing the leaching rates of different forms of Cu

from citrus leaves. While 93% of conventional ionic CuSO₄ applied to leaves is washed off with water after application on citrus leaves, 98% of the Cu in CuO NPs is retained under the same conditions. Further, the Cu leaching from the leaves treated with nanoparticulate Cu forms (Cu(OH)₂, CuO, Cu₂O, and Cu₂O coated-silica NP, or Cu²⁺ sorbed on graphene oxide) was mainly ionic (>75%), demonstrating that the nanostructures were better retained on the leaves than Cu ions.³¹ A similar observation was made with Au NPs and Au ions on wheat leaves.²⁷ Other work on Cu-based NPs has shown that the particle size distribution remains relatively constant over time and persisted at the leaf surface,³² supporting the assertion that metal and metal oxide NP species adhere to leaves better than ionic forms of metal and can present a higher foliar retention.

NP size and surface properties can also be tuned to increase their affinities with the cuticle and/or target specific leaf features. NP adhesion on the leaf surface is higher for smaller NPs in comparison with larger sizes, likely due to their higher specific surface area.³³ This was demonstrated with AuNPs of various sizes, where smaller NPs (approximately 3 nm) were nearly 100% retained at the wheat surface, while larger size (50 nm) almost entirely washed off.²⁷ Similar observations were made with Cu-based NPs.³¹ Further, NPs on the leaf surface can interact with functional groups present at the interface (mainly methylene, carboxyls and hydroxyls, but also amine, thiol, and aromatics³⁴) through hydrogen or covalent bonding and electrostatic or hydrophobic interactions. Foliar adhesion of NPs could thus be controlled through modulation of the NP surface chemistry. For example, abamectin poly(lactic acid) NPs functionalized with positively charged groups (H₂N-R)²⁸ showed higher retention (~58% of NPs adhesion) on cucumber leaves after rinsing compared to neutral (CH₃CO-R; ~40% adhesion) and negative (–OOC-R) functionalized surfaces (~35% adhesion). The authors hypothesized that neutral NPs bind to the cucumber leaves by hydrogen bonding through the –OH groups on the leaf surface and that adhesion is weakened or strengthened in the case of the negatively or positively charged surfaces. Charge is not likely the only parameter that matters regarding leaf adhesion, as AuNPs of similar charge (apparent zeta potential) showed a higher affinity for hydrophobic wheat surfaces when coated with polyvinylpyrrolidone (higher molecular weight) than with citrate.²⁷ Tuning NP surface properties like charge and hydrophobicity can thus be an interesting strategy to tune their affinity to the leaf surfaces. These same properties may also be leveraged to target specific leaf features, such as protein-rich structures on the leaves (e.g., trichomes and stomata).³⁵

Plant leaf physiology will play a significant role on NP adhesion. Studies have shown a higher retention of Pt NPs for leaves with a higher wettability, or surface free energy (SFE).³⁶ SFE is affected by leaf hydrophobicity, cuticle composition (amount of cutin and waxes, presence of phenolic and polysaccharide compounds), and surface roughness (hair and stomata density and structure, presence of necrotic spots, vasculature morphology).³⁶ Surface roughness has also been demonstrated to lead to NP trapping.³⁷ It is important to note that the plant leaf traits that drive the SFE may change when exposed to NPs if they contain a toxicant.³⁸ For example, Fe₂O₃ particles led to necrosis in citrus leaves,³⁹ which changed the leaf surface composition and therefore likely the NP–leaf interactions. This suggests that formulations can be

developed to take advantage of biological changes due to spraying and the resulting complex NP–plant interactions.

Trichomes (leaf “hairs”) could also play a significant role in NP physical immobilization on the leaf surface, given their high density on some leaf surfaces and their significant role in exchanging gases and fluids at the leaf–atmosphere interface. Trichomes can be secretory hairs that release metabolic products, or nonglandular presenting a protective function.⁴⁰ They have not yet been demonstrated as a route of NP uptake, even though they have been shown to accumulate NPs that are applied to plant surfaces.^{27,35} Fluorescently tagged polymer based NPs (40–50 nm) were detected inside the glandular trichomes of grape leaves immersed in a NP suspension with surfactant.⁴¹ AuNPs coated with bovine serum albumin protein had a high affinity to the head and the base of glandular trichomes.³⁵ It is still not clear whether these exuding structures are part of the NP uptake pathways or if they adsorb NPs without internalization. According to Li et al., both Zn NPs and ZnSO₄ salt accumulated in nonglandular trichomes of soybeans but not in their surrounding tissues, suggesting that metal did not transport away from the trichomes.⁴² Other studies support the fact that trichomes could be a sink for metals (maybe as a depuration process) rather than a route of uptake.^{27,35,42} Indeed, root uptake of ionic^{43,44} or nanoparticulate^{45,46} forms of metals has been shown to accumulate in trichomes. A similar observation of trichome trapping was found following a foliar application of AuNPs, away from the deposition zone, suggesting that the NPs transported inside the leaf to trichomes where they were trapped or were being exuded.²⁷ To date, the role of trichomes in NP immobilization (or depuration) and/or uptake is underexplored.

3. ROUTES FOR NP FOLIAR UPTAKE

Following deposition and adhesion to the leaf surface, a fraction of the applied NPs will be taken up into the leaf. Two different uptake pathways are likely to occur: (i) a polar pathway, through the leaf surface polar apertures, e.g., trichomes, hydathodes, necrosis spots, and stomata, and (ii) a nonpolar pathway, through the leaf cuticle and its pores (Figure 1). In the latter sections, uptake is defined to include all of the elements from the NP and their products of transformation (i.e., dissolved metals) that enter the leaf and reach its mesophyll.

It can be difficult to conclusively determine the amount of NP that is taken up into the leaf. This is largely due to methodological limitations to quantitatively distinguish NP uptake from surface adsorption, as plant leaf tissue digestion and metal measurement will not distinguish the two. Extensive rinsing of the leaf surface (including several washing steps and acidic solutions) can potentially remove most adhering metal and metal oxide NPs,³¹ but this may not be fully effective. Thus, quantitatively determining the precise size exclusion limits for NP foliar uptake or for comparing the effect of different NP properties on their route of uptake remains challenging.

Despite these limitations, NPs' primary size and aggregation state are factors that appear to affect uptake. Even though objects as big as 50–60 nm have been shown to be taken up after leaf surface deposition,^{27,47} and quite large structures have been found inside plant leaves following NP foliar deposition, these were lipid-based⁴⁸ (150–300 nm) or liposomal⁴⁹ (100 nm), and thus not rigid objects and potentially not indicative

of the size cutoffs for rigid metal NPs that are proposed as fertilizers. No direct evidence has demonstrated the inhibition or reduction of leaf uptake due to aggregation of NPs, but studies have reported that aggregates are retained on the leaf surface. For instance, the presence of large CeO₂ aggregates correlated with an absence of uptake on maize leaves⁵⁰ and with NPs trapped on cucumber leaf surface.⁵¹ Research has also shown that aerosol application of AuNPs increased their uptake in comparison to aqueous drop deposition,⁵² likely because of a decrease in the maximum size of the NP aggregates in the droplets. Therefore, formulations that limit aggregation will likely be more efficient at delivery, although the size limitations for leaf uptake remain unknown and will likely depend on the type of plants, the NP properties, and their pathways of uptake.

3.1. Hydrophilic Pathways of NP Uptake. Nanoparticle uptake can occur through the pores on the leaf surfaces, including stomata and hydathodes. Hydathodes are guttation-secreting pores,⁵³ directly connected to the vasculature system.⁵⁴ They can be up to several micrometers in size, large enough for some bacteria to enter plant leaves.⁵⁵ Though limited information is available concerning the potential entry of NPs in plant leaves through hydathodes, a study demonstrated CeO₂ NP (8 nm) accumulation inside hydathodes of lettuce leaves⁵⁶ and the negatively charged polymer poly(ϵ -caprolactone) NP penetrated *Brassica juncea* leaves through hydathode apertures.⁵⁷ Given the size and hydrophilicity of hydathodes, this route of entry on leaves could be significant for hydrophilic NPs depending on the plant species and their density of hydathodes, but studies are yet to investigate this question.

The role of stomata in NP uptake, in contrast, has been more studied and is hypothesized to be a primary route of entrance of NPs into plant leaves. Accumulation of NP on the stomata, in its vicinity, or in the stomatal cavity has been shown numerous times for a large range of NP types: fluorescent polystyrene NPs accumulating on the stomata and in the stomata cavities of *Vicia faba*,⁴⁷ Pb NPs in parsley close to a stomata,⁵⁸ Ag from AgNPs deposited on lettuce on stomata and in the subcavity,⁵⁹ aggregates of CeO₂ near maize leaf stomata,⁵⁰ Cu from CuO NPs in the center of the stomata aperture,⁶⁰ and silica NPs in the guard cells of spinach, arugula, and watercress.⁶¹ It thus seems that hydrophilic NPs enter the leaves through the stomatal openings.^{47,62,63} However, stomatal uptake of NPs will likely be dependent on plant morphology and physiological status. The stomatal density on plant leaves is dependent on the plant species, as well as environmental conditions.⁶⁴ Stomatal activity is also dependent on environmental conditions (e.g., CO₂ concentration,⁶⁵ air humidity,⁶⁶ light intensity⁶⁴) and the time of day at which objects are applied to the leaves due to stomatal aperture cycling. Therefore, both plant species and the environmental conditions at the time of application may influence the extent of NP uptake through the stomatal pathway. These are all important considerations in developing efficient delivery routes.

Furthermore, the large number of studies showing evidence of a stomatal uptake pathway does not preclude other routes of entrance, even though many publications state otherwise. Conceptually, the large aperture of stomata (from 10 up to 100 μm ⁶⁷) and the ease of visualization on a leaf surface are good explanations for the high incidence of this observation, and it is conventionally described in articles as the major (if not the

only) uptake route for NPs.⁶⁸ However, the accumulation of metal and NPs in stomata does not necessarily reflect a route of entrance, and could also be considered as a “dead-end” if the NPs do not translocate to other parts of the plants. Further, NPs in suspension are likely not taken up by mass flow of the suspension, since stomata are protected against infiltration of aqueous solutions with high ($>30 \text{ mN m}^{-1}$) surface tension.⁶⁹ This could explain why tagged-polystyrene NP of $1.1 \mu\text{m}$ could not enter the large ($\sim 25 \mu\text{m} \times 10 \mu\text{m}$) stomata of *Vicia faba*.⁴⁷ The formulation surface tension can be lowered by the use of surfactants, enhancing leaf wettability and/or stomatal infiltration.⁶² But the same surfactants also degrade the cuticle and epidermis layers and could thus increase NP uptake.⁷⁰ It is also noteworthy that stomatal infiltration may be influenced by the experimental protocol used for exposure. Keeping the atmosphere saturated with water during the experiments⁴⁷ or keeping the leaves moist continuously after foliar application^{39,42,71} to avoid evaporation of the suspension on the leaf surface can increase the time of interaction between NPs in suspension and the leaf surface, and therefore uptake. It may also prevent aggregation of the NPs that would occur with evaporation. Overall, increasing humidity may decrease the diffusive resistance of the fully hydrated cuticle,⁷² lowering the solution surface tension, potentially modifying stomata suspension infiltration⁶² and thus the scenarios of uptake. Comparison results from studies using different exposure protocols should be interpreted with care, as these confounding factors affecting uptake have not been systematically explored.

The size-exclusion limit for NP uptake through stomata may not be dictated by the stomatal aperture alone but rather by an inability to translocate from the stomatal cavity into the mesophyll and vasculature, either by symplastic and/or apoplastic pathways (more on this later, see also Figure 1 and 2). Other routes of uptake directly through the cuticle have been described in few studies as an important route of entrance, as described below. These cuticular pathways were hypothesized in studies showing that the association of CeO_2 NPs with maize leaves after rinsing was not improved when stomata were open (day exposure) or closed (night exposure).⁵⁰ Another example is the absorption of Zn from ZnSO_4 or ZnO NP on soybeans that seemed to be independent of the stomata pathway since no accumulation of Zn in the stomata was observed.⁴² Overall, the studies to date do not support the assertion that stomatal uptake is the only route of NP uptake into leaves.

3.2. Hydrophobic Pathways. The significantly greater surface area of plant leaves being covered by the cuticle compared to stomata or hydathodes makes it a major structure for NP delivery to plants through foliar application. While absorption of ionic species through cuticle pores is known,⁴² these pores are small (nanometer-range), and only NPs $< \sim 2 \text{ nm}$ are expected to enter plant leaves through that route.⁴⁷ However, several studies have suggested the potential for uptake of NPs directly through the waxy cuticle. Studies have hypothesized that 4 to 100 nm TiO_2 , Ag, or Pb NP could cross the waxy layer on the cuticle.^{58,59,73} Another reported that polymer-tagged NPs ($>50 \text{ nm}$) accumulated in the anticlinal cell walls of the epidermal cell layers of *A. thaliana* and *L. angustifolius*, in areas devoid of stomata.⁴⁸ Furthermore, 12 nm PVP-coated AuNPs appeared to accumulate in the mesophyll of wheat leaves, in areas devoid of stomatal apertures,²⁷ while similar sized citrate-coated AuNPs did not, demonstrating that

PVP-coated NPs were able to cross and/or disrupt the cuticle and enter wheat leaves.²⁷

All in all, the notion of cuticle permeability for NPs larger than $\sim 0.5\text{--}2 \text{ nm}$, or that are not strictly hydrophobic, is being recognized, and more effort is needed to understand the role of epicuticular wax and cutin in foliar metal transfers, along with the parameters and mechanisms that allow for some (even large, 50 nm) NPs to enter plant leaves through that route. Some surfactants (e.g., Silwet L-77) can also solubilize cuticular waxes, enhancing the hydration of the cuticle,⁷⁴ and in some cases the epidermis cells,⁷⁰ which could further facilitate NP uptake into the leaf mesophyll.^{48,70} However, the solubilization induced by surfactants can also lead to the formation of necrotic spots and phytotoxic symptoms,^{48,62} which may also be routes of entry.

Necrotic spots could also be generated by the release of ions from deposited NPs as has been described with Fe_2O_3 ,⁷⁵ Pb,³⁸ or CuO ⁶⁰ NPs. Molecules released from necrosis are a mix of oligosaccharides and enzymes.⁷⁶ These may form a physical barrier and decrease NP uptake into the mesophyll (see Figure 1). Alternatively, leaf injury apertures can be gates for pathogen entry and might increase NP internalization as shown with laser perforation in citrus leaves.⁷⁷ Understanding if metals taken up from NPs are the ions released by the NP (as hypothesized for ZnO ⁴²), the pristine—nondissolved—form of NP (as demonstrated for AuNPs²⁷), or a mix of both (as suggested for Ag³⁹ or ZnO ⁷⁸ for instance) is still an active area of research, and understanding the form by which metals can be taken up (as NP or dissolved ions) is critical to predicting foliar metal uptake, along with their beneficial or detrimental impacts.

4. TRANSLOCATING THROUGH THE MESOPHYLL AND LOADING THE PHLOEM

After crossing the cuticle, NPs will have to cross several barriers through the mesophyll before reaching the vasculature (see Figure 1 for a simplified diagram of monocotyledon and dicotyledon leaves morphologies). First is the epidermis layer. This outer layer of tightly packed cells is just beneath the cuticle and covers both the upper and lower surfaces of the leaf. Beyond the epidermis is the mesophyll. The mesophyll constitutes most of the inside leaf tissues, where the chloroplasts reside and where photosynthesis occurs. Mesophyll cells form a cell wall continuum (the apoplast), and a cytoplasmic continuum (symplast) connected through channels called plasmodesmata. NPs can move through symplastic (via the cytoplasm continuum) or apoplastic (through the cell wall continuum) pathways to the bundle sheath cells that are connected to companion cells, and further to sieve-tube elements (STE) of the phloem (Figure 2). Symplastic pathways will require movement through a large number of plasmodesmata, channels that connect the cell symplasts. In contrast, apoplastic pathways have fewer, but not zero, membrane barriers to cross to reach the phloem.

Distinguishing between apoplastic and symplastic transport pathways in plants is challenging. This is mainly due to the difficulty to image NP in plant tissues with sufficient spatial resolution to resolve these pathways, and to the lack of sample preparation methods that can avoid artifacts, e.g., NPs moving during tissue fixation, or thin sectioning of the tissues.²⁵ There are two categories of tool that can be used to study NP distribution within plants with sufficient resolution (below $1 \mu\text{m}$) to distinguish these pathways: (i) one approach requires

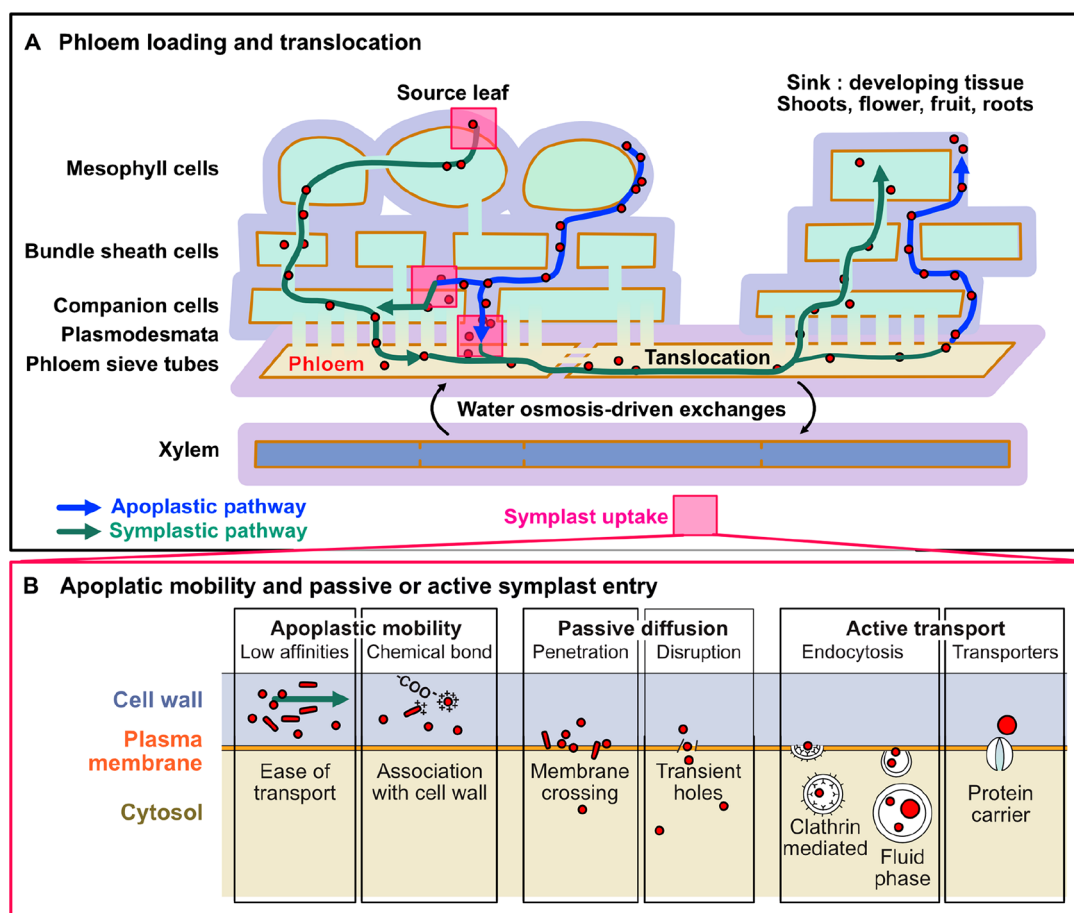


Figure 2. Pathways and routes for phloem loading. Scheme of A: Apoplastic and symplastic routes of nanoparticle transport from the exposed leaf to different plant compartments (sinks) through phloem. B: Processes driving NP apoplast mobility or symplast uptake.

tissue fixation and sometimes thin sections to study cellular distributions using electronic microscopy, laser-ablation ICP-MS, X-ray fluorescence mapping, particle or tissues induced X-ray emission and/or absorption, and nanoscale secondary ion mass spectrometry, and (ii) the second approach uses fresh tissues but requires light penetration into those tissues to study in vivo behaviors, using confocal microscopy and enhanced dark-field microscopy. Several reviews have been written on this subject.^{79,80} While preparing the samples, chemical fixation, drying, and cutting can displace NPs or alter their speciation. Transformations can be minimized using cryogenic fixation without chemical treatment but still require cutting that could mobilize the NPs. Confocal microscopy can also require that the NPs be conjugated with a fluorophore that can modify the NP behavior, leach, or bleach during the experiment. Enhanced-dark field microscopy with hyper-spectral imaging can map the locations of the NP in the plant tissue, but it is not quantitative and can lead to false negative observations. Typically, several of these tools must be applied in parallel to determine and confirm the locations of the NPs.

Studies investigating the impact of plant species and/or NP physical-chemical parameters on apoplast/symplastic transport and phloem loading after foliar exposure are rare, as described below. Yet, unraveling the parameters that influence the NPs translocation pathways in the mesophyll is critical to predicting their transport within the leaf epidermis and mesophyll, as well as their ability to enter the phloem and their further transport

to different sinks in the plant (e.g., shoots, flowers, roots, seeds). A better understanding of these pathways is needed to enable rational design of NPs for targeted delivery to selected plant locations.

4.1. NP Properties Influencing the Apoplastic Transport Pathway. The apoplast is a highly flexible continuum of extracellular matrix consisting of cellulose, hemicellulose, pectins (polysaccharides), and proteins with up to three layers: the middle lamella, the primary cell wall, and the secondary cell wall. The middle lamella (mainly found in dicotyledons and in some monocotyledons) is made of pectin. NP size and surface charge are both likely to impact their potential for mobility in the apoplast. Cellulose/hemicellulose networks have pores calculated to be in the 5–20 nm range,^{25,81} but this porosity can change with plant age and cell differentiation.⁸² Further, metal ions (e.g., those derived from NP dissolution) may also impact these pore sizes.⁸³ This continuum is likely a bottleneck for NP transport. Studies have shown structures being trapped in the cell walls, as observed for TiO₂NP (~270 nm) after atmospheric exposure of birch trees,⁸⁴ 40–50 nm poly(acrylic acid) in grape vine cells,⁴¹ or AgNPs aggregated and trapped in a cell wall and cytosol.⁸⁵ NP surface charge can affect transport in the apoplast. Cell walls are mainly negatively charged due to free carboxyl groups from the pectin, so movement of positively charged NPs through the apoplast is expected to be limited. While this has not been extensively studied for NPs, it has been for ionic species, and cations have been shown to accumulate in the apoplastic

space.⁸¹ This suggests that an NP property such as charge or the pK_a of surface functional groups or NP dissolution in the cytosol (with released cations interacting with cell walls) could be engineered to influence their apoplastic transport, likely in a plant species-specific manner.

4.2. NP Properties Affecting Interaction with and Transport through the Symplast. An NP in the cell wall may contact the plasma membrane, where it could be internalized into the cell symplast.⁸⁶ NPs can also be internalized in cell vacuoles or in cytosol through active transport or passive diffusion.⁸⁷ However, reaching and crossing the symplast seems to be a necessary step to load the phloem (Figure 2).

Direct, passive diffusion of NPs through the phospholipid bilayer that constitutes the plasma membrane is possible.^{88,89} Calculations indicate that anisotropic shapes^{90,91} and irregular patterns of hydrophilic and hydrophobic groups at the surface of NPs⁹² could increase their ability to cross the plasma membrane due to a disruption of the transmembrane potential. Models suggest that as a result of crossing this phospholipid membrane, some lipids will attach to the NP surface.⁹³ Several studies looking at *in vitro* NP internalization in protoplasts agreed with this model prediction, for NPs of different core compositions, shapes, sizes, and zeta potentials.^{89,92–94} Some work tends to validate this model *in planta*, as well.⁶³ These studies suggest that (i) NPs (even with the lowest dimension >30 nm) can passively cross the plasma membrane and enter the protoplasts, (ii) protoplasts of larger diameter (i.e., higher membrane tension) induced more NP uptake, and (iii) increasing the charge density at the NP surface through shape modification and/or a specific coating will increase the potential for crossing plasma membranes.

Active transport of NPs through the plasma membrane could also occur via endocytosis. Size selectivity seems to be low for endocytosis, as protoplasts have been shown to be able to internalize particles as large as 1 μm .^{95,96} The charge of the NP could influence the type of endocytosis that takes place (clathrin-dependent or -independent pathways), inducing different management of the NP by the cells (internalization vs degradation vs recycling pathways), which will further modulate an NP's potential for symplast transport. For instance, AuNP surfaces have been shown to mediate the endocytic path of internalization, with positively charged AuNPs being delivered in tubular vesicles and in vacuoles, while negatively charged AuNPs were transported to inner vesicles.⁹⁷ Other active pathways, such as protein carriers and ion channels, are less likely to be a major route of NP entrance to the plant symplast without any transformation, giving their very restrictive size (<1 nm⁹⁸).

This symplastic transport route is generally more restrictive for NPs than the apoplastic transport route, as plasmodesmata are rather small in diameter, generating bottlenecks reported to be in the 2–20 nm range. The size exclusion limit of this bottleneck can vary depending on the plant species, the type of cells, and the environmental stresses the plants are subject to.^{99,100} Studies have hypothesized that a higher NP aspect ratio and smaller aggregate size *in planta* could favor a symplastic pathway and that it could be tuned through foliar exposure methods (droplets or aerosols).⁵² Thus, size, shape (aspect ratio), and colloidal stability should affect cell-to-cell mobility. Both synthesis approaches and methods of application seem to be strategies allowing tuning for apoplastic

mobility. This mobility will play a major role with respect to favoring or limiting phloem loading.

4.3. Phloem Loading and Translocation to Other Plant Compartments. Several studies have demonstrated that foliarly applied NPs can spread through the entire plant, reaching unexposed plant compartments, such as shoots, stems, fruits, seeds, and roots, and even be exuded into the rhizosphere soil. This transport to other compartments via the plant vasculature could occur through the xylem or the phloem. Xylem mainly transports water and nutrients from roots to shoots.⁴⁰ Phloem transports the photosynthetates (sugar and amino acids and peptides) produced during the photosynthesis where they are needed: upward to the shoot apical meristem, fruits, and other newly formed leaves or downward to the roots.⁴⁰ Metals from foliarly applied NPs were detected in roots (TiO_2 , ZnO ,¹⁰¹ CaO ,¹⁰² Au ,²⁷ CeO_2 ,⁵¹ Pt ³⁶), or even in rhizosphere soil.²⁷ This downward translocation indicates that phloem transport is occurring. The transport of relatively insoluble NPs like TiO_2 and Au from leaves to roots also implies that NPs, not just dissolved metals derived from them, are transported in particulate forms in the phloem.

Together, apoplastic and symplastic transport of photosynthetates will generate a flow toward plant sinks¹⁰³ that the NPs are likely following. It is likely that NP transport is being modulated by both their size and surface functional groups. Eventually, mobile NPs in the mesophyll reach and load the phloem, transporting to the other parts of the plant as described below. The parameters influencing NPs entrance to the companion cells and their entrance in the STE¹⁰⁴ (referred to as “phloem loading”) are yet to be fully characterized. Loading in the phloem can be made through symplast diffusion or apoplastically by active processes (although the later is not found in all plant species¹⁰⁵). Better understanding the factors that influence these pathways, both the NP properties and the plant physiology, are required for the design of effective nanoenabled carriers for agriculture.

5. TRANSPORT OF NANOPARTICLES TO OTHER PLANT COMPARTMENTS

Several publications have shown the transport of metals from NP-exposed leaves to nonexposed plant shoots, flower, roots, and rhizosphere. Metal species have been shown to translocate from the NP-exposed leaves to flowers (CeO_2 ⁵¹), fruits (CeO_2 ,⁵¹ Ag ¹⁰⁶), seeds and grains (Se ,^{107,108} MnO ,¹⁰⁹ ZnO ¹¹⁰), non-exposed shoots (Fe_2O_3 ,¹¹¹ Ag ,^{112,113} CeO_2 ,⁵¹ Au ,²⁷ TiO_2 ,¹⁰¹ ZnO ,¹⁰¹ $\text{Cu}(\text{OH})_2$,¹¹⁴ PbO ¹¹⁵), roots (TiO_2 ,^{101,116} ZnO ,¹⁰¹ CaO ,¹⁰² Au ,^{27,52} CeO_2 ,^{51,56} Pt ,³⁶ Ag ,¹¹⁷ CuO ¹¹⁸), and even in rhizosphere soil ($\text{Cu}(\text{OH})_2$ ¹¹⁹ and Au ²⁷). Some studies comparing roots and foliar application of NPs have shown differences of metal concentration in specific sinks, such as Mn from Mn_2O_3 in wheat grains.¹⁰⁹ As to the exudation of metals derived from labile NP¹¹⁹ or from stable NP,²⁷ these leaf-to-soil transports are rarely investigated, but this approach could represent a means of delivering micro- and macronutrients or pesticides directly to plant roots and increasing efficiency over non-targeted methods like soil drenching.

Further, the NP applied should at some point release ions *in planta* to become bioavailable. It remains unclear if NP transformations resulting from a root exposure will undergo similar transformation than a foliarly absorbed one. As of today, the number of studies addressing the later are too scarce

to be able to highlight which particle would have similar behavior, and under which conditions. More work is needed to study these aspects. Moreover, questions about the optimal rate of dissolution for NPs to maximize bioavailability and/or beneficial impacts still need to be addressed. This understanding is crucial as the type, the dose, and the rate of ionic release can be either neutral, beneficial, or harmful for the plants. Additionally, nontransforming NPs can also trigger specific plant responses that may bring benefits regarding plant yield, and protections against biotic and abiotic stresses, e.g., ref 89. However, investigations of whether the metal translocated in different plant compartments is the original NP or a transformed species (i.e., dissolved and/or reprecipitated metal species) is rarely reported, as discussed in section 5.2.

It thus remains unclear if the majority of the metal transport is from NPs or from their products of transformation. However, studies using relatively insoluble NPs indicate that the particles may transport broadly throughout the plant via the vasculature. This implies significant potential for targeted and efficient delivery of fertilizers using foliar application of inorganic NPs. It may also be possible to deliver nitrogen, phosphorus, and potassium using appropriate nanocarriers.

5.1. Moving between Phloem and Xylem in the Vascular System. After foliar-applied NPs reach phloem, they can be translocated from the leaves to other parts of the plant through sieve tube elements.^{104,105} Concerning photosynthetates, when sieve tube elements have a high local photosynthetates concentration, and thus a lower water potential compared to nearby xylem cells, water will move from xylem to phloem by osmosis.¹²⁰ The excess water generates a higher pressure, resulting in the generation of a multidirectional flow that drives the photosynthetates upward or downward to the developing tissues (leaves, flower, fruits or downward to roots). Then photosynthetates unload occurs through active transport, or passive unloading.¹²⁰ After the photosynthetates concentration drops, osmotic exchanges takes place between the phloem and the xylem, with water moving from the former to the latter (see Figure 2). The water exchanges between the xylem and the phloem suggest that NP exchanges between the two are also possible, as demonstrated for photosynthetates.¹²⁰ Indeed, carbon nanotubes foliarly applied as suspensions on French bean leaves were found both in phloem sieve tubes and xylem vessels.¹²¹ While the phloem sieve tubes present large pore sizes, xylem vessels have smaller pores of a maximum of hundreds of nanometers, depending on plant species,¹²² making xylem transport of larger NPs more difficult. The size limit for transport in phloem is presumably dictated by their sieve plates, made of widened plasmodesmata which are quite large, e.g., 0.405 μm .¹²³ The most important limiting factor for NP translocation that entered the phloem will likely be the nominal size and those of their aggregates.

This analysis indicates that the studies detecting metals in plant tissues away from the NPs-exposed leaves are likely a result of phloem translocation. However, it could also be the results of exchanges between the phloem and the xylem. For instance, xylem-phloem-xylem transfer of CuO NPs has been described following root uptake.¹²⁴ The ultimate distribution in the plant may thus also be a result of their phloem-to-xylem transport in the shoots, and/or exudation into rhizosphere soil, followed by uptake into the xylem. Better understanding of these processes for NPs is critical to optimize their delivery to specific plant organs.

5.2. In Planta Transformations. Metals and metal NPs detected in plant compartments away from the foliar deposition area may not necessarily be the original applied NPs, but transformed species. This is especially true for labile NPs (like CuO and ZnO) that readily dissolve at slightly acidic pH, or in the presence of organic phases.^{125,126} During transport, NPs will face gradients in ionic strength and different redox agents, chelating molecules, and pH. Depending on the compartment they reach, the NP could aggregate, dissolve, precipitate, or gain an organic coating (through protein corona formation).

Aggregation of NPs in synthetic sap has been studied with AgNPs of different coatings.¹¹³ This study demonstrated that AgNPs of comparable sizes but different surface coatings could either be stable in sap (when coated with gum arabic) or aggregated into small (coated with PVP) or large aggregates (when coated with citrate). This aggregation, likely driven by steric stabilization, led to different sedimentation rates. Changes of aggregate sizes and sedimentation due to different coating hydrophobicities and surface charges will likely influence the *in planta* translocation of these objects.¹¹³

NP dissolution and precipitation *in planta* after foliar exposure has also been reported. *In vitro* tests of the dissolution of AgNPs in synthetic sap showed that Ag dissolution could be 4 times higher for inorganic sap (xylem-like sap, mineral nutrient rich) compared to organic sap (phloem-like, sugar rich).¹¹³ Larue et al. demonstrated by speciation measurements using micro-X-ray absorption near-edge structure that Ag NPs applied as a suspension on lettuce were oxidized inside the epidermis layer, and most of the Ag species were bound to thiols or found as AgCl precipitates.⁵⁹ Similarly, observations made with energy dispersive spectroscopy indicated that Ag was present as AgCl in soybeans.⁸⁵ Zhang et al. indicated that Zn from ZnO NPs accumulating within the wheat grain was present at 70–80% as Zn phosphate and 20–30% in other unknown forms.¹¹⁰ As discussed below, tracking for these speciation changes is critical to understand the mechanisms leading to bioavailable forms of metals from the NPs to predict for uptake, transport, and plant fertilization. This is relevant because the ability to fortify grains and fruits with nutrients like iron and zinc would increase their nutritional value. Further, assessing the persistence of metal in their nanoform in edible parts of the plant is critical to assessing for human exposure to nanoparticles.

Concerning translocation of more stable forms of NPs (e.g. TiO₂,¹⁰¹ CeO₂,⁵¹ Au⁵²), several studies hypothesized that a biocorona may coat the NP.⁵² This could facilitate translocation or just be a result of the multiple biological barriers they would be crossing.⁹³ For instance, Larue et al. demonstrated that translocating TiO₂ NPs applied on lettuce were surrounded by an uncharacterized organic coating.⁷³ Evidence for a protein corona following foliar application of NPs is limited in the literature but has been described following root uptake. For instance, AuNPs (50 nm) translocating from roots to xylem were found to be surrounded by some unknown substances.¹²⁷ Thus, NP translocation may be related to the ability of *in planta* organics to bind to NP surfaces.¹²⁸ This corona formation is likely to happen following foliar deposition and phloem loading, as the phloem contains approximately 40 times more amino acids (concentrations can reach 10 g/L) than the xylem.¹²⁹ NP surface properties could thus be engineered to decrease or increase

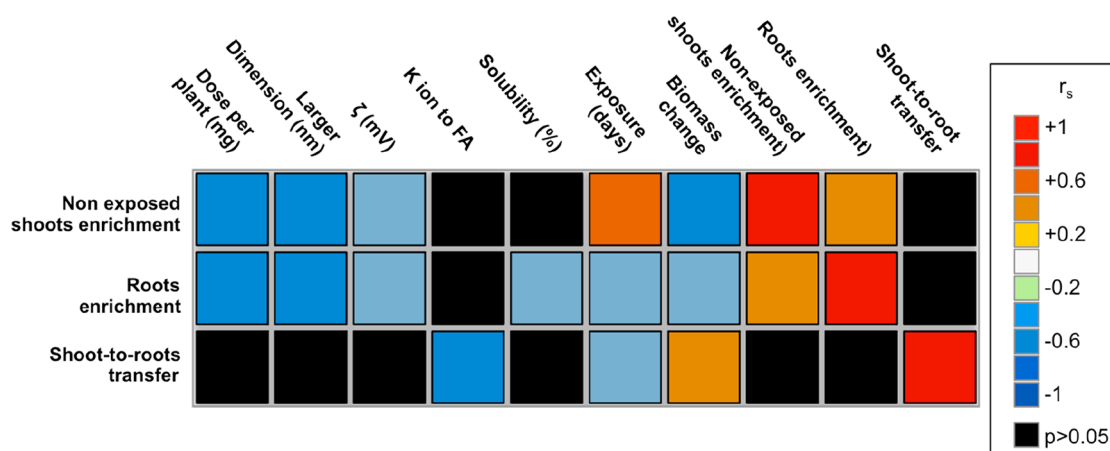


Figure 3. Correlation map of the Spearman coefficient ($p < 0.05$) between the NPs physical-chemical parameters used in the studies (size, zeta potential, solubility, and binding ion affinity to Fulvic acid), the experimental conditions (dose used for the foliar deposition, time of exposure), and the results of biomass changes or transport to other plant compartments (enrichment of nonexposed shoots or root, as well as shoot-to-root transfer ratio). Black indicates an absence of significant correlation ($p > 0.05$). From blue to red: negative ($r_s < 0$) to positive ($r_s > 0$) Spearman correlation coefficient (r_s). Details about values, calculations, correlations, and references used can be found in Figure S1 and Tables S1–S4.

this potential for a biocorona formation, affecting their potential for translocation.

Better understanding these transformations inside the plant is critical to understanding NP–plant interactions, for designing NPs for targeted delivery to selected locations, and for designing NPs that can transform to become more bioavailable once inside the plant, e.g., designing nanoenabled slow release (micro)nutrients.

5.3. Translocation of Metal from Exposed Leaves to Sinks, a Meta-Analysis. **5.3.1. Studies with Heterogeneous Data.** While questions remain about the translocation mechanisms of NP uptake and translocation in plants, analysis of the existing literature enables formation of hypotheses as to how NP properties can modulate translocation (see Table S1 for references and data used). Of the existing literature, 20 studies reported sufficient data for statistical analysis, i.e., metal concentration or content changes in exposed or unexposed plant tissues, time of exposure, and plant biomass. The resulting data set was used to calculate (where possible) the metal dosage from NPs (in mg) applied per plant, the percentage of total dosed metal recovered, changes of plant biomass (in mg), daily uptake of metal (ng of metal per mg of plant per day), metal accumulation in unexposed plant compartments (shoots or roots) relative to undosed controls, and shoot-to-root transfer. See the section Calculations for Dataset Analysis in the SI for details about the calculations to normalize and compare the data.

It should be noted that the exposure conditions vary from one study to another, with various NP types and formulations, different metals, plant species, protocols, and times of exposure, making direct quantitative comparisons of the metal translocation difficult (see Table S2). For example, foliar metal dosage from NPs varied from 42 ng to 423 mg per plant ($n = 64$). The time of exposure following leaf deposition of NPs varied from 1 to 70 days ($n = 119$). Interestingly, 75% of reports used dicotyledon species, even though the top three crops worldwide (wheat, maize, and rice) are monocotyledons, preventing the ability to assess the impact of plant type on NPs' fate. Finally, across all studies, the average percentage of metal recovery in all plant tissues regarding the dosed NPs was only $45.9\% \pm 13.2\%$ ($n = 72$; see Table S3). This low recovery

could indicate (i) a loss of metal when NPs are applied (especially when they are sprayed), (ii) undetected metals due to biomass dilution effects, or (iii) non-investigated sinks (flowers, roots, or rhizosphere), underlying the importance of systematically calculating and reporting metal recovery to ensure that the translocation sinks are identified and to allow comparisons between studies.

5.3.2. Factors Influencing Metal Uptake and in Planta Translocation. The data set produced correlations between (i) NP properties (largest dimension, zeta potential, metal ion binding affinity to Fulvic acid (log K), and solubility in water), (ii) their transport (or that of their transformed metallic species) to nonexposed shoots or roots, along with shoot-to-root transfer, and (iii) experimental parameters (applied NP dose on leaves, time of exposure, plant biomass) as shown in Figure 3. It should be noted that proxies chosen for NPs reactivity (solubility in water, binding affinity to Fulvic acid) were selected based on the hypothesis that transformation might play a significant role in metal transport. However, all of the metals are not transported in planta following similar strategies. Further, NP solubility in water, though partially informative, is not necessarily an adequate proxy for in planta transformations as it ignores the effects of solutes in cytoplasm, phloem, and xylem, but was used as a proxy because it is available for most of the metal NPs studied. See the calculation details in the SI.

The transfer of metal from NPs exposed leaves to nonexposed shoots (n-e shoots) and roots was significantly ($p < 0.01$) negatively correlated to (i) the dose of NPs per plant (n-e shoots $r_s = -0.43$, roots $r_s = -0.49$), (ii) the NPs primary size (n-e shoots $r_s = -0.42$, roots $r_s = -0.45$), and (iii) their zeta potential magnitudes (n-e shoots $r_s = -0.32$, roots $r_s = -0.34$). Relative accumulation of metals from NPs in the roots vs in the shoots (i.e., shoot-to-root transfer) was negatively correlated with metal ion binding affinity to Fulvic acid ($r_s = -0.513$), while root enrichment was (mildly) negatively correlated with the NPs' solubility in water ($r_s = -0.249$, $p = 0.016$). Finally, the time of exposure and the associated change in biomass (also impaired by the different treatments) significantly correlated ($p < 0.01$) positively with the nonexposed shoot enrichments (time $r_s = +0.62$, biomass r_s

= -0.51) and negatively with the root enrichment (time r_s = -0.25, biomass r_s = -0.26) and the shoot-to-root transfer (time r_s = -0.51).

Altogether, these correlations suggest that in planta mobility of metal from NP (as its capacity to enter the leaf and translocate to nonexposed tissues) is greater for smaller doses (as a mass per plant), negative zeta potential, and smaller NP sizes. This meta-analysis is consistent with the individual studies described earlier in the manuscript, suggesting that while higher doses may lead to higher metal uptake, it reduces the mobility *in planta*. This unexpected dose-transport correlation could be due to (homo- or hetero-) aggregation of NPs either on the leaf surface or *in planta*, decreasing the mobility (as described in section 3), along with metal toxicity responses and storage and/or immobilization strategies used by the plants. It is also likely that metal translocation from exposed leaves will be metal dependent, as plants have different physiological responses to various metals. It also suggests that the higher efficiency of uptake should occur with the lowest applied doses for NPs onto the leaves.

The negative correlation of metal mobility with increasing NP size could be due to a combination of factors: larger sizes being retained by several barriers and bottle necks and/or smaller sizes undergoing higher dissolution rates. The present state of the art characterization tools cannot determine if the lower mobility of large NPs is due to a smaller dissolution rate, a lower capacity to cross cellular barriers, or both. Further, the drivers for the translocation of NPs or their transformed species and how this impacts their bioavailability and translocation into sinks, roots, leaves, fruit, or grains has yet to be determined.

Further, the capacity of the metal to accumulate in the roots rather than in the shoots seems to be influenced by the NP solubility and by the binding affinity of the metals to organic acids (here proxied by the log(K) with Fulvic acid, the rationale is provided in the SI). Studying the initial speciation and reactivity of the dosed NP, along with its change in surface chemistry and dissolution *in vivo*, appears necessary to predict their fate *in planta*. It should also be noted that the average shoot-to-root ratio across all studies was $20.6 \pm 4.7\%$ ($n = 57$). This indicates that a large fraction of the metal from NPs applied on leaves reached the roots, independently from the type of metal or the plant species being used. Although shoot-to-root translocation of metals is an important process following foliar exposure, the number of studies quantifying metal or metal NP exudation into the rhizosphere remains very limited. Some studies demonstrate that the metal is exuded from the roots and suggest that the rhizosphere could be a major sink for metal from foliarly deposited NPs.^{27,113,119} This is of particular interest, as the plant could be used as an efficient vector to target chemicals (e.g., antifungals like CuO NPs) to rhizosphere soil without having to disturb the “bulk soil” integrity, composition, or functions.¹³⁰

Finally, the time of exposure and plant growth is a critical experimental trait that is overlooked in most studies. Plant strategies and biological activity to store, depurate, transform, and use metals will vary along their growth cycle. Although time and associated plant growth seems to be a driving factor for *in vivo* accumulation, the majority of the studies report only a snapshot of metal movement after a specified time period. Studies that analyze the impact of time on metal translocation from leaves into other sinks are scarce.^{60,131–133} Xiong and collaborators, for instance, showed that translocation of Cu

from CuO NPs to roots significantly increased with time (at 5, 10, or 15 days after NP deposition).⁶⁰ Similar observations were made with ZnHN, whose translocation to roots continuously increased over the 3 weeks of investigation.¹³³ An increase of nutrient content (N, P, K) with time was also described in soybean grains after foliar treatment with a nanonutrient composite, while respective concentrations in exposed shoots were decreasing.¹³² This impact of time on metal mobility could relate to the transformation of the NPs and/or to physiological and morphological change over plant growth.

It is noteworthy that we could not find a quantitative trait to assess the role of plant anatomy on metal uptake and transfer following NP deposition on plant leaves. Some studies specifically looked at the impact of plant variety and species impact on metal translocation for Cu(OH)₂-based nanopesticide in basil¹¹⁴ and demonstrated differences in copper translocation to roots and in biochemical responses. Similarly, silver transport and plant responses from Ag NPs sprayed on leaves varied for different tree species.¹¹² Despite the lack of studies currently available, this suggests that plant activity, defense strategies against metallic toxicity, morphology, and physiology can significantly impact NP fate after foliar deposition.

6. FUTURE DIRECTIONS AND RESEARCH NEEDS

The existing literature suggests that foliar application of nanoparticulate forms can increase the foliar retention of metals in comparison to dissolved or chelated forms. This is in part due to increased adhesion of NPs compared to dissolved metals that prevents wash-off by rain, and the resulting environmental contamination, but also a result of the lower doses applied, thus saving energy and resources. However, several unknowns remain before one could accurately predict the fate of NP following foliar deposition.

The influence of the plant physiology, morphology constraints, and environmental stressors is clearly overlooked. The present review describes the multilayered biological barriers faced by NPs when foliarly applied and identifies the main NP physical and chemical properties that determine their translocation *in planta*. However, while plant physiological responses to environmental stresses, time of exposure, and plant age seem to be modulating NPs' behavior, these questions remain largely underexplored drivers of nanoparticles' fate in the leaf–plant–rhizosphere continuum.

Several strategies seem achievable to modulate NP mobility in the mesophyll, facilitate phloem loading, and target specific plant compartments. From cuticle interactions to foliar uptake, mesophyll translocation pathways, phloem loading, and rhizosphere exudation, we highlighted here how size, surface charge, hydrophobicity, solubility, and binding affinity to organic phases seems to be major drivers for foliar uptake and *in vivo* mobility. Other aspects such as the bioavailability of nanoparticulate nutrients for plant growth, capacity to cross cellular barriers (cells walls and cell membranes), and corona formation following foliar uptake and translocation, along with nanospecific effects on plant parameters, remain poorly understood, and their importance for nanoparticle mobility and transport in the plant remains unknown.

Further, a better rationale for the experimental conditions used is needed. Studies generally aims at reaching two distinct goals, either (a) quantifying the performance benefits and/or detrimental impact of NP foliar application on plant health

and/or crop production and nutritional quality, or (b) gaining mechanistic insight into NP uptake and translocation *in planta*; although the majority of literature focused primarily on the former. While these assessments provide a valuable foundation for future research, they tend to under-report the mechanistic understanding of the described effects and fates, particularly related to the path of foliar uptake, their mobility in leaves, and the processes that occur during phloem loading. This lack of understanding of NP behavior in the leaf–plant–soil continuum hinders rational design for nanoagrochemical and fertilizer targeted delivery. A lack of standardization of the experimental design regarding the applied dosages, exposure times, and metal detection in upstream and downstream plant compartments (i.e., unexposed tissues, shoot-to-root transport, sink tissues) is evident. This makes it difficult to assess how the chemical and physical characteristics of applied nanoparticles overall influence the observed outcomes. Out of the 120 articles exploring the impact and/or the uptake and/or the translocation of foliarly applied NPs, only 16% presented (partial) quantitative data on the applied metal dose and their transfer to the different plant compartments. We also noted that the speciation of metal inside the plant was rarely investigated. However, this is of particular importance to determining the various metal species responsible for the translocation (i.e., pristine NP, metal ions, metal–organic complexes) and impacts. These would provide essential information not only on NPs uptake mechanism and chemical behavior, but also on how the plant responds to the applied materials.

These limitations were expected since nanoenabled plant fertilization and protection research is still in its infancy when compared to other areas of nanotechnology. Plant nanobiotechnology research is beginning to focus on key questions, driven by earlier studies that demonstrated the viability of such strategies. Recent work is starting to focus unravel the biophysicochemical aspects to modulate NPs behavior *in planta*. These studies demonstrated that improved foliar adhesion, uptake, and targeted delivery to specific cell compartments and/or plant tissues is achievable. Further, while this review focused on inorganic compounds, a lot of opportunities also arise for organic materials (as mRNA, nanopolymers, carbon nanotubes) that were not discussed here. These strategies could also benefit from a better understanding of the fate of inorganic NPs discussed in the present review. Finally, more mature bio-nano-technological fields could be leveraged to improve the design and efficacy of nanoenabled fertilizers and plant protection products, an example being the nanocarrier design and strategies adopted for biomedical applications.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.1c00178>.

(Figure S1) Correlation map of the Spearman coefficient ($p < 0.05$) between the NPs physical-chemical parameters used in the studies, the experimental conditions, and the results of biomass changes or transport to other plant compartments. (Table S1) Literature reference extracted from the literature and used for multivariate analysis presented in Figure 3. (Table S2) Statistic summary on selected papers

presenting comparable quantitative data. (Table S3) Average calculated parameters from the entire pool of articles. (Table S4) Correlation matrix (Spearman correlation with pairwise detection) used in Figure 3 and Figure S1. (Table S5) p values of the correlation matrix (Spearman correlation with pairwise detection) used in Table S4. (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Astrid Avellan – Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States; Biogeochemical Processes and Pollutants, Center for Environmental and Marine Studies, Universidade de Aveiro, 3810-193 Aveiro, Portugal; orcid.org/0000-0001-6081-4389; Email: aavellan@ua.pt

Gregory V. Lowry – Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States; orcid.org/0000-0001-8599-008X; Email: glowry@andrew.cmu.edu

Authors

Jie Yun – Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States; Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge 02139, United States

Bruno P. Morais – Biogeochemical Processes and Pollutants, Center for Environmental and Marine Studies, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Emma T. Clement – Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States

Sonia M. Rodrigues – Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States; Biogeochemical Processes and Pollutants, Center for Environmental and Marine Studies, Universidade de Aveiro, 3810-193 Aveiro, Portugal; orcid.org/0000-0002-3969-0972

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acs.est.1c00178>

Notes

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