Electronic Supplementary Information

Are nanomaterials leading to more efficient agriculture? Outputs from 2009-2022 research metadata analysis

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Manuscript search and selection

The present study was carried out by consulting 996 Web of Science indexed research articles related to nanomaterials and plants published between January, 2009 and September, 2020. For article selection at the Web of Science platform, we employed the following and booleans (TOPIC = nanoparticle OR nanomaterial OR nanotechnology) AND (TOPIC = seed OR plant OR agriculture).

The search returned around 4000 papers. The 996 papers employed in the present study were selected because they presented studies either dealing with the uptake and fate of nanoparticles in plants or depicted how nanoparticles affected plants. Despite interferences with plant development and the metabolism itself, important issues like diseases, salt and drought stresses were also considered. Papers that observed how nanomaterials interact with the plant surrounding environment, like the soil microbiota, were also taken into account. Some examples of papers that were rejected during the search are those related to synthesis of nanoparticles using plant extracts, use of nanomaterials in soil remediation and those focused only on pest control. Several rejected papers were related to the release of pesticides, essential oils, and other substances. Review articles were also excluded from the search. All selected articles were examined individually, and the selected information was used for composing the database.

The criteria used for data mining were as follows. First, spreadsheets were filled with the following information: digital object identifier (DOI) code, publication year, applied concentration, nanoparticle composition, particle and hydrodynamic size, plant species, exposure time, type of control used (soluble ionic compounds, bulk/micrometric counterparts, negative control), way of exposure, soil pH, effects on soil microorganisms, experimental environment, and effects on plants. Thereafter, each information was separated into different worksheets with data organization-specific criteria.

Nanoparticle composition

The following criteria were adopted to organize the nanoparticle composition data in the worksheets (data corresponding to Figure 1). The DOI code of each article and its respective nanoparticle composition were placed in the first two columns, then the frequency of each kind of NP was calculated using the function COUNTIF on Excel software. NPs were classified as "nutrient" or "non-nutrient" to the plants and categorized into metal oxide, elementary form, carbon nanomaterial, sulphide and sulfate, phosphate, carbon, core-shell nanoparticle, and others (hydroxides, mixed oxides, polymeric). The core-shell group refers to NPs coated with another chemical element. However, NPs coated with organic molecules, such as citrate-coated Ag NPs, were grouped following their elementary composition. Also, it is important to highlight that some articles studied more than one kind of NP, thus the DOI code of these articles is repeated in the worksheet, for this reason, the number of data entries (n) in Figure 1 is 1282 even though the number of consulted articles is 996.

We based the classification of plant nutrients according to Marschner[1] to categorize the NPs as "nutrient" or "non-nutrient". However, we did not consider silicon as a nutrient since this element is not essential for all plants. Besides that, potentially toxic NPs like CdS/ZnS were also categorized as "non-nutrient" even though they contain a mineral nutrient.

Table S1. Nanoparticle composition found in 1282 entries of 966 manuscripts.

Composition	chemical formula	Frequency	Frequency (%)
Metal oxide, 54%	ZnO	191	14.9
Metal oxide, 54%	CeO2	93	7.3
Metal oxide, 54%	TiO2	124	9.7
Metal oxide, 54%	Fe3O4	37	2.9
Metal oxide, 54%	CuO	94	7.3
Metal oxide, 54%	SiO2	33	2.6
Metal oxide, 54%	Fe2O3	40	3.1
Metal oxide, 54%	Al2O3	19	1.5
Metal oxide, 54%	Other	57	4.4
Elementary form, 25%	Au	38	3.0
Elementary form, 25%	Ag	153	11.9
Elementary form, 25%	Cu	47	3.7
Elementary form, 25%	Fe	27	2.1
Elementary form, 25%	Other	54	4.2
Carbon Nanomaterial, 10%	Carbon	20	1.6
Carbon Nanomaterial, 10%	C-Fullerene	14	1.1
Carbon Nanomaterial, 10%	Graphene Oxide	33	2.6
Carbon Nanomaterial, 10%	Carbon Nanotube	63	4.9
Carbon Nanomaterial, 10%	Other	4	0.3
Sulphide & sulfate, 2%	Sulphide & sulfate	25	2.0
Phosphates, 1%	Phosphates	15	1.2
Core-shell nanoparticle, 2%	Core-shell nanoparticle	23	1.8
Others (hydroxides, mixed oxides, polymeric), 6%	other	78	6.1
Total		1282	100

Plant species

The plant species worksheet displays the plants used on each of the selected articles. It was organized by DOI code, scientific name, and its respective family, as represented in Figure 2. It is important to highlight that, for the studies that mention just the plant's common name, we searched its scientific name and used only the first name of the binomial nomenclature which identifies its genus. Thus, we computed 1189 plants used in the 996 researched articles. The frequency of each species was calculated by the COUNTIF function as well. The species with a frequency below 1% are not reported in the corresponding figure.

Nanoparticle size

The worksheet that shows the NP size was arranged according to the size given by the manufacturer or measured by the authors using electronic microscopy or other methods, followed by the hydrodynamic diameter. In articles that reported both manufacture and authors measured particle size, the latter was considered. For those papers reported polydisperse nanoparticles of a certain size range, the average value was considered. For example, Güllüce et al.[2] reported ZnO particles between 50-150 nm, in this case, the worksheet presents 100 nm.

The standard deviation informed by some authors was disregarded. For the articles that reported size values with a signal of greater (>) or less than (<), we just considered the values. For example, Du et al.[3] reported that their ZnO NPs have a size "<100 nm", in which only 100 nm was maintained in the worksheet.

The size of carbon nanotubes, graphene, and nano-chitosan were considered as 'not applicable', since the articles reported two size values (length and width) or not informed any.

Articles that did not report size, or in which the information was not clear were considered as 'not applicable'.

For obtaining the information on NP primary diameter we employed 982 papers and 223 were considered as 'not applicable'. For the hydrodynamic diameter we employed 982 papers and 624 were considered as 'not applicable'.

Positive experimental controls

We verified the use of both bulk (microparticles) and soluble compounds and characterized it as a positive control. Bulk particles were considered positive controls when presenting the same composition as the NPs. On the other hand, the soluble control is stated as the effect of the elements ionic form studied on the plants. Some authors used different elements, but with a similar effect as the soluble control.[4] One should mention that, in principle, there is no soluble control for carbon-based nanomaterials such as nanotubes, graphene or carbon quantum dots, however graphite could be used as a proxy for micrometric control. Likewise, one should acknowledge that soluble titanium salts are not common and hence it is difficult to employ such positive control. When nanoparticles were used to sorb other nutrients, complete growth solutions were not considered as positive dissolved control.

Experimental environment

Four groups of the experimental environment were formed: i) greenhouse, which includes the experiments maintained inside a greenhouse; glasshouse, or screen house with some conditions controlled, such as temperature, photoperiod, watering, and humidity; ii) field, considering experiments that were maintained under field conditions with plants grown in soil without any control of temperature, photoperiod, and humidity; iii) growth chamber, which determines the experiments maintained in a growth chamber, germination chamber, laboratory, or *in vitro* conditions with some controlled parameters, such as temperature, photoperiod, watering, and humidity; iv) ambient conditions refers to all the experiments performed on environments that did not fit into the previous categories, such as experiments in a recipient (vase, tube, plastic bag) maintained under environmental conditions such as sunlight, weather or ambient atmosphere, indoor or outdoor with room temperature and natural conditions. It also included a few mesocosms experiments.

A total of 991 documents were analyzed. Among them, 892 were employed to build Figure 3c and 99 did not inform, or it was not clear, the experimental environment in which the plants were subjected to the treatments.

Way of exposure

Way of exposure indicates how plants were exposed to NPs and the criteria used for that. This section was also organized into four groups: i) leaf for foliar application of dispersed NP; ii) root includes NP applied in the soil, substrate, sand, river sediments, and hydroponics; iii) seeds for seed treatment-like procedures, and iv) plant tissue.

The most common forms of foliar application consisted in spraying and leaf dipping in NP dispersion. Aquatic plants were considered as leaf exposure.

Root exposure included all forms of contact with NP trough roots.

Seed exposure enconpasses studies that mimic conventional seed treatment. It included the following processes: soaking, priming and coating with NP.[5] Studies in which seeds were only germinated over media such as filter paper, cotton pads, soil or agar-like, containing NP were not considered as seed treatment. These papers were classed as root exposure. On the other hand, studies in which seeds were soaked, primed or coated with NP, and then germinated in a media containing NP were considered as seed exposure.

A total of 991 articles were consulted and data from 987 were employed to make Figure 4a. The form of exposure in four articles could not be determined.

Tissue exposure includes plant callus, cells, or organs cultivated *in vitro* conditions with culture medium containing NPs. Articles in which the way exposure was not clear were classified as "Not clear".

Time of exposure

The time of exposure expresses the time that plants were exposed to NPs. The criteria adopted to organize the data were as follows. Articles that cited the exposure time as "until maturity" were not considered, and those that presented plant exposure to less than 24 hours were considered equal to one day.

Likewise, some studies gave the approximate exposure time, for example nearly 'two days', in such cases numbers were rounded. When the manuscript informed a time range, the rounded average to the highest value was considered, for example, "5 to 10 days" = 7.5 days = 8 days. For articles that presented more than one exposure time, all the exposed values were taken into the database.

To build the Figure 4b, concentration reported in mg L^{-1}, we consulted 788 manuscripts, among which 68 did not inform or it was not clear which was the exposure time. For Figure b, concentration reported in mg kg^{-1}, we consulted 209 manuscripts and 16 among them did supply or could not find clear information regarding the exposure time.

Concentration of Nanoparticles

The concentration of nanoparticles was separated into group i) NP dispersed in liquid which concentration could be standardized in units of mg L^{-1}; group ii) NP dispersed in solid which could be standardized as mg kg^{-1}. Articles that cited the concentration in other units such as ppm, %, wt.%, molarity, mg/mL, mg/g were converted to mg/L or mg/kg of NPs. On the other hand, articles that cited concentration as the amount applied per area (mg/hectare, for example) were considered as 'not applicable'.

We consulted 752 articles to produce Figure 4c mg L^{-1}, among those 12 articles were considered as 'not applicable'. The considered papers employed 2344 treatments.

We also consulted 210 articles to produce Figure 4c mg kg^{-1} and 7 of them were considered ‘not applicable’. The considered papers employed 1175 treatments.

Effects on plants and correlation

Effect on plants was evaluated only on articles that dispersed NPs in soil, substrate, and sand (194 original articles and a total of 1060 treatments were identified, among those 133 treatments were considered as ‘not applicable’).

The effects were classified as “Beneficial”, “Harmful”, “Beneficial & Harmful”, and “No effect”.

The articles that did not evaluate plant responses as an effect of NP exposure in terms of development and physiology were identified as ‘Not Applicable”, this data was not added to Figure 5a. Instead, it was just reported in the worksheet "Figure 5a- effect". Additionally, we correlated the data of concentration of NPs in soil, species, and soil pH with the effect individually.

Regarding the effects on enzymes, we avoided reinterpreting the statements made by the authors. For example, an increase of enzymatic activity can either be considered a positive or negative effect depending on the context. We followed the interpretation given by the authors.

Most of the data uncertainty (57%) was related to incorrect effect evaluation itself, and 43% was due to errors on the number of treatments evaluated. Particularly, the data from Figure 5a was one of the most difficult of mining. Many studies did not clearly state whether the effect is beneficial, harmful or absent over the treatments, and consequently causing an increasing chances of error occurrence by human evaluation.

Moreover, the effects of NPs on soil microorganisms population were characterized. In the present study such effects were classified as increase, decrease, not change, and increase/decrease. For example, increase in either richness or abundance was considered as “increase”. Some papers reported an increase of certain phylum and decrease in another, which reflects a modification in the community structure, in this case the effect was “increase and decrease”. in which they were classified as “increase”, “decrease” or “not change” (Figure 5b). For this figure, we evaluated 194 articles and 1060 treatments.

Error estimation & data availability

Since we went through a large amount of data and considering that the worksheets were filled out manually, human errors occurred during the data mining process. Since writing is also a matter of style, some papers were much clearer than others. In general, the least clear information was related to the type of environment where the experiments were carried out, effects of NP on plants, particularly on enzymatic responses, and imprecise concentration units such as % and ppm.

Before plotting the data, at least 20% of the mined data of each worksheet was randomly double-checked the percentage of errors was measured. Then, the incorrect entries were corrected, which decreased the reported error. Each Figure in the manuscript is accompanied by an upper value of error estimation.

Finally, for sake of clarity and expecting that other scientists could reuse the mined data, the worksheets used to plot the Figures are available at https://doi.org/10.6084/m9.figshare.14575185.v1.

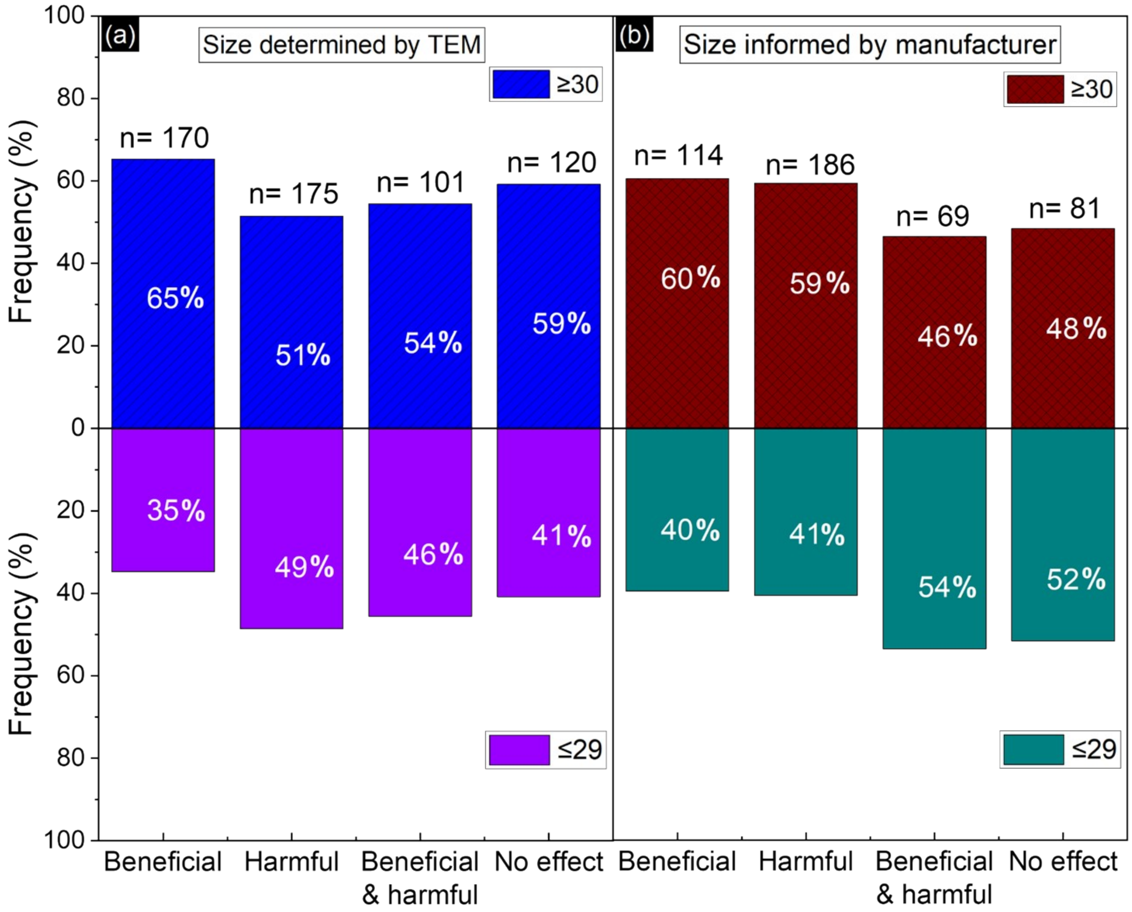

Figure S1. Frequency of reported effects of NPs on plants whose roots were exposed to the treatments in solid media, *i.e.*, soil or sand, as a function of the NPs size smaller or larger than 30 nm either determined by transmission electron microscopy (TEM) or informed by the manufacturer. The amount of data computed (n) from the studies herein employed is presented in each bar

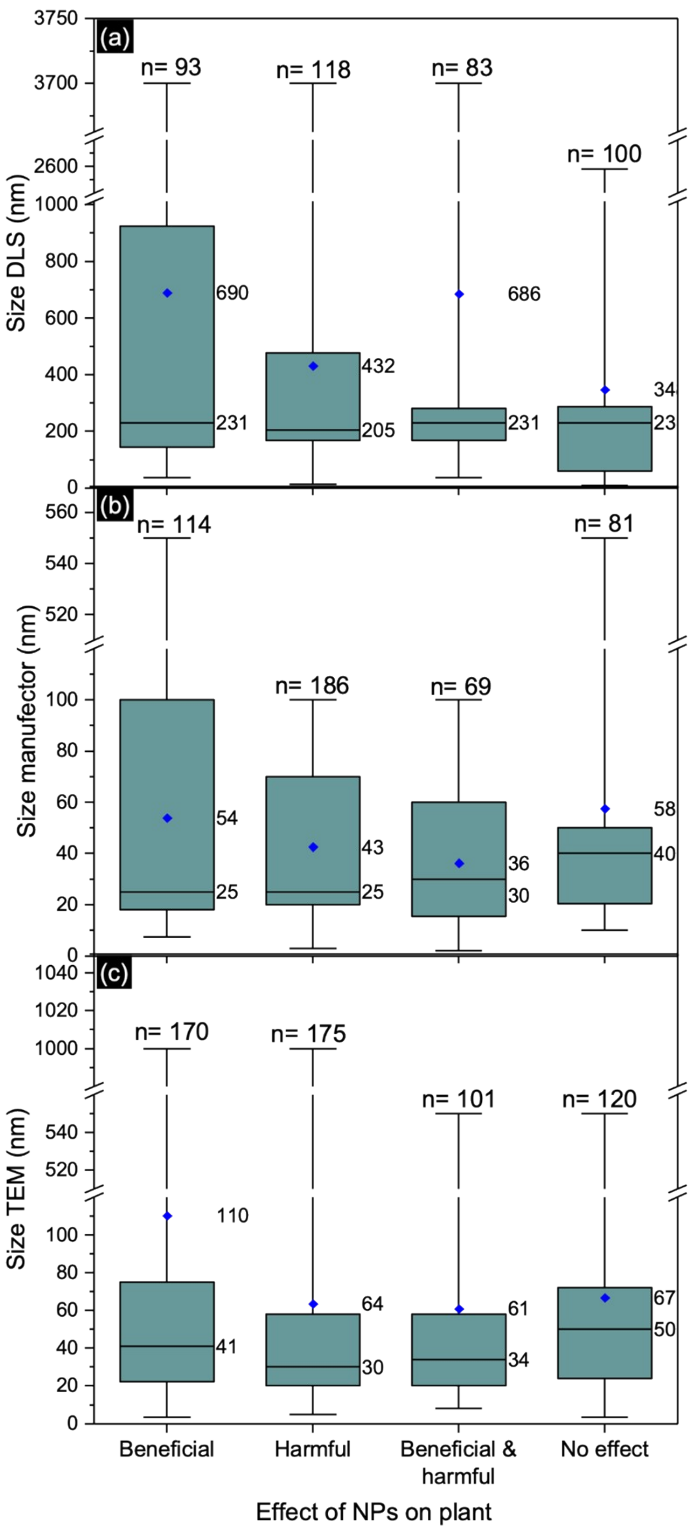

Figure S2. Boxplots of reported effects of NPs on plants whose roots were exposed to the treatments in solid media, *i.e.*, soil or sand, as a function of NPs size determined either through dynamic light scattering (DLS), transmission electron microscopy (TEM), or informed by the manufacturer. The amount of data computed (n) from the studies herein employed is presented in each bar.

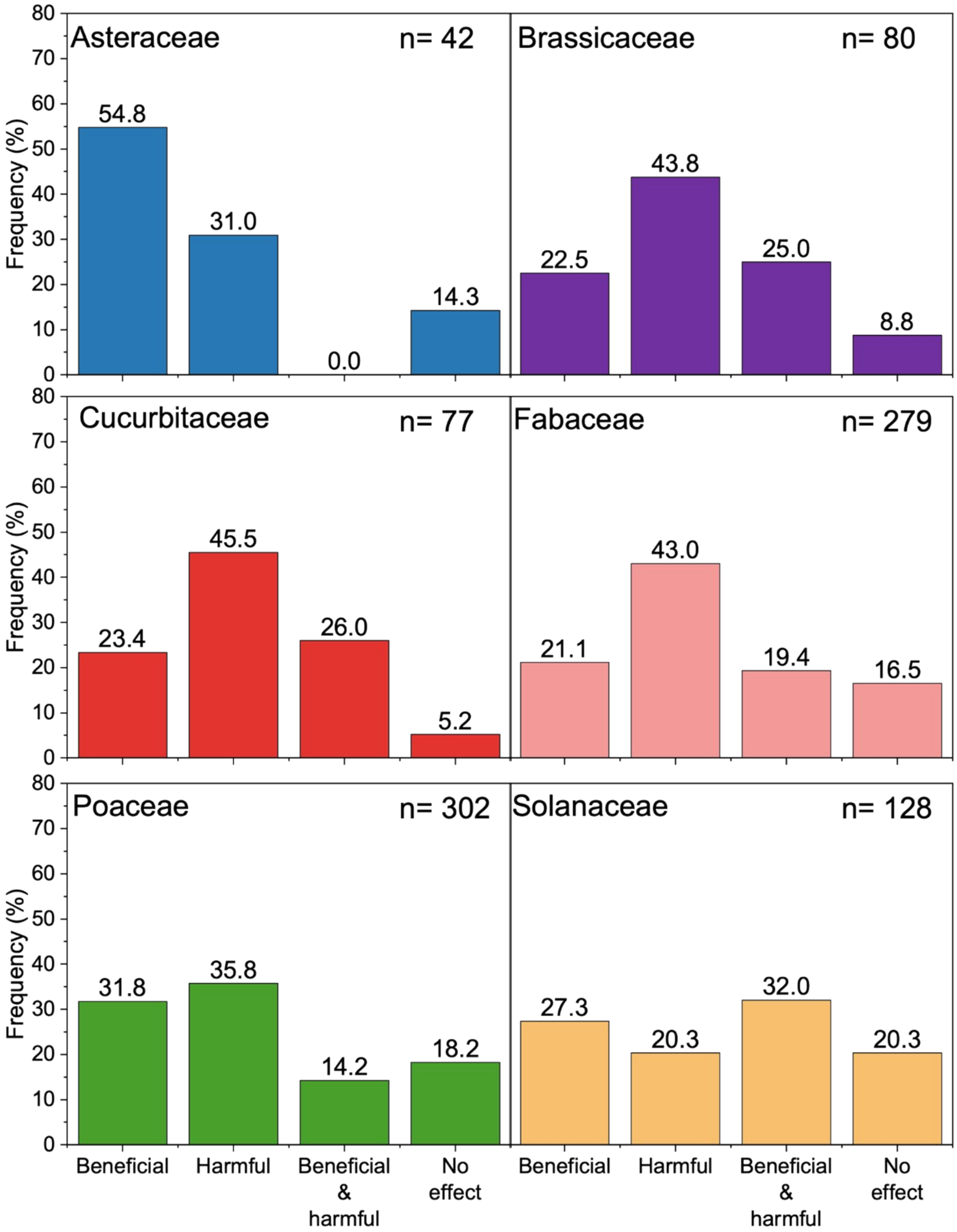

Figure S3. Frequency of reported effects of NPs on plants whose roots were exposed to the treatments in solid media, *i.e.*, soil or sand, across the plant families most reported in the studies herein evaluated (n > 50), *i.e.*, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Poaceae, and Solanaceae. The amount of data computed (n) from the studies herein employed is presented in each chart.

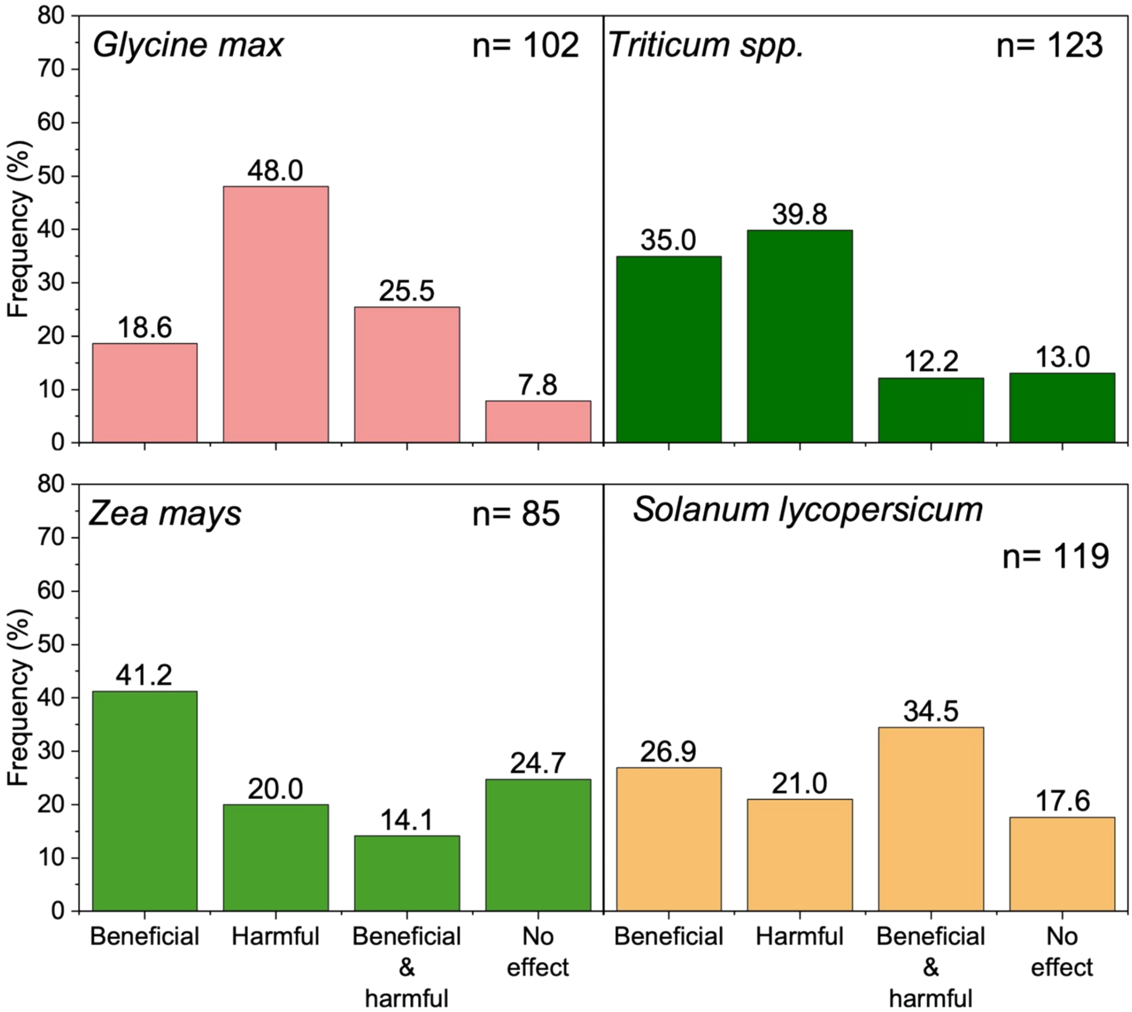

Figure S4. Frequency of reported effects of NPs on plants whose roots were exposed to the treatments in solid media, *i.e.*, soil or sand, across the plant families most reported in the studies herein evaluated (n > 50), *i.e.*, *Glycine max*, *Triticum* spp. (*T. aestivum* and *T. durum*), *Zea mays*, and *Solanum lycopersicum*. The amount of data computed (n) from the studies herein employed is presented in each chart.

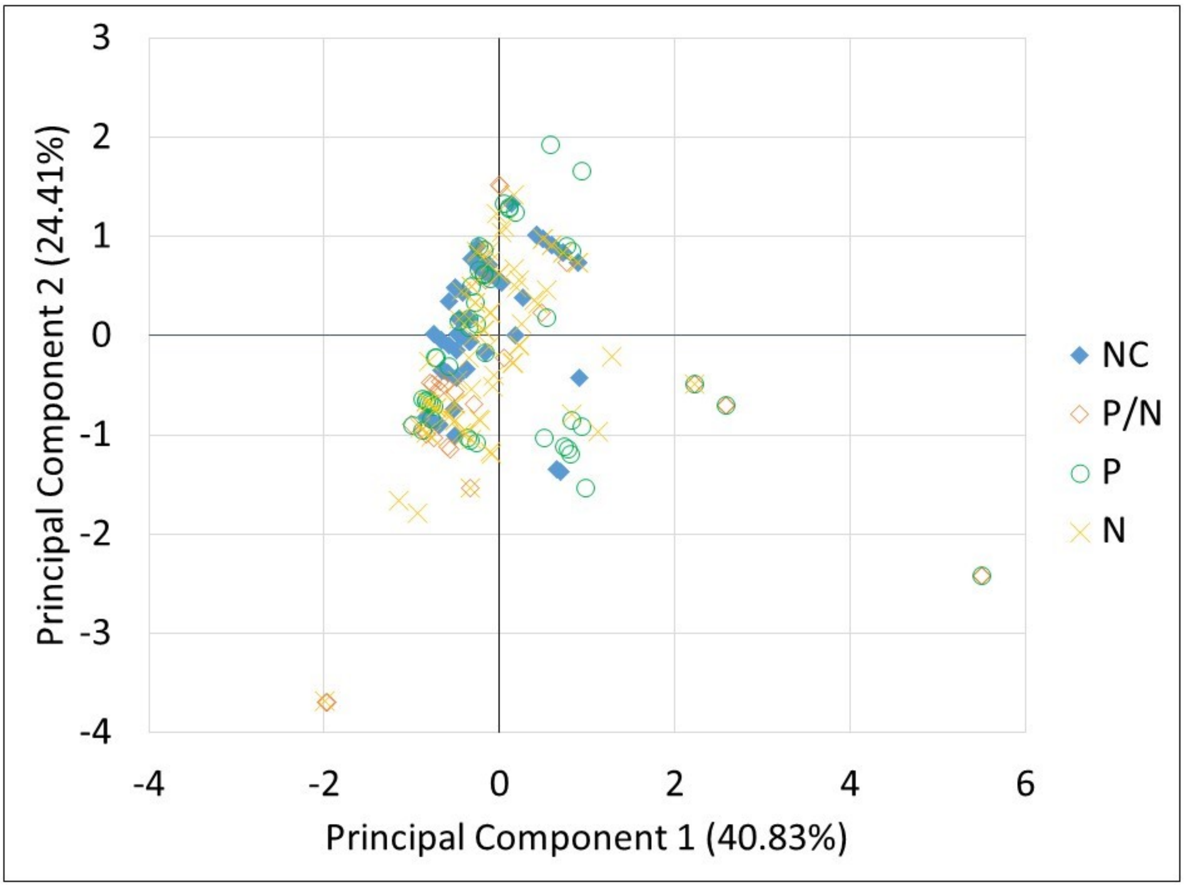

Figure S5. Scatter plot of the scores of principal components (PC) 1 and 2 obtained from the variables concentration, NP size, soil pH and exposure time as function of the effects, i.e., positive (P), negative (N), positive and negative (P/N) and no changes. PC 1 and 2 covered, respectively, a total of 40.83 and 24.41% of the variance in the data. The loadings of concentration, exposure time, soil pH, and NPs size with CP1 were respectively 0.81, -0.42, -0.42, and 0.79, and with CP2 were respectively -0.29, -0.64, 0.59, and -0.36.

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