Influence of soil type on TiO₂ nanoparticle fate in an agro-ecosystem

Larue C^{1*}, Baratange C¹, Vantelon D², Khodja H³, Surblé S³, Elger A¹, Carrière M⁴

¹ECOLAB, Université de Toulouse, CNRS, Toulouse, France (camille.larue@ensat.fr, arnaud.elger@univ-tlse3.fr)

²Synchrotron SOLEIL, L'Orme des Merisiers 48, St Aubin, 91192 Gif-sur-Yvette Cedex, France (delphine.vantelon@synchrotron-soleil.fr)

³LEEL, NIMBE, CEA/CNRS, Université Paris Saclay, 91191, Gif sur Yvette, France (hicham.khodja@cea.fr, suzy.surble@cea.fr)

⁴Univ. Grenoble Alpes, INAC, SyMMES, Chimie Interface Biologie pour l'Environnement, la Santé et la Toxicologie (CIBEST), F-38000 Grenoble, France (marie.carriere@cea.fr)

* corresponding author, camille.larue@ensat.fr

Abstract:

Nanoparticles (NPs) and in particular TiO₂-NPs are increasingly included in commercial goods leading to their accumulation in sewage sludge which is spread on agricultural soils as fertilizers in many countries. Crop plants are thus a very likely point of entry for NPs in the food chain up to humans. So far, soil influence on NP fate has been under-investigated. In this article, we studied the partitioning of TiO₂-NPs between soil and soil leachate, their uptake and biotransformation in wheat seedlings and their impact on plant development after exposure on 4 different types of soil with different characteristics: soil texture (from sandy to clayey), soil pH, cationic exchange capacity, organic matter content. Results suggest that a NP contamination occurring on agricultural soils will mainly lead to NP accumulation in soil (increase of Ti concentration up to 302 % in sand) but to low to negligible transfer to soil leachate and plant shoot. In our experimental conditions, no sign of acute phytotoxicity has been detected (growth, biomass, chlorophyll content). Clay content above 6% together with organic matter content above 1.5% lead to translocation factor from soil to plant leaves below 2.5% (*i.e.* below

30 13 mg Ti.kg⁻¹ dry leaves). Taken together, our results suggest low risk of crop contamination in an agro-
31 ecosystem.

32

33 Keywords: agriculture, bioavailability, ecotoxicology, food safety, plant, transfer

1. Introduction:

For more than a decade, **nanotechnologies** have attracted the interest of industrials and scientists throughout the world. The manipulation of matter at the nanometer scale opens up new possibilities in many different fields, such as industry, medicine and sustainable development. Nanotechnologies imply the use of a huge amount of nanoparticles (NPs), *i.e.* particles with at least one dimension below 100 nm. The new properties exhibited by NPs are also a source of questions about a potentially different behavior and toxicity.

The number of **commercial goods** available on the market and (officially) containing NPs has increased from 54 in 2005 to 2850 in 2016 in all fields of everyday life (cosmetics, food containers, sporting goods, personal care, medicine, construction, agriculture)^{1,2}. **TiO₂-NPs** are one of the most used NPs, appearing in about 25% of the products according to nano-databases^{1,2}. One can estimate a yearly production of TiO₂-NPs comprised between 1 000 and 10 000 tons³. Under their nanometric form, they are mainly used for their photocatalytic properties for instance in construction materials (self-cleaning materials) or for their UV-adsorbing properties in cosmetics (sunscreens)³. Besides, they can be found in small proportions in food additives^{4,5}.

This expansion of the use of TiO₂-NPs is inevitably leading to an increased **dissemination in the environment**. The release may be accidental, during the production or transport of NPs, or chronic, during the life cycle of products containing NPs. It has been shown that, TiO₂-NPs contained in certain textiles and paints are detached from their matrix during laundry or weathering and finally end up in wastewater treatment plants⁷⁻¹⁰ and in particular in sewage sludge, where they can reach a concentration of 2 g.kg⁻¹¹¹. Moreover, the spreading of sewage sludge as soil fertilizer is a common practice in agriculture with quantities reaching 3 tons (dry weight)/ha/year and is regulated for instance in the US, in Australia and in Europe for sludge heavy metal content but nothing about NPs¹²⁻¹⁵. This leads to the dissemination of TiO₂-NPs in agricultural soils. In addition, the expected environmental concentrations could be much higher locally with the intentional introduction of NPs in

the environment. For instance, thanks to their unique UV properties, TiO₂-NPs are used for purification purposes in both soil and water^{16–19}. Another critical case is the use of nanopesticides^{20,21}. Indeed, TiO₂-NPs are used in crop protection products for some years in order to optimize the product efficiency and then to eventually reduce the amount of inputs into the environment (sustainable development). The introduction of these products on the market is also expected to increase in the coming years^{22–25}.

Until very recently, most of the NP risk assessment studies have been carried out on a single organism in simplified conditions (hydroponics), exposed to high concentrations of NPs during short time, simulating acute contaminations^{26,27}. Over the last years, studies related to the fate of NPs in the environment evidenced a trend towards more realistic exposure scenario: soil exposure, longer exposure period and lower NP concentrations^{26,27}.

Studying the fate of TiO₂-NPs in soil is challenging. Indeed, soil is a very complex matrix. Moreover, the contribution of TiO₂ engineered nanomaterials is challenging to measure in the soil because of the high geogenic Ti background (≈0.6% of the terrestrial crust). Accordingly, to date, only modelling studies can help approximate the amount of engineered TiO₂-NPs that is ending up in the environment. The last article estimation forecasts the presence of 61 mg of TiO₂.kg⁻¹ of soil amended with sewage sludge¹¹, making **agricultural soil** the main sink for nanomaterials in the environment and crop plants a privileged point of entry for NPs in the food chain up to humans.

Currently, knowledge about the interactions of NPs with soil is very limited. Once NPs reach the soil, they can be either physically retained or chemically adsorbed onto the surface of soil particles²⁷. These interactions could mitigate NP phytotoxicity and bioavailability²⁷, meaning that a same contamination could lead to very distinct consequences according to soil type. The literature published on plants exposed in soils suggested a very limited transfer of NPs to aerial parts. For a better risk assessment, it is thus of primary importance to understand how soil characteristics control NP fate in a crop system. For this purpose we exposed wheat, a widely grown crop over the world, to TiO₂-NPs

(0, 100, 500 mg.kg⁻¹) in 4 different types of soil already containing geogenic Ti at different concentrations. Ti quantification in soil, soil leachates and wheat leaves was assessed. Ti distribution in roots and leaves and speciation in leaves were also determined using large scale research facilities. Finally NP phytotoxicity was evaluated through plant development parameters: height, fresh and dry weights and chlorophyll content.

2. Material and Methods

2.1. Experimental set-up

TiO₂-NPs with a nominal diameter of 25 nm and a crystalline phase mainly anatase (89%) were used (see Figure S1 for more details).

A sand and 3 agricultural soils provided by Lufa Speyer (Germany) were chosen with different characteristics (Table 1): texture ranging from sand to clayey loam, organic carbon content from 0 to 2.09%, cation exchange capacity (CEC) from 0 to 10 meq/100 g and pH from 5.1 to 7.2.

	Sand	Silty sand	Loamy sand	Clayey loam
Particle size (%)				
<0.002 mm	0.0 ± 0.0	2.9 ± 0.8	6.4 ± 0.9	41.7 ± 1.3
0.002-0.05 mm	0.0 ± 0.0	9.1 ± 1.4	11.6 ± 0.7	36.1 ± 2.2
0.05 – 2 mm	100.0 ± 0.0	88.0 ± 1.0	82.0 ± 0.7	22.2 ± 1.5
Water holding capacity				
g/100 g	25.3 ± 2.8	31.8 ± 3.0	46.5 ± 6.0	39.6 ± 5.0
Weight per Volume				
g/1000 mL	1756 ± 6	1430 ± 57	1220 ± 78	1330 ± 96
Organic Carbon				
%	0.0 ± 0.0	0.74 ± 0.14	2.09 ± 0.40	1.69 ± 0.17

pH value				
0.01M CaCl ₂	6.3 ± 0.0	5.1 ± 0.5	5.5 ± 0.1	7.2 ± 0.1
Cation Exchange Capacity				
Meq/100g	<2	4.0 ± 1.0	10.0 ± 0.5	22.0 ± 6.0
Leachates				
mL	10.6 ± 0.1 ^c	9.1 ± 0.2 ^{bc}	8.0 ± 0.3 ^{ab}	6.3 ± 0.6 ^a

Table 1. Soil characteristics (soil provided by Lufa Speyer - Germany: silty sand: soil 2.1, loamy sand: soil 2.2, clayey loam: soil 6S). Characteristics were provided by the supplier. For leachates, different lowercase letters indicate significant differences ($p < 0.05$) among all treatments.

Exposure concentrations of TiO₂-NPs represented a realistic condition (100 mg.kg⁻¹) and a higher and plausible concentration after several years of sewage sludge spreading (500 mg.kg⁻¹). TiO₂-NP suspensions (100 or 500 mg.L⁻¹ and ultrapure water as control) were mixed with soil with a ratio of 1:1 (w:w) for 12 hours on a shaker (200 rpm) in containers wrapped with aluminum foil. This mixing strategy was chosen to ensure the most homogeneous matrix as possible for plant exposure. After this step, the mixture was poured in a folded standard filter paper (Fisherbrand, Dia/Size 330 mm) and separated in soil leachates and soil (after 8h of settling). After weighting, soil leachates were evaporated and prepared for Ti quantification to determine the Ti mobility.

6 individual replicates for each type of soils (4) and each level of contamination (3) were set-up with each a wheat seed (*Triticum aestivum* L., cv. Courtaud). All experiments were carried out in a growth chamber under controlled conditions: day/night photoperiod (16/8 h), 100 µmol/m²/s, day/night temperature, (24/20±1 °C) and day/night relative humidity (70/75%). Leaf height and chlorophyll content (determined using a SPAD-502 chlorophyll meter, Minolta Camera Co., Japan) were assessed every day for 3 weeks until harvest. At the end of the experiment fresh leaf weight and dry leaf weight after 48h in an oven at 70°C were recorded.

120

121 **2.2. Bulk Ti quantification by ICP-AES**

122 Different tests have been performed to ensure a proper acidic digestion of TiO₂-NPs which represents
123 a critical step to avoid underestimation of Ti concentration in the samples. Several digestion protocols
124 adapted from the literature have been compared (Figure S2A) with different acid cocktails,
125 temperatures and evaporation steps. The best digestion yield was obtained with a protocol adapted
126 from Mariet *et al.* (2008) with a mix of HNO₃, NH₄F, HClO₄ and H₂O₂ with several evaporation steps at
127 100 °C in a DigiPrep® system (see Supporting Information for more details)²⁸. The yield was about 88%
128 and reproducible. This test demonstrates that the digestion protocol needs to be re-optimized for each
129 TiO₂-NP. Indeed, in one of our previous experiments using 4 nm anatase TiO₂-NPs, the protocol from
130 Mariet *et al.* was not efficient ²⁹. The linearity of the digestion was also assessed with increasing TiO₂-
131 NP concentrations (Figure S2B). Finally the stability of the solution after digestion over a month was
132 monitored (Figure S2C). After digestion, samples (soils, leachates, plant shoots) were analyzed using
133 Inductively Coupled Plasma – Mass Spectrometry (ICP-AES IRIS, Thermo Scientific). Quality of sample
134 preparation was checked with blank samples containing only chemicals and with reference samples
135 (tomato leaves, NIST RM 1573a) and permitted to validate sample preparation and ICP analysis (Figure
136 S2bis, Table S1).

137

138 **2.3. Spatially resolved Ti semi-quantification by μ PIXE/RBS**

139 A combination of micro-particle induced X-ray emission (μ PIXE) and Rutherford Backscattered
140 spectroscopy (RBS) has been used for Ti semi-quantification. μ PIXE/RBS quantification of Ti was chosen
141 as a spatially resolved technique to avoid mixing the Ti signal coming from NPs adsorbed at the surface
142 of roots with Ti really absorbed inside the root tissues.

143 Wheat roots were thoroughly washed with deionized water to take off NPs lightly bound to the surface
144 and immersed in a droplet of resin (Tissue Teck Sakura®) to be immediately cryo-fixed by plunging the

sample in isopentane cooled with liquid nitrogen. Samples were then cut in thin cross-sections (50 μm) using a cryo-microtome (Leica) and finally freeze-dried (48h, -52°C , 0.01 mbars). Freeze-dried sections were analyzed at the nuclear microprobe available at the Atomic Energy Commission (CEA) Center of Saclay (France) with a proton source of 3 MeV, a beam focused to 2.5 μm and a current intensity of 500 pA. Data processing was performed using Rismin³⁰ software to define regions of interest and extract spectra, and SIMNRA³¹ and GUPIX³² codes to reduce RBS and PIXE data, respectively. The Ti/Ca ratio was used as a Ti enrichment indicator as Ca is an abundant endogenous element.

2.4. Ti distribution and speciation by μXRF and μXANES

Samples were prepared as described previously for nuclear microprobe experiments (section 2.3.). Synchrotron experiments were carried out on LUCIA beamline at SOLEIL (Saint Aubain, France)³³. Micro X-ray fluorescence (μXRF) maps were recorded above the Ti-K edge (5.1 keV) with a step size of 5 μm and a dwell time of 2000 ms. μXRF data were fitted using PyMCA software³⁴. Ti-K edge (4.5 to 5.5 keV energy range, 0.5 eV step) micro X-ray absorption near edge spectroscopy (μXANES) spectra were recorded in regions of interest of the fluorescence maps. XANES spectra on reference compounds were recorded during previous experiments^{29,35}. For the fitting, 3 groups of references were created: anatase, rutile and others (containing Ti-oxysulfate, amorphous Ti oxide and Ti acetate). XANES data treatment was performed using Athena software³⁶ as previously^{29,35}.

2.5. Statistical analysis

Data were checked for normality (Shapiro test) and homoscedasticity (Bartlett or Levene's tests). They were compared using two-way ANOVA, with soil type and TiO_2 -NP concentration as main effects, and the interaction (soil type x NP concentration). If the whole set of data did not follow a normal distribution even after transformation, Student T test, Wilcoxon test, one-way ANOVA or Kruskal–Wallis test were performed on subset of data (according to soil type or NP concentration). Post hoc

tests (Tukey's HSD or Dunn's test) were applied to detect differences between groups of samples. Differences were considered significant when $P < 0.05$. All statistical analyses are gathered in Table S21.

To study the global influence of TiO_2 -NPs on plant elemental content, MANOVA based on Pillai's trace test was used on the whole set of micro- and macronutrients measured by ICP-AES.

All statistical analyses were performed using the R statistical software (version 3.1.3)³⁷, with *car*³⁸, *multcompView*³⁹, *pgirmess*⁴⁰ and *lsmeans*⁴¹ packages.

3. Results and discussion

3.1. Ti partitioning between soil and soil leachates

As stated earlier, Ti is a geogenic element of the earth crust. As such, the 4 natural soils (control condition) contained Ti concentrations ranging from 43 to 1671 mg.kg^{-1} with sand having the lowest concentration followed by the group of silty and loamy sands and finally the clayey loam being the richest in Ti ($P < 0.0001$, Figure 1A). Ti concentration in natural soils was positively correlated with the fraction of soil particles in the 0.002 to 0.005 mm size range ($r^2 = 0.9997$, Table 1) and to a lesser extent to the fraction < 0.002 mm ($r^2 = 0.9618$). After addition of 100 mg.kg^{-1} of TiO_2 -NPs, the Ti concentration in soils did not significantly increase in any soil. After addition of 500 mg.kg^{-1} of TiO_2 -NPs, the increase in Ti was only significant for sand and silty sand ($P < 0.0001$), *i.e.* soils with the lowest clay fraction.

Those results can be confronted with Ti concentrations in soil leachates (Figure 1B). In natural soils, Ti concentrations in the leachates after 12h mixing were respectively 0.03, 0.18, 0.07 and 0.23 mg.L^{-1} for sand, silty sand, loamy sand and clayey loam. After addition of 100 mg.kg^{-1} of TiO_2 -NPs, only the sand leachate had a significantly higher Ti concentration (13.18 mg.L^{-1} , $P = 0.0379$) with a more than 400 fold increase in comparison with the control sand. This is also the soil in which the leachate volume recovered was the highest (Table 1): 71% of the 15 mL of the contamination suspension was collected in the leaching compartment. This fraction was decreased to 42% in the case of the clayey loam soil.

Crossing this result with Ti concentration in soil, one can conclude that most of the TiO₂-NP suspension added in the 100 mg.kg⁻¹ condition went through the sand column and ended up in the soil leachate. After addition of 500 mg.kg⁻¹ of TiO₂-NPs, Ti concentration in soil leachate was significantly increased as compared to the control for all soils except for the clayey loam soil ($P=0.0019$ for silty sand and $P=0.0031$ for loamy sand).

Figure 1. Ti concentration in the soil system: soil (A) and soil leachate (B) determined by ICP-AES. Different lowercase letters indicate significant differences ($p<0.05$) among all treatments in A or among treatment within a soil type in B. Capital letters in A highlight significant differences among soil types. ($n=6$).

If a contamination occurs on a sand, most TiO₂-NPs will likely go through the soil and reach the aquifers. Only a small fraction will remain in the soil if a high dose (500 mg.kg⁻¹) of NPs is applied. In the silty sand, Ti increase in both soil and soil leachate was only detectable for the highest concentration added. For the loamy sand, the high Ti natural background (> 500 mg.kg⁻¹) shadowed the addition of NPs in the soil and Ti increase was visible only in the leachate at the highest NP concentration added. Finally, in the clayey loam, all added Ti was retained in the soil but did not increase significantly Ti concentration also because of the very high geogenic Ti level. Depth column studies would be needed to confirm these results about NP mobility and gain a deeper understanding.

Soils with coarser texture, lower organic carbon content as well as lower CEC (sand and silty sand) were the ones in which NPs displayed the highest availability. This result is in agreement with previous literature^{42–45}. Authors suggested that soils with a higher clay content have also smaller pore size and larger surface area which increase both electrostatic interactions and physical straining explaining the lower transport of NPs to soil leachates. Likewise, Cu-NP translocation was observed to be low in soils enriched in organic matter and high in clay⁴⁶. Another important point that could be considered is the interaction of NPs with other potential contaminants of the soil. Indeed, it has been shown in the literature that addition of TiO₂-NPs in sandy soils can improve Cu and Pb mobility in soils^{43,47}. Sewage sludge also often contains a certain amount of heavy metals¹⁵; NP contamination of sewage sludge amended soils could thus represent a threat to both terrestrial and aquatic ecosystems through higher heavy metal bioavailability and leaching towards aquifers. Finally, different microbial communities likely occur in different soils, and might have a role on TiO₂ fate and speciation over long term exposure.

3.2. Ti uptake in wheat roots

Spatially resolved techniques were employed to investigate Ti uptake in wheat roots exposed in control soil and soil contaminated with 100 mg.kg⁻¹ of TiO₂-NPs (the most environmentally relevant condition).

233 Figure 2A-D displays Ti distribution in roots of wheat seedlings grown in the 4 different natural soils. Ti
234 was mainly detected adsorbed at the surface of the roots but also in the parenchyma with maximum
235 fluorescence intensity of 42 counts in average for sand, loamy sand and clayey loam and up to 133
236 counts in silty sand roots. In roots grown in contaminated soils (Figure 2E-H), the same distribution
237 pattern appeared, with Ti mainly detectable on root surface and less Ti observed adsorbed in the clayey
238 loam soil in comparison with the 3 other ones. However, the maximum fluorescence intensities were
239 increased in root inner tissues (parenchyma and vascular cylinder) especially for roots grown in sand
240 and silty sand. In first approach, these results seem to correlate with those obtained in the Ti
241 partitioning experiment: Ti added to sandy soil is more available and thus internalized in roots, while
242 Ti added in soils with more clay and organic matter is more retained by the soil matrix and lead only
243 to a slight Ti fluorescence signal increase in wheat roots.

244 ICP-AES measurements of Ti content in roots would not be reliable because Ti can be heavily adsorbed
245 on their surface, leading to over-estimation of Ti absorption. Thus, to have a proper quantitative
246 assessment of Ti accumulation in roots, thin sections were analyzed using μ PIXE coupled to μ RBS,
247 which permitted the quantification of all the chemical elements of the periodic table, light elements
248 (C, H, O, N) included (Figure 2I). The main difference evidenced by the analysis was the one related to
249 soil type ($P = 0.0003$): roots from clayey soils contained significantly less Ti than the roots grown in
250 loamy sand and silty sand. Wheat roots accumulated significantly more Ti after addition of TiO_2 -NPs in
251 silty sand only ($P = 0.0008$). In this condition, Ti content was multiplied by a factor of 7. Surprisingly, in
252 contrast with μ XRF results, Ti content in roots grown on sand did not show any significant increase (P
253 $= 0.4699$). However, this is in agreement with the fact that Ti content in sand after addition of 100
254 mg.kg^{-1} TiO_2 -NPs did not increase significantly. One possible explanation for this discrepancy is that
255 μ XRF technique has a lower limit of detection than μ PIXE/RBS⁴⁸. Overall, no correlation was detectable
256 between Ti concentration in soil and Ti/Ca content in roots ($r=0.111$, $P = 0.742$).

Figure 2. Ti in wheat roots after a 3 week exposure: elemental distribution by μ XRF of Ti (in red) and Ca (in green) in root sections of wheat grown in control soils (A-D) or in contaminated soils (E-H). Numbers in red (fluorescence counts) and arrows indicate the highest fluorescence intensity detected inside the root. ep. epidermis, p. parenchyma, v.c. vascular cylinder. Scale bar = 50 μ m. Semi-quantitative analysis of Ti by μ PIXE/RBS in root sections of wheat grown in natural soils and soils contaminated with 100 mg.kg⁻¹ TiO₂-NPs (I). Capital letters indicate significant differences among soil types and * indicate significant differences between exposed plants and their corresponding control ($p < 0.05$) ($n = 3$).

Ti speciation was then analyzed by μ XANES on high intensity spots identified by μ XRF (Figure 3). This analysis was performed in roots grown in the 4 different types of soils and in both natural and contaminated (100 mg.kg⁻¹ added NPs) conditions. Different chemical forms of Ti were detected in wheat grown in natural soils from mainly rutile in clayey loam and sand (with respectively 73 and 77%

of rutile crystalline form) to mainly anatase in loamy sand (63% anatase). Anatase and rutile forms of TiO_2 correspond to an octahedral arrangements of the O atoms around Ti. In the natural silty sand, there is a shift of the A3 peak towards the lower energies (about 0.5 eV) with an increase of the peak height and area which suggests a different conformation of the TiO unit going from octahedral towards trigonal bipyramidal (5 coordinations) or tetrahedral (4 coordinations) conformations ⁴⁹. After exposure to TiO_2 -NPs (89% anatase, 11% rutile, Figure S1), the ratio between anatase and rutile in comparison with the same ratio in the control roots increased in the roots of all 4 conditions highlighting the contribution of the mainly anatase added NPs. The increase was more important in the case of plants grown in the soils with coarser texture (sand and silty sand).

There are not many studies evaluating NP fate in plants after soil exposure even though this number has been increasing over the last few years. But there are even fewer articles reporting the fate of NPs in roots (Majumdar *et al.* 2016 on CeO_2 -NPs ⁵⁰ or Gao *et al.*, 2018 on CuO -NPs⁵¹). Indeed it is very challenging to obtain this type of data and only spatially resolved techniques are able to provide reliable data getting rid of the geogenic signal surrounding the roots. Moreover, if one wants to perform a comparative study, the technique also needs to be quantitative which leaves the scientific community with few tools mainly based on spectroscopy. In this study by combining 4 different techniques namely μXRF , $\mu\text{PIXE/RBS}$ and μXANES , we were able to map Ti distribution in wheat roots with a good sensitivity, quantify it and investigate its speciation *in vivo*. Our results demonstrate that the added TiO_2 -NPs can be taken up by plant roots when exposure occurs in soil and in particular in sand and silty sand. Considering the concentration of NPs added to natural soils (no significant increase of Ti concentration in soils after addition of 100 mg.kg^{-1} , Figure 1A), our results also suggest that Ti from NPs was taken up by roots preferentially than geogenic Ti (no significant Ti concentration increase in soil vs. increase in roots). This highlights that the added Ti had a different behavior than the natural one and a different bioavailability which justifies the fact to study the impact of a TiO_2 -NP contamination in the environment even though plants have evolved with high Ti concentrations in soils for ages.

Figure 3. Ti speciation assessed by μ XANES in wheat roots grown in control soils (Ctl) or soils contaminated with 100 mg.kg^{-1} TiO_2 -NPs (+ TiO_2) after a 3 week exposure. A. anatase, R. rutile, other gathers Ti-acetate, Ti-oxysulfate and amorphous Ti oxyde. In colors: experimental spectra, in dashed black: fits. Red dashed vertical line shows the position of A3 peak in $[\text{Ti}]_6$ spectra. The fit of Ctl silty sand is not displayed since a major reference compound was not available (trigonal bipyramidal and/or tetrahedral form(s)) for a proper fitting.

Moreover, the results obtained in this work are also in agreement with several similar articles of the literature. For instance, in a study carried out with the same NPs (but much higher concentration) and a soil similar to our silty sand, Ti based NPs have been detected (TEM-EDX) in the roots of red clover exposed to 1000 mg.kg^{-1} after 14 weeks of exposure⁵². Previously in 2011, Du *et al.*, also detected by TEM, TiO_2 -NPs in the roots of wheat grown for 2 weeks in an artificially contaminated loamy clay soil⁵³. Finally those results are also in line with those we previously demonstrated for wheat seedlings

exposed for 7 days to the same nanomaterial in hydroponics: anatase TiO₂-NPs were also evidenced in the roots by various techniques ^{54,55}.

3.3. Ti translocation to wheat leaves

Ti accumulation in aerial parts was quantified by ICP-AES (Figure 4A). Wheat leaves had a basal Ti concentration of 9 mg.kg⁻¹ in average in control soils. The only significant Ti concentration increase in leaves after exposure was detected for seedlings grown in sand after addition of 500 mg.kg⁻¹ TiO₂-NPs ($P < 0.0001$) with a concentration reaching more than 100 mg Ti.kg⁻¹ DW. The same trend was also noticeable in silty sand but not significant (from 7 in natural soil to 19 mg.kg⁻¹ in the most contaminated condition). Plants grown on clayey loam had the lowest Ti concentration in all conditions (4.4 mg.kg⁻¹ on average) even though this was the soil with the highest Ti concentration.

Another factor of interest after contamination of an agro-ecosystem is the translocation factor which corresponds to the fraction of Ti the plant is able to transfer from the soil to its leaves (Figure 4B). Even though the clayey loam soil was the richest in Ti – mainly geogenic Ti as demonstrated earlier – it was also the one in which Ti translocation factors were significantly lower than in other soils with an average of 0.4% ($P < 0.0001$). The addition of TiO₂-NPs to silty sand, loamy sand and clayey loam had no influence on Ti translocation factors whatever the concentration added. Translocation factor was only increased after addition of NPs in sand ($P = 0.0108$). Sand was the soil with the lowest Ti content, but with the highest translocation factors ranging from 23.4 to 61.9% meaning that Ti was more bioavailable in sand soil. All conditions considered, a negative significant correlation was evidenced between Ti in soil and Ti in leaves ($r = -0.374$, $P = 0.0130$).

Figure 4. A. Ti quantification in leaves and B. Translocation factors ($[Ti]_{leaf}/[Ti]_{soil}$) determined by ICP-AES in seedlings grown in control soils and soils contaminated with 100 or 500 mg.kg⁻¹ TiO₂-NPs, after a 3 week exposure. Lowercase letters indicate significant differences ($p<0.05$) among NP concentrations (control, 100 or 500 mg.kg⁻¹) within a soil type in A or among all treatments in B. Capital letters indicate significant differences among soil types. (n=6)

Results in the literature also report very little to no Ti translocation to leaves when plants were exposed in soils. For instance, tomato plants exposed to 250 mg.kg⁻¹ 25 nm TiO₂-NPs in calcinated fuller earth for 66 days accumulated between 25 to 83 mg Ti.kg⁻¹ approximately in their aerial parts with no significant difference with the control condition⁵⁶. Likewise, Gogos *et al.* did not evidenced any significant increase in red clover shoot and wheat grain after exposure to the same TiO₂-NPs as ours in a silty sand contaminated with concentrations ranging from 1 to 1000 mg.kg⁻¹⁵². TiO₂-NPs were also not detected in the leaves of wheat plants grown on a contaminated loamy clay (clay: 32.6%, OM content: 4.6%, added NPs: 90 mg.kg⁻¹) for 2 weeks⁵³. Ti was detected up to the fruits of cucumber plants after exposure to TiO₂-NPs in a sandy loam soil contaminated with 750 mg.kg⁻¹ for 150 days but with no quantitative data or comparison to control⁵⁷. Results were also similar for other NPs and plant species (CuO and lettuce⁵⁸, CeO₂ and radish⁵⁹), unless very high concentrations were introduced (>1000 mg.kg⁻¹).

Others in the literature also investigated soil characteristic influence on NP translocation, in particular the impact of organic matter. Interestingly most of the available studies concluded that increasing organic matter leads to higher metal concentration in plant shoots (Ce in kidney bean ⁵⁰, Ce in fescue ⁶⁰, Zn in cucumber ⁶¹). However, in another study, about 10 times more Zn was found in the shoot of wheat grown on “acid soil” (with 1.6% OM and 8% clay) than in “calcareous alkaline soil” (with 4.1% OM and 28% clay) after exposure to ZnO NPs. This was related to a higher proportion of soluble Zn in the acidic soil (200 times more) ⁶². Authors tried to confirm the relationship between OM and metal translocation by comparing Zn internalization in plants grown on sand with increasing concentrations of humic acid, but they were not able to reproduce the results obtained in natural soil: adding humic acid did not influenced Zn internalization. Finally, corn plants exposed to CeO₂ NPs in untreated soil contained significantly less Ce in roots but more Ce in shoot than plants exposed in the same soil enriched in organic matter. In parallel, ICP measurements demonstrated that the organic soil contained more Ce in the soil solution than the untreated soil (375 vs. 18 mg/100 mL soil solution) ⁶³ which is in contradiction with the previous study. Thus it seems that the correlation between OM content and NP/metal uptake is still not clear. OM could also have an indirect effect on plant development, possibly leading to an increased foliar area and thus plant evapotranspiration which could in turn increase NP uptake by plants. Those discrepancies might also be related to plant species and their different strategies for metal uptake (monocot vs. dicot ⁶⁴) or related to NP surface charge or biotransformation. Indeed, XANES studies have demonstrated that Zn is mainly detected in soil and plant shoot as dissolved ions recomplexed to either phosphate, phytate, citrate or histidine ^{65,66} while Ce has been detected as Ce(IV)O₂-NPs in roots and Ce(III) carboxyl in the stem and leaves, suggesting that the dissolution of CeO₂-NPs took place in the plants rather than in the soil. Ce would then be present in the soil as NPs while Zn would be under ionic form which implies different interactions with the soil particles and thus different uptake dynamics. Ti XANES spectra in plant leaves, in this study and in our previous work, were dominated by the anatase NP crystalline form which indicates that

TiO₂-NPs are more inert than ZnO-NPs and would behave in the soil more like CeO₂-NPs. Obviously more studies are needed to really understand how OM impacts different types of NP uptake by plants.

Soil texture, and in particular the amount of clay, is another parameter of interest to determine the fate of NPs. Our results showed that an increased percentage of clay in the soil lead to a decrease of Ti uptake in plant shoots. They are in agreement with a study from Zhang *et al.*, in which radish roots and shoots tend to accumulate more Ce after exposure to CeO₂-NPs in the loamy sand (76% sand) than in the silty loam (16% sand) ⁵⁹. Likewise, Layet *et al.*, concluded that increasing clay fraction enhanced the retention of CeO₂-NPs in soil and thus decreased its uptake by fescue ⁶⁰.

3.4. TiO₂-NP phytotoxicity

TiO₂-NP toxicity was evaluated during the time course of the exposure via seedling shoot length and chlorophyll content. The main noticeable difference was linked to soil type with plants growing in sand being the smallest followed by plants grown in silty sand, loamy sand and clayey loam (for instance at day 16, $P < 0.0001$) (Figure S3) but no significant difference was highlighted between NP treatment (for instance at day 16, $P = 0.528$). Results for chlorophyll content were similar (data not shown): the only difference was related to soil type ($P = 0.0080$) but not to NP addition ($P = 0.9290$).

After 3 weeks, leaf fresh and dry biomasses were recorded (Figure 5). Fresh leaf biomass was only influenced by soil type ($P < 0.0001$) with 3 groups of individuals: sand < silty sand and loamy sand < clayey loam (Figure 5A). Dry leaf biomass results were similar ($P = 0.0008$) with plants grown in sand having the lowest biomass with 20 mg on average, followed by plants grown in silty sand, loamy sand and clayey loam with respectively 36 mg, 41 mg and 43 mg (Figure 5B). The ratio between fresh matter and dry matter seems to be a more sensitive parameter which evidenced a soil effect ($P = 0.0080$): plants grown on clayey loam had 19% of dry matter vs. plants grown in loamy sand with 25% (Figure 5C). Moreover, this ratio also highlighted a NP concentration effect ($P = 0.0410$) with plants exposed in

soil with 500 mg.kg⁻¹ TiO₂-NPs having a lower content in dry matter as compared with plants grown in control soils. It has already been shown previously that NPs are able to interact with water uptake processes⁶⁷.

Figure 5. TiO₂-NP phytotoxicity on wheat biomass (fresh: A, dry: B, ratio between fresh and dry: C) after a three week exposure to different concentrations of NPs (0, 100, 500 mg.kg⁻¹) in different types of soils. Capital letters indicate significant differences (p<0.05) among soil types (n=6).

TiO₂-NP impact on plant hydromineral nutrition was also investigated at harvest after ICP-AES measurements of plant macro- (Ca, K, P, S) and micro- (Al, B, Ba, Cu, Fe, Mg, Mn, Na, Si, Zn) nutrient concentrations. Again, the main effect detected was a soil effect (Manova with Pillai's trace, F_{3,32}= 10.4, P < 0.0001) but no NP specific effect was highlighted (Manova with Pillai's trace, F_{2,33}= 1.5, P = 0.1210).

In the literature, no major acute toxicity has been reported after plant exposure to TiO₂-NPs in soil. For instance, there was no significant difference in catalase and ascorbate peroxidase activities in cucumber exposed to up to 750 mg.kg⁻¹ TiO₂-NPs. Likewise, no effect on chlorophyll content was detected until 500 mg.kg⁻¹ NPs (but an increase was seen at 750 mg.kg⁻¹)⁵⁷. The same results were also obtained previously in hydroponics for short term exposure^{29,54,55}. It would be interesting to confirm this lack of toxicity through multigenerational tests and through more in-depth endpoints (-omics approaches).

Conclusion

The results obtained in this study demonstrated that TiO₂-NPs can be significantly taken up by wheat seedlings after a 3 week exposure in a sandy soil in which 500 mg.kg⁻¹ of engineered NPs have been added. This is also in this soil type that NPs were able to reach in higher quantities the leachates. Thus if a contamination occurs in a sand, NPs will be very mobile (towards aquifers) and bioavailable to crop plants representing a risk for trophic transfer. However, sand is not a relevant medium for agriculture. The same trend has been highlighted in silty sand but in smaller proportions. In contrast, in loamy sand and clayey loam no difference has been evidenced after addition of NPs to the system: no Ti concentration increase in the soil, partly due to the high Ti geogenic background, no increase in plant roots and leaves and little to no increase in the soil leachates. This difference in TiO₂-NP behavior can be related to 3 characteristics of the soil used: organic carbon content, clay content and CEC, but the experimental plan do not allow to go further. In all conditions, no acute sign of phytotoxicity was detected in the endpoints analyzed. The overall conclusion for agro-ecosystems is that TiO₂-NP contamination (after a short term exposure: 3 weeks) seems to have little impact on plant health and low chance of uptake as well as low risk of leaching to the aquifers. The soil displaying the highest risk for food safety would be the silty sand but this soil has little nutrients for plant growth and is not the best featured for agriculture. However, those results also imply that TiO₂-NPs will remain in the soil with possible implications for soil micro and macro-organisms. It is thus important to understand NP

fate in soils upon weathering on a long term experiment. Finally, an experiment covering the full life cycle of different types of crop plants (fruit crop, leaf crop, root crop) is also needed to confirm data obtained here and further investigate the potential risk on silty sand soil.

Acknowledgement: We acknowledge SOLEIL for provision of synchrotron radiation facilities and we would like to thank LUCIA staff for assistance in using beamline LUCIA. Authors would also like to thank David Baqué, R&D Engineer on the ICP platform of EcoLab, for operating the ICP-AES instrument.

Funding sources: Camille Larue received a grant from the Atomic Energy Commission and the French Environment and Energy Management Agency (ADEME). This work was financially supported by the Region Ile de France through the framework of C’Nano Ile de France (project PLUTON: Plant Localization and Uptake of Nanoparticles). Camille Larue and Clément Baratange’s work was supported by the Toulouse IdEx Recherche (UNITI - programme “Nouveaux entrants”).

Authors have no competing interests to declare.

Supporting Information is available, it contains 4 figures and 2 tables.

References

1. www.nanotechproject.org. The project on emerging nanotechnologies. (2015).
2. consumer products. *the nanodatabase* Available at: <http://nanodb.dk/en/analysis/consumer-products/>.
3. Piccinno, F., Gottschalk, F., Seeger, S. & Nowack, B. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. *J. Nanoparticle Res.* **14**, 1109 (2012).
4. Jovanovic, B. Critical Review of Public Health Regulations of Titanium Dioxide, a Human Food Additive. *Integr. Environ. Assess. Manag.* **11**, 10–20 (2015).
5. Weir, A., Westerhoff, P. K., Fabricius, L. & von Goetz, N. Titanium Dioxide Nanoparticles in Food and Personal Care Products. *Env. Sci Technol* **46**, 2242–2250 (2012).
6. Robichaud, C. O., Uyar, A. E., Darby, M. R., Zucker, L. G. & Wiesner, M. R. Estimates of Upper Bounds and Trends in Nano-TiO₂ Production As a Basis for Exposure Assessment. *Env. Sci Technol* **43**, 4227–4233 (2009).
7. Windler, L. *et al.* Release of Titanium Dioxide from Textiles during Washing. *Environ. Sci. Technol.* **46**, 8181–8188 (2012).
8. Mackevica, A. & Foss Hansen, S. Release of nanomaterials from solid nanocomposites and consumer exposure assessment - a forward-looking review. *Nanotoxicology* **10**, 641–653 (2016).
9. Zhang, Y., Leu, Y.-R., Aitken, R. J. & Riediker, M. Inventory of Engineered Nanoparticle-Containing Consumer Products Available in the Singapore Retail Market and Likelihood of Release into the Aquatic Environment. *Int. J. Environ. Res. Public. Health* **12**, 8717–8743 (2015).
10. Mitrano, D. M., Motellier, S., Clavaguera, S. & Nowack, B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products. *Environ. Int.* **77**, 132–147 (2015).
11. Sun, T. Y., Bornhöft, N. A., Hungerbühler, K. & Nowack, B. Dynamic probabilistic modeling of environmental emissions of engineered nanomaterials. *Env. Sci Technol* 4701–4711 (2016).

12. Gottschalk, F., Sonderer, T., Scholz, R. W. & Nowack, B. Modeled Environmental Concentrations of Engineered Nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerenes) for Different Regions. *Environ. Sci. Technol.* **43**, 9216–9222 (2009).
13. Kim, B., Murayama, M., Colman, B. P. & Hochella, M. F. Characterization and environmental implications of nano- and larger TiO₂ particles in sewage sludge, and soils amended with sewage sludge. *J. Environ. Monit.* **14**, 1128–1136 (2012).
14. Gottschalk, F., Sun, T. & Nowack, B. Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. *Env. Pollut* **181**, 287–300 (2013).
15. Sharma, B., Sarkar, A., Singh, P. & Singh, R. P. Agricultural utilization of biosolids: a review on potential effects on soil and plant grown. *Waste Manag.* **64**, 117–132 (2017).
16. Zimbone, M., Cacciato, G., Boutinguiza, M., Privitera, V. & Grimaldi, M. G. Laser irradiation in water for the novel, scalable synthesis of black TiO_x photocatalyst for environmental remediation. *Beilstein J. Nanotechnol.* **8**, 196–202 (2017).
17. Bessa da Silva, M., Abrantes, N., Nogueira, V., Gonçalves, F. & Pereira, R. TiO₂ nanoparticles for the remediation of eutrophic shallow freshwater systems: Efficiency and impacts on aquatic biota under a microcosm experiment. *Aquat. Toxicol. Amst. Neth.* **178**, 58–71 (2016).
18. Wu, M. *et al.* Simultaneous biological-photocatalytic treatment with strain CDS-8 and TiO₂ for chlorothalonil removal from liquid and soil. *J. Hazard. Mater.* **320**, 612–619 (2016).
19. Sanz Lobón, G. *et al.* Efficient electrochemical remediation of microcystin-LR in tap water using designer TiO₂@carbon electrodes. *Sci. Rep.* **7**, (2017).
20. Bergeson, L. L. Nanosilver pesticide products: What does the future hold? *Environ. Qual. Manag.* **19**, 73–82 (2010).
21. Sastry, K., Rashmi, H. & Rao, N. Nanotechnology patents as R&D indicators for disease management strategies in agriculture. *J. Intellect. Property Rights* **15**, 197–205 (2010).
22. Khot, L. R., Sankaran, S., Maja, J. M., Ehsani, R. & Schuster, E. W. Applications of nanomaterials in agricultural production and crop protection: A review. *Crop Prot.* **35**, 64–70 (2012).

- 505 23. Ghormade, V., Deshpande, M. V. & Paknikar, K. M. Perspectives for nano-biotechnology enabled
506 protection and nutrition of plants. *Biotechnol Adv* **29**, 792–803 (2011).
- 507 24. Gogos, A., Knauer, K. & Bucheli, T. D. Nanomaterials in plant protection and fertilization: current
508 state, foreseen applications, and research priorities. *J. Agric. Food Chem.* **60**, 9781–9792 (2012).
- 509 25. Nair, R. *et al.* Nanoparticulate material delivery to plants. *Plant Sci.* **179**, 154–163 (2010).
- 510 26. Cox, A., Venkatachalam, P., Sahi, S. & Sharma, N. Silver and titanium dioxide nanoparticle toxicity
511 in plants: A review of current research. *Plant Physiol. Biochem.* **107**, 147–163 (2016).
- 512 27. Laxma Reddy, P. V., Hernandez-Viezcas, J.-A., Peralta-Videa, J. R. & Gardea-Torresdey, J. L.
513 Lessons learned: Are engineered nanomaterials toxic to terrestrial plants? *Sci. Total Environ.* **568**,
514 470–479 (2016).
- 515 28. Mariet, C., Belhadj, O., Leroy, S., Carrot, F. & Metrich, N. Relevance of NH₄F in acid digestion
516 before ICP-MS analysis. *Talanta* **77**, 445–450 (2008).
- 517 29. Larue, C. *et al.* Fate of pristine TiO₂ nanoparticles and aged paint-containing TiO₂ nanoparticles
518 in lettuce crop after foliar exposure. *J Hazard Mater* **273**, 17–26 (2014).
- 519 30. Daudin, L., Khodja, H. & Gallien, J. P. Development of ‘position-charge-time’ tagged spectrometry
520 for ion beam microanalysis. *Nucl. Instrum. Methods Phys. Res. Sect. B-Beam Interact. Mater. At.*
521 **210**, 153–158 (2003).
- 522 31. Mayer, M. SIMNRA, a simulation program for the analysis of NRA, RBS and ERDA. in *Application*
523 *of Accelerators in Research and Industry, Pts 1 and 2* (eds. Duggan, J. L. & Morgan, I. L.) **475**, 541–
524 544 (Amer Inst Physics, 1999).
- 525 32. Campbell, J. L., Hopman, T. L., Maxwell, J. A. & Nejedly, Z. The Guelph PIXE software package III:
526 Alternative proton database. *Nucl. Instrum. Methods Phys. Res. Sect. B-Beam Interact. Mater. At.*
527 **170**, 193–204 (2000).
- 528 33. Vantelon, D. *et al.* The LUCIA beamline at SOLEIL. *J Synchrotron Radiat* **23**, 635–640 (2016).

34. Sole, V. A., Papillon, E., Cotte, M., Walter, P. & Susini, J. A multiplatform code for the analysis of energy-dispersive X-ray fluorescence spectra. *Spectrochim. Acta Part B-At. Spectrosc.* **62**, 63–68 (2007).
35. Larue, C. *et al.* Foliar exposure of the crop *Lactuca sativa* to silver nanoparticles: Evidence for internalization and changes in Ag speciation. *J. Hazard. Mater.* **264**, 98–106 (2014).
36. Ravel & Newville. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrotron Radiat.* **12**, 537–541 (2005).
37. R Core Team. R: A language and environment for statistical computing. (2015).
38. Fox, J. & Weisberg, S. *An {R} Companion to Applied Regression*. (Thousand Oaks CA: Sage, 2011).
39. Graves, S., Piepho, H.-P. & Selzer, L. *Visualizations of Paired Comparisons*. (2015).
40. Giraudoux, P. *pgirmess: Data Analysis in Ecology*. (2017).
41. Lenth, R. V. Least-Squares Means: The R Package lsmeans. *J. Stat. Softw.* **69**, 1–33 (2016).
42. Fang, J., Shan, X. Q., Wen, B., Lin, J. M. & Owens, G. Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. *Environ. Pollut.* **157**, 1101–1109 (2009).
43. Fang, J. *et al.* Transport of copper as affected by titania nanoparticles in soil columns. *Environ. Pollut. Barking Essex 1987* **159**, 1248–1256 (2011).
44. Pachapur, V. *et al.* Behavior and characterization of titanium dioxide and silver nanoparticles in soils. *Science of the Total Environment* 933–943 (2016).
45. Wang, M., Gao, B. & Tang, D. Review of key factors controlling engineered nanoparticle transport in porous media. *Journal of Hazardous Materials* 233–246 (2016).
46. Shah, V. *et al.* Fate and impact of zero-valent copper nanoparticles on geographically-distinct soils. *Science of the Total Environment* 661–670 (2016).
47. Fang, J. *et al.* Co-transport of Pb(2+) and TiO₂ nanoparticles in repacked homogeneous soil columns under saturation condition: Effect of ionic strength and fulvic acid. *Sci. Total Environ.* **571**, 471–478 (2016).

48. Larue, C. *et al.* Innovative combination of spectroscopic techniques to reveal nanoparticle fate in a crop plant. *Spectrochim. Acta Part B At. Spectrosc.* **119**, 17–24 (2016).
49. Farges, F., Brown, G. E. & Rehr, J. J. Ti K-edge XANES studies of Ti coordination and disorder in oxide compounds: Comparison between theory and experiment. *Phys. Rev. B* **56**, 1809–1819 (1997).
50. Majumdar, S. *et al.* Soil organic matter influences cerium translocation and physiological processes in kidney bean plants exposed to cerium oxide nanoparticles. *Sci. Total Environ.* **569–570**, 201–211 (2016).
51. Gao, X. *et al.* CuO Nanoparticle Dissolution and Toxicity to Wheat (*Triticum aestivum*) in Rhizosphere Soil. *Env. Sci Technol* (2018). doi:DOI: 10.1021/acs.est.7b05816
52. Gogos, A. *et al.* Vertical transport and plant uptake of nanoparticles in a soil mesocosm experiment. *Journal of Nanobiotechnology* (2016).
53. Du, W. C. *et al.* TiO₂ and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. *J. Environ. Monit.* **13**, 822–828 (2011).
54. Larue, C. *et al.* Accumulation, translocation and impact of TiO₂ nanoparticles in wheat (*Triticum aestivum* spp.): influence of diameter and crystal phase. *Sci Total Env.* **431**, 197–208 (2012).
55. Larue, C. *et al.* Comparative Uptake and Impact of TiO₂ Nanoparticles in Wheat and Rapeseed. *J. Toxicol. Environ. Health A* **75**, 722–34 (2012).
56. Raliya, R., Nair, R., Chavalmane, S., Wang, W.-N. & Biswas, P. Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.) plant. *Metallomics* 1584–1594 (2015).
57. Servin, A. D. *et al.* Synchrotron verification of TiO₂ accumulation in cucumber fruit: a possible pathway of TiO₂ nanoparticle transfer from soil into the food chain. *Env. Sci Technol* **47**, 11592–11598 (2013).
58. Servin, A. D. *et al.* Weathering in soil increases nanoparticle CuO bioaccumulation within a terrestrial food chain. *Nanotoxicology* 98–111 (2017).

59. Zhang, W. *et al.* Bioavailability of cerium oxide nanoparticles to *Raphanus sativus* L. in two soils. *Plant physiology and biochemistry: PPB* 185–193 (2017).
60. Layet, C. *et al.* Evidence that Soil Properties and Organic Coating Drive the Phytoavailability of Cerium Oxide Nanoparticles. *Env. Sci Technol* **51**, 9756–9764
61. Moghaddasi, S. *et al.* Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. *Ecotoxicology and Environmental Safety* 543–551 (2017).
62. Watson, J.-L. *et al.* The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties. *Biometals* **28**, 101–112 (2015).
63. Zhao, L. *et al.* Effect of surface coating and organic matter on the uptake of CeO₂ NPs by corn plants grown in soil: Insight into the uptake mechanism. *J. Hazard. Mater.* **225–226**, 131–138 (2012).
64. Palmer, C. M. & Guerinot, M. L. Facing the challenges of Cu, Fe and Zn homeostasis in plants. *Nat. Chem. Biol.* **5**, 333–340 (2009).
65. Dimkpa, C. O. D. C. O. *et al.* Fate of CuO and ZnO Nano- and Microparticles in the Plant Environment. *Env. Sci Technol* **47**, 4734–4742 (2013).
66. Wang, P. *et al.* Fate of ZnO Nanoparticles in Soils and Cowpea (*Vigna unguiculata*). *Env. Sci Technol* (2013). doi:10.1021/es403466p
67. Khodakovskaya, M. *et al.* Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *Acs Nano* **3**, 3221–3227 (2009).