Influence of soil type on TiO_2 nanoparticle fate in an agro-ecosystem

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Abstract:

Nanoparticles (NPs) and in particular TiO$_2$-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 TiO$_2$-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

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

31   ecosystem.

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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]. **TiO$_2$-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 TiO$_2$-NPs comprised between 1 000 and 10 000 tons [3]. 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 TiO$_2$-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, TiO$_2$-NPs contained in certain textiles and paints are detached from their matrix during laundry or weathering and finally end up in wastewater treatment plants[7–10] and in particular in sewage sludge, where they can reach a concentration of 2 g.kg^{-1} [11]. 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 NPs [12–15]. This leads to the dissemination of TiO$_2$-NPs in agricultural soils. In addition, the expected environmental concentrations could be much higher locally with the intentional introduction of NPs in

60   the environment. For instance, thanks to their unique UV properties, TiO_2-NPs are used for purification

61   purposes in both soil and water[16–19]. Another critical case is the use of nanopesticides[20,21]. Indeed, TiO_2-

62   NPs are used in crop protection products for some years in order to optimize the product efficiency

63   and then to eventually reduce the amount of inputs into the environment (sustainable development).

64   The introduction of these products on the market is also expected to increase in the coming years[22–

65   25].

66   Until very recently, most of the NP risk assessment studies have been carried out on a single

67   organism in simplified conditions (hydroponics), exposed to high concentrations of NPs during short

68   time, simulating acute contaminations [26,27]. Over the last years, studies related to the fate of NPs in the

69   environment evidenced a trend towards more realistic exposure scenario: soil exposure, longer

70   exposure period and lower NP concentrations [26,27].

71   Studying the fate of TiO_2-NPs in soil is challenging. Indeed, soil is a very complex matrix. Moreover,

72   the contribution of TiO_2 engineered nanomaterials is challenging to measure in the soil because of the

73   high geogenic Ti background (≈0.6% of the terrestrial crust). Accordingly, to date, only modelling

74   studies can help approximate the amount of engineered TiO_2-NPs that is ending up in the environment.

75   The last article estimation forecasts the presence of 61 mg of $TiO_2.kg^{-1}$ of soil amended with sewage

76   sludge [11], making **agricultural soil** the main sink for nanomaterials in the environment and crop plants

77   a privileged point of entry for NPs in the food chain up to humans.

78   Currently, knowledge about the interactions of NPs with soil is very limited. Once NPs reach the

79   soil, they can be either physically retained or chemically adsorbed onto the surface of soil particles [27].

80   These interactions could mitigate NP phytotoxicity and bioavailability [27], meaning that a same

81   contamination could lead to very distinct consequences according to soil type. The literature published

82   on plants exposed in soils suggested a very limited transfer of NPs to aerial parts. For a better risk

83   assessment, it is thus of primary importance to understand how soil characteristics control NP fate in

84   a crop system. For this purpose we exposed wheat, a widely grown crop over the world, to TiO_2-NPs

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85 (0, 100, 500 mg.kg^{-1}) in 4 different types of soil already containing geogenic Ti at different

86 concentrations. Ti quantification in soil, soil leachates and wheat leaves was assessed. Ti distribution

87 in roots and leaves and speciation in leaves were also determined using large scale research facilities.

88 Finally NP phytotoxicity was evaluated through plant development parameters: height, fresh and dry

89 weights and chlorophyll content.

90

91 **2. Material and Methods**

92

93 **2.1. Experimental set-up**

94 TiO$_2$-NPs with a nominal diameter of 25 nm and a crystalline phase mainly anatase (89%) were used

95 (see Figure S1 for more details).

96 A sand and 3 agricultural soils provided by Lufa Speyer (Germany) were chosen with different

97 characteristics (Table 1): texture ranging from sand to clayey loam, organic carbon content from 0 to

98 2.09%, cation exchange capacity (CEC) from 0 to 10 meq/100 g and pH from 5.1 to 7.2.

99

	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]

100

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 TiO$_2$-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}). TiO$_2$-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.

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2.2. Bulk Ti quantification by ICP-AES

Different tests have been performed to ensure a proper acidic digestion of TiO_2-NPs which represents a critical step to avoid underestimation of Ti concentration in the samples. Several digestion protocols adapted from the literature have been compared (Figure S2A) with different acid cocktails, temperatures and evaporation steps. The best digestion yield was obtained with a protocol adapted from Mariet *et al*. (2008) with a mix of HNO_3, NH_4F, $HClO_4$ and H_2O_2 with several evaporation steps at 100 °C in a DigiPrep® system (see Supporting Information for more details)[28]. The yield was about 88% and reproducible. This test demonstrates that the digestion protocol needs to be re-optimized for each TiO_2-NP. Indeed, in one of our previous experiments using 4 nm anatase TiO_2-NPs, the protocol from Mariet *et al*. was not efficient [29]. The linearity of the digestion was also assessed with increasing TiO_2-NP concentrations (Figure S2B). Finally the stability of the solution after digestion over a month was monitored (Figure S2C). After digestion, samples (soils, leachates, plant shoots) were analyzed using Inductively Coupled Plasma – Mass Spectrometry (ICP-AES IRIS, Thermo Scientific). Quality of sample preparation was checked with blank samples containing only chemicals and with reference samples (tomato leaves, NIST RM 1573a) and permitted to validate sample preparation and ICP analysis (Figure S2bis, Table S1).

2.3. Spatially resolved Ti semi-quantification by μPIXE/RBS

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

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

145   sample in isopentane cooled with liquid nitrogen. Samples were then cut in thin cross-sections (50 μm)

146   using a cryo-microtome (Leica) and finally freeze-dried (48h, -52°C, 0.01 mbars).

147   Freeze-dried sections were analyzed at the nuclear microprobe available at the Atomic Energy

148   Commission (CEA) Center of Saclay (France) with a proton source of 3 MeV, a beam focused to 2.5 μm

149   and a current intensity of 500 pA. Data processing was performed using Rismin[30] software to define

150   regions of interest and extract spectra, and SIMNRA [31] and GUPIX [32] codes to reduce RBS and PIXE

151   data, respectively. The Ti/Ca ratio was used as a Ti enrichment indicator as Ca is an abundant

152   endogenous element.

153

154   **2.4. Ti distribution and speciation by μXRF and μXANES**

155   Samples were prepared as described previously for nuclear microprobe experiments (section 2.3.).

156   Synchrotron experiments were carried out on LUCIA beamline at SOLEIL (Saint Aubain, France)[33]. Micro

157   X-ray fluorescence (μXRF) maps were recorded above the Ti-K edge (5.1 keV) with a step size of 5 μm

158   and a dwell time of 2000 ms. μXRF data were fitted using PyMCA software [34].

159   Ti-K edge (4.5 to 5.5 keV energy range, 0.5 eV step) micro X-ray absorption near edge spectroscopy

160   (μXANES) spectra were recorded in regions of interest of the fluorescence maps. XANES spectra on

161   reference compounds were recorded during previous experiments [29,35]. For the fitting, 3 groups of

162   references were created: anatase, rutile and others (containing Ti-oxysulfate, amorphous Ti oxide and

163   Ti acetate). XANES data treatment was performed using Athena software [36] as previously [29,35].

164

165   **2.5. Statistical analysis**

166   Data were checked for normality (Shapiro test) and homoscedasticity (Bartlett or Levene's tests). They

167   were compared using two-way ANOVA, with soil type and TiO_2-NP concentration as main effects, and

168   the interaction (soil type x NP concentration). If the whole set of data did not follow a normal

169   distribution even after transformation, Student T test, Wilcoxon test, one-way ANOVA or Kruskal–

170   Wallis test were performed on subset of data (according to soil type or NP concentration). Post hoc

171 tests (Tukey's HSD or Dunn's test) were applied to detect differences between groups of samples.

172 Differences were considered significant when $P < 0.05$. All statistical analyses are gathered in Table

173 S21.

174 To study the global influence of TiO_2-NPs on plant elemental content, MANOVA based on Pillai's trace

175 test was used on the whole set of micro- and macronutrients measured by ICP-AES.

176 All statistical analyses were performed using the R statistical software (version 3.1.3) [37], with car [38],

177 multcompView [39], pgirmess [40] and lsmeans [41] packages.

178

179

180 **3. Results and discussion**

181 **3.1. Ti partitioning between soil and soil leachates**

182 As stated earlier, Ti is a geogenic element of the earth crust. As such, the 4 natural soils (control

183 condition) contained Ti concentrations ranging from 43 to 1671 mg.kg^{-1} with sand having the lowest

184 concentration followed by the group of silty and loamy sands and finally the clayey loam being the

185 richest in Ti ($P<0.0001$, Figure 1A). Ti concentration in natural soils was positively correlated with the

186 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

187 to the fraction <0.002 mm (r^2=0.9618). After addition of 100 mg.kg^{-1} of TiO_2-NPs, the Ti concentration

188 in soils did not significantly increase in any soil. After addition of 500 mg.kg^{-1} of TiO_2-NPs, the increase

189 in Ti was only significant for sand and silty sand ($P<0.0001$), *i.e.* soils with the lowest clay fraction.

190 Those results can be confronted with Ti concentrations in soil leachates (Figure 1B). In natural soils, Ti

191 concentrations in the leachates after 12h mixing were respectively 0.03, 0.18, 0.07 and 0.23 mg.L^{-1} for

192 sand, silty sand, loamy sand and clayey loam. After addition of 100 mg.kg^{-1} of TiO_2-NPs, only the sand

193 leachate had a significantly higher Ti concentration (13.18 mg.L^{-1}, P=0.0379) with a more than 400 fold

194 increase in comparison with the control sand. This is also the soil in which the leachate volume

195 recovered was the highest (Table 1): 71% of the 15 mL of the contamination suspension was collected

196 in the leaching compartment. This fraction was decreased to 42% in the case of the clayey loam soil.

9

197 Crossing this result with Ti concentration in soil, one can conclude that most of the TiO$_2$-NP suspension

198 added in the 100 mg.kg^{-1} condition went through the sand column and ended up in the soil leachate.

199 After addition of 500 mg.kg^{-1} of TiO$_2$-NPs, Ti concentration in soil leachate was significantly increased

200 as compared to the control for all soils except for the clayey loam soil (*P*=0.0019 for silty sand and *P*=

201 0.0031 for loamy sand).
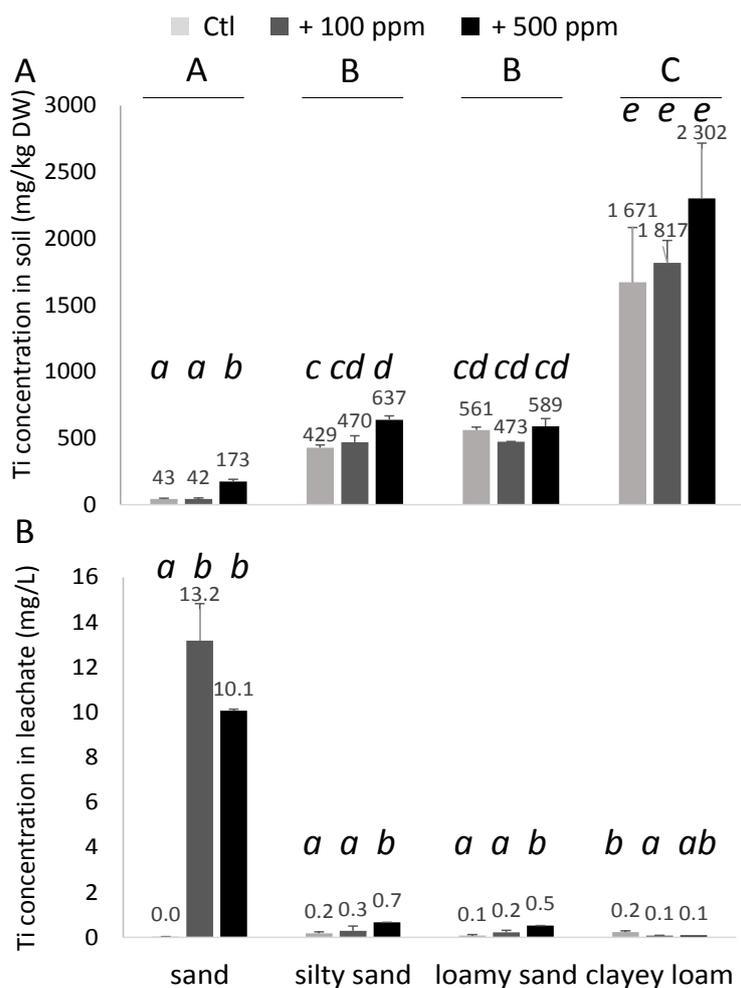
202

203 *Figure 1. Ti concentration in the soil system: soil (A) and soil leachate (B) determined by ICP-AES.*

204 *Different lowercase letters indicate significant differences (p<0.05) among all treatments in A or among*

205 *treatment within a soil type in B. Capital letters in A highlight significant differences among soil types.*

206 *(n=6).*

207

208   If a contamination occurs on a sand, most TiO_2-NPs will likely go through the soil and reach the aquifers.

209   Only a small fraction will remain in the soil if a high dose (500 mg.kg^{-1}) of NPs is applied. In the silty

210   sand, Ti increase in both soil and soil leachate was only detectable for the highest concentration added.

211   For the loamy sand, the high Ti natural background (> 500 mg.kg^{-1}) shadowed the addition of NPs in

212   the soil and Ti increase was visible only in the leachate at the highest NP concentration added. Finally,

213   in the clayey loam, all added Ti was retained in the soil but did not increase significantly Ti

214   concentration also because of the very high geogenic Ti level. Depth column studies would be needed

215   to confirm these results about NP mobility and gain a deeper understanding.

216   Soils with coarser texture, lower organic carbon content as well as lower CEC (sand and silty sand) were

217   the ones in which NPs displayed the highest availability. This result is in agreement with previous

218   literature [42–45]. Authors suggested that soils with a higher clay content have also smaller pore size and

219   larger surface area which increase both electrostatic interactions and physical straining explaining the

220   lower transport of NPs to soil leachates. Likewise, Cu-NP translocation was observed to be low in soils

221   enriched in organic matter and high in clay [46]. Another important point that could be considered is the

222   interaction of NPs with other potential contaminants of the soil. Indeed, it has been shown in the

223   literature that addition of TiO_2-NPs in sandy soils can improve Cu and Pb mobility in soils [43,47]. Sewage

224   sludge also often contains a certain amount of heavy metals [15]; NP contamination of sewage sludge

225   amended soils could thus represent a threat to both terrestrial and aquatic ecosystems through higher

226   heavy metal bioavailability and leaching towards aquifers. Finally, different microbial communities

227   likely occur in different soils, and might have a role on TiO_2 fate and speciation over long term
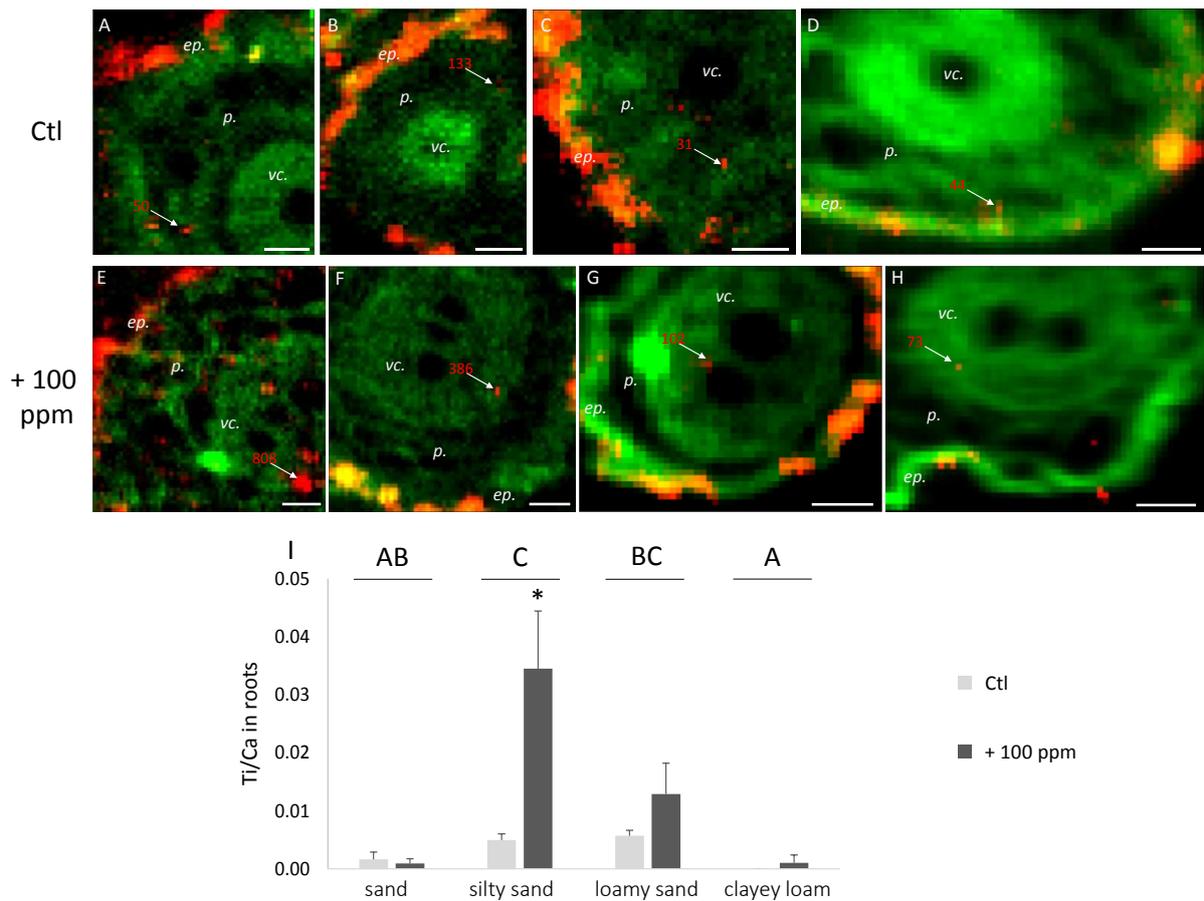
228   exposure.

229

230   **3.2. Ti uptake in wheat roots**

231   Spatially resolved techniques were employed to investigate Ti uptake in wheat roots exposed in control

232   soil and soil contaminated with 100 mg.kg^{-1} of TiO_2-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 µPIXE coupled to µRBS,

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 µXRF 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   µXRF technique has a lower limit of detection than µPIXE/RBS[48]. Overall, no correlation was detectable

256   between Ti concentration in soil and Ti/Ca content in roots (r=0.111, *P* = 0.742).

257

Figure 2. Ti in wheat roots after a 3 week exposure: elemental distribution by µXRF of Ti (in red) and Ca

(in green) in root sections of wheat grown in control soils (A-D) or in contaminated soils (E-F). 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 µm. Semi-quantitative analysis

of Ti by µPIXE/RBS in root sections of wheat grown in natural soils and soils contaminated with 100

mg.kg^{-1} TiO$_2$-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).

265

Ti speciation was then analyzed by µXANES on high intensity spots identified by µXRF (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%

13

270   of rutile crystalline form) to mainly anatase in loamy sand (63% anatase). Anatase and rutile forms of

271   TiO_2 correspond to an octahedral arrangements of the O atoms around Ti. In the natural silty sand,

272   there is a shift of the A3 peak towards the lower energies (about 0.5 eV) with an increase of the peak

273   height and area which suggests a different conformation of the TiO unit going from octahedral towards

274   trigonal bipyramidal (5 coordinations) or tetrahedral (4 coordinations) conformations [49]. After

275   exposure to TiO_2-NPs (89% anatase, 11% rutile, Figure S1), the ratio between anatase and rutile in

276   comparison with the same ratio in the control roots increased in the roots of all 4 conditions

277   highlighting the contribution of the mainly anatase added NPs. The increase was more important in

278   the case of plants grown in the soils with coarser texture (sand and silty sand).

279   There are not many studies evaluating NP fate in plants after soil exposure even though this number

280   has been increasing over the last few years. But there are even fewer articles reporting the fate of NPs

281   in roots (Majumdar *et al.* 2016 on CeO_2-NPs [50] or Gao *et al.,* 2018 on CuO-NPs[51]). Indeed it is very

282   challenging to obtain this type of data and only spatially resolved techniques are able to provide

283   reliable data getting rid of the geogenic signal surrounding the roots. Moreover, if one wants to

284   perform a comparative study, the technique also needs to be quantitative which leaves the scientific

285   community with few tools mainly based on spectroscopy. In this study by combining 4 different

286   techniques namely µXRF, µPIXE/RBS and µXANES, we were able to map Ti distribution in wheat roots

287   with a good sensitivity, quantify it and investigate its speciation *in vivo*. Our results demonstrate that

288   the added TiO_2-NPs can be taken up by plant roots when exposure occurs in soil and in particular in

289   sand and silty sand. Considering the concentration of NPs added to natural soils (no significant increase

290   of Ti concentration in soils after addition of 100 mg.kg^{-1}, Figure 1A), our results also suggest that Ti

291   from NPs was taken up by roots preferentially than geogenic Ti (no significant Ti concentration increase

292   in soil *vs.* increase in roots). This highlights that the added Ti had a different behavior than the natural

293   one and a different bioavailability which justifies the fact to study the impact of a TiO_2-NP

294   contamination in the environment even though plants have evolved with high Ti concentrations in soils

295   for ages.

14
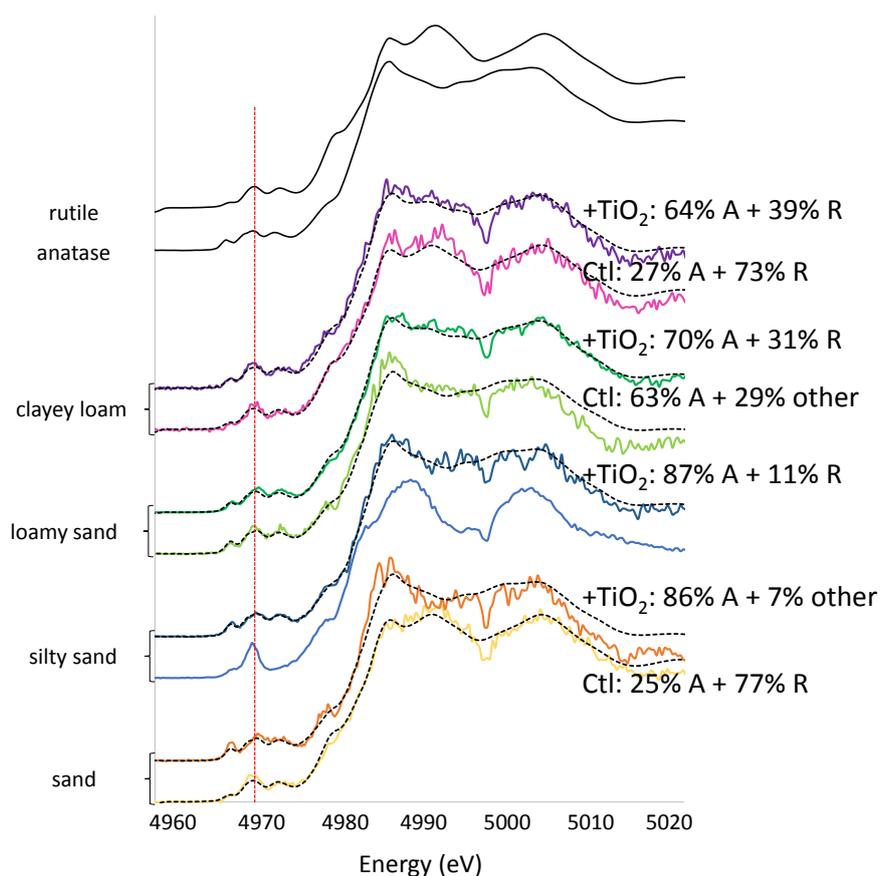
Figure 3. Ti speciation assessed by µXANES in wheat roots grown in control soils (Ctl) or soils contaminated with 100 mg.kg^{-1} TiO$_2$-NPs (+TiO$_2$) after a 3 week exposure. A. anatase, R. rutile, other gathers Ti-acetate, Ti-oxysulfate and amorphous Ti oxyde. In colors: experimental spectra, in dashed black: fits. Red dashed vertical line shows the position of A3 peak in [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 exposure[52]. 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 soil[53]. Finally those results are also in line with those we previously demonstrated for wheat seedlings

310   exposed for 7 days to the same nanomaterial in hydroponics: anatase TiO_2-NPs were also evidenced in

311   the roots by various techniques [54,55].

312

3.3. Ti translocation to wheat leaves

314   Ti accumulation in aerial parts was quantified by ICP-AES (Figure 4A). Wheat leaves had a basal Ti

315   concentration of 9 mg.kg^{-1} in average in control soils. The only significant Ti concentration increase in

316   leaves after exposure was detected for seedlings grown in sand after addition of 500 mg.kg^{-1} TiO_2-NPs

317   (P<0.0001) with a concentration reaching more than 100 mg Ti.kg^{-1} DW. The same trend was also

318   noticeable in silty sand but not significant (from 7 in natural soil to 19 mg.kg^{-1} in the most contaminated

319   condition). Plants grown on clayey loam had the lowest Ti concentration in all conditions (4.4 mg.kg^{-1}

320   on average) even though this was the soil with the highest Ti concentration.

321   Another factor of interest after contamination of an agro-ecosystem is the translocation factor which

322   corresponds to the fraction of Ti the plant is able to transfer from the soil to its leaves (Figure 4B). Even

323   though the clayey loam soil was the richest in Ti – mainly geogenic Ti as demonstrated earlier – it was

324   also the one in which Ti translocation factors were significantly lower than in other soils with an

325   average of 0.4% (P < 0.0001). The addition of TiO_2-NPs to silty sand, loamy sand and clayey loam had

326   no influence on Ti translocation factors whatever the concentration added. Translocation factor was

327   only increased after addition of NPs in sand (P= 0.0108). Sand was the soil with the lowest Ti content,

328   but with the highest translocation factors ranging from 23.4 to 61.9% meaning that Ti was more

329   bioavailable in sand soil. All conditions considered, a negative significant correlation was evidenced

330   between Ti in soil and Ti in leaves (r= -0.374, P=0.0130).

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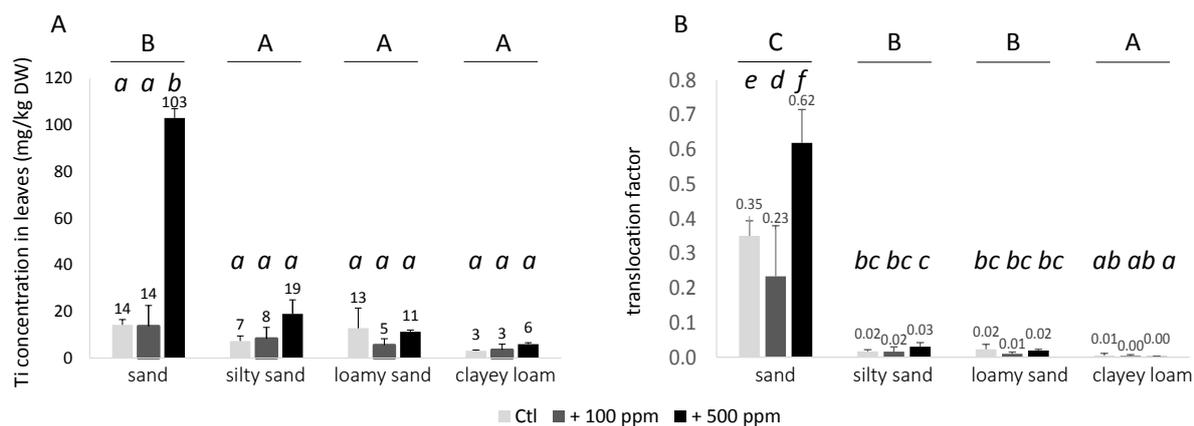
Figure 4. A. Ti quantification in leaves and B. Translocation factors ($[Ti]_{leaf}$ /$[Ti]_{soil}$) determined by ICP-AES in seedlings grown in control soils and soils contaminated with 100 or 500 mg.kg^{-1} TiO$_2$-NPs, after a 3 week exposure. Lowercase letters indicate significant differences (p<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 TiO$_2$-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 condition[56]. Likewise, Gogos *et al.* did not evidenced any significant increase in red clover shoot and wheat grain after exposure to the same TiO$_2$-NPs as ours in a silty sand contaminated with concentrations ranging from 1 to 1000 mg.kg^{-1} [52]. TiO$_2$-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 weeks [53]. Ti was detected up to the fruits of cucumber plants after exposure to TiO$_2$-NPs in a sandy loam soil contaminated with 750 mg.kg^{-1} for 150 days but with no quantitative data or comparison to control [57]. Results were also similar for other NPs and plant species (CuO and lettuce[58], CeO$_2$ and radish[59]), unless very high concentrations were introduced (>1000 mg.kg^{-1}).

350   Others in the literature also investigated soil characteristic influence on NP translocation, in particular

351   the impact of organic matter. Interestingly most of the available studies concluded that increasing

352   organic matter leads to higher metal concentration in plant shoots (Ce in kidney bean [50], Ce in fescue

353   [60], Zn in cucumber [61]). However, in another study, about 10 times more Zn was found in the shoot of

354   wheat grown on "acid soil" (with 1.6% OM and 8% clay) than in "calcareous alkaline soil" (with 4.1%

355   OM and 28% clay) after exposure to ZnO NPs. This was related to a higher proportion of soluble Zn in

356   the acidic soil (200 times more) [62]. Authors tried to confirm the relationship between OM and metal

357   translocation by comparing Zn internalization in plants grown on sand with increasing concentrations

358   of humic acid, but they were not able to reproduce the results obtained in natural soil: adding humic

359   acid did not influenced Zn internalization. Finally, corn plants exposed to CeO_2 NPs in untreated soil

360   contained significantly less Ce in roots but more Ce in shoot than plants exposed in the same soil

361   enriched in organic matter. In parallel, ICP measurements demonstrated that the organic soil

362   contained more Ce in the soil solution than the untreated soil (375 *vs.* 18 mg/100 mL soil solution) [63]

363   which is in contradiction with the previous study. Thus it seems that the correlation between OM

364   content and NP/metal uptake is still not clear. OM could also have an indirect effect on plant

365   development, possibly leading to an increased foliar area and thus plant evapotranspiration which

366   could in turn increase NP uptake by plants. Those discrepancies might also be related to plant species

367   and their different strategies for metal uptake (monocot *vs.* dicot [64]) or related to NP surface charge

368   or biotransformation. Indeed, XANES studies have demonstrated that Zn is mainly detected in soil and

369   plant shoot as dissolved ions recomplexed to either phosphate, phytate, citrate or histidine [65,66] while

370   Ce has been detected as Ce(IV)O_2-NPs in roots and Ce(III) carboxyl in the stem and leaves, suggesting

371   that the dissolution of CeO_2-NPs took place in the plants rather than in the soil. Ce would then be

372   present in the soil as NPs while Zn would be under ionic form which implies different interactions with

373   the soil particles and thus different uptake dynamics. Ti XANES spectra in plant leaves, in this study

374   and in our previous work, were dominated by the anatase NP crystalline form which indicates that

375   TiO$_2$-NPs are more inert than ZnO-NPs and would behave in the soil more like CeO$_2$-NPs. Obviously

376   more studies are needed to really understand how OM impacts different types of NP uptake by plants.

377   Soil texture, and in particular the amount of clay, is another parameter of interest to determine the

378   fate of NPs. Our results showed that an increased percentage of clay in the soil lead to a decrease of

379   Ti uptake in plant shoots. They are in agreement with a study from Zhang *et al.*, in which radish roots

380   and shoots tend to accumulate more Ce after exposure to CeO$_2$-NPs in the loamy sand (76% sand) than

381   in the silty loam (16% sand) [59]. Likewise, Layet *et al.*, concluded that increasing clay fraction enhanced

382   the retention of CeO$_2$-NPs in soil and thus decreased its uptake by fescue [60].

383

384   **3.4. TiO$_2$-NP phytotoxicity**

385   TiO$_2$-NP toxicity was evaluated during the time course of the exposure via seedling shoot length and

386   chlorophyll content. The main noticeable difference was linked to soil type with plants growing in sand

387   being the smallest followed by plants grown in silty sand, loamy sand and clayey loam (for instance at

388   day 16, $P < 0.0001$) (Figure S3) but no significant difference was highlighted between NP treatment (for

389   instance at day 16, $P = 0.528$). Results for chlorophyll content were similar (data not shown): the only

390   difference was related to soil type ($P= 0.0080$) but not to NP addition ($P=0.9290$).

391   After 3 weeks, leaf fresh and dry biomasses were recorded (Figure 5). Fresh leaf biomass was only

392   influenced by soil type ($P<0.0001$) with 3 groups of individuals: sand < silty sand and loamy sand <

393   clayey loam (Figure 5A). Dry leaf biomass results were similar ($P=0.0008$) with plants grown in sand

394   having the lowest biomass with 20 mg on average, followed by plants grown in silty sand, loamy sand

395   and clayey loam with respectively 36 mg, 41 mg and 43 mg (Figure 5B). The ratio between fresh matter

396   and dry matter seems to be a more sensitive parameter which evidenced a soil effect ($P=0.0080$):

397   plants grown on clayey loam had 19% of dry matter *vs.* plants grown in loamy sand with 25% (Figure

398   5C). Moreover, this ratio also highlighted a NP concentration effect ($P=0.0410$) with plants exposed in

399   soil with 500 mg.kg^{-1} TiO$_2$-NPs having a lower content in dry matter as compared with plants grown in

400   control soils. It has already been shown previously that NPs are able to interact with water uptake

401   processes [67].
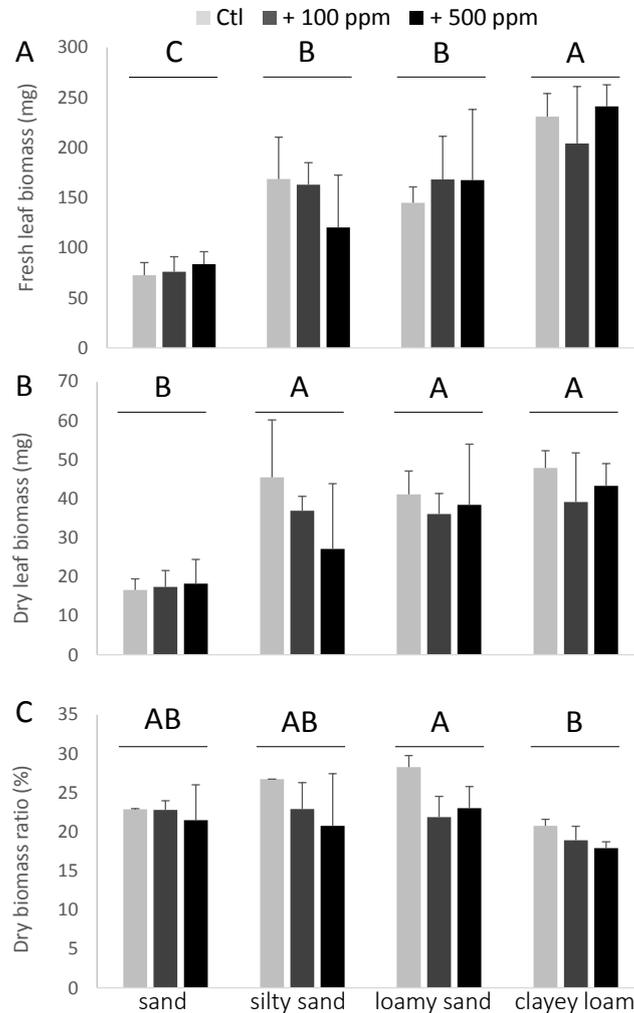
402

403   *Figure 5. TiO$_2$-NP phytotoxicity on wheat biomass (fresh: A, dry: B, ratio between fresh and dry: C) after*

404   *a three week exposure to different concentrations of NPs (0, 100, 500 mg.kg^{-1}) in different types of soils.*

405   *Capital letters indicate significant differences (p<0.05) among soil types (n=6).*

406

407   TiO$_2$-NP impact on plant hydromineral nutrition was also investigated at harvest after ICP-AES

408   measurements of plant macro- (Ca, K, P, S) and micro- (Al, B, Ba, Cu, Fe, Mg, Mn, Na, Si, Zn) nutrient

409   concentrations. Again, the main effect detected was a soil effect (Manova with Pillai's trace, $F_{3,32}$= 10.4,

410   P < 0.0001) but no NP specific effect was highlighted (Manova with Pillai's trace, $F_{2,33}$= 1.5, P = 0.1210).

411 In the literature, no major acute toxicity has been reported after plant exposure to TiO_2-NPs in soil. For

412 instance, there was no significant difference in catalase and ascorbate peroxidase activities in

413 cucumber exposed to up to 750 mg.kg^{-1} TiO_2-NPs. Likewise, no effect on chlorophyll content was

414 detected until 500 mg.kg^{-1} NPs (but an increase was seen at 750 mg.kg^{-1}) [57]. The same results were also

415 obtained previously in hydroponics for short term exposure [29,54,55]. It would be interesting to confirm

416 this lack of toxicity through multigenerational tests and through more in-depth endpoints (-omics

417 approaches).

418

419 **Conclusion**

420 The results obtained in this study demonstrated that TiO_2-NPs can be significantly taken up by wheat

421 seedlings after a 3 week exposure in a sandy soil in which 500 mg.kg^{-1} of engineered NPs have been

422 added. This is also in this soil type that NPs were able to reach in higher quantities the leachates. Thus

423 if a contamination occurs in a sand, NPs will be very mobile (towards aquifers) and bioavailable to crop

424 plants representing a risk for trophic transfer. However, sand is not a relevant medium for agriculture.

425 The same trend has been highlighted in silty sand but in smaller proportions. In contrast, in loamy sand

426 and clayey loam no difference has been evidenced after addition of NPs to the system: no Ti

427 concentration increase in the soil, partly due to the high Ti geogenic background, no increase in plant

428 roots and leaves and little to no increase in the soil leachates. This difference in TiO_2-NP behavior can

429 be related to 3 characteristics of the soil used: organic carbon content, clay content and CEC, but the

430 experimental plan do not allow to go further. In all conditions, no acute sign of phytotoxicity was

431 detected in the endpoints analyzed. The overall conclusion for agro-ecosystems is that TiO_2-NP

432 contamination (after a short term exposure: 3 weeks) seems to have little impact on plant health and

433 low chance of uptake as well as low risk of leaching to the aquifers. The soil displaying the highest risk

434 for food safety would be the silty sand but this soil has little nutrients for plant growth and is not the

435 best featured for agriculture. However, those results also imply that TiO_2-NPs will remain in the soil

436 with possible implications for soil micro and macro-organisms. It is thus important to understand NP

21

437   fate in soils upon weathering on a long term experiment. Finally, an experiment covering the full life

438   cycle of different types of crop plants (fruit crop, leaf crop, root crop) is also needed to confirm data

439   obtained here and further investigate the potential risk on silty sand soil.

440

450

451   Authors have no competing interests to declare.

452

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

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