# Long-term outdoor lysimeter study with cerium dioxide nanomaterial

Martin Hoppe[a,*], Karsten Schlich[b], Jonas Wielinski[c,d], Jan Köser[a], Daniel Rückamp[a], Ralf Kaegi[c], Kerstin Hund-Rinke[b]

[a] Federal Institute for Geosciences and Natural Resources (BGR), Germany
[b] Fraunhofer Institute for Molecular Biology and Applied Ecology, Auf dem Aberg 1, 57392 Schmallenberg, Germany
[c] Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland
[d] ETH Zürich, Institute of Environmental Engineering, 8093 Zürich, Switzerland

## ARTICLE INFO

## ABSTRACT

Fate and impact of cerium dioxide nanomaterials ($CeO_2$ NM) in soil remains uncertain. Most of the recent environmental studies used high doses of $CeO_2$ NM in short-term laboratory experiments and stated soil as large sink for NM. Recent studies covering the life cycle of plants found evidence for particle uptake in crop plants, and triggered concern about NM entering the food chain. Here, we present a 25 months outdoor lysimeter study that investigates the translocation, biological impact, and transformation of $CeO_2$ NM in soils cultivated with different crops (wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), and barley (*Hordeum vulgare* L.)). Low doses of $CeO_2$ NM (NM-212, 10 and 50 mg $CeO_2$ NM $kg^{-1}$ soil) were applied to a Cambisol by sewage sludge or artificial rainwater. To simulate ploughing, the $CeO_2$ NM was mixed into the first 20 cm of the lysimeter soil. Three weeks after the NM application by sewage sludge and five days after the latest NM application by artificial rainwater wheat seeds were sown.

We found no vertical Ce translocation to deeper soil layers as well as negligible release of Ce into percolating water, confirming that soil is a sink for $CeO_2$ NM. Furthermore, no inhibition of the NM sensitive ammonium oxidizing bacteria (AOB) was observed which implies a low bioavailability of $CeO_2$ NM in soil. The detected Ce root uptake for canola (Ce < 35.7 mg $kg^{-1}$) and barley (Ce < 61.8 mg $kg^{-1}$) indicates that physico-chemical conditions in the rhizosphere or the aging of the NM in soil can remobilize the applied $CeO_2$ NM to some extent. One can speculate that the obtained reduction of $Ce^{4+}$ to $Ce^{3+}$ ($\approx$ 50% after 25 months) was coupled to different conditions (ionic strength, pH, water content, soil organic matter content) in the rhizosphere and necessary for the root accumulation of Ce.

Due to negligible mobility in the soil-water interface, as well as non-toxicity to AOB, the environmental risk of the tested $CeO_2$ NM appears to be low, compared to other highly mobile emergent pollutants. Nonetheless, further long-term investigations need to focus on the speciation and localization of NM in plants to clarify uptake and impact of NM to crops.

## 1. Introduction

Cerium dioxide nanomaterials ($CeO_2$ NM) show many useful properties because of their unique nano-sized structure, surface reactivity, and redox activity (Andreescu et al., 2014). The global production of $CeO_2$ NM is approximately 10,000 t $a^{-1}$ with various applications (diesel fuel additive, electronic and optical devices, metallurgy, polishing agents for glass and silicon wafers, and exterior paints) (Collin et al., 2014). These widespread applications led to concerns about the environmental impact of $CeO_2$ NM and related consequences for human health (Zhang et al., 2011). Atmospheric deposition and sewage sludge

applications have been discussed as possible pathways for the release of $CeO_2$ NM to different environmental compartments (Gottschalk et al., 2013). In a model wastewater treatment plant, uncoated $CeO_2$ NM were removed from wastewater by attachment (heteroaggregation) to sewage sludge flocs (Limbach et al., 2008). Hence, the application of sewage sludge as a fertilizer will result in a $CeO_2$ NM input into agricultural soils which are supposed to be a final sink for NM (Pan and Xing, 2012). However, only a few studies addressed the fate and effects of $CeO_2$ NM in soil under environmentally relevant conditions. Batch experiments with five Australian soils showed a wide range in the non-equilibrium retention coefficients for $CeO_2$ NM (Cornelis et al., 2010).

* Corresponding author at: Federal Institute for Geosciences and Natural Resources, Stilleweg 2, 30655 Hannover, Germany.
  *E-mail address:* martin.hoppe@bgr.de (M. Hoppe).

The positive correlation of the non-equilibrium retention coefficients (median $K_r = 9.6\,l\,kg^{-1}$, $n = 16$) with the soil clay content indicates heteroaggregation between $CeO_2$ NM and soil colloids (Cornelis et al., 2011). Tella et al. (2015) found that bare $CeO_2$ NM homo- and heteroaggregated and settled out of the water column of an aquatic mesocosm. A seven-month lasting outdoor lysimeter study showed no vertical transport of Ce ions or $CeO_2$ NM within the lysimeter profile (Du et al., 2015). These results from Du et al. (2015) suggest that $CeO_2$ NM dominantly remained immobile in the soil solid phase. However, the authors found enhanced Ce root concentration that could have caused the observed toxicity to wheat seedlings, affecting their grain development. Further reports indicate that $CeO_2$ NM can be taken up from soil by several food crops (soybean, corn), suggesting a translocation to the food chain (Hernandez-Viezcas et al., 2013; Zhao et al., 2015). Moreover, López-Moreno et al. (2010) found evidence for $CeO_2$ NM root-uptake and genotoxicity in hydroponic experiments with soybeans. A soil microcosm study showed beneficial (biomass production) and harmful effects (spike production) of $CeO_2$ NM in barley (Rico et al., 2015). Synchrotron based X-ray absorption spectroscopy (XAS) and micro X-ray fluorescence (XRF) have proven useful to investigate the plant uptake of $CeO_2$ NM and the Ce speciation in plants and soil (López-Moreno et al., 2010; Hernandez-Viezcas et al., 2013; Arai and Dahle, 2018; Rico et al., 2017). Soybeans accumulated $CeO_2$ NM in their roots (López-Moreno et al., 2010). In addition, Hernandez-Viezcas et al. (2013) found that Ce was mainly stored as $CeO_2$ NM in the nodules of soybeans with little translocation to the pods. One of the first studies, investigating Ce speciation in the rhizosphere of wheat roots by Rico et al. (2017), revealed root adsorption but no uptake of $CeO_2$ NM with limited transformation ($Ce^{4+}$ to $Ce^{3+}$). Furthermore, the authors showed $CeO_2$ NM agglomeration with low $Ce^{4+}$ to $Ce^{3+}$ transformation in soil. In contrast, a high reduction (98%) of $CeO_2$ NM occurred at the root-soil interface of barley which was suggested to facilitate the $Ce^{3+}$ root uptake (Rico et al., 2018).

The $CeO_2$ NM attachment and uptake to plant roots resulting in growth and yield responses of several plants suggest that the particles are bioavailable in soil, which is why also other important terrestrial organisms, such as microorganisms, might be affected by the particles. Currently, very little information is available on how $CeO_2$ NM affect soil microorganisms (Collin et al., 2014). Hamidat et al. (2016) added different $CeO_2$ NM to soil and found reduced microbial activity in the rhizosphere of canola, highlighting the impact of these NM on soil microorganisms. In addition, Vittori Antisari et al. (2013) found a concentration-dependent long-term effect on the C/N ratio of the microbial biomass and short-term effects on the metabolic quotient, a commonly used stress indicator.

Despite few studies addressed the fate and effects of $CeO_2$ NM in soil and towards different soil organisms, there is a general lack of knowledge regarding the long-term fate and bioavailability of $CeO_2$ NM in soil under realistic exposure conditions. In this study, we therefore investigated the long-term fate and bioavailability of $CeO_2$ NM over three growing seasons (wheat, canola, barley) in an outdoor lysimeter experiment. Two different exposure pathways (rain, sewage sludge) to soil were evaluated in outdoor lysimeters applying low doses of standard $CeO_2$ NM (NM-212, European Commission - Joint Research Center). The horizontal and vertical Ce distribution was measured in four soil layers in each of the lysimeters. Moreover, the Ce concentrations of the percolate water, the plant roots as well as the effect of the applied $CeO_2$ NM to ammonium oxidizing bacteria (AOB) was determined. Synchrotron based XAS measurements were conducted for two soil samples and the plant roots (barley) of the lysimeter with the highest $CeO_2$ NM application to assess the speciation changes of Ce that occurred during the experiment.

## 2. Material and methods

### 2.1. Soil sampling and soil application to the lysimeter

The lysimeters are located at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME, Schmallenberg, Germany). The soil for the five outdoor lysimeters was collected from the uppermost layer of an arable soil in Lower Saxony (Refesol 01A, Hagen, Germany, Ce concentration = $13.8\,mg\,kg^{-1}$, sd = $2.2\,mg\,kg^{-1}$, $n = 5$). This soil was sieved to 4 mm and filled into the cubical lysimeters (90 cm, 90 cm, 90 cm) two years prior to the beginning of the experiments. The lysimeters were kept open as fallow land until the first sowing of wheat in May 2015. In January 2015, the top 20 cm of the soils were limed with 200 g CaO (Sigma Aldrich, St. Louis, USA) to achieve pH values of 5.5 to 6.0.

### 2.2. Application of $CeO_2$ NM to the outdoor lysimeters

NM powders were dispersed in deionized water following established dispersion protocols (Taurozzi et al., 2012). In brief, 50 mg of the $CeO_2$ NM powders were added to 50 ml of deionized water ($H_2O_{dd}$) and treated for 1 min with a 70 W ultrasonic homogenizer (Bandelin Sonoplus HD 2070, Berlin, Germany) to disperse the $CeO_2$ NM. Using the described dispersion protocol, a hydrodynamic diameter (HDD) of 224 nm (sd = 1 nm, $n = 3$) and a zeta potential (ZP) of 43.5 mV ($n = 1$) were determined for the $CeO_2$ NM. Refer to Singh et al. (2014) for a more detailed characterization of the applied $CeO_2$ NM. Artificial rainwater (Siemens et al., 2008) was used to dilute the dispersed NM to a concentration of $25\,mg\,l^{-1}$. The diluted $CeO_2$ NM was added to lysimeter 9 (L 9) by a watering can three times per week for the duration of four weeks ($V_{total} = 120\,l$, $m_{total}$ ($CeO_2$ NM) = 3000 mg). After four weeks, the uppermost 20 cm of the soil were dug over to simulate ploughing. The control lysimeter 8 (L 8) received artificial rainwater without $CeO_2$ NM following the same procedure as described for L 9.

Lysimeter 3 (L 3) and lysimeter 7 (L 7) received $CeO_2$ NM spiked sewage sludge from the wastewater treatment plant in Schmallenberg (Germany). The one-time application of the NM via sewage sludge into soil is described in Schlich et al. (2017). In brief, lysimeter 10 served as control and received pristine sewage sludge. The target $CeO_2$ NM concentrations in the uppermost 20 cm of the soil were $10\,mg\,kg^{-1}$ (L 7) and $50\,mg\,kg^{-1}$ (L 3), respectively. Therefore, two sewage sludge batches (405 g dry matter) were mixed with 2.5 l and 12.5 l dispersed $CeO_2$ NM ($1000\,mg\,l^{-1}$), respectively. This achieved concentrations of 6170 mg and 30,860 mg $CeO_2$ NM per kg dry matter sewage sludge. Hence, the application of 1.67 g dry matter sewage sludge per kg soil, resulted in the above mentioned concentrations ($10\,mg\,kg^{-1}$ and $50\,mg\,kg^{-1}$), which is in accordance to the German sewage sludge ordinance (Schlich et al., 2017). After addition of the $CeO_2$ NM to the sewage sludge, the sewage sludge was aerated, stirred for 24 h, and subsequently treated with a 0.2% cationic polyacrylamide solution (Sedifloc 154, Kemira Germany GmbH, Frankfurt, Germany) to dewater the sewage sludge. The dewatered sewage sludge was passed over a fine gauze to remove as much water as possible. Afterwards, the spiked sewage sludge was mixed to an aliquot (25 kg) of the lysimeter soil which was subsequently applied to the lysimeters. The uppermost 20 cm of all lysimeters (L 3, L 7, L 10) were dug over after the application of sewage sludge to simulate ploughing.

For more details regarding the timeline of NM application to the lysimeters refer to Table S1.

### 2.3. Sampling of soil, plants, and percolating water

A detailed time line regarding plant cultivation, soil sampling, and plant sampling is presented in Table S1. The collection of the first soil samples was conducted with a Pürckhauer boring rod before the sowing of the wheat, and after the application of the $CeO_2$ NM and simulated

ploughing. From each of the five boreholes per lysimeter, we took four soil samples (0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm). The boreholes were refilled with Refesol 01A after each sampling campaign.

In the three growing seasons, we planted wheat (*Triticum aestivum* L., May 2015–August 2015), canola (*Brassica napus* L., September 2015–July 2016), and barley (*Hordeum vulgare* L., September 2016–July 2017), which represents a typical local crop rotation. The lysimeters (L 8, L 9) without sewage sludge application received fertilizer (130 g per lysimeter, Hakaphos-soft-plus, Compo GmbH, Münster, Germany) before the sowing of the wheat. No additional fertilizers, and no additional water were added to the lysimeters during the experiment. All plants were removed from the lysimeters after maturation. Three random samples of ten plants were taken from the total sample of each lysimeter, separated into roots, shoots, and grains, and subsequently cleaned with $H_2O_{dd}$ before drying. After each harvesting, a spade was used to simulate ploughing of the uppermost 20 cm of the lysimeters.

After each of the three vegetation periods, soil samples were collected as described above. To test the inhibition of AOB, the soil (0–20 cm) was sampled periodically (Table S2).

Percolating water samples were collected between May 2015 and February 2017. One percolating water sample was taken per month to differentiate between the total Ce concentration ($Ce_{total}$), the Ce concentration in the fraction < 0.45 μm ($Ce_{0.45μm}$), and the dissolved Ce concentration ($Ce_{dissolved}$). If percolating water occurred more than once per month, only the $Ce_{0.45μm}$ fractions were analyzed in these additional samples. For details regarding the treatment of the percolating water, refer to Section 2.5.

### 2.4. Recovery of $CeO_2$ NM from soil

To ensure a complete $CeO_2$ NM recovery from the lysimeter soil, three different digestion methods were tested. For that purpose, the NM was dry weighed to the soil samples to achieve $CeO_2$ NM concentrations of 10 mg kg$^{-1}$ and 100 mg kg$^{-1}$, respectively. Subsequently, the soil samples were milled with a vibration mill (Retsch MM 400, Haan, Germany) at 28 Hz for 4 min. Acid-hydrogen-peroxide digestion (AHD) was tested with a microwave digestion device, and with a hot plate vessel with a reflux cooler. In addition, the effectiveness of aqua regia digestion (ARD) was tested in a hot plate vessel with a reflux cooler. For the AHD with reflux cooler, 5 ml of nitric acid (65% $HNO_3$, Suprapur, Merck, Darmstadt, Germany) and 2.5 ml of hydrogen peroxide (30% $H_2O_2$, pa ISO EMSURE, Merck, Darmstadt, Germany) were transferred to 1 g of the soil and heated for 2 h at 110 °C in the digestion vessel. Afterwards, the samples were diluted with $H_2O_{dd}$ (electrical conductivity (EC) = 0.055 μS cm$^{-1}$, TKA, Niederelbert, Germany).

The ARD was performed according to EN 16174 (2012) using 21 ml of HCl (37%, p. a., Merck, Darmstadt, Germany) and 7 ml of HNO3 (65%, p. a., Merck, Darmstadt, Germany). Three grams of milled soil were digested for 120 min at 180 °C, filtered (595½, Whatman, Dassel, Germany) and diluted with $H_2O_{dd}$ (EC = 0.055 μS cm$^{-1}$, TKA, Niederelbert, Germany).

For the microwave assisted AHD, 200–500 mg of the milled samples were weighed into Teflon vessels. Subsequently, 4 ml $HNO_3$ (69%, supra, Carl Roth GmbH & Co. KG, Karlsruhe, Germany und 2 ml $H_2O_2$ (35%, supra, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) were added. After a digestion time of 45 min at 205 °C in a MLSW-Ethos 1800 (1000 W, MLS GmbH, Leutkirch, Germany) the samples were centrifuged (3000 g, 15 min, Heraeus Cryofuge 8500i, Hanau, Germany) and diluted with $H_2O_{dd}$ (EC = 0.055 μS cm$^{-1}$, TKA, Niederelbert, Germany).

The Ce concentrations after digestion were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES, Ciros Vision, Spectro, Kleve, Germany) and inductively coupled plasma optical mass spectrometry (ICP-MS, 7500 Series, Agilent, Santa Clara, USA). For more details regarding the quality control and quality assurance of the ICP-MS measurements refer to the supplementary

information (SI).

### 2.5. Standard analytics for soil, plants, and percolating water

The soil organic carbon content ($C_{org}$) was determined at the beginning (May 26th, 2015) and during the experiment (July 13th, 2016). The soil was air dried and milled (28 Hz for 4 min, Retsch MM 400, Haan, Germany) before the measurement with a CNS analyzer (vario MAX CNS, Elementar GmbH, Hanau, Germany). The pH was measured in the same sampling interval. For that purpose, the air-dried and sieved (≤ 2 mm) soil was mixed with $H_2O_{dd}$ (1:5 v/v, EC = 0.055 μS cm$^{-1}$, TKA, Niederelbert, Germany) and shaken for 10 min. Afterwards, the pH (SenTix 41 electrode, WTW, Weilheim, Germany) and the electrical conductivity (InLab 731, Seven Easy, Mettler Toledo GmbH, Gießen, Germany) were measured.

Fractionation was applied to the percolating water to achieve information regarding the colloidal speciation of Ce. According to DIN 38402-11 (2009), the percolating water was sampled, filtered (0.45 μm, Graphic Controls, Buffalo, USA), and acidified (50 μl $HNO_3$ (65%), ROTIPURAN, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). This fraction is labeled as $Ce_{0.45μm}$ concentration. A subsample of the percolating water was digested with 5 ml $HNO_3$ (65%, Suprapur, Merck, Darmstadt, Germany) and 2.5 ml $H_2O_2$ (30%, p. a. ISO EMSURE, Merck, Darmstadt, Germany) in an open vessel digestion to retrieve information about the total Ce concentration ($Ce_{total}$). Another subsample was ultracentrifuged (60 min at 390,000 g, Beckman Coulter, Brea, USA) to determine the dissolved Ce concentration ($Ce_{dissolved}$). This samples were subsequently acidified with $HNO_3$ (80 μl, 65%, Suprapur, Merck, Darmstadt, Germany) before measurement. All Ce concentrations ($Ce_{total}$, $Ce_{0.45μm}$, $Ce_{dissolved}$) were measured with ICP-MS (7500 Series, Agilent, Santa Clara, USA).

The milled soil and plant material was digested with the microwave assisted AHD as described in Section 2.4.

### 2.6. X-ray absorption spectroscopy

Soil and root samples were air dried and milled in a vibration ball mill (Retsch MM 400, Haan, Germany) at 28 Hz for 4 min. About 60 mg of sample were mixed with 4 mg of boron carbide (Alfa Aesar, USA), 4 mg quartz sand (Sigma-Aldrich, Schnelldorf, Germany), and 15 mg of cellulose (Sigma-Aldrich, Schnelldorf, Germany) in a mortar. A 50 mg aliquot thereof was pressed into a 7 mm pellet using a hand-held press for XAS measurements. Ce L$_3$-Edge ($E_0$ = 5723 eV) XAS measurements were conducted at the X10DA (SuperXAS) beamline at the Swiss Light Source operated with a 2.4 GeV storage ring energy by the Paul-Scherrer-Institute (Villigen, Switzerland). All samples were measured in fluorescence mode using a 5-channel silicon drift detector (SGX Sensortech, Chelmsford, UK). The X-ray absorption near edge spectroscopy (XANES) was recorded up to 240 eV above the edge. Cerium dioxide (99.995%, Sigma-Aldrich, Gallen, Switzerland), $CePO_4$ (99% min, Alfa Aesar, Karlsruhe, Germany), Ce-Allanite (Sieber & Sieber AG, Aathal, Switzerland) and a $CeO_2$ NM dispersion (Envirox, Energenics, Begbroke, UK) were prepared as reference materials for transmission measurement. All samples and standards were measured at 100 K facilitated by a cryo jet (Oxford Instruments, Abington, UK). For energy calibration, a $CeO_2$ reference pellet was mounted between the second and the third ion chamber and a spectrum was recorded in transmission mode for every measurement. Three spectra were recorded for every sample and merged to increase the data quality. Data treatment and linear combination fitting (LCF) of reference materials to sample spectra was performed using Athena (Ravel and Newville, 2005).

### 2.7. Inhibition of ammonium oxidizing bacteria

In accordance with the European Chemical Agency guidance on information requirements and chemical safety assessment, the nitrogen
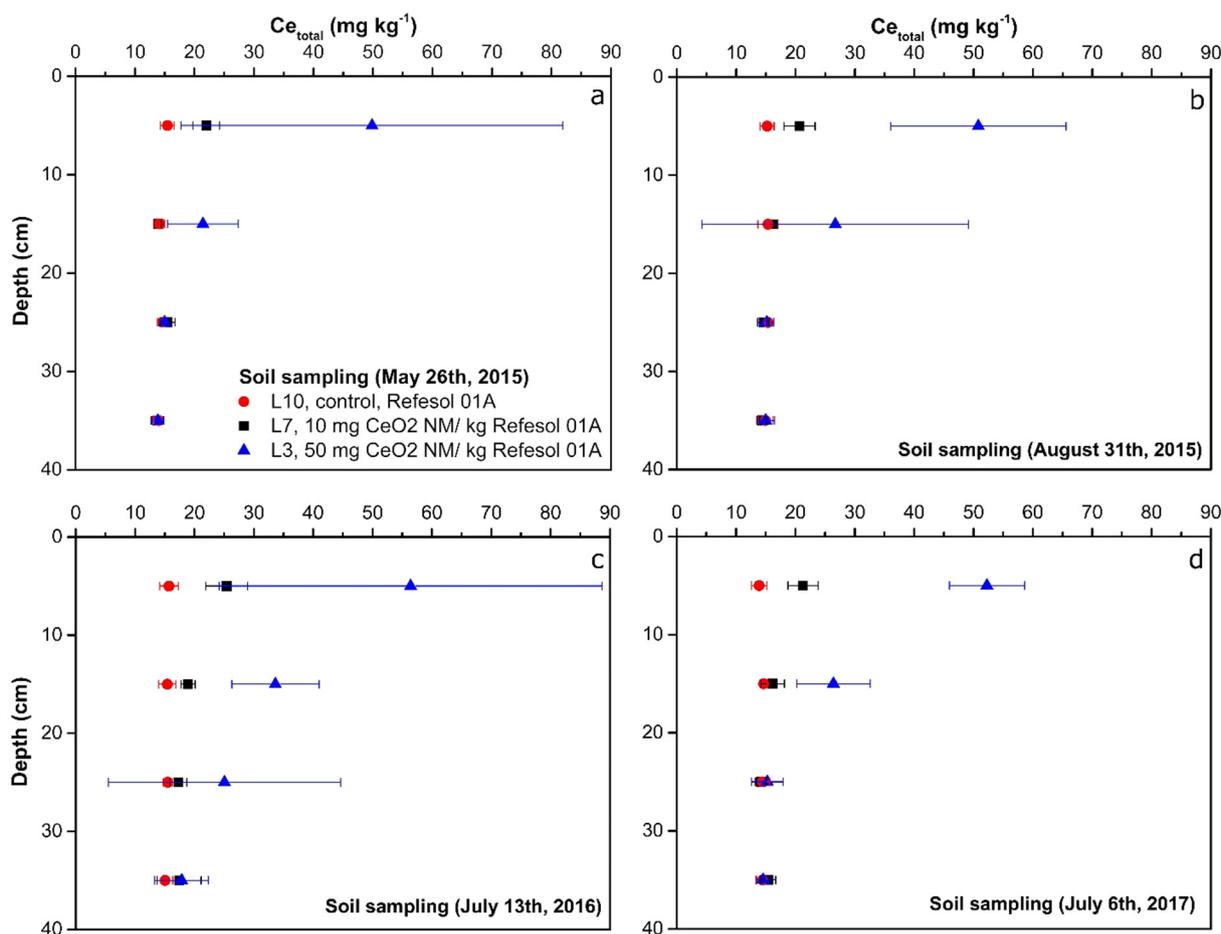
**Fig. 1.** Total Ce concentrations in the lysimeter soil: The cerium dioxide nanomaterial ($CeO_2$ NM) was added via spiked sewage sludge to lysimeter 3 (L 3) and Lysimeter 7 (L 7). Lysimeter 10 (L 10) received sewage sludge without $CeO_2$ NM (control). From each lysimeter, five borehole samples were collected and divided in four layers (0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm). The bars show the standard deviation of the five replicated Ce measurements per layer.

transformation was used to evaluate the effect of chemicals on soil microorganisms. Hund-Rinke et al. (2016) recommends the ISO 15685 (2012) for the testing of nanoparticulate ion releasing metals or metal oxides in the context of regulation, which is more suitable than the OECD TG 216 (2000). A detailed description of the method is presented in Schlich and Hund-Rinke (2015).

### 2.8. Statistical analysis

Statistical analyses were conducted with SPSS 22.0.0.0 (IBM Corp., Armonk, USA). The non-parametric Mann-Whitney $U$ test was applied to check for differences between the control lysimeters and the spiked lysimeters (L 8 vs. L 9, L 10 vs. L 7, L 10 vs. L 3) in the three different fractions of percolating water ($Ce_{total}$, $Ce_{0.45\mu m}$, $Ce_{dissolved}$). The Mann-Whitney U test was chosen because of the low number of measurements and their lack of normal distribution. The t-test was used to evaluate normal distributed data with n ≥ 40.

### 3. Results and discussion

#### 3.1. Soil characterization

The soil (Refesol 01A) was classified as a sandy Cambisol according to Ad-hoc-AG Boden (2006). Initial experiments showed a pH of 5 for the lysimeter soil which is a relatively low value compared to German arable soils (Scheffer and Schachtschabel, 2002). Furthermore, ISO Guideline 15685 (2012) emphasizes a soil pH above 5 for testing ammonium oxidizing bacteria (Section 3.5). Thus, the lysimeters were

limed four months before starting the experiments. The pH in all lysimeters was 5.26 (standard deviation (sd) = 0.41, n = 100) at the beginning of the experiment. Due to the buffering action of lime, the mean pH increased to 5.58 (sd = 0.51, n = 100) after 15 months. The raise in pH was not detected in the deepest soil layer (35 cm) for the rainwater treated L 8 and L 9 (Fig. S1) as well as for the sewage sludge treated L 3, L 7 and L 10 (Fig. S2). These results suggest that the neutralization front has not reached the deepest sampling layer.

The mean $C_{org}$ content for the lysimeter without sewage sludge application decreased significantly ($P < 0.001$, t-test) from 1.04% (sd = 0.03%, $n = 40$) to 0.95% (sd = 0.05%, n = 40) in the 15 months after the beginning of the experiment (Fig. S3). This suggests a degradation of soil organic matter (SOM) that might have been induced by enhanced microbial activity as a result of liming. For the lysimeter with sewage sludge application, the $C_{org}$ content decreased slightly from 1.04% (sd = 0.05%, $n = 60$) at the beginning of the experiment to 0.99% (sd = 0.07%, $n = 59$) after 15 months (Fig. S4). However, the decrease of the $C_{org}$ content was significant according to the t-test (P < 0.001). Possibly, the effect of lime derived SOM degeneration was partially compensated in L 3, L 7, and L 10 by the applied sewage sludge.

Further soil characteristics for the sandy Cambisol can be found elsewhere (Hoppe et al., 2015).

#### 3.2. Recovery of $CeO_2$ NM from soil

The Ce recovery was measured in the mixtures of $CeO_2$ NM with the Cambisol. The highest recovery was achieved by microwave assisted

AHD (Fig. S5). With this method, the recovery for the $10\,mg\,kg^{-1}$ preparation was 97% (sd = 15%, $n = 3$), and the recovery for the $100\,mg\,kg^{-1}$ preparation was 94% (sd = 9%, n = 3). Thus, the microwave assisted AHD was used to determine the Ce concentration in all soil samples from the lysimeters (Section 3.3.1).

### 3.3. Fate of $CeO_2$ NM in the lysimeters

#### 3.3.1. Cerium concentrations in the lysimeter profile

The natural Ce background was $14.4\,mg\,kg^{-1}$ (sd = $1.5\,mg\,kg^{-1}$, $n = 20$) in the rainwater treated control lysimeter (L 8) at the beginning of the experiment. For both approaches, the sewage sludge application (Fig. 1) as well as the rainwater application (Fig. S6), almost no vertical Ce translocation was detected in the soil samples over two years. The highest Ce concentrations were found in the first soil layers for the sewage sludge and rainwater treated lysimeters (L 3, L 7, L 9). This can be explained by the sewage sludge and rainwater application to the topsoil with subsequent digging of the first 20 cm by spade which is not supposed to generate a complete equilibration of the Ce concentrations. Fig. 1c shows an enhanced Ce concentration in the third layer for the high application ($50\,mg\,kg^{-1}$), which was not found during the other three measurement campaigns. In addition, the lower $CeO_2$ NM application ($10\,mg\,kg^{-1}$) showed no enhanced Ce concentration below the second sampling layer during the experiment. Thus, the enhanced Ce concentration was very likely caused by an inaccuracy of the soil sampling.

Despite the differences in the $CeO_2$ NM application ($10\,mg\,kg^{-1}$, rainwater vs. sewage sludge), both approaches resulted in a nearly complete retention of the $CeO_2$ NM in the uppermost 20 cm of the lysimeters. For more details regarding the Ce transport with the percolating water, refer to Sections 3.3.2 and 3.6. In general, our findings are in line with (Cornelis et al., 2014) stating that soil act as sink for NM. For a holistic data interpretation, the Ce concentrations in the percolating water were measured to assess the transport of $CeO_2$ NM through the lysimeters (Section 3.3.2).

#### 3.3.2. Cerium concentrations in the percolating water

The lower number of monthly collected and separated percolating water samples ($Ce_{total}$, $n = 8$, $Ce_{0.45\mu m}$, $n = 7$), compared to the higher number of filtered percolating water samples ($Ce_{0.45\mu m}$, $n = 15$, Sections 2.3, 2.5) measured according to DIN 38402-11 (2009) are explicable by the fact that field capacity is necessary to retrieve percolating water. Especially, in soils with no preferential flow, which is expectable for the sandy Cambisol, no percolating water will occur until field capacity is reached. During vegetation periods (June 24th–September 14th, 2015; March 8th–July 18th; 2016) and periods with low rainfall rates (July 18th–October 26th, 2016; November 21st, 2016–January 27th, 2017) no percolating water emerged. If once field capacity was reached, then possibly more than one percolating water sample was received per month. This explains the higher number of $Ce_{0.45\mu m}$ samples.

Compared to the digestion of the soil matrix, the measurements of Ce concentrations in the percolating water is more sensitive because of the lower limit of quantification (ppt vs. ppm). However, the daily sampling of percolation water cannot show the accuracy of an online monitoring. After heavy rainfall events, the sampling container overflowed a few times which might have caused alteration of the observed Ce concentrations. Fig. 2 shows that the highest amount of Ce was associated with the particulate fraction ($Ce_{total}$) larger than $0.45\,\mu m$. Small amounts of Ce were also present in the particulate fraction smaller than $0.45\,\mu m$ ($Ce_{0.45\mu m}$). As expected, the ionic Ce fraction showed very low concentrations ($Ce_{dissolved} < 100\,ng\,l^{-1}$) reflecting that lanthanides are sparingly soluble as earlier derived from laboratory experiments by Cornelis et al. (2011). None of the three fractions ($Ce_{total}$, $Ce_{0.45\mu m}$, $Ce_{dissolved}$) showed significant differences between the control and the highest $CeO_2$ NM application in L 3 (Mann-Whitney $U$
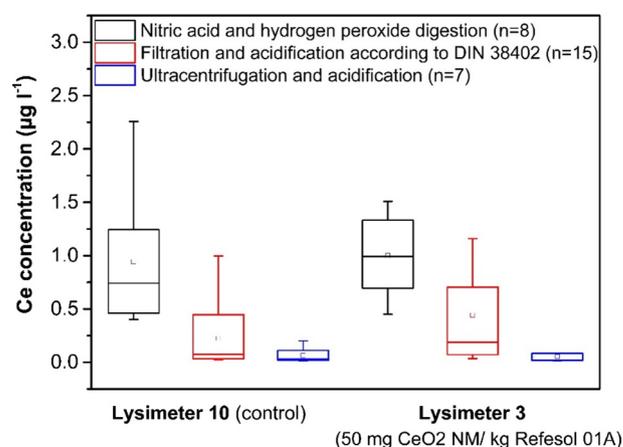


**Fig. 2.** The Ce concentrations in the different fractions of percolating water: The cerium dioxide nanomaterial ($CeO_2$ NM) was spiked to sewage sludge which was subsequently added to lysimeter 3 (L 3). Lysimeter 10 (L 10) received sewage sludge without $CeO_2$ NM (control). From each lysimeter, the percolating water was sampled after every rainfall event and subsequently filtered and acidified before measurement of the Ce concentrations ($n = 15$, red boxplots, $Ce_{0.45\mu m}$). The monthly collected fresh water samples were digested ($n = 8$, black boxplots, $Ce_{total}$) or ultracentrifuged and acidified ($n = 7$, blue boxplots, $Ce_{dissolved}$) before measurement of the of the Ce concentrations. According to the Mann-Whitney $U$ test, no significant differences were found between L 10 and L 3 in the similarly treated groups (Table S3).

test, Table S3). Thus, even for the sewage sludge applied lysimeter with the highest $CeO_2$ NM concentration ($50\,mg\,kg^{-1}$), no release of NM was detected. The same results were obtained for the lower $CeO_2$ NM sewage sludge application ($10\,mg\,kg^{-1}$) to L 7 (Fig. S7, Table S3), which confirms that the sewage sludge applied $CeO_2$ NM became largely retained in the lysimeter soil. The application of $CeO_2$ NM by artificial rainwater showed no significant release of $Ce_{0.45\mu m}$ and $Ce_{dissolved}$ to the percolating water (Fig. S8, Table S3). However, after the application of the dispersed NM, an increased Ce concentration ($Ce_{total} = 16\,\mu g\,l^{-1}$, May 21st, 2015) was measured in the percolating water of L 9 recently after the rainwater application. This value is clearly above the median of the $Ce_{total}$ fraction (median $Ce_{total} < 1\,\mu g\,l^{-1}$), which indicates a transport of $CeO_2$ NM with percolating water. Apart from this outlier, no significant difference of Ce was detected between the control and L 9 in any of the three groups ($Ce_{total}$, $Ce_{0.45\mu m}$, $Ce_{dissolved}$). Considering the large $CeO_2$ NM retention in soil after rainwater application (L 9, Fig. S6), one can conclude that even for the worst-case scenario, of a direct $CeO_2$ NM application to the Cambisol, the soil was a sink for the NM. To our knowledge, there is only one other study measuring the Ce concentrations in the percolating water of $CeO_2$ NM spiked outdoor lysimeters (Du et al., 2015). The authors found no vertical transport of $CeO_2$ NM and ionic Ce (Du et al., 2015), which is in line with the presented results. In addition, Schlich et al. (2018) showed that Ag NM applied by sewage sludge remained largely in the introduced soil layers during a long-term outdoor experiment. In summary, our measurements in soil and percolating water confirm that soil act as sink for NM (Pan and Xing, 2012).

#### 3.3.3. Cerium concentrations in the plant roots

The uptake of $CeO_2$ NM applied via spiked sewage sludge by plant roots increased with increasing $CeO_2$ NM concentration in the lysimeters (Fig. 3). The uptake of sewage sludge applied $CeO_2$ NM to plant roots increased with time from no uptake for wheat (August 31st, 2015), to a slightly increasing uptake from canola (July 13th, 2016) to barley (July, 6th, 2017). Therefore, it remains unclear whether the increasing uptake is a plant-specific or a time-dependent effect. The information contained in quantifying Ce in the total digestions of plant roots are limited as they cannot resolve whether the $CeO_2$ NM were
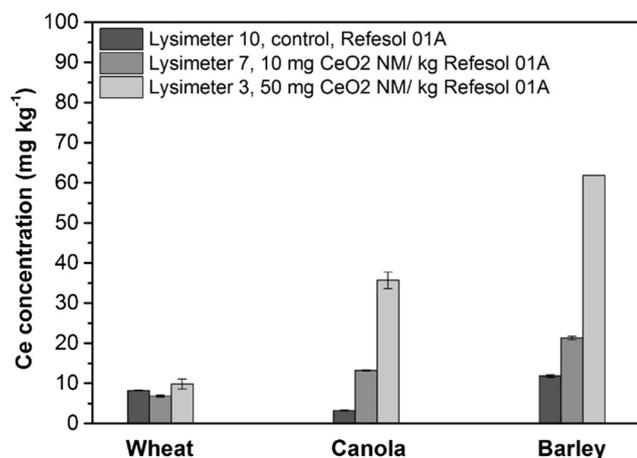
**Fig. 3.** Total Ce concentrations in the plant roots: The cerium dioxide nano-material (CeO$_2$ NM) was added via sewage sludge to lysimeter 3 and lysimeter 7. Lysimeter 10 received sewage sludge without CeO$_2$ NM (control). The washed roots were digested with HNO$_3$ and H$_2$O$_2$. Afterwards, the Ce concentrations were determined in triplicates, bars show the standard deviation.

located at the root surfaces or were taken up into the roots. One can speculate that the microbiome-derived aging of the sewage sludge combined with the special physicochemical conditions in the rhizosphere might transform and/or remobilize the CeO$_2$ NM. Moreover, Zhang et al. (2012) considered that CeO$_2$ NM were first attached to the root surface of cucumbers, dissolved and reduced to Ce$^{3+}$ by root exudates, and subsequently precipitated with phosphate on the root surface and in intercellular spaces, or form carboxyl compounds on the way to the shoots.

The rainwater approach also shows no root uptake of Ce to wheat but explicit uptake for canola and barley (Fig. S9). The root uptake in the 10 mg kg$^{-1}$ approaches (L 7, L 9) with sewage sludge vs. rainwater showed a low root accumulation in the same order of magnitude. This implies that the way of application did not impact the root uptake. The low but detectable Ce uptake to the plant roots of canola and barley suggests that despite of a large CeO$_2$ NM retention potential in soil (Fig. 1, Fig. 2), the NM are bioavailable to some extent in the rhizosphere (Fig. 3). In general, our findings are in line with other studies, reporting that CeO$_2$ NM can be taken up by plants (e.g. López-Moreno et al., 2010; Hernandez-Viezcas et al., 2013; Rico et al., 2017). In more detail, Rico et al. (2018) discussed a large reduction of Ce at the root-soil interface that possibly facilitates the entry of Ce$^{3+}$ into the roots with subsequent re-oxidation to Ce$^{4+}$.

### 3.4. Cerium speciation in soil and plant roots

The determination of total Ce concentrations provides valuable information regarding the fate of the added CeO$_2$ NM during the lysimeter experiments (Section 3.3). More detailed information about the speciation of Ce in soil and plant roots and the speciation changes of the spiked CeO$_2$ NM were obtained from XAS measurements.

The spectra of the Ce reference material can be divided into two groups, (i) spectra of Ce$^{3+}$ containing reference materials exhibiting an intense white line at 5727.5 eV and (ii) Ce$^{4+}$ containing reference material showing a double peak with their highest intensities at 5732.5 eV and 5739 eV (Fig. 4a, vertical lines) as described by Bianconi et al. (1987). Low Ce concentrations (50–62 mg kg$^{-1}$) led to considerable noise in the recorded data, which precluded the identification of minor speciation differences e.g. the differentiation between bulk- and nano-CeO$_2$. Ce concentrations below 50 mg kg$^{-1}$ were insufficient for XAS measurements. Thus, only samples from the lysimeter 3 (L 3), which showed the highest Ce concentration ($\approx$ 50 mg kg$^{-1}$), were considered for XAS measurements.

The spectrum of the unified A-horizon (0–20 cm) of CeO$_2$ NM spiked lysimeter soil in the beginning of the experiment shows a strong Ce$^{4+}$ signature indicated by the double peaks at 5732.5 eV and 5739 eV (L 3, Refesol 01A, May 25th, 2015). Through aging for 25 months, the soil loses the Ce$^{4+}$ signature, and the respective XAS spectrum is much better represented by Ce$^{3+}$, as indicated by the intense white line at 5727.5 eV (L 3, Refesol 01A, July 6th, 2017). The shape of the spectrum of the root (L 3, barley root, July 6th, 2017) is closer to the spectra of Ce$^{4+}$ (oscillations at 5732.5 eV and 5739 eV), but with higher contribution of Ce$^{3+}$ (oscillation at 5727.5 eV) compared to the soil in the beginning of the experiment (L 3, Refesol 01A, May 25th, 2015).

Although the CeO$_2$ (nano) reference peak at 5732.5 eV was broadened compared to the CeO$_2$ (bulk), the spectra of the two Ce$^{4+}$ references were very similar. Similarly, the two Ce$^{3+}$ reference spectra (Ce-Allanite and CePO$_4$) show the same features. Thus, the XAS analyses of the Ce-L3 edge in this study were limited to the extraction of the oxidation state of the Ce. Therefore, the fractions of the Ce$^{4+}$ or Ce$^{3+}$ reference materials determined by LCF (Table S4) were combined for the discussion.

The LCF to the spectrum of the fresh soil (L 3, Refesol 01A, May 25th, 2015) suggested 10% of Ce to be represented by Ce$^{3+}$ and 90% by Ce$^{4+}$ (Fig. 4b). The fraction of Ce$^{3+}$ might originate from either (i) partial reduction of the CeO$_2$ NM in the activated sewage sludge, in line with previously reported results (Barton et al., 2014), or (ii) was already present in the soil prior to spiking (Takahashi et al., 2005). In the aged soil (L 3, Refesol 01A, July 6th, 2017), Ce$^{3+}$ and Ce$^{4+}$ reference spectra contribute about 50% each. This indicates that approximately half of the spiked CeO$_2$ NM was reduced to Ce$^{3+}$ during the two years of CeO$_2$ NM aging in the lysimeter soil (L 3, 50 mg kg$^{-1}$). The LCF to the barley root sample spectrum (L 3, barley root, July 6th, 2017) indicated about 85% of Ce being represented by Ce$^{4+}$ references and 15% by Ce$^{3+}$ references (Table S4).

Rico et al. (2018) observed an almost complete reduction of Ce$^{4+}$ to Ce$^{3+}$ at the root - soil interface already after 60 d which may be explained by differences in the experimental designs (climate chamber vs. outdoor lysimeter). It was speculated that Ce is taken up by the roots as Ce$^{3+}$, thus requiring a reduction of Ce (Rico et al., 2018). Other authors also found that aging of a CeO$_2$ NM spiked soil triggered an enhanced Ce$^{3+}$ concentration in the soil as well as an increasing Ce concentration (87%) in radish roots (Zhang et al., 2016). In the barley root, we found $\approx$80% of the sample spectra represented by Ce$^{4+}$ references and $\approx$ 20% represented by Ce$^{3+}$. These results are in line with the findings reported by Rico et al. (2018), suggesting a reoxidation of Ce$^{3+}$ to Ce$^{4+}$ inside the roots.

### 3.5. Response of ammonium oxidizing bacteria to the application of CeO$_2$ NM

The measured activities of the AOB and the determined inhibition over the entire test period from May 2015 to July 2017 are presented in Table S2. There were no differences in the AOB activity of the CeO$_2$ NM spiked lysimeters (L 3, L 7, L 9) and the control lysimeters (L 8, L 10). Neither the concentrations (10 mg kg$^{-1}$ vs. 50 mg kg$^{-1}$) nor the type of application (simulated rainwater vs. sewage sludge) affected the activity of the AOB. Very little information is available on effects of CeO$_2$ NM to soil microorganisms, but contradictory results were reported (Hamidat et al., 2016; Vittori Antisari et al., 2013; Li et al., 2017). Hamidat et al. (2016) showed that CeO$_2$ NM decreased the catalase activity in the rhizosphere but did not affect the microbial biomass. Vittori Antisari et al. (2013) observed CeO$_2$ NM concentration-dependent long-term effects on the C/N ratio of the microbial biomass and concluded that the bacterial/fungal biomass ratio changed. In addition, concentrations ranging from 100 to 1000 mg kg$^{-1}$ of CeO$_2$ NM in a soil-grass microcosm system lead to exposure-dependent toxicity to soil microorganisms (Li et al., 2017). These findings are to some extent contradictory to our study, but may be explained by differences in the
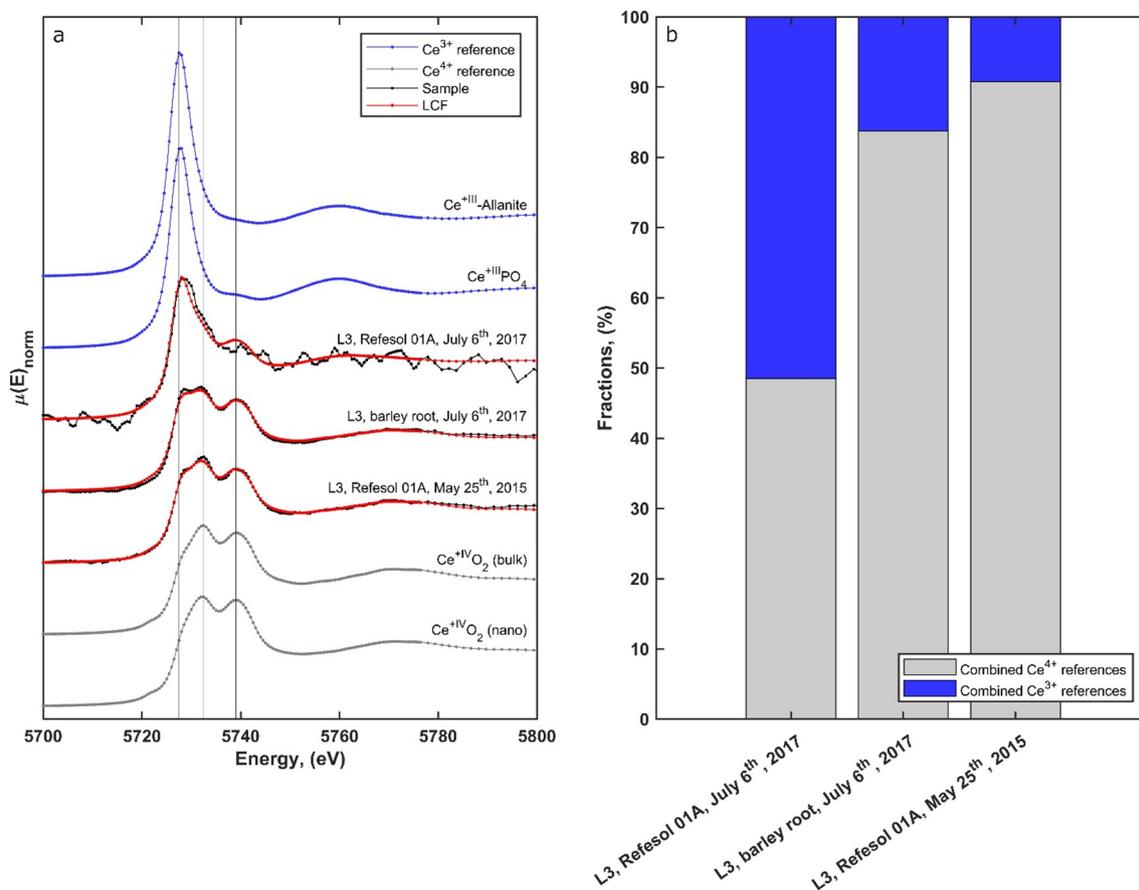
**Fig. 4.** Speciation of Ce in soil and plant roots: The X-ray absorption near edge spectroscopy results including reference material spectra, sample spectra and linear combination fitting (LCF) results to the sample spectra (a). Blue curves indicate references containing $Ce^{3+}$, gray curves indicate reference spectra containing $Ce^{4+}$, black curves the sample spectra and red the LCF results. LCFs were performed from $-20 < E_0 < 50$ eV. (b) shows the fractions found to describe the LCFs in (a) where $Ce^{4+}$ (gray) and $Ce^{3+}$ (blue) fractions were combined. Detailed LCF results including fit quality parameters are given in Table S3.

experimental designs (outdoor vs. laboratory) or the higher $CeO_2$ NM concentrations up to 1000 mg kg$^{-1}$ in previous studies.

### 3.6. Implications

For both $CeO_2$ NM spiking approaches (sewage sludge and artificial rainwater), no vertical Ce translocation was detected over two years and except for one measurement after the application by rainwater, no release of Ce to the percolating water was detected during this time. In addition, no inhibition of AOB was observed, though AOB are very sensitive to other NM (Schlich et al., 2017). Hence, the investigated $CeO_2$ NM are possibly non-toxic for soil microorganism up to a concentration of 50 mg kg$^{-1}$. This value is above the predicted environmental concentrations (PEC) for $CeO_2$ NM in sewage sludge amended soil ($\approx$ 1 mg kg$^{-1}$, Gottschalk et al., 2013), which currently suggest a limited risk of $CeO_2$ NM to soil microorganism.

The summarized results indicate that the $CeO_2$ NM remained largely immobile in the spiked soil layer which is in good agreement with other recent findings (Cornelis et al., 2014). According to Servin and White (2016), experimental results on plant NM interactions are mainly derived from studies conducted with high doses and short-term exposures in model media. The presented results are in accord with the current state of knowledge about the environmental fate of $CeO_2$ NM retrieved from short-term exposure applying higher NM concentrations. However, the measured Ce uptake by the plant roots of canola and barley indicates that despite of the efficient $CeO_2$ NM retention by the soil matrix, the NM are to some extent bioavailable in the rhizosphere. This is in line with López-Moreno et al. (2010) demonstrating $CeO_2$ NM root

uptake for higher NM concentrations under short-term conditions. Species-specific plant root exudates will directly affect the physicochemical soil properties, and the specific microbiome will produce biomolecules that may affect the fate of NM (Servin and White, 2016). These processes may explain the observed differences in the Ce root-uptake from no uptake for wheat to notable uptakes for canola and barley. On the contrary, Rico et al. (2017) found evidence for $CeO_2$ NM root adsorption for wheat with almost no transformation of $Ce^{4+}$ to $Ce^{3+}$. Especially the different climate conditions between the climate chamber (Rico et al., 2017) and the outdoor lysimeter might explain the somehow ambiguous findings. Based on XAS measurements Rico et al. (2018) concluded that $CeO_2$ NM accumulated on barley roots and that the $Ce^{4+}$ was reduced to $Ce^{3+}$ which then facilitated root uptake. Our results from XAS measurements indicate a partial reduction of $Ce^{4+}$ to $Ce^{3+}$ in the Cambisol. Hence, the transformation of the $CeO_2$ NM ($Ce^{4+}$ to $Ce^{3+}$) might have induced the increased root-uptake of Ce over the 25 months of our experiment. Barley might have other root exudates than wheat (Rico et al., 2018), which may explain the different Ce uptake observed for barley and wheat in this study.

In summary, negligible mobility and toxicity was found for the investigated $CeO_2$ NM in soil. However, Ce accumulation in roots was observed for canola and barley suggesting trophic transfer of $CeO_2$ NM to the terrestrial food chain. Trophic NM transfer is still debated controversially (Servin and White, 2016) and further long-term investigations need to focus on the speciation and localization of NM in plants (e.g. sugar beet) under environmentally relevant conditions.

## Funding

## Acknowledgements

## Appendix A. Supplementary information

Supplementary information related to this article can be found at: https://www.journals.elsevier.com/nanoimpact. https://doi.org/10.1016/j.impact.2019.100170

## References

Ad-hoc-AG Boden, 2006. In: BGR in Zusammenarbeit mit den Staatlichen Geologischen Diensten (Ed.), Bodenkundliche Kartieranleitung. 5. verbesserte und erweiterte Auflage, 5th edition. Schweizerbart Science Publishers, Stuttgart.

Andreescu, D., Bulbul, G., Ozel, R.E., Hayat, A., Sardesai, N., Andreescu, S., 2014. Applications and implications of nanoceria reactivity: measurement tools and environmental impact. Environ. Sci.: Nano, The Royal Society of Chemistry 1, 445–458. https://doi.org/10.1039/C4EN00075G.

Arai, Y., Dahle, J.T., 2018. Redox-ligand complexation controlled chemical fate of ceria nanoparticles in an agricultural soil. J. Agric. Food Chem. 66, 6646–6653. https://doi.org/10.1021/acs.jafc.7b01277.

Barton, L.E., Auffan, M., Bertrand, M., Barakat, M., Santaella, C., Masion, A., Borschneck, D., Olivi, L., Roche, N., Wiesner, M.R., Bottero, J.-Y., 2014. Transformation of pristine and citrate-functionalized CeO2 nanoparticles in a laboratory-scale activated sludge reactor. Environ. Sci. Technol. 48 (13), 7289–7296. https://doi.org/10.1021/es404946y.

Bianconi, A., Marcelli, A., Dexpert, H., Karnatak, R., Kotani, A., Jo, T., Petiau, J., 1987. Specific intermediate-valence state of insulating 4f compounds detected by L₃ x-ray absorption. Phys. Rev. B 35 (2), 806–812. https://doi.org/10.1103/PhysRevB.35.806.

Collin, B., Auffan, M., Johnson, A.C., Kaur, I., Keller, A.A., Lazareva, A., Lead, J.R., Ma, X., Merrifield, R.C., Svendsen, C., White, J.C., Unrine, J.M., 2014. Environmental release, fate and ecotoxicological effects of manufactured ceria nanomaterials. Environ. Sci.: Nano, The Royal Society of Chemistry 1, 533–548. https://doi.org/10.1039/C4EN00149D.

Cornelis, G., Kirby, J.K., Beak, D., Chittleborough, D., McLaughlin, M.J., 2010. A method for determination of retention of silver and cerium oxide manufactured nanoparticles in soils. Environ. Chem. 7 (5440), 298–308. https://doi.org/10.1071/EN10013.

Cornelis, G., Ryan, B., McLaughlin, M.J., Kirby, J.K., Beak, D., Chittleborough, D., 2011. Solubility and batch retention of CeO2 nanoparticles in soils. Environmental Science & Technology 45 (7), 2777–2782. https://doi.org/10.1021/es103769k.

Cornelis, G., Hund-Rinke, K., Kuhlbusch, T., van den Brink, N., Nickel, C., 2014. Fate and bioavailability of engineered nanoparticles in soils: a review. Crit. Rev. Environ. Sci. Technol. 44, 2720–2764. https://doi.org/10.1080/10643389.2013.829767.

DIN 38402-11. German standard methods for the examination of water, waste water and sludge - General information (group A) - Part 11: Sampling of waste water (A 11). 2009, Beuth Verlag; Berlin, https://dx.doi.org/10.31030/1442438.

Du, W., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Zhu, J., Peralta-Videa, J.R., Guo, H., 2015. Physiological and biochemical changes imposed by CeO2 nanoparticles on wheat: a life cycle field study. Environmental Science & Technology 49, 11884–11893. https://doi.org/10.1021/acs.est.5b03055.

EN 16174, 2012. Sludge, Treated Biowaste and Soil - Digestion of Aqua Regia Soluble Fractions of Elements; German Version EN 16174: 2012. Beuth Verlag, Berlin.

Gottschalk, F., Sun, T., Nowack, B., 2013. Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. Environ. Pollut. 181, 287–300. https://doi.org/10.1016/j.envpol.2013.06.003.

Hamidat, M., Barakat, M., Ortet, P., Chanéac, C., Rose, J., Bottero, J.-Y., Heulin, T., Achouak, W., Santaella, C., 2016. Design defines the effects of nanoceria at a low dose on soil microbiota and the potentiation of impacts by the canola. *Plant.* Environmental Science & Technology 50 (13), 6892–6901. https://doi.org/10.1021/acs.est.6b01056.

Hernandez-Viezcas, J.A., Castillo-Michel, H., Andrews, J.C., Cotte, M., Rico, C., Peralta-Videa, J.R., Ge, Y., Priester, J.H., Holden, P.A., Gardea-Torresdey, J.L., 2013. In situ synchrotron X-ray fluorescence mapping and speciation of CeO2 and ZnO nanoparticles in soil cultivated soybean (glycine max). ACS Nano 7 (2), 1415–1423. https://doi.org/10.1021/nn305196q.

Hoppe, M., Mikutta, R., Utermann, J., Duijnisveld, W., Kaufhold, S., Stange, C.F.,

Guggenberger, G., 2015. Remobilization of sterically stabilized silver nanoparticles from farmland soils determined by column leaching. Eur. J. Soil Sci. 66, 898–909. https://doi.org/10.1111/ejss.12270.

Hund-Rinke, K., Baun, A., Cupi, D., Fernandes, T.F., Handy, R., Kinross, J.H., Navas, J.M., Peijnenburg, W., Schlich, K., Shaw, B.J., Scott-Fordsmand, J.J., 2016. Regulatory ecotoxicity testing of nanomaterials - proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. Nanotoxicology 10 (10), 1442–1447. https://doi.org/10.1080/17435390.2016.1229517.

ISO Guideline 15685. 2012. Soil Quality - Determination of Potential Nitrification and Inhibition of Nitrification - Rapid Test by Ammonium Oxidation, in: International Organization for Standardization (Ed.), (Geneva, Switzerland).

Li, B., Chen, Y., Liang, W.-z., Mu, L., Bridges, W.C., Jacobson, A.R., Darnault, C.J.G., 2017. Influence of cerium oxide nanoparticles on the soil enzyme activities in a soil-grass microcosm system. Geoderma 299, 54–62. https://doi.org/10.1016/j.geoderma.2017.03.027.

Limbach, L.K., Bereiter, R., Müller, E., Krebs, R., Gälli, R., Stark, W.J., 2008. Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency. Environmental Science & Technology 42, 5828–5833. https://doi.org/10.1021/es800091f.

López-Moreno, M.L., de la Rosa, G., Hernández-Viezcas, J.Á., Castillo-Michel, H., Botez, C.E., Peralta-Videa, J., Gardea-Torresdey, J.L., 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (Glycine max) plants. Environmental Science & Technology 44, 7315–7320. https://doi.org/10.1021/es903891g.

OECD TG 216, 2000. OECD guideline for the testing of chemicals. In: Test No. 216: Soil Microorganisms: Nitrogen Transformation Test. Organisation for Economic Co-operation and Development, Paris.

Pan, B., Xing, B., 2012. Applications and implications of manufactured nanoparticles in soils: a review. Eur. J. Soil Sci. 63 (4), 437–456. https://doi.org/10.1111/j.1365-2389.2012.01475.x.

Ravel, B., Newville, M., 2005. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. J. Synchrotron Radiat. 12 (4), 537–541. https://doi.org/10.1107/S0909049505012719.

Rico, C.M., Barrios, A.C., Tan, W., Rubenecia, R., Lee, S.C., Varela-Ramirez, A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2015. Physiological and biochemical response of soil-grown barley (Hordeum vulgare L.) to cerium oxide nanoparticles. Environ. Sci. Pollut. Res. 22, 10551–10558. https://doi.org/10.1007/s11356-015-4243-y.

Rico, C.M., Johnson, M.G., Marcus, M.A., Andersen, C.P., 2017. Intergenerational responses of wheat (Triticum aestivum L.) to cerium oxide nanoparticles exposure. Environ. Sci.: Nano. 4, 5635–5642. https://doi.org/10.1039/C7EN00057J.

Rico, C.M., Johnson, M.G., Marcus, M.A., 2018. Cerium oxide nanoparticles transformation at the root-soil interface of barley (Hordeum vulgare L.). Environ. Sci.: Nano. 5, 1807–1812. https://doi.org/10.1039/C8EN00316E.

Scheffer, F., Schachtschabel, P., 2002. Lehrbuch der Bodenkunde, 15th edition. Spektrum Akademischer Verlag Heidelberg, Berlin.

Schlich, K., Hund-Rinke, K., 2015. Influence of soil properties on the effect of silver nanomaterials on microbial activity in five soils. Environ. Pollut. 196, 321–330. https://doi.org/10.1016/j.envpol.2014.10.021.

Schlich, K., Hoppe, M., Kraas, M., Fries, E., Hund-Rinke, K., 2017. Ecotoxicity and fate of a silver nanomaterial in an outdoor lysimeter study. Ecotoxicology 26, 738–751. https://doi.org/10.1007/s10646-017-1805-4.

Schlich, K., Hoppe, M., Kraas, M., Schubert, J., Chanana, M., Hund-Rinke, K., 2018. Long-term effects of three different silver sulfide nanomaterials, silver nitrate and bulk silver sulfide on soil microorganisms and plants. Environ. Pollut. 242, 1850–1859. https://doi.org/10.1016/j.envpol.2018.07.082.

Servin, A.D., White, J.C., 2016. Nanotechnology in agriculture: next steps for understanding engineered nanoparticle exposure and risk. NanoImpact 1, 9–12. https://doi.org/10.1016/j.impact.2015.12.002.

Siemens, J., Ilg, K., Pagel, H., Kaupenjohann, M., 2008. Is colloid-facilitated phosphorus leaching triggered by phosphorus accumulation in sandy soils? J. Environ. Qual. 37. https://doi.org/10.2134/jeq2007.0544.

Singh, C., Friedrichs, S., Ceccone, G., Gibson, P., Jensen, K., Levin, M., Goenaga Infante, H., Carlander, D., Rasmussen, K., 2014. Cerium Dioxide, NM-211, NM-212, NM-213. Characterisation and Test Item Preparation. European Commission Joint Research Centre Institute for Health and Consumer Protection, Ispra, Italy. https://doi.org/10.2788/80203.

Takahashi, Y., Yuita, K., Kihou, N., Shimizu, H., Nomura, M., 2005. Determination of the CeIVCeIII ratio by XANES in soil horizons and its comparison with the degree of Ce anomaly. Phys. Scr. 936. https://doi.org/10.1238/physica.topical.115a00936.

Taurozzi, J.S., Hackley, V.A., Wiesner, M.R., 2012. Preparation of a Nanoscale TiO2 Aqueous Dispersion for Toxicological or Environmental Testing (Version 1.2). NIST Special Publication, pp. 1200–1203. https://doi.org/10.6028/NIST.SP.1200-3.

Tella, M., Auffan, M., Brousset, L., Morel, E., Proux, O., Chaneac, C., Angeletti, B., Pailles, C., Artells, E., Santaella, C., Rose, J., Thiéry, A., Bottero, J.-Y., 2015. Chronic dosing of a simulated pond ecosystem in indoor aquatic mesocosms: fate and transport of CeO2 nanoparticles. Environ. Sci.: Nano. 2 (6), 653–663. https://doi.org/10.1039/C5EN00092K.

Vittori Antisari, L., Carbone, S., Gatti, A., Vianello, G., Nannipieri, P., 2013. Toxicity of metal oxide (CeO2, Fe3O4, SnO2) engineered nanoparticles on soil microbial biomass and their distribution in soil. Soil Biol. Biochem. 60, 87–94. https://doi.org/10.1016/j.soilbio.2013.01.016.

Zhang, H., He, X., Zhang, Z., Zhang, P., Li, Y., Ma, Y., Kuang, Y., Zhao, Y., Chai, Z., 2011. Nano-CeO2 exhibits adverse effects at environmental relevant concentrations. Environmental Science & Technology 45, 3725–3730. https://doi.org/10.1021/es103309n.

Zhang, P., Ma, Y., Zhang, Z., He, X., Zhang, J., Guo, Z., Tai, R., Zhao, Y., Chai, Z., 2012. Biotransformation of ceria nanoparticles in cucumber plants. ACS Nano 6, 9943–9950. https://doi.org/10.1021/nn303543n.

Zhang, W., Dan, Y., Shi, H., Ma, X., 2016. Effects of aging on the fate and bioavailability of cerium oxide nanoparticles to radish (Raphanus sativus L.) in soil. ACS Sustain. Chem. Eng. 4, 5424–5431. https://doi.org/10.1021/acssuschemeng.6b00724.

Zhao, L., Sun, Y., Hernandez-Viezcas, J.A., Hong, J., Majumdar, S., Niu, G., Duarte-Gardea, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2015. Monitoring the environmental effects of CeO2 and ZnO nanoparticles through the life cycle of corn (zea mays) plants and in situ μ-XRF mapping of nutrients in kernels. Environmental Science & Technology 49 (5), 2921–2928. https://doi.org/10.1021/es5060226.