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Effect of CeO_2 Nanoparticles on Plant Growth and Soil Microcosm in a Soil-Plant Interactive System

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Highlights:

Both CeO_2 NPs and Ce^{3+} ions stimulated cucumber roots growth.

Biotransformation of CeO_2 NPs occurred in root rhizosphere.

CeO_2 NPs and Ce^{3+} ions altered bacterial taxonomic and compositions.

CeO_2 NPs showed particle-specific effects.

Abstract

The impact of CeO_2 nanoparticles (NPs) on plant physiology and soil microcosm and the underlying mechanism remains unclear to date. This study investigates the effect of CeO_2 NPs on plant growth and soil microbial communities in both the rhizosphere of cucumber seedlings and the surrounding bulk soil, with $CeCl_3$ as a comparison to identify the contribution of the particulate and ionic form to the phytotoxicity of CeO_2 NPs. The results show that Ce was significantly accumulated in the cucumber tissue after CeO_2 NPs exposure. In the roots, 5.3% of the accumulated Ce has transformed to Ce^{3+}. This transformation might take place prior to uptake by the roots since 2.5% of CeO_2 NPs was found transformed in the rhizosphere soil. However, the transformation of CeO_2 NPs in the bulk soil was negligible, indicating the critical role of rhizosphere chemistry in the transformation. CeO_2 NPs treatment induced oxidative stress in the roots, but the biomass of the roots was significantly increased, although the Vitamin C (Vc) content and soluble sugar content were decreased and mineral nutrient contents were altered. The soil enzymatic activity and the microbial community in both rhizosphere and bulk soil samples were altered, with rhizosphere soil showing more prominent changes. $CeCl_3$ treatment induced similar effects although less than CeO_2 NPs, suggesting that Ce^{3+} released from CeO_2 NPs contributed to the CeO_2 NPs induced impacts on soil health and plant physiology.

Keywords: CeO_2 NPs; Transformation; Soil enzymes; Soil bacterial community; Rhizosphere; Cucumber seedlings

1. Introduction

The UN Food and Agriculture Organization and the World Bank are promoting the use of nanotechnology as a sustainable technology to increase crop yields to feed the growing population (Asadishad *et al.*, 2018). Engineered nanomaterials (ENMs) present great potentials in the agricultural application (Chen *et al.*, 2021). For example, CeO_2 nanoparticles (NPs) have shown their potential in crop protection due to their intrinsic antioxidative capacity (Dai *et al.*, 2020). However, unlike application in other fields, the agricultural application requires large scale and high quantities which raises concerns about their adverse effects on the agricultural ecosystem (e.g. soil and plant health) as well as on animal and human health (Zhang *et al.*, 2021).

The interactions between CeO_2 NPs and terrestrial plants have been extensively studied. Priester *et al.* (2012) demonstrated that high concentrations (1000 mg/kg) of CeO_2 NPs significantly reduced the yield of soybean by 22.5%. Lower concentration (200 mg/kg) of CeO_2 NPs was reported to reduce the photosynthetic rate and CO_2 assimilation efficiency of *Clarkia unguiculata*, possibly by disrupting energy transfer from photosystem II to the Calvin cycle (Conway *et al.*, 2015). However, contradictory results found no phytotoxic effects on *Cucumis sativus* in the Hoagland solution at concentrations up to 2000 mg/kg (Ma *et al.*, 2015). Moreover, at lower doses (100 mg/kg) nano-CeO_2 showed positive impacts on the photosynthesis and growth of *Lactuca sativa* (Gui *et al.*, 2015).

Soil ecosystem is the most important sink of nanomaterials (Nowack and Bucheli, 2007). Soil microorganisms are essential to many ecological functions, particularly in soil organic matter decomposition and nutrient mineralization, which has greatly impact the growth of terrestrial plants (Delgado-Baquerizo *et al.*, 2016). Wang *et al.* (2018b) reported that long-term exposure (210 days) of activated sludge to 1 mg/L CeO_2 NPs induced the deterioration of denitrifying process by reducing the abundance of some dominant denitrifying bacteria such as *Acinetobacter* and *Flexibacter*. Pan *et al.* (2020) found that CeO_2 NPs exposure with Fe amendment enhanced the abundances of several functionally significant bacterial phyla including *Proteobacteria* and *Bacteroidetes*, which was associated with C and N cycling. The microorganism in the soil plays important roles in maintaining plant health. However, so far, most research has focused only on the impact of CeO_2 NPs on plant species in the hydroponic culture system, with limited study investigating effect in the soil-plant system.

The present study aims to evaluate the effect of CeO_2 NPs on plant growth and soil microcosm in a soil-plant interactive system. Effects of the ionic form of Ce was studied as a comparison to identify the contribution of the particulate and ionic form to the CeO_2 NPs toxicity. The chemical species of Ce was determined by X-ray absorption near-edge spectroscopy (XANES) to examine the role of biotransformation in the observed biological effects. Bulk and rhizosphere soils were compared to understand the role of the rhizosphere in the response of bacteria to exposure.

2. Materials and Methods

2.1 Chemicals and Nanomaterials

$Ce(NO_3)_3·6H_2O$ and $CeCl_3·7H_2O$ (purity of 99.9%) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (China). CeO_2 NPs were synthesized using a precipitation method described previously (Xie *et al.*, 2021). Briefly, 10 mmol of $Ce(NO_3)_3·6H_2O$ was added to 320 mL of NaOH solution (78 mmol·L^{-1}), followed by vigorous stirring using a magnetic stirrer for 48 h. The resulting precipitate was collected by centrifugation (15000 × *g*), followed by several washes with deionized (DI) water and ethanol. The particle morphology, size, crystal structure, hydrodynamic diameter, and zeta potential in DI water and Hoagland nutrient solution, surface chemical valence states were characterized using Transmission electron microscopy (TEM, JEM 200CX, Japan), powder X-ray diffraction (XRD, X'pert PRO MPD, Holland), and X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250XI, USA) and dynamic light scattering (DLS, Zetasizer Nano ZS90, UK), respectively.

2.2 Dissolution of CeO_2 NPs

The dissolution of CeO_2 NPs in DI water was analyzed by measuring the Ce^{3+} released into the solution. Briefly, CeO_2 NPs suspensions (100 mg/L) in 25 mL deionized H_2O were prepared and incubated for 48 h at 37 °C, followed by centrifuging at 11,000 *g* for 15 min. The supernatants were collected and diluted with 2% nitric acids for ICP-MS analysis (Thermo Elemental X7). A range of Ce standard solutions (0.1, 1, 5, 10, 50, 100, 500 μg/L) were also measured for calibration. The recovery rates of Ce was tested to be 99.87%.

2.3 Plant-Soil System Exposure and Sample Collection

Silt loam soil (13% clay, 55% silt, 30.9% sand, and 1.1% organic matter content, pH 7.85) was collected from the Shangzhuang Experimental Station of China Agricultural University and air-dried, followed by sieving through a 2 mm mesh and stored at 4°C. Cucumber seeds (*Cucumis sativus*, Zhongnong NO.16) were purchased from the Chinese Academy of Agricultural Sciences. 30 plastic pots (6.0 cm diameter×5.3 cm height) filled with 60 g of the sieved soil were divided into six equal groups for different treatments: unamended control, CeO_2 NPs treatment at 5.8 mmol kg^{-1} (1000 mg/kg), and $CeCl_3 \cdot 6H_2O$ treatment at 0.6 mmol kg^{-1} (100 mg/kg), with and without plant seedling. The concentration of 100 mg/kg ionic Ce was chosen under the assumption that 10% of the CeO_2 NPs would be dissolved (Pagano *et al.*, 2016).

Cucumber seeds were germinated on moist paper towels for 4 d. Then 15 uniform seedlings were selected and transferred to the corresponding pots (planted pots). The remaining 15 pots were left unplanted. Then 10 mL CeO_2 NPs suspension, $CeCl_3$ solutions, and Hoagland solution (control group) were applied in each treatment (day 1). Hoagland's solution was used to water the pots every day. Both planted and unplanted pots were cultivated in a climate chamber with 16 h photoperiod (light intensity of 1.76×10^4 μmol/m^2 s), 25°C/18°C day/night temperature and 50%/70% day/night humidity.

Twenty days after transplanting (day 21), samples of soil and plants were harvested. The soil on the root surface was manually removed and collected as rhizosphere soil. Soils from the unplanted treatments were used as bulk soil samples. A portion of one soil sample was stored at 4 ºC for enzymatic activity measurements, and the remainder was stored at -80 ºC to characterize the soil bacterial community structure. Fresh plants were collected and the physiological response was measured immediately. For other measurements, the plants are washed, dried at 60 ºC, and then weighed to acquire constant weight.

2.4 Plant Physiology

At day 21, the relative chlorophyll content of the cucumber leaves was measured before harvest using a Konic Minolta SPAD-502 Plus (Konica Minolta Optics, Japan). Total soluble sugar was determined according to the method described by Buysse and Merckx (1993). Leaf nitrate-N content was analyzed by a colorimetric method (Cataldo *et al.*, 1975). Soluble protein concentrations in the roots and leaves were determined using the Pierce BCA Protein assay kit (Thermo Scientific). The content of Vitamin C was analyzed using an assay kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

2.5 Element Measurement of Ce and Mineral Nutrient in plant tissues

To quantify the macro-and micro-nutrient contents (K, Ca, Na, Mg, Fe, S, P, Cu, Zn, Mn, and Mo) and concentration of Ce in plants, dried roots, stems, and leaves were ground into fine powders and digested with a 3:1 (v:v) mixture of HNO_3 (75%) and H_2O_2 (30%) on a heating plate (80 °C for 1 h, 120 °C for 3 h, and 160 °C for 0.5 h). Elemental concentrations in the digestion solution were then analyzed by ICP-MS or inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer). Standard solutions (0.5-50 mg/L) containing all of the selected elements were used for external calibration. Blanks were analyzed between every six samples. Spiking recovery experiments and analysis of certified reference materials (GBW 07602 and GBW07603 Bush Branches and Leaves) were performed for analytical method validation. Recoveries and detection limits for all of the elements are reported in **Table S1**. The recoveries for all elements were between 93.1% and 111.5% with a relative standard deviation of < 1.5% (**Table S2**).

2.6 Stress Response of Cucumber to CeO_2 NPs and $CeCl_3$

Fresh roots, stems and leaves were excised, homogenized with cold phosphate-buffered saline (PBS) (50 mM, pH 7.8), and centrifuged at 10000 × *g* at 4 °C for 10 min. The supernatants were collected for analyses of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities, and the malondialdehyde (MDA) contents according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7 Ce Speciation Analysis by XANES

To analyze the chemical species of Ce in plant roots and soils, all samples were ground to fine powders and pressed into thin slices (~2 mm). Ce L_{III} edge (5723 eV) spectra were recorded at ambient temperature in fluorescence mode at beamline 1W1B of the Beijing Synchrotron Radiation Facility. The storage ring was run at 2.5 GeV with a current intensity of 50 mA during the spectra collection. XANES spectra of the reference compound CeO_2 NPs and $CePO_4$ were also collected. Linear combination fitting (LCF) analyses of the XANES spectra were performed on the software program ATHENA to identify and quantify Ce species.

2.8 Determination of Enzymatic Activity in Soil

Acid phosphatase, β-D-glucosidase, and arylsulfatase activities were quantified using the method described by Saiya-Cork *et al.* (2002). Urease activity was evaluated by measuring the release of NH_3-N (mg) per gram of dry soil in 24 h (Yang *et al.*, 2007). Dehydrogenase activity was tested by a method for reductive generation of triphenyl formazan (TF), expressing as TF (mg) per gram dry soil in 24 h (Ross, 1971). Peroxidase activity was expressed as the amount of quinone in mg formed per g dry soil in 2 h (Mi and Kim, 1994). Invertase activity was determined with sucrose as a substrate, based on 3,5-dinitrosalicylic acid colorimetry to detect glucose (mg) per gram dry soil in 24 h (Yang *et al.*, 2006).

2.9 DNA extraction analysis

Total DNA was extracted from a 0.3 g soil sample using a Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). The 16S rDNA V4 region of the sample is amplified by the specific primers with Barcode in the designated sequence area, which is 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The polymerase chain reactions (PCR) were conducted using the following temperature profiles: denaturation at 94 ºC for 5 min, followed by 30 cycles of amplification at 94 ºC for 1 min, 48 ºC for 1 min and 72 ºC for 1 min, followed by a final extension at 72 ºC for 10 min, and finally held at 4 ºC. In addition, soil samples from triplicates were mixed, with the DNA extracted and amplified. The amplified products were separated by 1.5 % agarose electrophoresis. Purified amplicons were obtained using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA), and concentrations were determined on GE NanoVue System (Thermo Scientific). Then a library was constructed using Illumina TruSeq DNA PCR-Free Sample Prep Kit. The paired-end sequencing was performed at Beijing Genome Institute, Beijing, China, using a paired 150 bp MiSeq 2000 sequencing system (Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

2.10 Statistical analysis

All statistical analyses were conducted using the SPSS 19.0 statistical software package for Windows (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was performed to compare the significance of differences between different groups. The significance levels ($^{*,\,\#}P < 0.05$, $^{**,\,\#\#}P < 0.01/0.001$) between the different treatments and the control were determined by the Fisher Least Significant Difference (LSD) test.

3. Results and Discussion

3.1 Characterization of CeO_2 NPs

The average particle size of CeO_2 NPs is 5.1 ± 0.8 nm (**Fig. S1a**). XRD analysis showed that the CeO_2 NPs have a cubic fluorite structure (**Fig. S1b**). XPS spectra show that the percentage of surface Ce^{3+} is 4.7% (**Fig. S1c**). The hydrodynamic size in DI water and Hoagland's solution were 653 ± 166 nm and 1059 ± 139 nm, respectively. The ζ potential of CeO_2 NPs in DI water and Hoagland's solutions were 5.75 ± 0.13 mV and -1.38 ± 0.26 mV, respectively (**Fig. S1d**). The solubility of CeO_2 NPs (100 mg/L) in water was very low (< 0.1%).

3.2 Plant physiological responses to CeO_2 NPs and $CeCl_3$ exposure

As shown in **Fig. 1**, CeO_2 NPs significantly increased the biomass (fresh and dry weight) of cucumber roots but not stems and leaves (**Fig. 1a, b**). However, the organic nutrient contents were reduced. The contents of Vc and soluble sugar in leaves were reduced by 54% ($P < 0.01$) and 32% ($P < 0.05$), respectively, by CeO_2 NPs treatment (**Fig. 1e, f**). The total soluble protein, nitrate-N content, and chlorophyll contents in cucumber leaves were not affected (**Fig. 1c, d, g, h**). Similar to CeO_2 NPs, $CeCl_3$ induced similar trends of changes in the biomass of roots and Vc contents, suggesting that CeO_2 NPs and $CeCl_3$ share a similar effect and indicating that dissolution might partly contribute to the impacts of CeO_2 NPs. Therefore, the transformation of CeO_2 NPs in soil and plant was analyzed next.

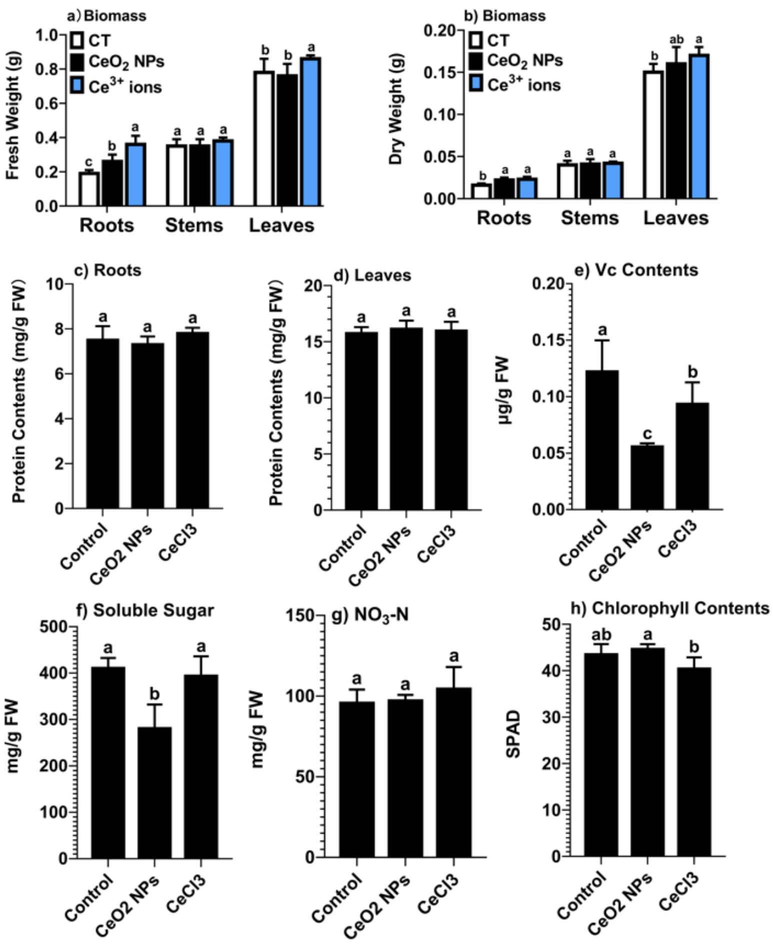

Fig. 1 Phenotypes and contents of organic nutrients after CeO_2 NPs and $CeCl_3$ exposure for 20 days. **a)** Fresh weight and **b)** dry weight of plant roots, stems, and leaves, respectively. **c)** and **d)** Soluble protein of the cucumber seedlings treated with CeO_2 NPs and $CeCl_3$ ions in roots and leaves. **e)**, **f)**, **g)**, and **h)** are the contents of Vc, soluble sugar, and nitrate-N content of the cucumber seedlings and relative chlorophyll contents (SPAD) in leaves treated with CeO_2 NPs and $CeCl_3$ ions. Different lowercase letters indicate

significant difference between different groups at $p < 0.05$ (n = 6).

3.3 Distribution and chemical species of Ce in plant tissues

Ce accumulated in the roots (812.2±100.8 mg/kg), stem (19.5±4.9 mg/kg) and leaves (19.1±3.9 mg/kg) of cucumber after CeO_2 NPs exposure. However, $CeCl_3$ treatment only led to the accumulation of Ce in the root (57.6±9.9 mg/kg), no upward translocation was observed (**Fig. 2a**). Such difference might be related to the different translocation behavior of particles and ions. Most of the Ce^{3+} can be easily fixed as $CePO_4$ on the root surface by the PO_4^{3-} from the nutrients in soils, therefore, there was little chance to go upward. However, the NPs usually can move upward easily with water flow (Zhang *et al.*, 2011), as demonstrated by the XANES data showing that most of the Ce entering the plant roots was in the form of particles (94.7%) (**Fig. 2b**).

Biotransformation of CeO_2 NPs is more likely to occur around the rhizosphere than in the region far away from the root because it mainly occurs in acidic environment and usually requires reducing agents (Rico *et al.*, 2018; Xie *et al.*, 2019). Root exudates and soil microorganisms in the small rhizosphere region are considered to play crucial roles in the reduction of CeO_2 NPs and the release of Ce^{3+} ions (Zhang *et al.*, 2012; Zhang *et al.*, 2017). Our study found that, in the rhizosphere soil, 2.5% of CeO_2 NPs was in the form of Ce(III), while only a little fraction of Ce(III) (0.6%) was observed in the bulk soil (**Fig. 2b**), suggesting the crucial role of rhizosphere chemistry in the transformation of CeO_2 NPs.

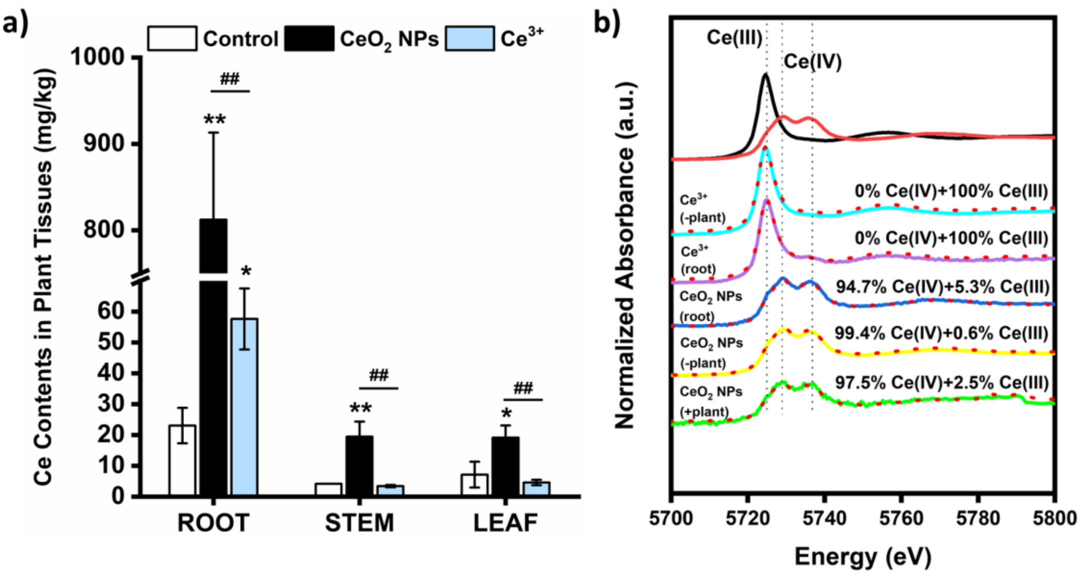

Fig. 2 a) Cerium contents in the root, stem, and leaf treated with CeO_2 NPs or $CeCl_3$ at day 21. Data are expressed as mean ± SD (n = 6) an average of six replicates. * and ** indicates a significant difference at $p < 0.05$ and $p < 0.01$ (n = 6) compared with the control, respectively. ## indicates a significant different at $p < 0.01$ (n = 6) between CeO_2 NPs and $CeCl_3$ treatments; **b)** XANES normalized Ce L_{III} edge spectra of reference compounds ($CePO_4$ and CeO_2) and samples. (-plant) and (+plant) indicates bulk soil and rhizosphere soil, respectively. (root) means CeO_2 or $CeCl_3$ enriched in the root and we detected the root samples by XANES.

3.4 Antioxidative Response in Plants

Since Ce accumulated significantly in plant tissues under CeO_2 NPs exposure, we next examined the antioxidative responses of cucumber shoot and root after exposure. In the shoots, neither treatment-induced any changes in SOD, POD, and CAT activities and the MDA contents (**Fig. 3B**). However, in the roots, the SOD and POD activities as well as the MDA contents were significantly increased after CeO_2 NPs treatment (**Fig. 3A**), indicating the oxidative damage of cell membrane and activation of the plant defense system. In the $CeCl_3$ group, only SOD activity was increased. This indicates that the antioxidative responses found for CeO_2 NPs were mainly particle-specific effects. The triggered antioxidative response and oxidative stresses by CeO_2 NPs contributed to the reduction of organic nutrients in shoots. However, Ce^{3+} ions only triggered little antioxidative responses which may explain the insignificant change of organic nutrient contents.

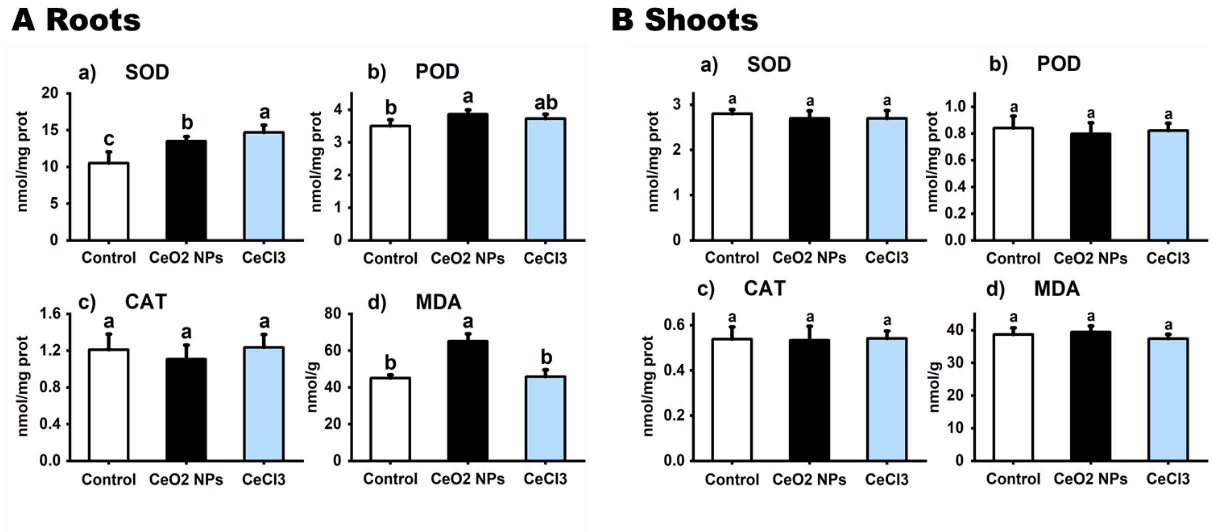

Fig. 3 SOD, POD, CAT activities, and MDA contents in root (**a**) and shoot (**b**) after exposure to CeO_2 NPs and $CeCl_3$ for 20 days. Different lowercase letters indicate significant difference between different groups at $p < 0.05$ ($n = 6$).

3.5 Alteration of Mineral Nutrient Homeostasis

Higher plants need at least 14 mineral elements to support their growth and reproduction (White and Brown, 2010; DalCorso *et al.*, 2014). Deficiency or overload of any elements may lead to growth impairment or physiological disorders such as necrosis or chlorosis. To further investigate the effect of CeO_2 NPs and Ce^{3+} ions, we measured the uptake of several key nutrient elements that are essential for plant growth. Our results showed that both CeO_2 NPs and $CeCl_3$ treatment influence the balance of the mineral element levels. Ce accumulation led to the imbalance of several key nutrient elements that are essential for plant growth. The effect on the element contents in different tissues was different as shown in the heatmap (**Fig. 4**). In roots, CeO_2 NPs significantly increased the K, Mg, and Mo contents while reduced the Ca, S, P, Cu, and Zn contents. In stems and leaves, results show that the amounts of Ca, Fe, Cu, Zn contents decreased while Mo increased. In general, the effects of CeO_2 NPs and $CeCl_3$ on the mineral homeostasis in the cucumber seedlings were similar.

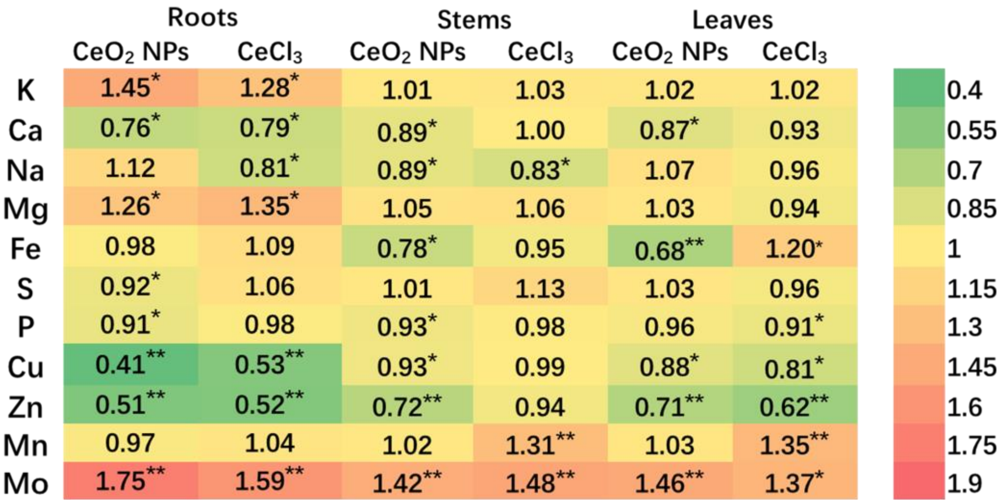

Fig. 4 Heatmap showing the changes of inorganic nutrients in roots, stems, and leaves after CeO_2 NPs and $CeCl_3$ exposure. K, Ca, Na, Mg, S, and P were determined by ICP-OES; Fe, Cu, Zn, Mn, and Mo were determined by ICP-MS. Numbers indicate the fold change of elemental content compared with the control group. < 1 indicates that the content was decreased; > 1 indicates that the content was increased. * and ** indicates a significant difference at $p < 0.05$ and $p < 0.01$ (n = 6) compared with the control, respectively.

3.6 Enzyme Activities in the Cucumber Rhizosphere and Bulk Soil

The activity of soil enzymes is a valuable indicator of overall soil health and functionality (Chaperon and Sauvé, 2007; Lessard *et al.*, 2013). In the bulk soil, CeO_2 NPs treatment significantly increased the activities of arylsulfatase (46.8%), peroxidase (8.3%), and phosphatase (93.0%). However, in the rhizosphere soil, CeO_2 NPs caused higher enhancement of phosphatase (37.4%) but less enhancement of peroxidase activities (18.6%), and didn't induce any change of arylsulfatase (**Table 1**). $CeCl_3$ treatment resulted in the enhanced activity of invertase in the bulk soil (14.6%), and decreased activity of dehydrogenase in the rhizosphere soil. These indicate responses of rhizosphere and bulk soils to CeO_2 NPs are different. Plant roots can release root exudates, which can coat the NP surface, potentially shielding the particles from reaction or chelating metal ions that are released from the metal oxide NPs, consequently lessening the toxicity of particles (Tong *et al.*, 2007; Philippot *et al.*, 2013). These may partially explain the less significant effect of CeO_2 NPs on the enzyme activity in the rhizosphere soil compared to that in the bulk soil. Urease activity was not affected by 1000 mg/kg CeO_2 NPs and 100 mg/kg Ce^{3+} ions exposure, which may be due to the fact that the microbial-secreted urease is very resistant to environmental breakdown in the soil (Zantua and Bremner, 1977) (**Table 1**). Soil phosphatase is an enzyme that can catalyze the mineralization of soil organophosphorus compounds, subsequently making phosphorus (P) available for uptake by plants (Margesin *et al.*, 2000; Belyaeva *et al.*, 2005). Interestingly, phosphatase activities in the rhizosphere in CeO_2 NPs group is much higher than that in the bulk soil in both control and $CeCl_3$ treatment. However, the P uptake was not increased by CeO_2 NPs (**Fig. 4**). In our study, we supplemented Hoagland solutions to the soil every day to provide nutrients including the P. The increase of soil phosphatase was thus not directly correlated with P uptake by plant. A recent study found that 100 mg/kg CeO_2 NPs inhibited urease and β-glucosidase activities but stimulate phosphatase activity (Li *et al.*, 2017). The authors hypothesized that the stimulation might be due to the changes in the phosphatase-associated microbes in the soil, potential from enhanced activity, or population size, which we will discuss in the following section.

Table 1. Enzyme activities in the cucumber rhizosphere and bulk soil after 20 days of exposure to CeO_2 NPs and $CeCl_3$. The data are means of six replicates ± standard deviation.

Enzyme activity	Bulk soil			Rhizosphere		
	CT	NPs	Ce^{3+}	R-CT	R-NPs	R-Ce^{3+}
Arylsulfatase (nmol MU g^{-1} soil h^{-1})	0.47±0.09	0.69±0.10**	0.44±0.03	0.46±0.08	0.55±0.11	0.52±0.09
Dehydrogenase (μmol /d/g soil)	464±54.9	469±32.3	501±9.51	501±17.4	461±42.1	462±20.3*
Invertase (mg/d/g soil)	6.59±0.58	6.59±0.21	7.55±0.69*	6.18±0.42	6.09±0.15$^{\#\#}$	6.41±0.28$^{\#}$
Peroxidase (mg/d/g soil)	65.2±1.63	70.6±4.37*	64.0±3.56	58.6±4.56	69.5±5.29*	65.4±9.09
Phosphatase (nmol MU g^{-1} soil h^{-1})	22.8±1.44	44.0±7.40*	20.7±6.62	33.7±0.80$^{\#\#}$	46.3±4.6**	31.7±2.27$^{\#\#}$
Urease (μg/d/g soil)	423±43.0	432±11.6	452±13.7	436±9.92	434±5.39	458±22.7
β-glucosidase (nmol MU g^{-1} soil h^{-1})	23.6±3.30	22.5±3.64	20.2±2.68	22.2±1.67	23.2±3.89	21.9±1.39

Note: CT=control, soil without CeO_2 NPs or Ce^{3+} ions added. R-CT means the soil planted cucumber seedlings. "*" means comparison with control at bulk soil and rhizosphere, respectively; "#" represents a comparison between bulk soil and rhizosphere under the same exposure conditions ($^{*,\#}$, p < 0.05; $^{**,\#\#}$, p

< 0.01).

3.7 Microbial Community Structure in Soil

The difference in the enzymatic activity in the soil may result from the different soil microbial communities (Brookes, 1995). The effects of metal and metal oxide NPs on soil microbial activity, diversity, and abundance have been studied (Miao *et al.*, 2018; Fang *et al.*, 2022). However, little is known on the effects of CeO_2 NPs and Ce^{3+} ions on soil microbial in the presence or absence of plants. For instance, our results showed that the operational taxonomic units (OTU) number in the rhizosphere group was significantly higher than that in bulk soil **(Fig**. **5**), highlighting the role of plant root rhizosphere. Plant root exudates in rhizosphere can provide nutrients for the microorganisms, promoting the metabolism and proliferation of these populations (Weinert *et al.*, 2011; Khodakovskaya *et al.*, 2013). Besides, the microbial composition in the rhizosphere soil showed difference from that in the bulk soil, although 81.74% of OTU were shared by each group (**Fig. 5).** CeO_2 NPs exerted acute toxicity to the bacterial community and reduced the bacterial diversity (**Fig. 5**), which was in accordance to previously reported result (Miao *et al.*, 2018). However, $CeCl_3$ increased the OTUs, which may be attributed to the unique physicochemical properties of Ce^{3+} which affects the soil enzyme activities and bacterial communities. In both the bulk and rhizosphere soil, the dominant phyla include *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Firmicutres*, accounting for ＞85% of the total microbial community (**Fig. 6a, Fig. S2**). The abundance of *Proteobacteria* (the most dominant phylum (>60%)) in the rhizosphere soil was lower than that in bulk soil (**Fig. 6a**). The difference in microbial composition between the bulk and rhizosphere soil group was also observed at the class and genus levels (**Fig. 6b, 6c**).

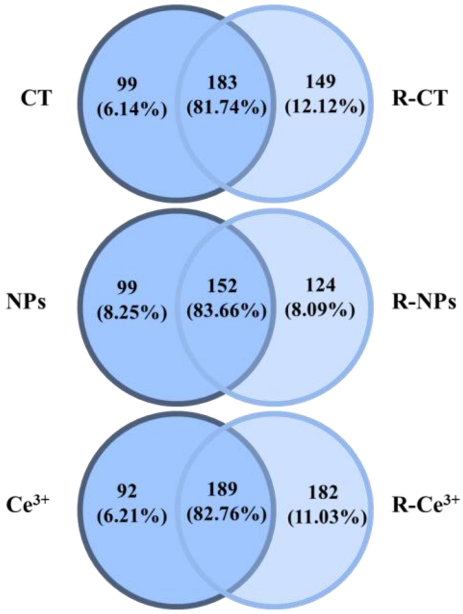

Fig. 5 The analysis of common and different OTUs between control and treated groups was obtained from the sequencing data. CT, NPs, and Ce^{3+} are the three treatments in bulk soil, and the prefix R- represents soil incubation with the plant.

CeO_2 NPs treatment significantly changed the microbial populations in the rhizosphere soil but not in the bulk soil (**Fig. 5, 6**). A previous study also found that CeO_2 NPs (100 mg/kg) altered soil bacterial communities in soybean-planted soil but did not affect bacterial communities in unplanted soil (Ge *et al.*, 2014). The relative abundance of *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* was increased while the abundance of *Acidobacteria* was decreased after CeO_2 NPs treatment in the rhizosphere soil (**Fig. 6**). Similarly, Pan *et al.* (2020) found that the abundances of *Proteobacteria* and *Bacterioidetes* at the phylum level increased after CeO_2 NPs exposure with the ferrous amendment. *Actinobacteria* plays vital roles in the decomposition of organic matter and the carbon cycle (Lewin *et al.*, 2016; Chen *et al.*, 2021). Phosphatase encoding gene (*pho D*) mainly existed in *Actinobacteria* (Luo *et al.*, 2017). Therefore, the increased enzymatic activity of phosphatase may result from the increased abundance of *Actinobacteria*. However, the relative abundances of *Nitrospira* at the phylum level were decreased under CeO_2 NPs and Ce^{3+} treatment in the rhizosphere (**Fig. 6**). Compared with R-CT (0.8%), the relative abundance of *Nitrospira* at the phylum level was significantly lower than in the CeO_2 NPs treatment (0.37%) and Ce^{3+} ions treatment (0.6%).

These findings are in accordance with previous studies that the CeO_2 NPs impaired the soil microbial community and soil organic carbon mineralization (Luo *et al.*, 2020). A recent study also found that the relative abundance of *Nitrospira* was reduced after exposure to 10 mg/L CeO_2 NPs for xx days (Wang *et al.*, 2018a). Negative effects on the relative abundance of *Nitrospira* were also reported for rGO, MWCNTs, and C60. *Nitrospira* is involved in plant's nitrification processes (Hao *et al.*, 2018). The effects of CeO_2 NPs and Ce^{3+} ions on *Nitrospira* indicate that they may negatively impact soil nitrogen cycling. However, because of the lack of long-term experiments, the relationship between the change of soil microbial communities and its soil enzyme activities, and whether it will eventually have a positive or a negative impact on plant growth remains still unknown. We found that the relative abundances of the rare bacteria (*e.g., Euryarchaeota*, *Fibrobacteres*) at the phylum level for the total bacterial community are also more sensitive to environmental factors because of their response to the soil rhizosphere. The bacterial composition at the class and genus level also shifted markedly upon CeO_2 NPs exposure (**Fig. S3** and **Fig. S4)**. Overall, although sharing some similarities, CeO_2 NPs showed particle-specific effects on soil microorganisms compared with Ce^{3+} ions.

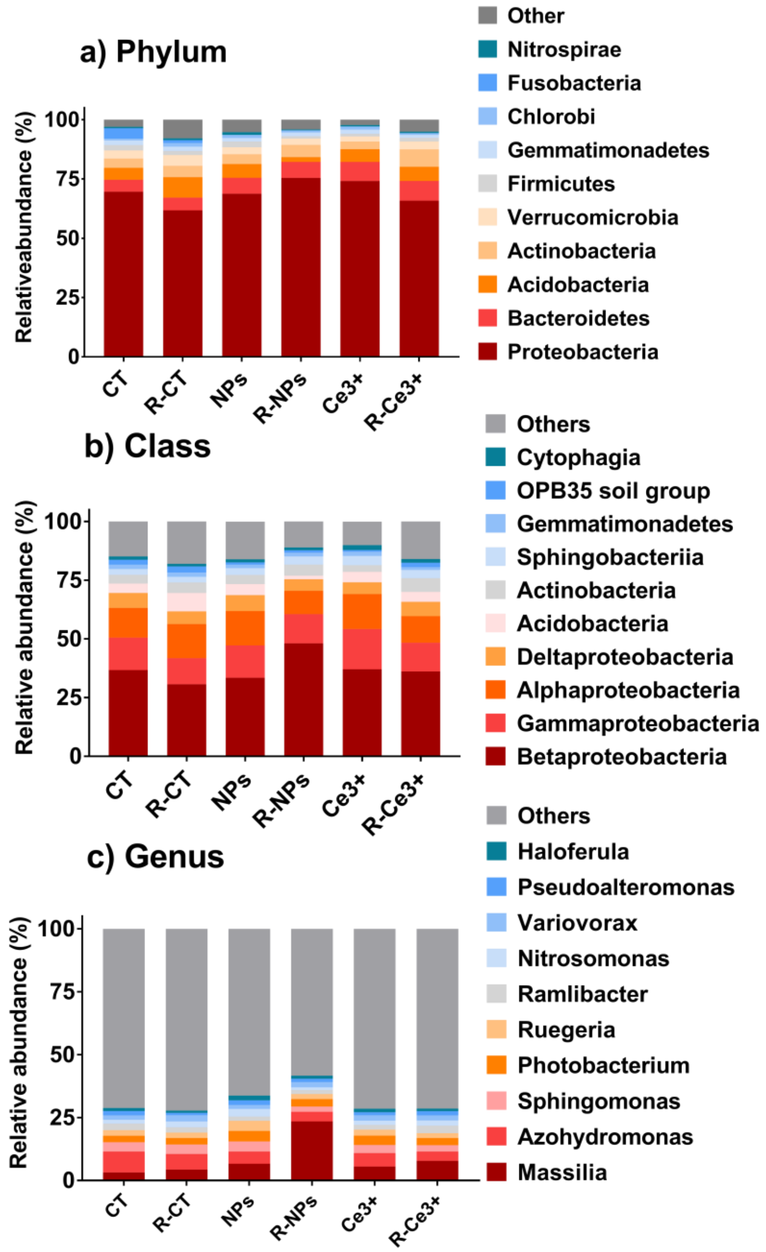

Fig. 6 Relative abundance of major phyla (**a**), class (**b**), and genus (**c**) in bulk soil, rhizosphere, or soil treated with CeO_2 NPs and $CeCl_3$ and the respective controls. CT, CeO_2 NPs, and Ce^{3+} are the three treatments in bulk soil, and Prefix R- represents soil incubation with the plant, respectively.

Conclusions

With the increasing application of nanomaterials in environmental remediation and agriculture, a thorough understanding of their environmental impacts are critical for their sustainable design and safe use. In this study, the responses of soil microbial communities, soil enzyme activities, and cucumber seedling growth in a soil-plant interactive system were systematically investigated. CeO_2 NPs shared some similarities in the effects with $CeCl_3$, which was attributed to the biotransformation of CeO_2 NPs in the rhizosphere. However, CeO_2 NPs also show distinct nano-specific effects on the antioxidant system, organic nutrient accumulation, and soil enzyme activities. Distinct microbial response in the rhizosphere with that in bulk soils highlights the critical role of rhizosphere chemistry in nanomaterial-induced soil impacts. This study indicates that any environmental factors that alter the rhizosphere chemistry may affect the behavior and biological effects of NPs in soil-plant system. It should be noted that the present study was a short-term study, the long-term effects of NPs exposure on the resiliency of soil microbial communities and their functions should be evaluated in the future.

CRediT authorship contribution statement

Changjian Xie: Investigation, Formal analysis, Writing-original draft, Funding acquisition. **Zhiling Guo:** Formal analysis, Writing-review & editing. **Peng Zhang:** Resource and Funding acquisition. **Jie Yang:** Investigation. **Junzhe Zhang:** Investigation. **Yuhui Ma**：Investigation. **Xiao He**：Investigation. **Iseult Lynch**：Investigation. **Zhiyong Zhang:** Conceptualization, Methodology, Formal analysis, Writing-original draft, Writing-review & editing, Resources, Funding acquisition.

Declaration of competing interest

We declare we have no competing interests.

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