Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary information

Rhizosphere regulation with Cerium oxide nanomaterials promoted carrot taproot thickening

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Text S1 Determination of CeO_2 NMs in plant tissues and soil

For the detection of Ce particle concentration, carrot taproots and shoots were digested using 2 mL of 5% macerozyme R-10 and 3 mL 20 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH = 5.0). The mixture was shaken at 37 °C for 24 h. After precipitation for 1 h, the supernatant was passed through a 0.45 μm filter membrane, diluted with ultrapure water and used for SP-ICP-MS analysis[1].

For the detection of Ce element content, 25 mg dry samples were digested in concentrated HNO_3 (3 mL) and 25% hydrogen peroxide (0.5 mL) in microwave digestion system (MARS 6, CEM, USA) at 190°C for 45 and 25 min. The digested samples were cooled down at ambient temperature and filtrated using a 0.22 μm membrane. The filtrate was diluted to 50 mL with ultrapure water prior to ICP-MS.

Text S2 Sequentially extraction and digestion method

Soil sample (0.5 g) was first extracted with 20 mL of 0.11 mol/L acetic acid solution (pH 2.81) by shaking with an oscillator at 250 rpm (25 ℃, 16 h) and centrifuged at 6,000×g for 10 min to obtain the exchangeable fraction (F1). The residue was then resuspended and extracted by 20 mL of 0.5 mol/L hydroxylamine hydrochloride solution (pH 1.5) and shaken with an oscillator at 250 rpm (25 ℃, 16 h). The mixture was centrifuged at 6,000×g for 10 min to obtain the reducible fraction (F2). The residue was then resuspended and mixed with 30% H_2O_2 and shaken at 250 rpm for 1 h at 25 ℃, followed by another hour at 85 ℃ in the water bath pot with a closed cap. The volume of the mixture was reduced to less than 1.5 mL by further heating at the same temperature without cap. Following the volume reduction, an aliquot of 5 mL of 30% w/v H_2O_2 was added and the heating process was repeated until the volume was reduced to about 0.5 mL. Afterwards, 20 mL of 1 mol/L ammonium acetate solution (pH 2) was mixed with the residue for 16 h at 25 ℃ and the mixture was centrifuged at 8,000×g for 10 min to extract the oxidizable fraction (F3). The residue fraction (F4) was extracted by microwave digestion; 0.1 g of residue from the third fraction was mixed with 5mL HNO_3 and 2mL HF for microwave digestion at 200℃ for1600W. The digested samples were cooled down at ambient temperature and filtered with a 0.45μm filter membrane[2].

Text S3 16S rRNA gene sequencing of rhizosphere soil microbial community

The purity of the extracted DNA was further measured and quantified by spectrophotometry using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). The 16S rRNA genes were amplified using the primer set of 515F (5'-GTGCCAGCMGCCGCGG-3')/806R (GGACTACHVGGGTATCTAAT) targeting the V3-V4 variable regions. The amplified products of the bacterial 16S rRNA gene were checked by 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U. S.) and quantified using ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (PE250) on an Illumina platform according to the standard protocols. The 16S rRNA genes were subsequently sequenced on an the PacBio Sequel platform. Sequences with ≥ 97% similarity were assigned to the same OTU, and the OTUs were aligned with the SILVA database (version 132) to classify the caroot rhizosphere soil microorganism community into phenotypes (bacterial families or phyla). The bacterial diversity and richness of the rhizosphere soil microbial community were evaluated via QIIME software (version 1.6.0).

Text S4 Operating parameters of LC–MS/MS and the data processing

The injection volume was 6 μL. The column kept at 40 °C throughout the run. Mobile phase A was 0.1 % v/v formic acid in water, and mobile phase B was 0.1 % v/v formic acid in acetonitrile. The linear elution gradient was set as follows: 0 min, 5% B; 1.5 min, 5 % B; 10 min, 100 % B; 11 min, 100 % B; 11.5 min, 5 % B; 14 min, 5 % B; flow rate: 0.35 mL·min−1. The compounds were detected using a quantitative time-of-flight mass spectrometer equipped with an Apollo II electrospray ion source (Bruker Daltonics, USA) in positive and negative ion modes. Capillary voltage was: +3.0 kV (−3.0 kV); sampling cone: +40 V (−23 V); source temperature: 120 °C; desolvation temperature: 350 °C; collision energy: 10-40 V; ion energy: 1 V; scan time: 0.03 s; inter scan time: 0.02 s; and scan range: m/z 70-1050. The quality control (QC) samples were injected at regular intervals (every five samples) throughout the analysis to assess repeatability.

Data preprocessing and statistical analysis. The raw data files were processed using Compound Discoverer 3.1 software coupled with the mzCloud and S-7 Chem Spider libraries. Principal components analysis (PCA), and supervised methods, such as partial least squares discriminant analysis (PLS-DA) were conducted on the LC–MS/MS data via MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/). All data was log-transformed and normalized before statistical analysis. Significantly changed metabolites were determined in univariate statistical analyses (Variable importance in projection (VIP) score > 1; and Student's *t*-test, $p < 0.05$)[3].

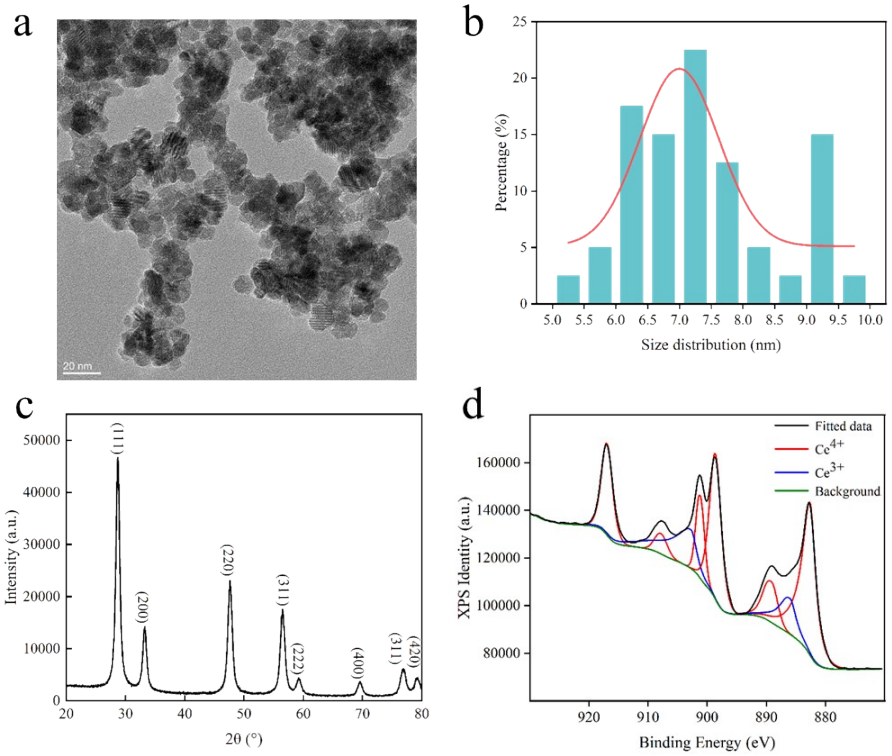

Fig.S1 TEM image (a), size distribution (b) of CeO_2 NMs, XRD analysis (c), and XPS spectra (d)

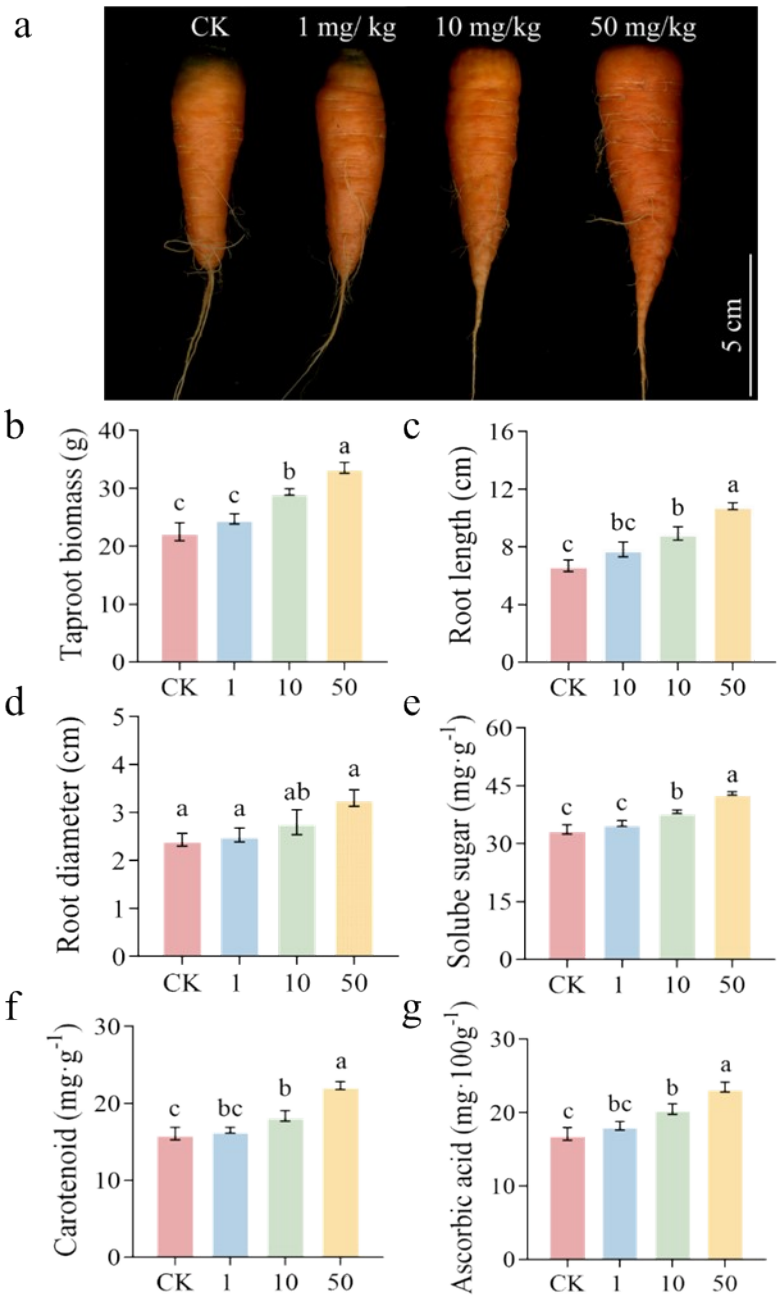

Fig.S2 Yield and quality of carrot after exposure CeO_2 NMs: (a) Carrot phenotypes, (b) Taproot biomass, (c) Root length, (d) Root diameter, (e) Solube sugar, (f) Carotenoid, (g) Ascorboic acid.

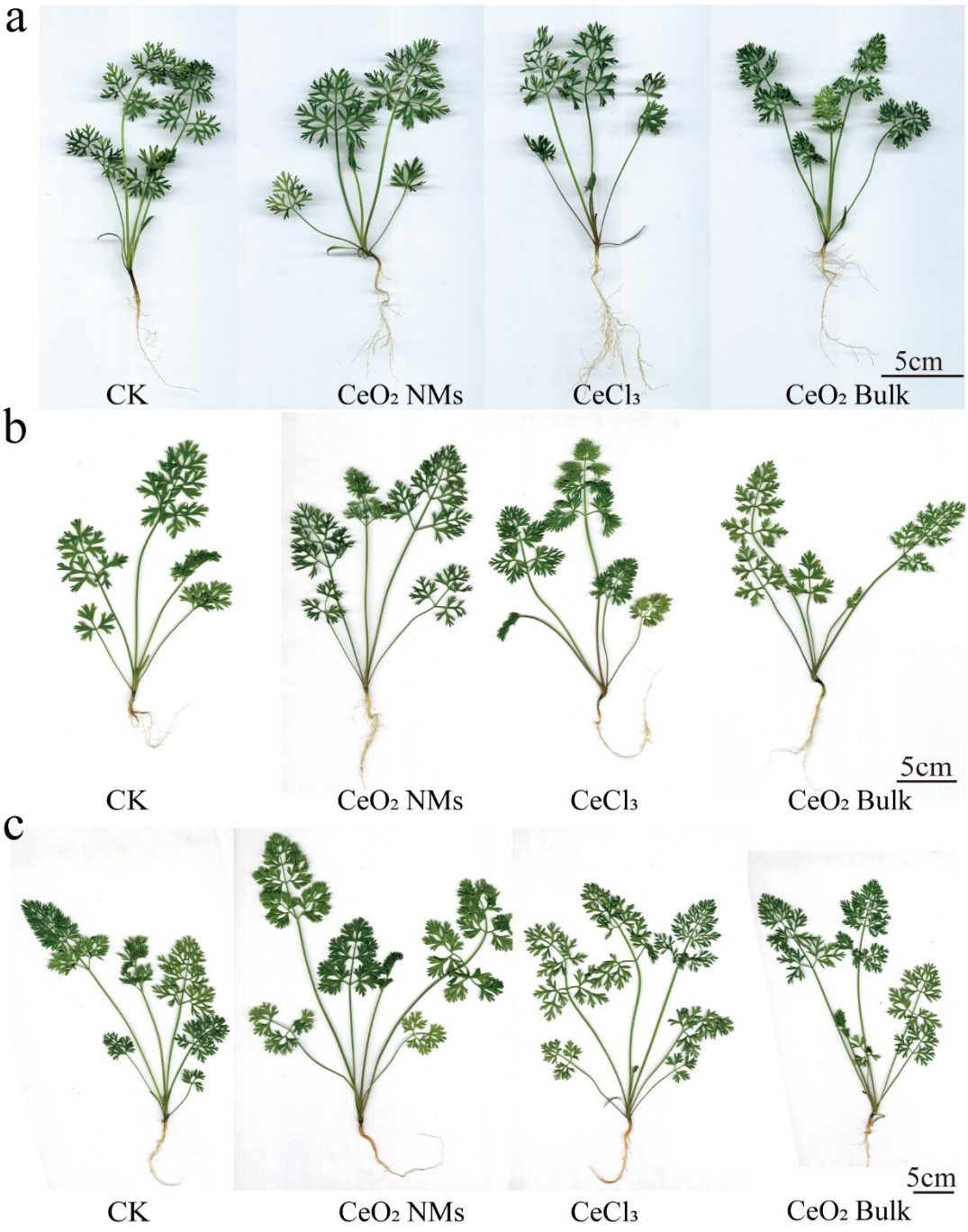

Fig S3. Carrot phenotypes at 1(a), 3(b) and 7(c) days after exposure.

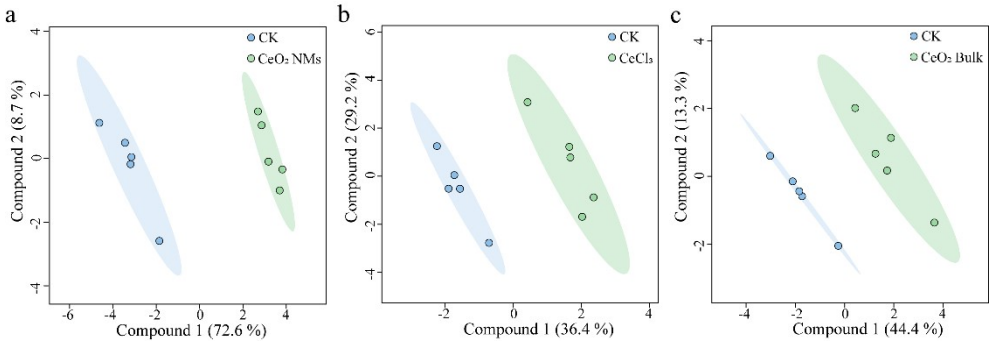

Fig.S4 Multivariate analyses of metabolite profiles in carrot root exudates at 5 days after exposure: (a) Partial least-squares discriminant analysis (PLS-DA) score plots of the differentially abundant metabolites between the control and CeO_2 NMs, (b) Partial least-squares discriminant analysis (PLS-DA) score plots of the differentially abundant metabolites between the control and $CeCl_3$, (c) Partial least-squares discriminant analysis (PLS-DA) score plots of the differentially abundant metabolites between the control and CeO_2 Bulk.

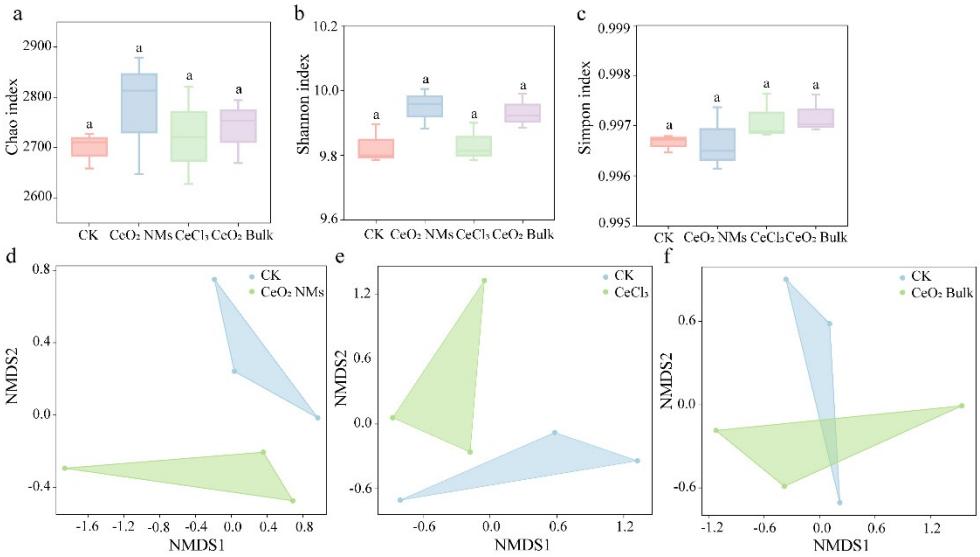

Fig.S5 Variation of carrot rhizosphere microbiome at 5 days after exposure: (a-c) Alpha diversity index (Chao1, Simpson, and Shannon, $p<0.05$), (d-f) Non-metric multidimensional scaling (NMDS) analysis.

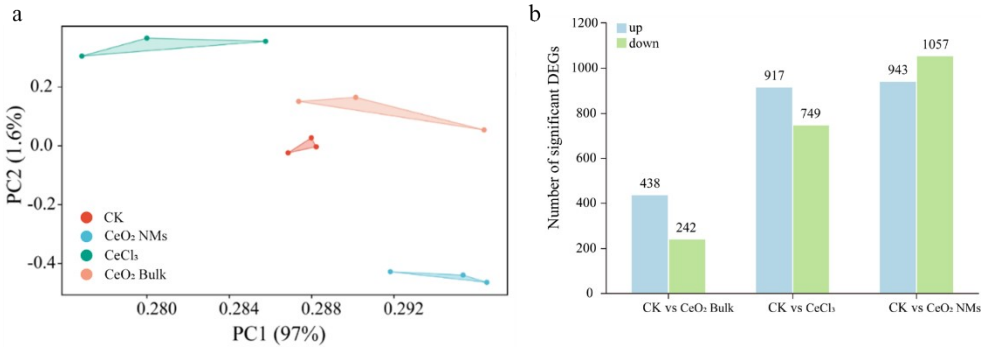

Fig. S6 Differentially expressed genes in carrot taproots at 5 days after exposure: (A) PCA analysis, (B) the number of differential genes up-regulated and down-regulated between groups.

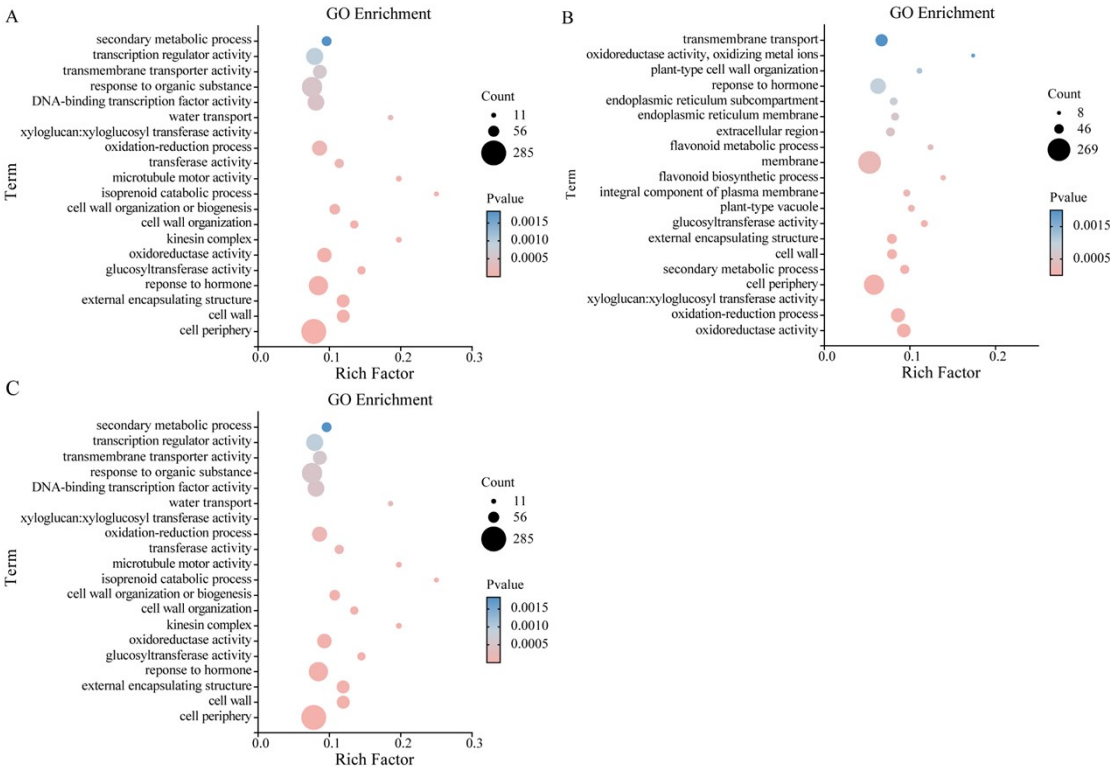

Fig. S7 GO enrichment of differentially expressed genes (DEGs). The different color depth tables and the size of points represent the Pvalue value and the number of DEGs in that pathway, respectively. (A) CK vs. CeO_2 NMs. (B) CK vs. $CeCl_3$. (C) CK vs. CeO_2 Bulk.

Table S1 The physicochemical properties of soil used in this research

pH	Organic matter ($g \cdot kg^{-1}$)	Ce content ($mg \cdot kg^{-1}$)	Available phosphorus ($mg \cdot kg^{-1}$)	Available potasiums ($mg \cdot kg^{-1}$)
7.1	19.14	53.2	30.42	410.13

Table S2 Zeta potential and hydrodynamic diameter of CeO_2 NMs in deionized water

NMs	Zeta potential (mV)	Hydrodynamic diameter (nm)
50 mg·$L^{-1}CeO_2$	15.87±0.09	335.9±18.82

References

[1] Wang J, Yue L, Zhao J, et al.Uptake and bioaccumulation of nanoparticles by five higher plants using single-particle-inductively coupled plasma-mass spectrometry[J].Environmental Science: Nano,2022, 9 (8): 3066-3080.
[2] Jiao C, Dong C, Dai W, et al.Geochemical cycle of exogenetic CeO2 nanoparticles in agricultural soil: Chemical transformation and re-distribution[J].Nano Today,2022, 46: 101563.
[3] Jiao L, Cao X, Wang C, et al.Crosstalk between in situ root exudates and rhizobacteria to promote rice growth by selenium nanomaterials[J].Sci Total Environ,2023, 878: 163175.