Supplementary Information

Nanomaterials-modulated cellular sodium extrusion and vacuolar sequestration for salt tolerance

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Number of pages: 14

Number of figures: 9

Number of supplementary texts: 4

Number of tables: 3

Number of video: 1

List of Supplementary Information

Text S4. LC-MS/MS analysis of metabolites.

Table S1. Hydrodynamic diameter and zeta potential of $nCeO_2$ and N-CDs.

Table S2. Primers used for qPCR.

Table S3. Evaluation of method accuracy for ICP-MS.

Video S1. NMT measurement of net Na^+ fluxes in BY-2 cells under salt stress (grey line, S) and after additional exposure to $nCeO_2$ for 6 h (red line, $nCeO_2$).

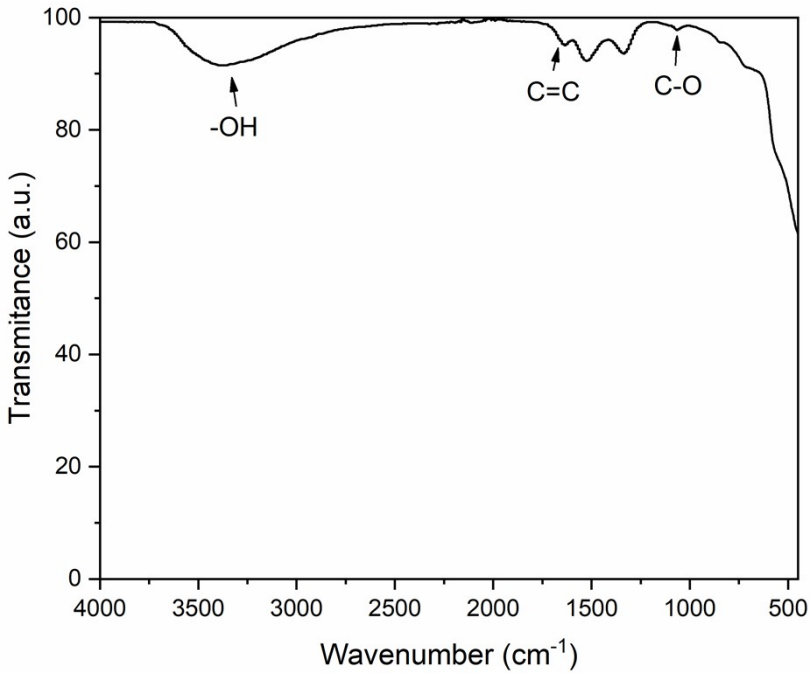

Figure S1. The functional groups of $nCeO_2$ characterized by FTIR.

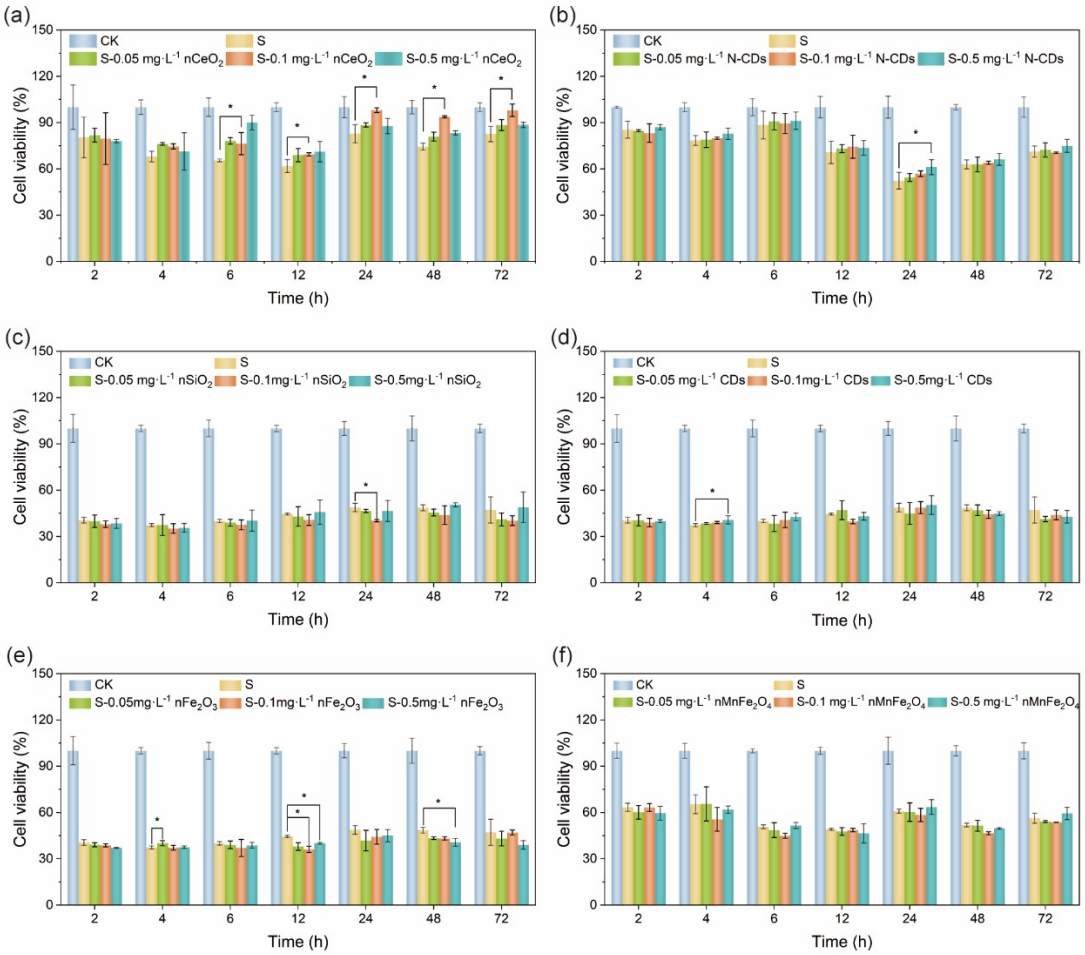

Figure S2. Cell viability tested by TTC after exposure to $nCeO_2$ (a), N-CDs (b), $nSiO_2$ (c), CDs (d), nFe_2O_3 (e), and $nMnFe_2O_4$ (f) during 2-72 h. CK: control BY-2 cells without any treatments, S: BY-2 cells treated with 100 mM NaCl, S-NMs: salt-stressed cells exposed to nanomaterials.

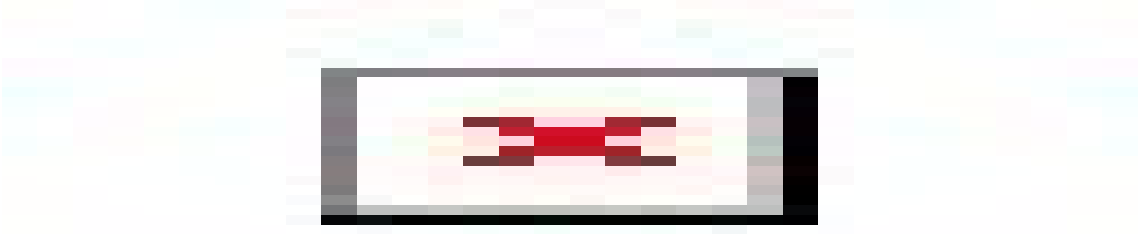

Figure S3. Ion release of $nCeO_2$ in 1/2 MS medium added with 100 mM NaCl (a). Viability of control cells (CK), cells exposed to NaCl alone (S), or NaCl + 0.1 mg L^{-1}

CeO_2 bulk particles (BPs) (b). Viability of control cells (CK), cells exposed to NaCl alone (S), or NaCl + 0.93 or 1.51 μg L^{-1} Ce^{3+} ions (c).

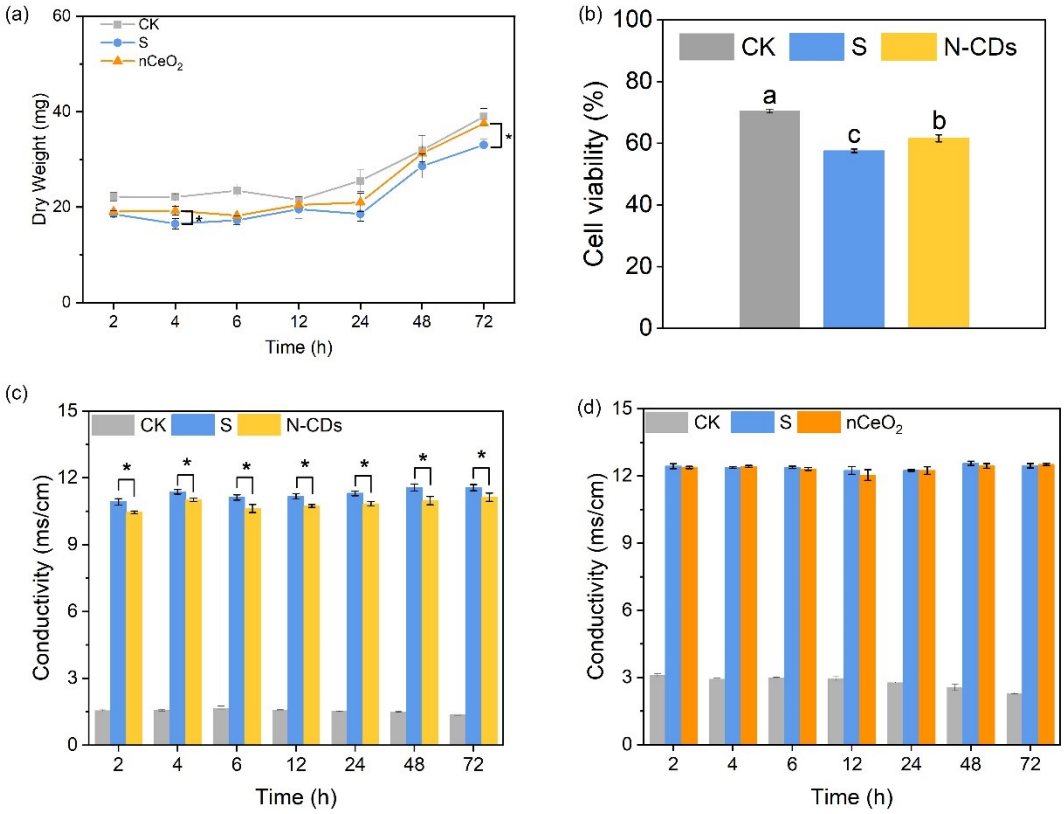

Figure S4. Dry weight of BY-2 cells after addition of 0.1 mg L^{-1} $nCeO_2$ or 0.5 mg L^{-1} N-CDs (a); Cell viability tested by PI/FDA staining of BY-2 cells after 24 h exposure in the presence or absence of N-CDs (b); Conductivity of BY-2 cells altered by 0.5 mg L^{-1} N-CDs (c) or 0.1 mg L^{-1} $nCeO_2$ (d).

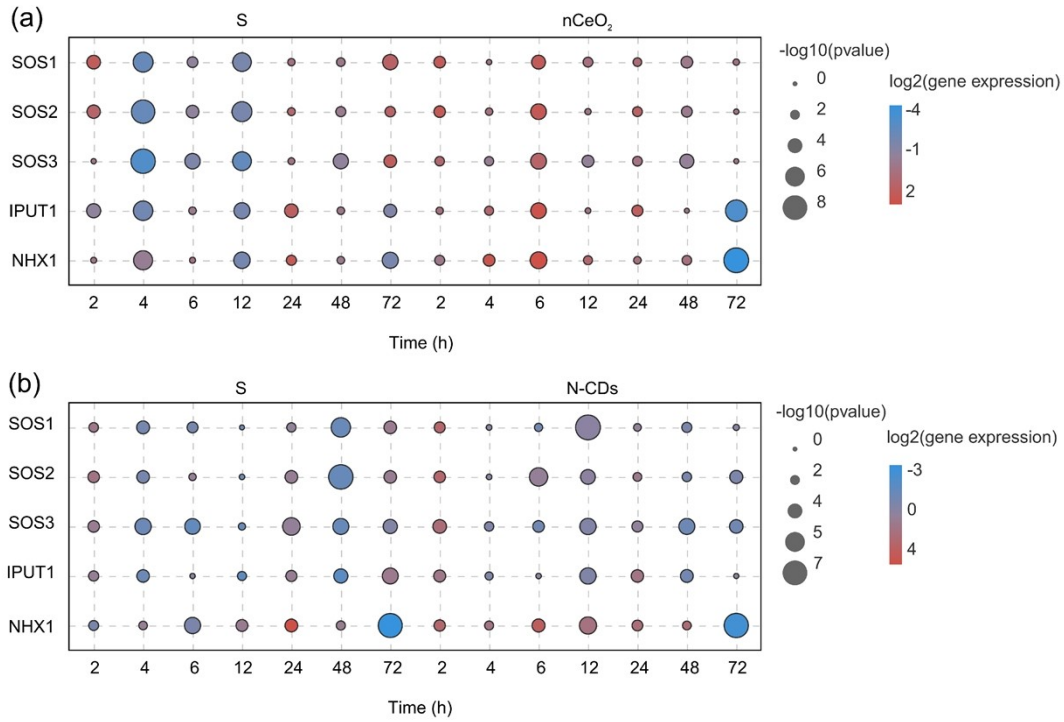

Figure S5. Relative gene expression of salt tolerance-related genes after exposure to 0.1 mg L^{-1} $nCeO_2$ (a) or 0.5 mg L^{-1} N-CDs (b). S: BY-2 cells treated with 100 mM NaCl, S-NM: salt-stressed cells exposed to either $nCeO_2$ or N-CDs.

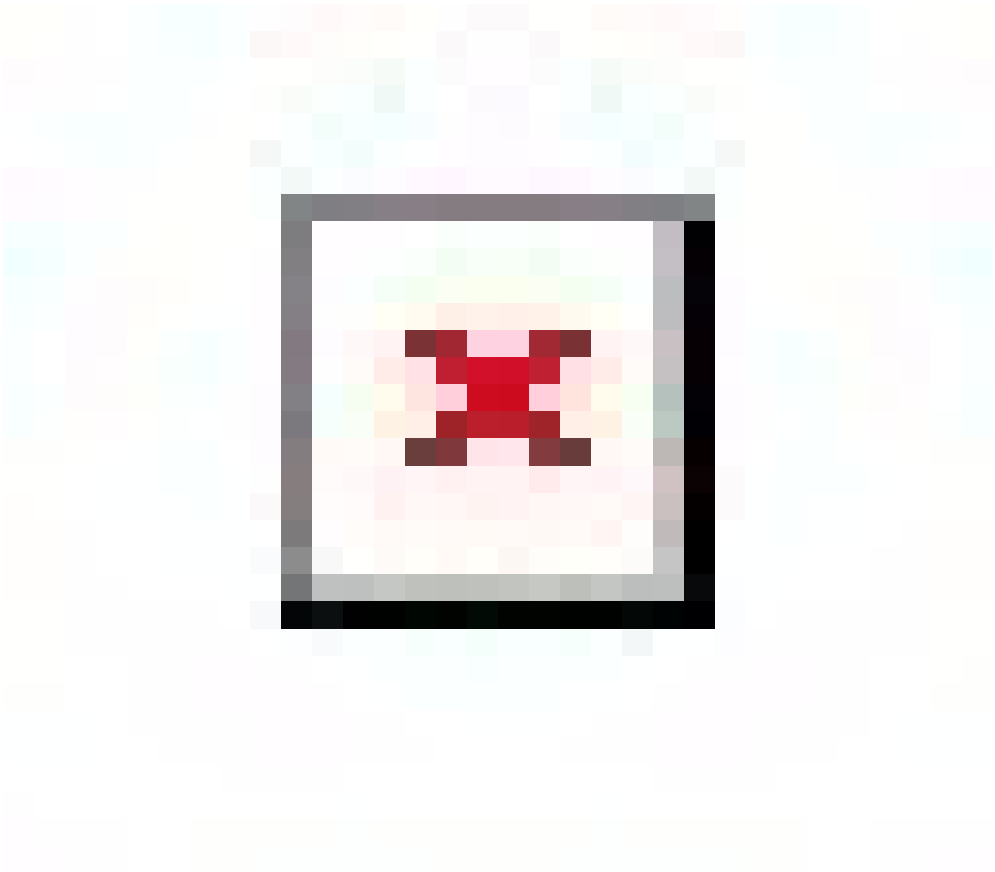

Figure S6. Cellular contents of Ca (a) and Na (b) after exposure to 0.1 mg L^{-1} $nCeO_2$ and contents of Na (c) and K (d) after exposure to 0.5 mg L^{-1} N-CDs.

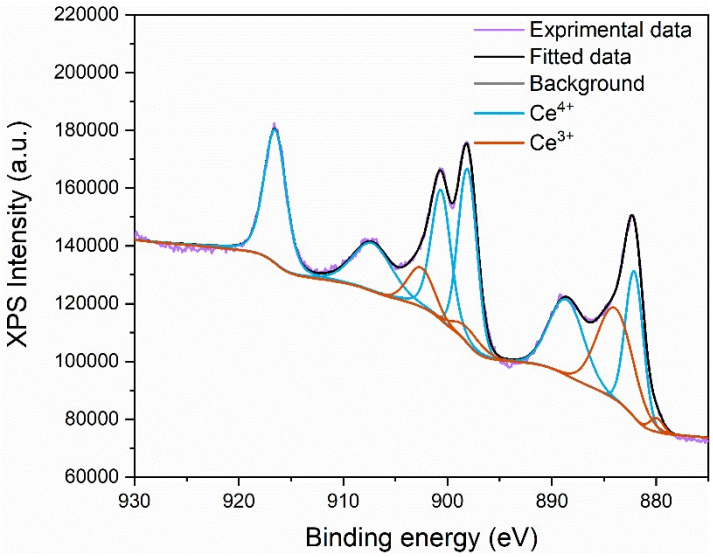

Figure S7. The surface electronic states of $nCeO_2$ characterized by XPS.

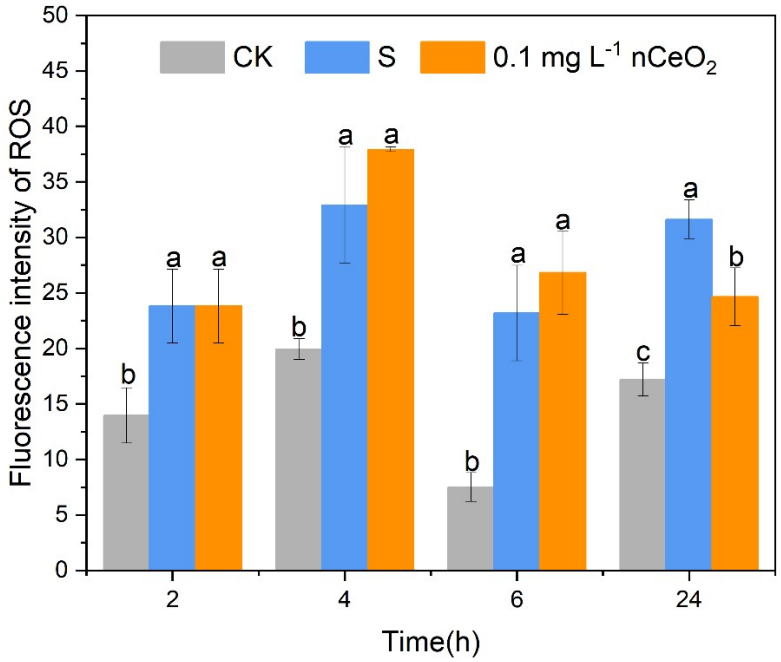

Figure S8. Intracellular ROS levels in salt-stressed BY-2 cells after addition of 0.1 mg L^{-1} $nCeO_2$.

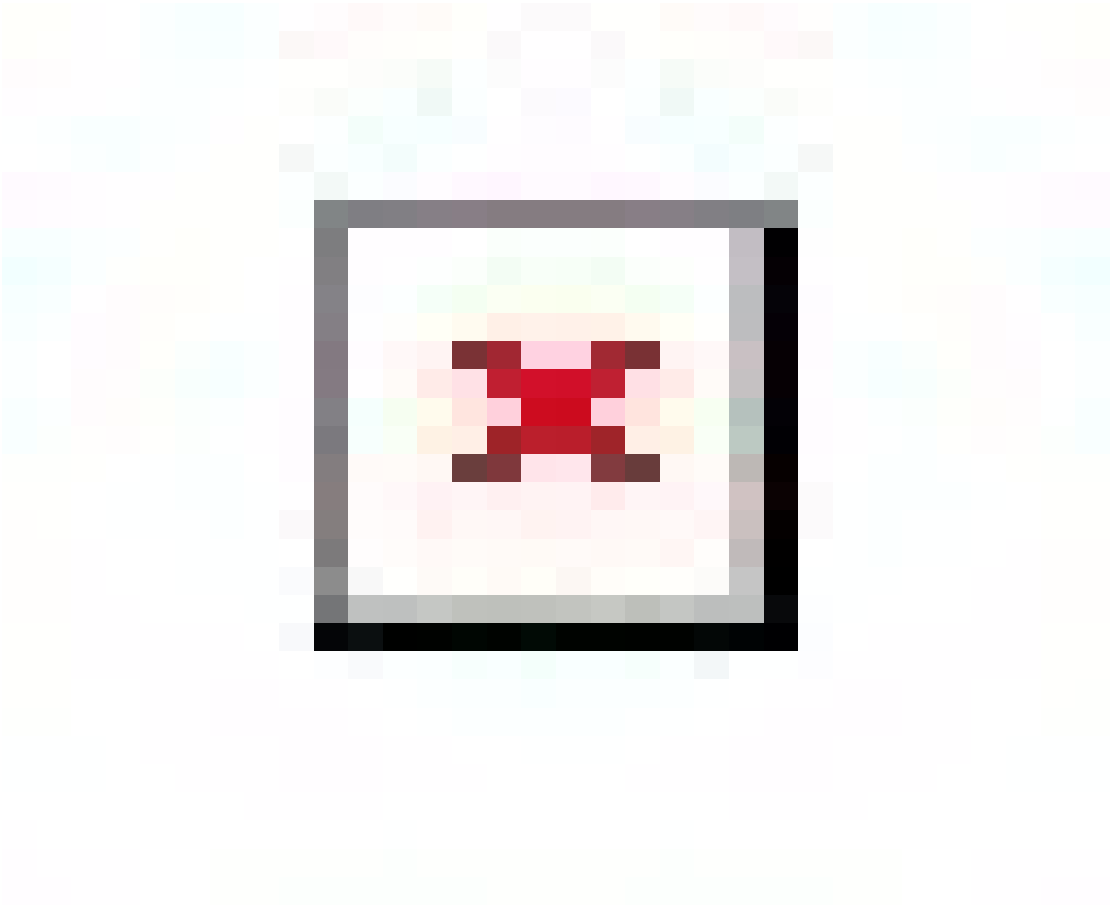

Figure S9. The separation between groups in principal component analysis (PCA) of

metabolites altered by $nCeO_2$ (a) or N-CDs (b) exposure; Number of common and distinctively changed metabolites between $nCeO_2$ and N-CDs treatment (c); VIP scores of metabolites in BY-2 cells changed by either $nCeO_2$ (d) or N-CDs (e) exposure. The color intensity of the scale bar indicates up-and down-regulation of metabolites.

Text S1. Measurement of cell viability after different NMs exposure

BY-2 cells at exponential stage were transferred into 1/2 MS medium spiked with 100 mM NaCl for 24 h, followed by addition of different NMs (0.05, 0.1, and 0.5 mg L^{-1} $nCeO_2$, N-CDs, $nTiO_2$, $nMnFe_2O_4$, and nFe_2O_3), and samples were collected at time intervals of 2, 4, 6, 12, 24, 48, and 72 h, respectively. One mL BY-2 cell suspension was washed with PBS buffer (0.1 M, pH 7.2) for three times and the resulting supernatants were removed. The cells were resuspended with 1 mL TTC (0.3% v/v, in 0.05 M PBS, pH 7.2) and incubated for 8 h in dark at 25 °C, the samples were centrifuged at 6000 rpm to remove PBS followed by addition of ethanol (95%), and placing in a water bath at 60 °C for 15 min to extract the formazan. The supernatant after 5 min centrifugation at 6000 rpm was quantified with a microplate reader (Thermo Scientific, USA) with absorbance at 485 nm. Based on the results of TTC measurement (Fig. S2) and results from our previous study,[1] $nCeO_2$ (0.1 mg L^{-1}) and N-CDs (0.5 mg L^{-1}) were selected as optimal NMs for the following experiments. For FDA/PI double staining, 100 μL cell suspension was mixed with FDA (20 mg L^{-1}) and PI (20 mg L^{-1}) for 5 min and evaluated with a fluorescent microscope (Nikon Ni-U, Japan), the cell viability was determined as the percentage of viable (FDA positive) and dead (PI positive) cells.

Text S2. ICP-MS measurement of $nCeO_2$ and cellular Ca, Na, and K content.

The particle concentration and size of $nCeO_2$ in BY-2 cells were quantified by single particle inductively coupled plasma-mass spectrometry (SP-ICP-MS). After washing with PBS buffer, 100 mg of cells were collected and added with 4 mL MES buffer (20 mM, pH 5.0) for further vortexing and sonication. One mL enzyme solution (Macerozyme R-10, 20 mg mL^{-1}) was then added and incubated on a shaker (37°C, 200 rpm) for 24 h. After enzymatic digestion, the samples were settled for 1 h and the supernatant was filtered (0.22 μm) and diluted two times before measurement on SP-ICP-MS (Thermo Scientific™ iCAP™ TQ ICP-MS, Germany). The quantification of cellular Ca, Na, and K content was conducted by an inductively coupled plasma mass spectrometer (ICP-MS, Thermo Scientific™ iCAP™ TQ ICP-MS, Germany). Dried cells (25 mg) were digested with 3 mL ultrapure water and 3 mL guaranteed reagent HNO_3 in a microwave accelerated reaction system (CEM corp, Matthews, NC), the digestions were filtered with 0.22 μm membrane before loading on the ICP-MS. A multi-element standard solution was inserted every five samples for conducting the quality control, and method accuracy was evaluated by using a standard reference material (tea leave, GBW10016a, Table S3).

Text S3. TEM imaging and measurement of ROS.

The subcellular morphology of BY-2 cells exposed to N-CDs was visualized by TEM. The samples were prepared as follows: cells were collected by centrifugation at 1,500 rpm under room temperature followed by two times of wash with 0.1 M PBS (pH 7.2), fixation and embedding were conducted as previously described.[2] The embedded cells were sectioned by an ultra-microtome (Leica, EM UC7) and then scanned by the TEM.

The ROS levels in salt-stressed BY-2 cells after addition of addition of 0.1 mg L^{-1} $nCeO_2$ were measured based on a previous literature.[3] Briefly, cells were collected after 2, 4, 6, and 24 h exposure, supernatants were removed after centrifugation at 12 000 rpm for 5 min. After three times washing with PBS buffer (0.1M， pH 7.2) and removal of supernatants, samples were incubated with DCFH-DA (Sigma-aldrich, USA, 10 μM) in the dark for 30 min. Cells were then washed three times with PBS buffer and centrifuged at 12 000 rpm for 5 min. The fluorescence intensity was measured by a microplate reader (Thermo Scientific, USA) at the excitation and emission wavelengths of 488 nm and 525 nm, respectively.

Text S4. LC-MS/MS analysis of metabolites.

An Acquity HSS T3 column (2.1 × 100 mm, 1.8 μm) was applied for rapid separation of analytes using the following mobile phases for elution: H_2O, 0.1% formic as mobile phase A, and acetonitrile, 0.1% formic acid as mobile phase B with following gradient: 0 min 5% B; 1.5 min 5% B; 15.5 min 100% B; 16 min 5% B; 18 min 5% B. Additionally, an Agilent Hilic Poroshell 120 column (2.1 × 100 mm, 2.7 μm) was also applied using the following mobile phases for elution: H_2O, 0.1% formic, 5 mM ammonium acetate as mobile phase A, and 95% acetonitrile, 0.05% formic acid, 5 mM ammonium acetate as mobile phase B with following gradient: 0 min 98% B; 7 min 75% B; 10 min 10% B; 13 min 10% B; 13.5 min 98% B; 15 min 98% B. The flow rate was 0.35 mL min^{-1}. The injection volume was 5 μL. Two QC samples were injected at the beginning of each sample sequence and re-injected every eight samples to evaluate the stability of the analytical system. The ESI interface was adjusted to the following

conditions: sheath gas pressure, 35 arbitrary units; aux. gas flow, 15 arbitrary units; sweep gas flow, 0 arbitrary units; capillary temperature, 320 °C; aux. gas heater temperature, 350 °C. A spray voltage of 3.5 kV and −3.0 kV for ESI (pos) and ESI (neg) was used, respectively. The raw data were processed using Compound Discoverer 3.1 software (Thermo Fisher Scientific, USA) coupled with the mzCloud and ChemSpider libraries. A supervised partial least-squares discriminant analysis (PLS-DA) clustering method was applied for LC-MS/MS data via online resources (http://www.MetaboAnalyst.ca/). Before PLS-DA analysis, data were normalized by median and log-transformed. The variable importance in projection (VIP) score was used to rank the metabolites based on their importance to the entire model. A VIP score > 1 or t-test p value < 0.05 is regarded as significant for the separation and is defined as a discriminating metabolite.[4]

Table S1. Hydrodynamic diameter and zeta potential of $nCeO_2$ and N-CDs.

NMs	Culture medium	Hydrodynamic diameter (nm)	Zeta potential (mV)
$nCeO_2$	water	355.53±38.40	5.70±0.54
	1/2 MS	91.16±0.39	-20.38±1.95
	1/2 MS +100 mM NaCl	250.87±36.10	-14.43±1.48
N-CDs	water	136.07±4.06	-6.48±0.06
	1/2 MS	353.33±2.81	-10.77±0.21
	1/2 MS +100 mM NaCl	205.27±22.02	-5.07±0.22

Table S2. Primers used for qPCR.

Gene Name	FW-Primer	RW-Primer
EF1α	TCACATCAACATTGTGGTCATTGGC	TTGATCTGGTCAAGAGCCTCAAG
SOS1	ACACTCATCATCAACGGCTCAACC	TCCAAGATCACCAAATGCTTCCAGAG
SOS2	ACGATTGAAGCAGCAGCAGAGTC	TGACCAGCCTTATTTGCCGTTACC
SOS3	TGTTCGGTCGTTAAGCCTATTCCAC	TCAGCTCTTCACGTTCTATGTATCCAG
IPUT1	CATCAGCGGCAGCAATCATGTTG	GACCACCTACCACCATCAAACCTAAC
NHX1	TGGTGTGGATATGGATTTGGACTTCAG	AGGCAGCAATGGCACAATAGGC

Table S3. Evaluation of method accuracy for ICP-MS.

Elements	Linearity range (mg/L)/R^2	Linearity equation	Detection limit (ng/L)	Tea (GBW10016a) Standard value (μg/g)	Tea (GBW10016a)ICP-MS measured value (μg/g, n = 3)
Ca	0-10/0.999	y = 5.795x + 438.342	20217	4700±200	4262±125
Na	0-10/1.000	y = 469.533x + 6329.130	3066	22.6±6.7	23.0±2.2
K	0-10/0.999	y = 85.732x + 3544.261	12862	14500±500	17401±380
Ce	0-0.1/1.000	y = 81303.777x– 40.017	19	1.3±0.2	1.6±0.1
Fe	0-10/1.000	y = 81.982x + 70.042	422	149±7	213±8
Mg	0-10/1.000	y = 199.001x + 258.414	29	1800±100	1498±4
P	0-10/1.000	y = 795.152x + 1876.021	419	2400±100	2546±23
S	0-10/1.000	y = 1271.860x + 17220.382	706	2600±200	2432±32
Zn	0-0.1/0.997	y = 775.7x + 1036.427	114	27±3	28.8±0.4
Mn	0-0.1/0.999	y = 1480.176x + 71.852	6	1250±40	1624±47

Video S1. NMT measurement of net Na^+ fluxes in BY-2 cells under salt stress (grey line, S) and after additional exposure to $nCeO_2$ for 6 h (red line, $nCeO_2$).

References:

1. L. Zhu, F. Chen, M. Tao, Y. Liu, X. Zhao, Z. Wang, Mechanisms of plant salt tolerance promoted by nanomaterials. *Research of Environmental Sciences*, 2022, Doi:10.13198/j.issn.1001-6929.2022.03.14.
2. J. Zhao, X. Cao, X. Liu, Z. Wang, C. Zhang, C. J. White, B. Xing, Interactions of CuO nanoparticles with the algae *Chlorella pyrenoidosa*: adhesion, uptake, and toxicity. *Nanotoxicology* 2016, **10**, 1297-1305.
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CuO nanoparticles with plant cells: internalization, oxidative stress, electron transport chain disruption, and toxicogenomic responses, *Environ. Sci.: Nano*, 2018, **5**, 2269-2281.

4. W. Ma, L. Yue, F. Chen, H. Ji, N. Fan, M. Liu, Z. Xiao, Z. Wang, Z. Silica nanomaterials and earthworms synergistically regulate maize root metabolite profiles via promoting soil Si bioavailability, *Environ. Sci.: Nano*, 2021, **8**, 3865-3878.