Supplementary materials for

Salinity alters the toxicity of copper nanoparticles on anammox consortia through modulating extracellular polymeric substances and membrane permeability

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TEXT S1. Determination of anammox activity.

The determination of SAA was conducted in serum bottles with an effective volume of 120.0 mL. $(NH_4)_2SO_4$ and $NaNO_2$ were added to maintain the final concentrations of NH_4^+-N and NO_2^--N in the inorganic medium at 100 mg N L^{-1}. A total of 100.0 mL of substrates and 1.25 mL L^{-1} of trace element solutions I and II were used to sustain cellular metabolism and prevent osmotic shock. Anammox biomass was collected from reactor, followed by three washes with inorganic salt solution and distilled water. Subsequently, 3.0 g VSS L^{-1} of anammox sludge was added to each serum bottle. The pH was adjusted to 7.5 ± 0.1 using 1 mol L^{-1} hydrochloric acid or sodium hydroxide. Then, argon gas (purity 99.9%) was bubbled through for 10 minutes to displace oxygen, ensuring anaerobic conditions in the serum bottles, which were immediately sealed with butyl rubber stoppers to prevent oxygen leakage. The serum bottles were then placed on a shaker at 35 °C and 180.0 rpm for oscillating cultivation. During this period, 2.0 mL samples were withdrawn from the serum bottles every 30 minutes using a syringe to determine the substrate consumption rate and stored at 4 °C for SAA determination. Each test was performed in triplicate. After sampling, the sludge samples in the serum bottles were filtered, oven-dried at 105 °C for 24 hours, and then calcined in a muffle furnace at 650 °C for 2 hours to determine the sludge concentration in the serum bottles. SAA was determined as the ratio of substrate consumption rate to sludge concentration. Normalized anammox activity (NAA, %) was calculated as the ratio of activity in the presence of the inhibitor to the activity of the control group.

Text S2. Excitation-Emission Matrix Fluorescence Spectra Scan.

The Excitation-emission matrix (EEM) fluorescence spectra were scanned using a fluorescence spectrophotometer (Hitachi F4600, Japan). The excitation and emission wavelengths were set to 200 - 580 nm and 220 - 600 nm, respectively, with a scan interval of 5 nm for both excitation and emission wavelengths. The spectral bandwidths for excitation and emission wavelengths were both 10 nm. The photomultiplier tube voltage was set to 500 V, and the scan rate was 30000 nm min^{-1}. Double-distilled water was used as a blank to eliminate the effects of Raman and Rayleigh scattering. MATLAB 2016 software was used to subtract the blank, and the DOMFluor toolbox was employed to remove the interference of Raman scattering.

TEXT S3. Determination of LDH Activity.

The determination of LDH activity in the supernatant followed established protocols outlined in the LDH assay kit (Nanjing Jiancheng Co., China). After centrifugation (10,000 g, 10 min), the supernatant was meticulously transferred to a 96-well plate. Subsequently, 50 μL of substrate mix was added, and the supernatant was incubated at 35 °C for 30 minutes. Following this, 50 μL of stop solution was added, and the absorbance at 450 nm was monitored.

Table S1. Composition of the synthetic wastewater.

Composition	Concentration
$MgSO_4 \cdot 7H_2O$	58.6 mg L^{-1}
NaH_2PO_4	10 mg L^{-1}
$NaHCO_3$	840 mg L^{-1}
$CaCl_2 \cdot 2H_2O$	73.5 mg L^{-1}
Trace element I[a]	1.25 mL L^{-1}[c]
Trace element II[b]	1.25 mL L^{-1}[c]
$(NH_4)_2SO_4$	Add as required[d]
$NaNO_2$	Add as required[d]

[a] The composition of trace element solution I was 5 g L^{-1} EDTA and 9.14 g L^{-1} $FeSO_4 \cdot 7H_2O$.

[b] The trace element solution II was composed of 15 g L^{-1} EDTA, 0.014 g L^{-1} H_3BO_4, 0.99 g L^{-1} $MnCl_2 \cdot 4H_2O$, 0.25 g L^{-1} $CuSO_4 \cdot 5H_2O$, 0.43 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.21 g L^{-1} $NiCl_2 \cdot 6H_2O$, 0.22 g L^{-1} $NaMoO_4 \cdot 2H_2O$ and 0.24 g L^{-1} $CoCl_2 \cdot 6H_2O$.

[c] 1.25 mL of trace element solutions I and II were added per liter of wastewater.

[d] Equimolar ammonium and nitrite were supplied.

Table S2. The dosing strategy of CR and SR.

Phase	Time (d)	CuNPs (mg L^{-1})	CR NaCl (g L^{-1})	SR NaCl (g L^{-1})
P0	1 - 33	0	0	0
P1	34 - 58	1	0	5
P2	59 - 87	2	0	5
P3	88 - 117	3	0	5
P4	118 - 137	3	0	8
P5	138 - 174	0	0	0

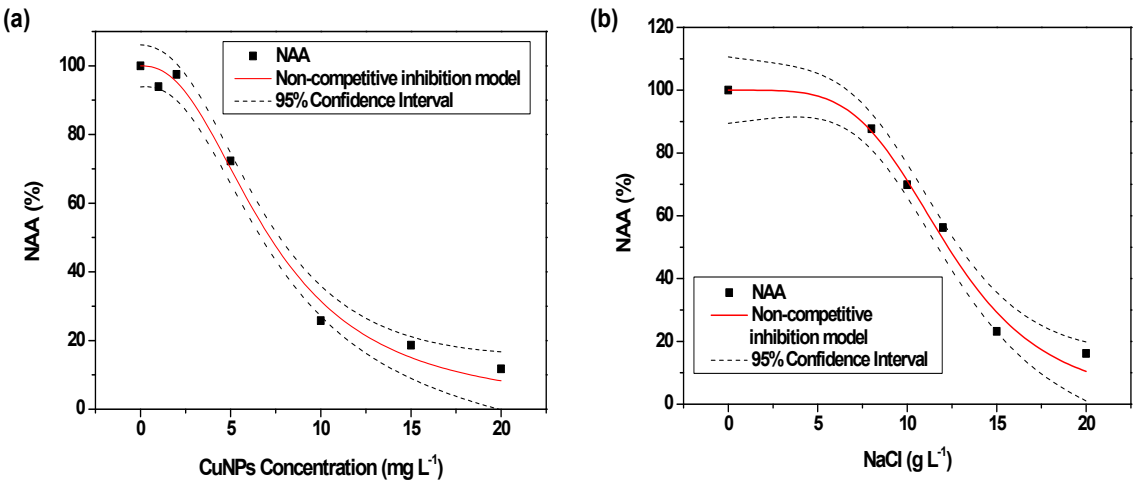

Fig. S1. Non-competitive inhibition model for the response of normalized anammox activity (NAA, %) to different exposure levels of CuNPs (a) and NaCl (b).

Fig. S2. Equivalent effect picture of the joint toxicity of NaCl and CuNPs. a_1 and a_2 constitute the 95% confidence interval of the IC_{50} of NaCl, whereas b_1 and b_2 constitute the 95% confidence interval of the IC_{50} of CuNPs.

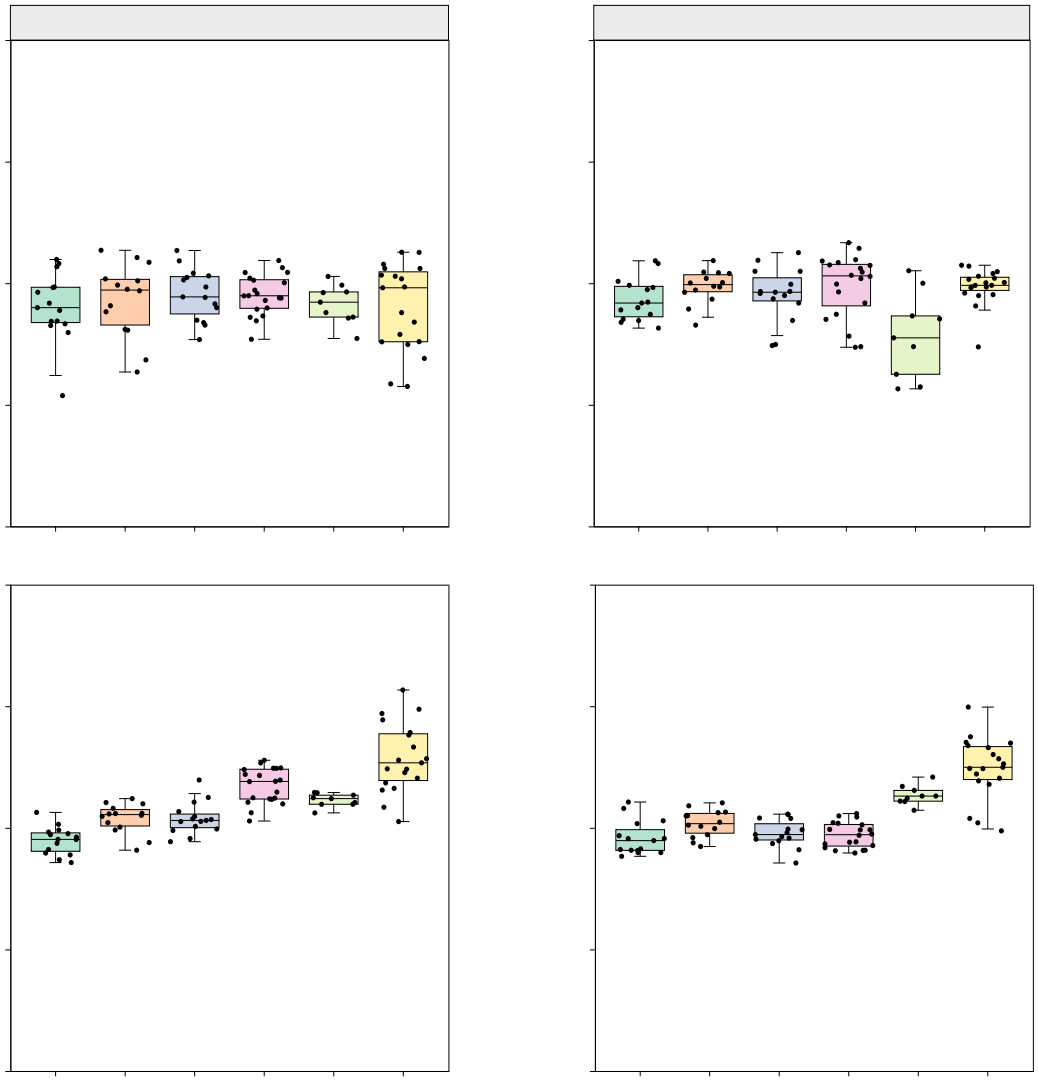

Fig. S3. The molar ratios of substrate reaction in the anammox biomass were analysed during different phases, and the estimated contribution of anammox bacteria to nitrogen loss was determined using R_S (NO_2^--N conversion/NH_4^+-N depletion) and R_P (NO_3^--N production/NH_4^+-N depletion).