Supporting Information

Species-dependent eco-corona dictates the aggregation of black phosphorus nanosheets: the role of protein and calcium

Linfeng Wei,[ab] Qing Zhang,[ad] Xingwang Hou,[ab] Guangbo Qu,[abc] Jiyan Liu,[*abc] Guibin Jiang[abc]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing, 100085, China

[b] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, 100049, China

[c] School of Environment, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou, 310000, China

[d] School of Environment, Beijing Normal University, Beijing, 100875, China

*Corresponding author

Tel: +86 10 62849334

Fax: +86 10 62849339

E-mail: liujy@rcees.ac.cn

Supporting information consisted of 12 pages, 11 figures, and 2 tables.

Contents:

Section 1. Experimental details

Section 1.1. Chemicals and reagents

1-Methyl-2-pyrrolidone (NMP) was purchased from J&K Scientific Ltd. (Beijing, China). NaCl, $CaCl_2 \cdot 2H_2O$, and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, USA) was used to determine the exudate protein concentrations. Amicon Ultra-15 centrifugal filters (Millipore, Darmstadt, Germany) with 3 kDa molecular weight cutoff were used to divide the root exudate into fractions containing different molecules. The water used in this study was ultrapure water (18.2 MΩ cm) prepared by a Milli-Q Advantage A10 system (Billerica, MA, USA).

Section 1.2. Preparation and characterization of BPNSs

BP crystals were thoroughly ground in NMP at a concentration of 1 g L^{-1}, followed by sonication with a power of 300 W for 10 h at temperature below 30°C. The dispersion solution was centrifuged at 4000 rpm for 15 min and 7000 rpm for 15 min in sequence. The resulting supernatant (containing BPNSs in NMP) was stored at 4°C as the stock solution of BPNSs.

The hydrodynamic size and zeta potential of the BPNSs were determined by a Zetasizer Nano ZS (Malvern, UK). The zeta potential is given as the average of three determinations, and each determination contained 20 measurements. X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific, USA), atomic force microscopy (AFM, Bruker, Germany) and high-resolution transmission electron microscopy (HR-TEM, JEM-2100F, Japan) were employed to characterize the surface property, thickness, and morphology of BPNSs, respectively.

Section 1.3. Germination and cultivation of rice, soybean, and pumpkin seedlings

Rice seeds were germinated by soaking in deionized (DI) water at 30°C for 3 days in the dark. The germinated seeds were cultivated on a floating net for 2 weeks and then transferred to 50-mL brown glass flasks for further 1-week cultivation with half strength and full strength Hoagland nutrient solution. Soybean seeds were first immersed in ultrapure water for 8 h, transplanted to sterile perlite beds with ultrapure water, and cultivated for 7 days to obtain seedlings. Pumpkin seeds were soaked in ultrapure water and kept moist for 2 days for germination. The germinated seeds were transplanted to a sterile perlite bed and watered with ultrapure water for 7 days. All the seedlings were cultivated in an illumination growth chamber at 28°C for 16 h and at 25°C for 8 h for light and dark treatments, respectively.

Section 1.4. Identification of exudate protein adsorbed on the surface of the BPNSs

The pellet sample was mixed with 2% sodium dodecyl sulfate (SDS), 20 mM Tris HCl, 10 mM dithiothreitol (DTT), and heated to 100°C to elute the adsorbed protein for 15 min. This mixture was centrifuged, and the supernatant was embedded in a gel composed of 10% polyacrylamide, 0.1% ammonium persulfate (APS), and 0.01% N,N,N',N'-tetramethylethylenediamine (TEMED). The gel was excised into pieces of 1 mm^3 after coagulation. All of the excised gel pieces were washed twice with ultrapure water and twice with 50% acetonitrile in water. Then, the gel pieces were dehydrated with acetonitrile until the color of the gel turned white, and the acetonitrile was subsequently removed under vacuum. The sample was incubated with 10 mM DTT at 56°C for 1 h. Excess DTT was discarded, and the gel pieces were placed in iodoacetamide (IAM, 55 mM) in the dark for 1 h.

After the excess IAM was removed, the sample was washed twice with NH_4HCO_3 (25 mM) for 10 min each time. The excess NH_4HCO_3 was discarded, and the sample was washed twice with 50% acetonitrile in water for 10 min each time, dehydrated with acetonitrile, and dried under vacuum. Subsequently, the gel pieces were placed in trypsin solution (0.1 mg mL^{-1}, 15×, diluted with NH_4HCO_3) and

digested for 16 h at 37°C. After the digestion, peptides were extracted with NH_4HCO_3 (50 mM, 50 μL), formic acid in H_2O (0.1%, 50 μL), formic acid in acetonitrile (0.1%, 50 μL), and acetonitrile (50 μL) in turn by centrifugation. All the extraction solutions were combined, freeze-dried, and dissolved in formic acid in H_2O (0.1%).

The peptide samples were analyzed by LC-MS/MS using the Ultimate 3000 Nano LC system (Dionex, USA) coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). A reversed phase C_{18} column (5 μm, 300 Å, Agela Technologies) was used for the separation of peptides. Gradient elution mode was used with 5–80% acetonitrile in 0.1% formic acid over 48 min at a flow rate of 400 nL min^{-1}.

A full MS scan was performed in a data-dependent manner in positive ion mode from 350 to 2000 *m*/*z* with a resolution of 70,000. In MS/MS mode, higher collision energy dissociation (HCD) was employed with a resolution of 17,500. The mass spectral data obtained from LC-MS/MS were analyzed by searching against the tiger_rice protein database (66,339 sequences and 29,610,149 residues). Peptide identification was performed with two missed cleavages allowed. The precursor mass and fragment mass tolerance were 15 ppm and 20 mmu, respectively. The identified rice exudate proteins on the surface of the BPNSs (peptide score > 27 searched in the Mascot database) are listed in Table S3. Protein databases such as UniProt and String were used for target protein matching.

Section 2. Figures and tables

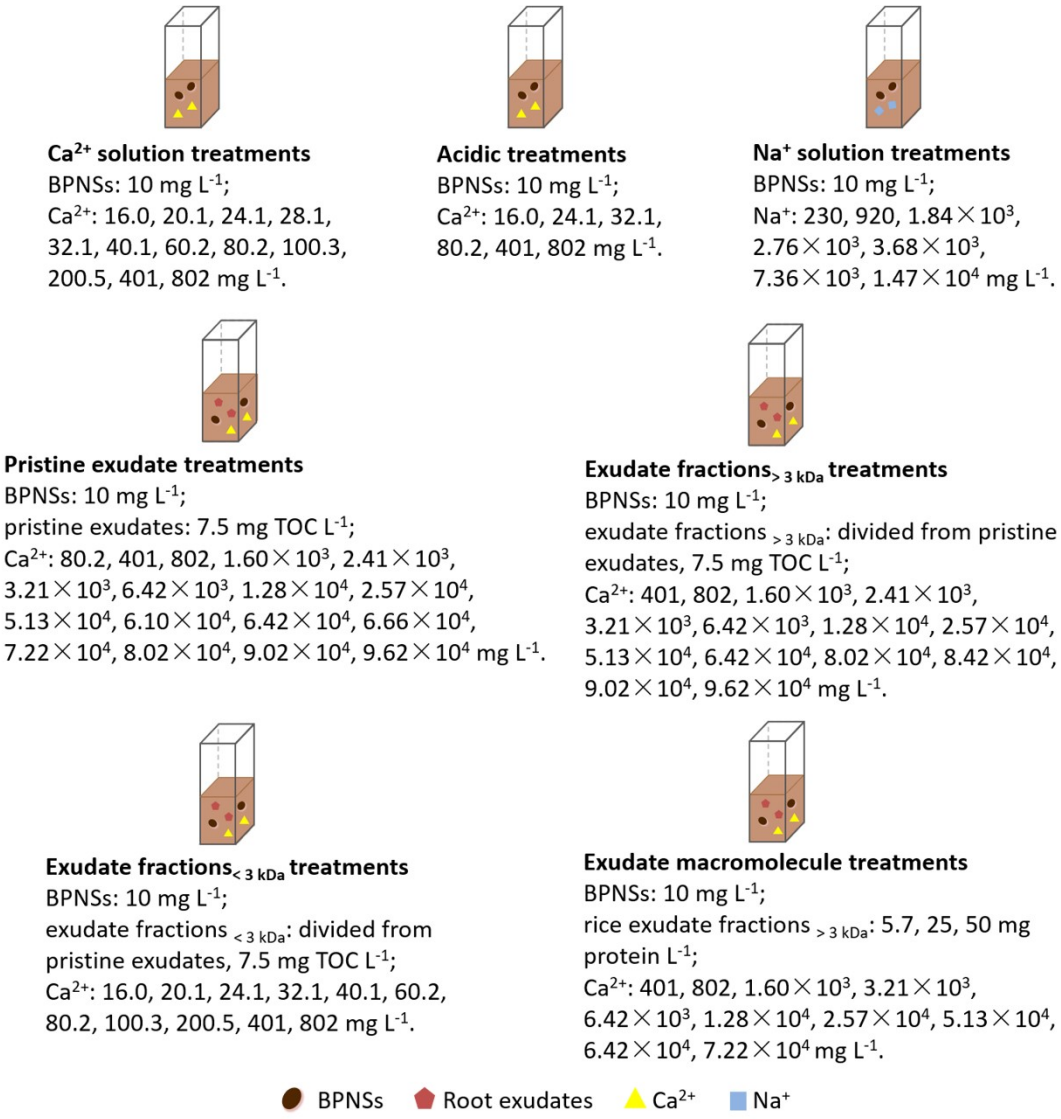

Fig. S1 Scheme of the treatment sets for the aggregation kinetics study of BPNSs. Experiments using the root exudates of rice, pumpkin, and soybean were individually conducted.

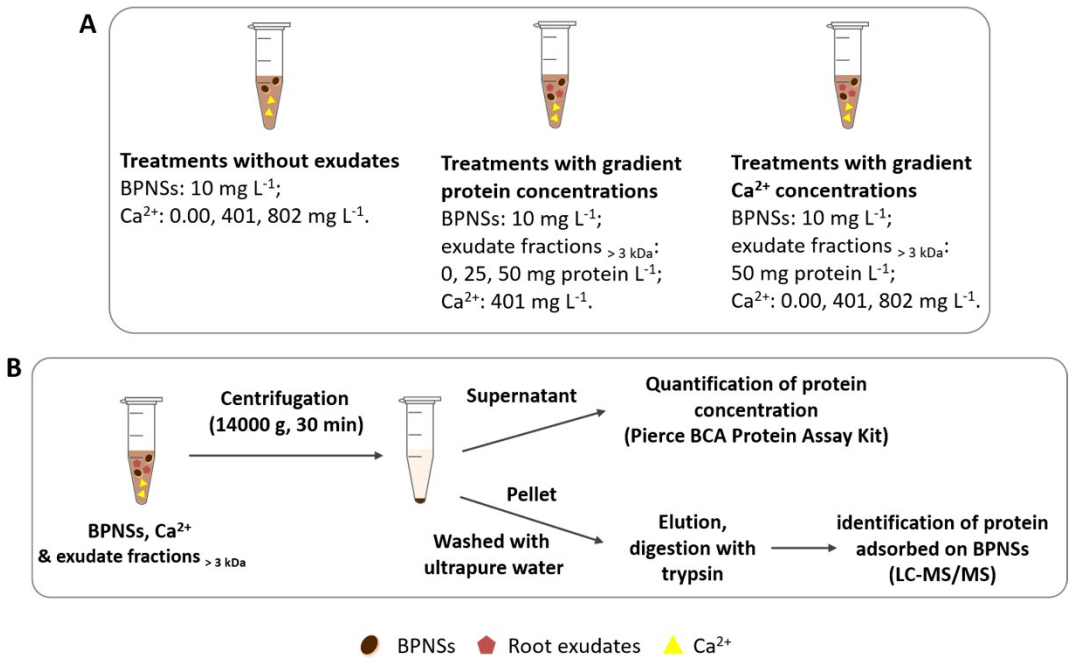

Fig. S2 Scheme of (A) treatments used to quantify protein adsorbed to BPNSs, and (B) experimental procedures for the quantification and identification of protein adsorbed on BPNSs. The experiments using root exudates of rice, pumpkin, and soybean were individually conducted.

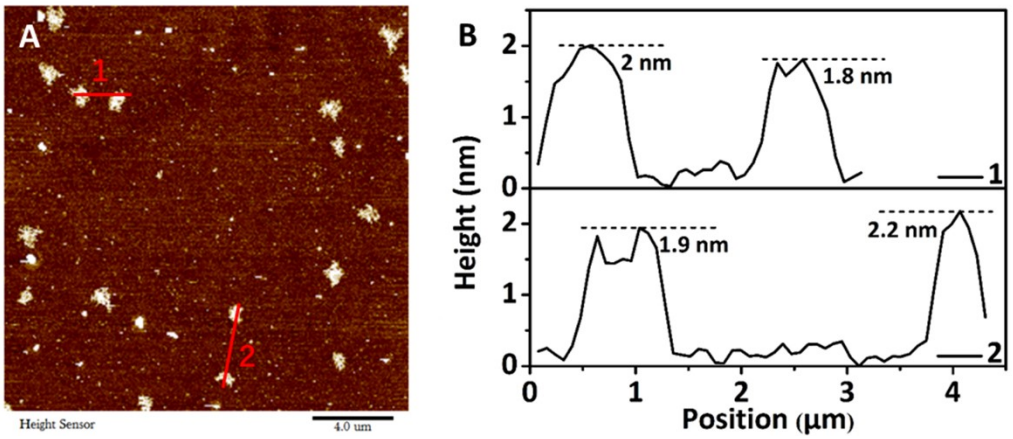

Fig. S3 Thickness characterization of BPNSs: (A) representative AFM image, and (B) thickness corresponding to the red line in (A).

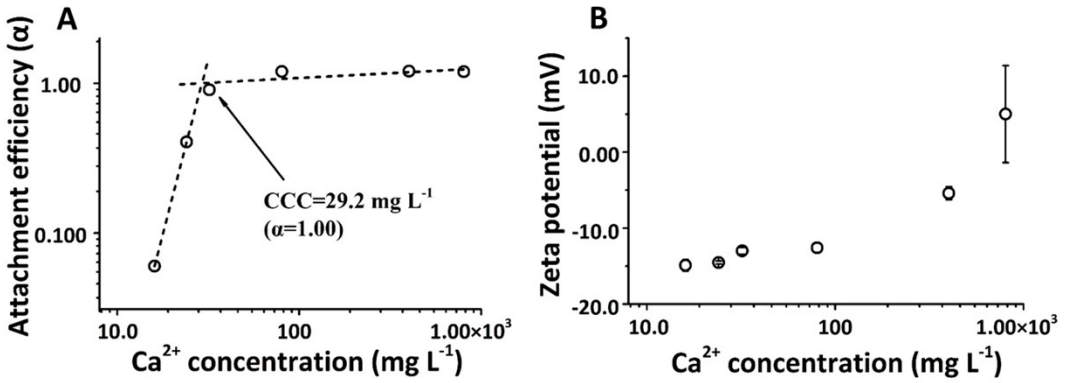

Fig. S4 Variations of (A) attachment efficiency (α) and (B) zeta potential of BPNSs over Ca^{2+} concentrations in HCl solution (pH 5.25) without root exudates.

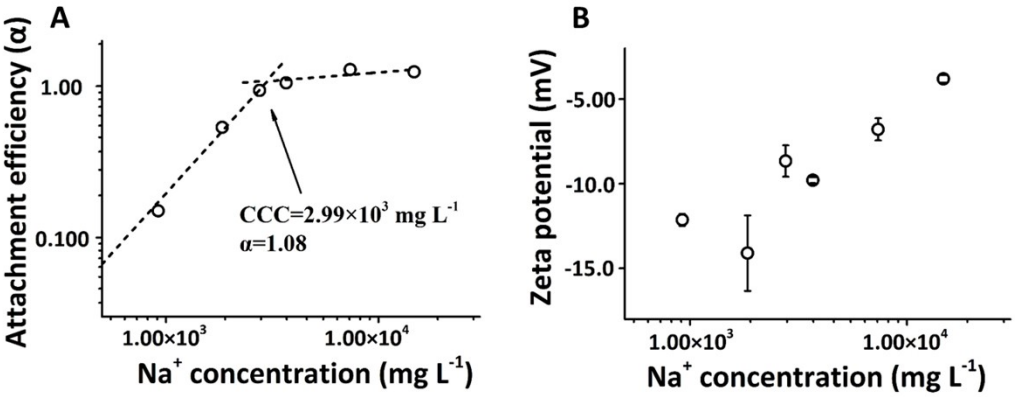

Fig. S5 (A) The attachment efficiency (α) and (B) zeta potential of BPNSs changed over Na^{+} concentrations without root exudates.

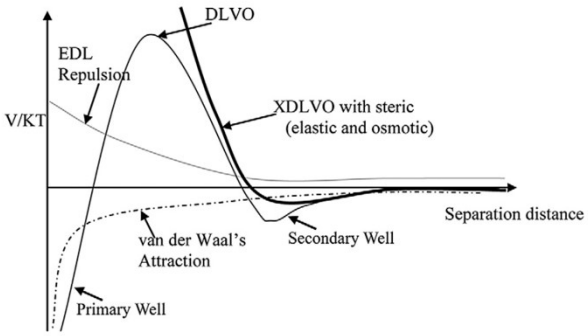

Fig. S6 Potential energy of particles as functions of separation distance. Electrostatic double layer (EDL) force (gray line) and van der Waals force (dashed line) were included in the DLVO (Derjaguin-Landau-Verwey-Overbeak) theory. Steric force was plotted together to determine the total potential energy as a function of separation distance in XDLVO (extended DLVO) theory. V/KT = potential energy divided by Boltzmann's constant and absolute temperature. In the plot, the potential energy (V) was negative or positive and illustrated that the forces between particles were net attractive or net repulsive, respectively. [1]

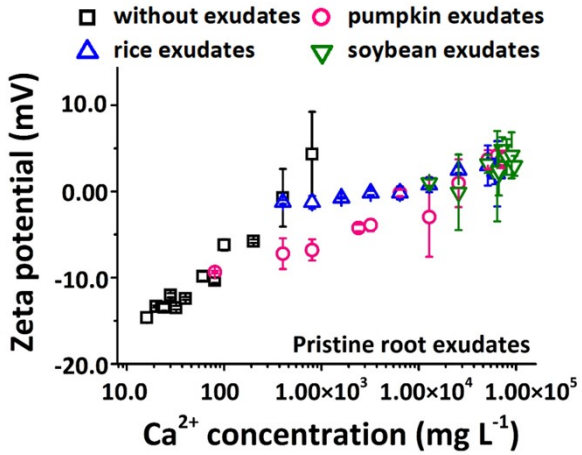

Fig. S7 The zeta potential of the BPNSs varied over Ca^{2+} concentrations with the absence and presence of root exudates (pumpkin, rice, and soybean).

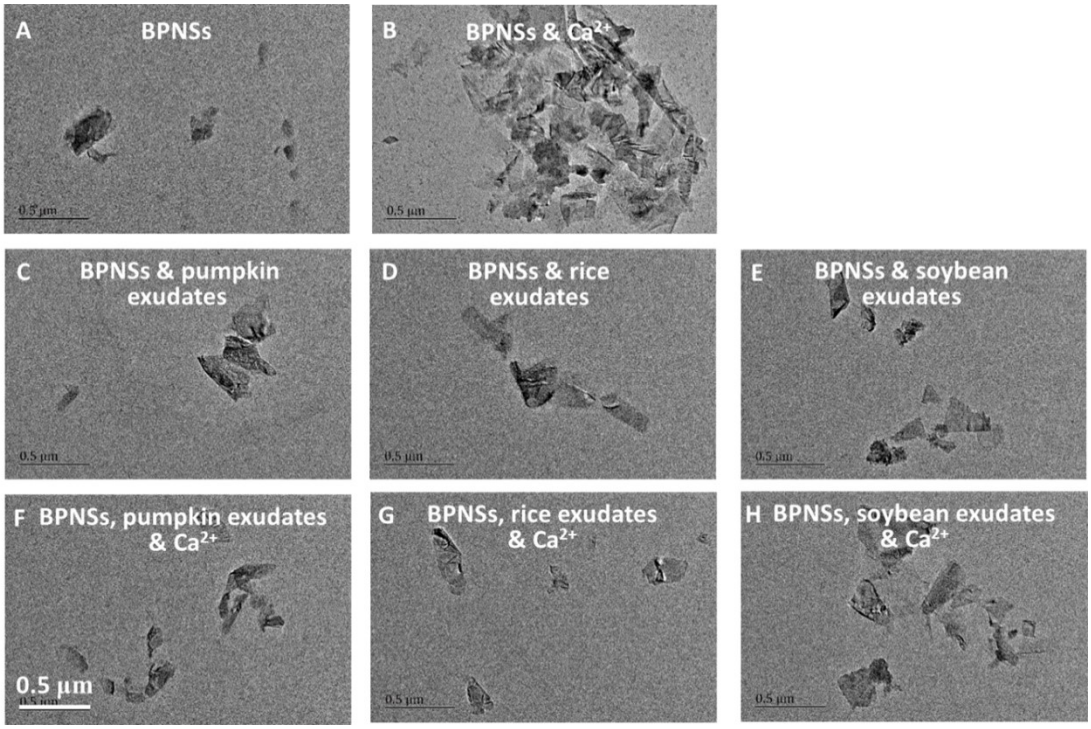

Fig. S8 Representative HR-TEM images of BPNSs after incubation for 30 min (A) without Ca^{2+} and exudates, (B) with only Ca^{2+} (40.1 mg L^{-1}), (C–E) with only root exudates, and (F–H) with both Ca^{2+} (40.1 mg L^{-1}) and pristine exudates, in which (C, F) are pumpkin, (D, G) are rice, and (E, H) are soybean.

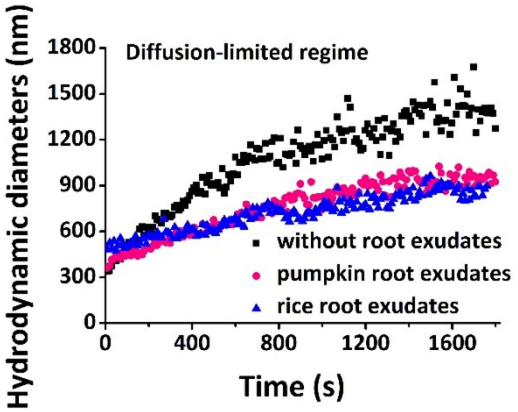

Fig. S9 Hydrodynamic diameters of BPNSs changed over time in rice and pumpkin exudates and without exudates with 2.57×10^4 mg L^{-1} of Ca^{2+}.

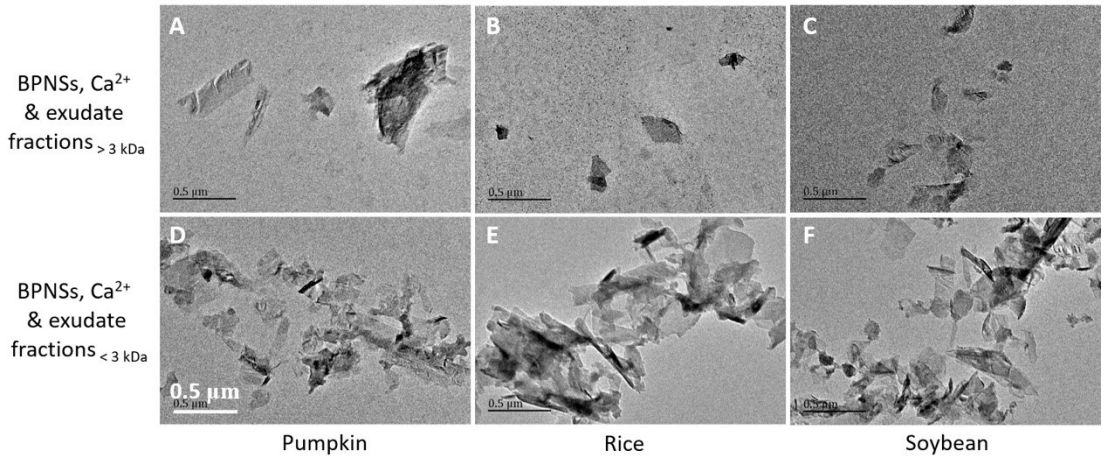

Fig. S10 Representative HR-TEM images of BPNSs in 40.1 mg L^{-1} Ca^{2+} solution with occurrences of (A–C) exudate fractions $_{> 3\ kDa}$, and (D–F) exudate fractions $_{< 3\ kDa}$, in which (A, D) are pumpkin, (B, E) are rice, and (C, F) are soybean. Each mixed solution was incubated for 30 min.

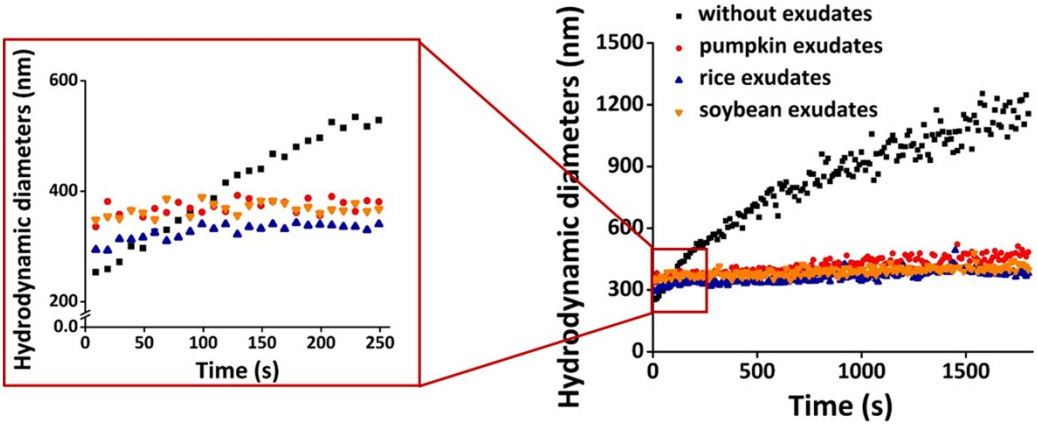

Fig. S11 Hydrodynamic diameters of BPNSs changed over time with or without root exudates (Ca^{2+} 802 mg L^{-1}).

Table S1. pH, total organic carbon (TOC), and protein concentrations of various root exudates of rice, pumpkin, and soybean. (Stock solutions were the root exudates collected after the primary centrifugation. Working solutions were obtained by diluting the stock exudate solutions to 15 mg TOC L^{-1}.)

	Rice	Pumpkin	Soybean
pH of working solution	5.25 ± 0.01	5.76 ± 0.02	6.07 ± 0.02
TOC of stock solution (mg TOC L^{-1})	53.0 ± 33.9	50.7 ± 9.93	35.3 ± 27.7
TOC of fraction $_{>3kDa}$ in working solution (mg TOC L^{-1})	6.51 ± 0.74	6.10 ± 0.13	9.44 ± 0.93
TOC of fraction $_{<3kDa}$ in working solution (mg TOC L^{-1})	8.43 ± 0.38	8.17 ± 0.22	6.73 ± 0.49
Protein concentration in working solution (mg L^{-1})	17.1 ± 0.71	15.4 ± 0.40	24.8 ± 1.11

Table S2. Hydrodynamic sizes of BPNSs in HR-TEM samples (nm)

	BPNSs	BPNSs and exudates	BPNSs, Ca^{2+} and exudates	BPNSs, Ca^{2+} and exudate fractions$_{>3kDa}$	BPNSs, Ca^{2+} and exudate fractions$_{<3kDa}$	BPNSs and Ca^{2+}
No exudates	209 ± 3.58					1312 ± 84.9
Pumpkin		221 ± 5.61	369 ± 6.29	335 ± 7.21	1270 ± 118	
Rice		227 ± 1.24	305 ± 5.13	313 ± 1.82	1144 ± 63.6	
Soybean		249 ± 4.84	356 ± 8.13	356 ± 11.6	1219 ± 74.4	

Reference

1. E. M. Hotze, T. Phenrat and G. V. Lowry, Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment, *J. Environ. Qual.*, 2010, **39**, 1909-1924.