Supplementary materials

Nanoplastic Aggravates CH_4 and N_2O Emission in Plant-Soil System

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Construction of the flooded soil-reed system; Photographs of the plant–soil system setup and the gas collection chambers; Conversion relationship between reed plant height and plant biomass; Detailed steps for preparation of PS-NPs; PS-NPs under a scanning electron microscope (SEM); Detailed steps for SOD activity, POD activity and MDA content Assay Kit; Sample processing and testing steps for root exudates; Air temperature, soil temperature, light intensity, plant biomass, and gas emissions; Physical and chemical properties of wetland soils.

Text SI-1. Detailed steps for preparation of PS-NPs

1. Add 900 L of deionized water to a three-neck flask. Purge with N_2 gas and heat to 80°C. Turn on the cooling water and set the stirring speed to 800 rpm.

2. Weigh 4.0 g of sodium dodecyl sulfate (SDS) and 4.0 g of potassium persulfate (KPS) separately. Dissolve each in 40 mL of the preheated water, then add both solutions to the flask after complete dissolution.

3. Place 6 mL of styrene (containing 5% divinylbenzene) in a 10 mL centrifuge tube, add 3 mL of n-butanol, and mix well. Gradually add this mixture dropwise to the flask and react for 60 minutes.

4. In a brown Erlenmeyer flask, combine 100 mL of styrene and 6 mL of divinylbenzene. Add 5 g of basic alumina and shake for 10 minutes. Allow the mixture to settle, then add it all at once to the main flask after completing the previous step.

5. After 60 minutes, add 2 mL of acrylic acid and raise the temperature to 85°C.

6. After another 120 minutes, stop heating and cool the flask.

7. Pour the cooled solution into a dialysis bag and place it in deionized water, changing the water twice daily for 3-5 days.

8. Adjust the pH of the PS-NPs solution to match that of the wetland soil using NaOH.

9. Repeat the above procedure until the total amount of PS-NPs meets the experimental requirements.

Text SI-2. Superoxide Dismutase(SOD) Activity Assay Kit

Determination of Significance: Superoxide dismutase (SOD, EC 1.15.1.1) is a kind of metalloenzyme widely found in organism. It is an important oxygen radical scavenger and can catalytic disproportionation of superoxide anion to form H_2O_2 and O_2. SOD is not only the superoxide anion scavenging enzyme, but also the main H_2O_2 producing enzyme, which plays an important role in the biological antioxidant system.

Measurement Principle: Superoxide anion (O_2^-) is produced by the xanthine and xanthine oxidase reaction system. O_2^- can reduce blue tetrazole to form blue formazan, which has absorbance in 560 nm. SOD can remove O_2^- and inhibit the formation of methionine. The darker the blue color of the reaction solution, the lower the SOD activity. The lighter the blue color of the reaction solution, the higher the activity of SOD.

Procedure: Weigh 0.1g of tissue and add 1mL of extraction solution. Extract thoroughly under liquid nitrogen. Centrifuge at 8000g for 10 minutes at 4°C, and collect the supernatant. Place it on ice for measurement. Preheat the microplate reader for at least 30 minutes and set the wavelength to 560nm. Incubate all reagents at 25°C for more than 5 minutes before measurement. Using Solarbio reagents, measure 20μL of each sample for both the test tube and the control tube, along with blank tubes 1 and 2, ensuring the total volume in each tube is 0.2mL. Mix thoroughly and incubate in a 37°C water bath for 30 minutes. Measure the absorbance at 560nm for each tube. When the inhibition percentage in the xanthine oxidase coupling reaction system is 50%, the SOD enzyme activity in the reaction system is defined as one unit of enzyme activity.

Calculation:

∆A Test = A Test - A Control

∆A Blank = A1 Blank - A2 Blank

Inhibition Percentage = (∆A Blank - ∆A Test) / ∆A Blank × 100%

SOD Activity (U/g tissue) = 10 × Inhibition Percentage / [(1 - Inhibition Percentage) × 0.1]

Text SI-3. Peroxidase(POD) Activity Assay Kit

Determination of Significance: Peroxidase (POD, EC 1.11.1.7) widely exists in animals, plants and microorganisms. It can catalyzes the oxidation of phenols and amines by hydrogen peroxide, and has the dual effect of eliminating toxicity of hydrogen peroxide, phenols and amines.

Measurement Principle: In the presence of hydrogen peroxide, POD can catalyzes H_2O_2 oxidize specific substrates to produce one substance which has a absorption at 470 nm.

Procedure: Weigh 0.1g of tissue and add 1mL of extraction solution. Extract thoroughly under liquid nitrogen. Centrifuge at 8000g for 10 minutes at 4°C, and collect the supernatant. Place it on ice for measurement. Preheat the microplate reader for at least 30 minutes and set the wavelength to 470nm. Incubate all reagents at 25°C for more than 10 minutes before measurement. In an EP tube, sequentially add 120μL of Solarbio Reagent 1, 30μL of Reagent 2 working solution, 30μL of Reagent 3, 60μL of distilled water, and 5μL of sample. Mix immediately and start timing. Immediately transfer 200μL to a 96-well plate. Record the absorbance at 470nm at 30 seconds (A1) and at 1 minute 30 seconds (A2). The change in absorbance at 470nm by 0.005 per minute per gram of tissue in the reaction system is defined as one unit of enzyme activity.

Calculation Method:

$\Delta A = A2 - A1$

POD Activity (U/g tissue) = $9800 \times \Delta A / 0.1$

Text SI-4. Malondialdehyde(MDA) Content Assay Kit

Determination of Significance: Lipid peroxide is produced by the action of oxygen free radicals on unsaturated fatty acid, then resolves to compounds, including malondialdehyde (MDA). The level of lipid peroxidation can be showed by detecting the level of MDA.

Measurement Principle: Under acidic and high temperature conditions, the brown red 3,5,5- three methyl sulfamethoxazole -2,4-two ketone is synthesized with MDA and thiobarbituric acid (TBA) taking place condensation reaction, and the largest absorption wavelength is 532 nm. After colorimetry, the MDA content in the sample can be estimated.

Procedure: Weigh 0.1g of tissue and add 1mL of extraction solution. Extract thoroughly under liquid nitrogen. Centrifuge at 8000g for 10 minutes at 4°C, and collect the supernatant. Place it on ice for measurement. Preheat the microplate reader for at least 30 minutes. In the test tube, mix 100μL of each sample, 300μL of detection working solution, and 100μL of Reagent 3. In the blank tube, mix 100μL of distilled water, 300μL of detection working solution, and 100μL of Reagent 3. Incubate the mixture in a 100°C water bath for 60 minutes (ensure the tubes are tightly covered to prevent water loss), then cool in an ice bath. Centrifuge at 10000g at room temperature for 10 minutes. Transfer 200μL of the supernatant to a micro glass cuvette or a 96-well plate, and measure the absorbance of each sample at 532nm and 600nm.

Calculation Method:

$\Delta A532 = A532\ Test - A532\ Blank$

$\Delta A600 = A600\ Test - A600\ Blank$

$\Delta A = \Delta A532 - \Delta A600$

$MDA\ Content\ (nmol/g\ tissue) = 53.763 \times \Delta A / 0.1$

Text SI-5. Sample processing and testing steps for root exudates

The samples (1 mL) were freeze-dried and resuspended with prechilled 80% methanol by well vortex. Then the samples were incubated on ice for 5 min and centrifuged at 15,000 g, 4°C for 15 min. Some of supernatant was diluted to final concentration containing 53% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and thenwere centrifuged at 15000 g, 4°C for 15 min. Finally, the supernatant was injected into the LC-MS/MS system analysis. UHPLC-MS/MS analyses were performed using a Vanquish UHPLC system (ThermoFisher, Germany) coupled with an Orbitrap Q ExactiveTM HF mass spectrometer or Orbitrap Q ExactiveTMHF-X mass spectrometer (Thermo Fisher, Germany) in Novogene Co., Ltd. (Beijing, China). The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.3 (CD3.3, ThermoFisher) to perform peak alignment, peak picking, and quantitation for each metabolite. And then peaks were matched with the mzCloud (https://www.mzcloud.org/), mzVault and MassList database to obtain the accurate qualitative and relative quantitative results. These metabolites were annotated using the KEGG database (https://www.genome.jp/kegg/pathway.html)

Table S1. Air temperature, soil temperature, light intensity, plant biomass, and gas emissions.

				Control			200 ppm			1000 ppm			1500 ppm		
1st Phase	Air temp.	0 ℃	Plant biomass	0	0	0	0	0	0	0	0	0	0	0	0
	Soil temp.	8 ℃	CH_4	0.022	0.064	0.038	0.077	0.061	0.044	0.054	0.067	0.041	0.061	0.054	0.086
	Light intensity	325 μmol/m^2s	N_2O	0.000	0.018	0.008	0.007	0.015	0.002	0.005	0.016	0.018	0.020	0.021	0.012
	Air temp.	6 ℃	Plant biomass	1.2	9.7	1.5	5.9	0.8	2.5	6.4	1	4.4	3.7	0.32	3
	Soil temp.	12 ℃	CH_4	0.079	0.103	0.052	0.072	0.074	0.070	0.078	0.074	0.081	0.057	0.098	0.063
	Light intensity	817 μmol/m^2s	N_2O	0.006	0.009	0.017	0.005	0.022	0.028	0.022	0.029	0.017	0.025	0.015	0.020
	Air temp.	12 ℃	Plant biomass	15.9	22.7	18.6	11.3	12.6	7	14.1	6.7	11.1	7.5	3.5	6.9
	Soil temp.	16 ℃	CH_4	0.162	0.255	0.205	0.131	0.226	0.161	0.120	0.171	0.173	0.123	0.109	0.129
	Light intensity	651 μmol/m^2s	N_2O	0.006	0.009	0.010	0.012	0.026	0.015	0.018	0.019	0.011	0.010	0.003	0.020
	Air temp.	13 ℃	Plant biomass	26.06	34.64	27.77	25.59	35.02	12.92	29.06	17.64	21.6	14.1	6.42	18.41
	Soil temp.	15 ℃	CH_4	0.244	0.247	0.324	0.207	0.281	0.205	0.233	0.206	0.195	0.177	0.158	0.175
	Light intensity	1120 μmol/m^2s	N_2O	0.004	0.021	0.013	0.005	0.017	0.012	0.014	0.012	0.011	0.023	0.017	0.012
	Air temp.	18 ℃	Plant biomass	112.2	108	107	80.61	88.72	58.8	50.51	22.28	84.07	54.06	13.67	60.05
	Soil temp.	18 ℃	CH_4	0.273	0.291	0.253	0.247	0.395	0.230	0.224	0.307	0.402	0.255	0.329	0.168
	Light intensity	1657 μmol/m^2s	N_2O	0.002	0.007	0.019	0.006	0.031	0.012	0.018	0.016	0.003	0.001	0.022	0.032
	Air temp.	27 ℃	Plant biomass	129.4	152.1	124.8	119.8	125.2	114.3	158.5	80.74	148.9	99.04	50.61	142.2
	Soil temp.	20 ℃	CH_4	0.278	0.394	0.251	0.336	0.404	0.345	0.445	0.376	0.393	0.411	0.348	0.418
	Light intensity	1223 μmol/m^2s	N_2O	0.021	0.012	0.010	0.005	0.044	0.042	0.051	0.027	0.018	0.042	0.040	0.024

Table S1. Air temperature, soil temperature, light intensity, plant biomass, and gas emissions.

				Control			200 ppm			1000 ppm			1500 ppm		
1st Phase	Air temp.	28 ℃	Plant biomass	175.8	173.2	162.5	178.9	172.2	174	178.8	148.3	176.2	169.1	122.1	169.9
	Soil temp.	22 ℃	CH_4	0.324	0.397	0.273	0.384	0.467	0.349	0.418	0.414	0.474	0.526	0.359	0.565
	Light intensity	1227 μmol/m^2s	N_2O	0.012	0.006	0.016	0.036	0.018	0.039	0.047	0.026	0.029	0.034	0.016	0.049
	Air temp.	29 ℃	Plant biomass	203.8	203.1	192.9	199.4	197.8	200.3	207.5	177.2	206.2	205.3	164.4	199.8
	Soil temp.	24 ℃	CH_4	0.366	0.385	0.376	0.549	0.476	0.452	0.614	0.535	0.552	0.641	0.563	0.619
	Light intensity	1635 μmol/m^2s	N_2O	0.024	0.006	0.007	0.020	0.045	0.042	0.032	0.042	0.035	0.037	0.046	0.052
2nd Phase	Air temp.	33 ℃	Plant biomass	226.5	216.6	223.7	221.3	222.9	208.7	224.4	215.9	216.4	220.1	213.5	223.5
	Soil temp.	26 ℃	CH_4	0.455	0.440	0.414	0.473	0.575	0.509	0.658	0.723	0.623	0.728	0.700	0.784
	Light intensity	1760 μmol/m^2s	N_2O	0.004	0.031	0.013	0.050	0.038	0.029	0.041	0.047	0.036	0.042	0.038	0.054
	Air temp.	34 ℃	Plant biomass	228.5	218.6	225.7	223.3	224.9	213.7	226.4	219.9	220.4	222.1	216.5	225.5
	Soil temp.	28 ℃	CH_4	0.403	0.521	0.417	0.523	0.583	0.510	0.754	0.836	0.700	0.743	0.717	0.791
	Light intensity	1536 μmol/m^2s	N_2O	0.022	0.021	0.024	0.030	0.034	0.050	0.045	0.051	0.035	0.037	0.045	0.052
	Air temp.	36 ℃	Plant biomass	229.5	220.6	227.7	225.3	226.9	216.7	228.4	221.9	222.4	224.1	218.5	227.5
	Soil temp.	30 ℃	CH_4	0.455	0.555	0.409	0.561	0.573	0.562	0.827	0.955	0.663	0.762	1.115	0.858
	Light intensity	1477 μmol/m^2s	N_2O	0.024	0.035	0.022	0.034	0.049	0.045	0.032	0.058	0.031	0.056	0.034	0.041

Table S2. Physical and chemical properties of wetland soils in different groups

	Control			200 mg/kg			1000 mg/kg			1500 mg/kg		
	1	2	3	1	2	3	1	2	3	1	2	3
ORP(mV)	-61	9.3	-33.7	0.6	-16.5	-5.2	-7.9	-29.8	9.4	-61.9	-17.3	-12.9
pH	7.83	7.93	7.94	7.84	7.89	7.97	7.86	8.03	7.81	7.7	7.77	7.87
θ_m	335%	325%	315%	379%	368%	334%	350%	348%	366%	357%	344%	330%
SOC(g/kg)	5.76	5.88	5.91	5.83	5.77	5.97	5.71	5.76	5.6	5.8	5.73	5.54
SON(g/kg)	3.05	2.98	2.96	3.14	3.05	3.11	3.02	2.98	3.08	2.94	3.05	3.02

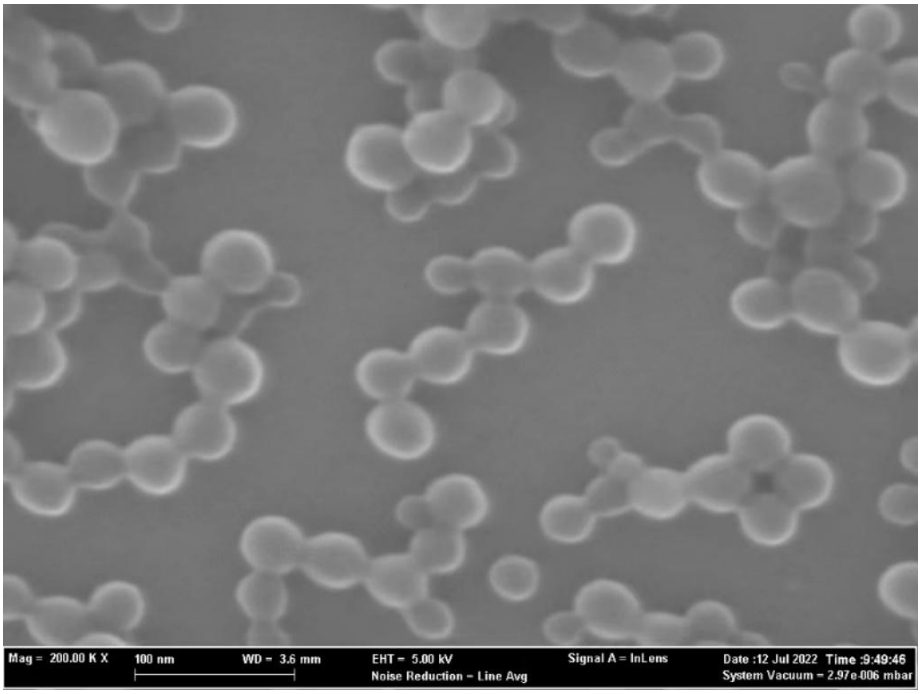

Figure S1. PS-NPs under a scanning electron microscope (SEM)

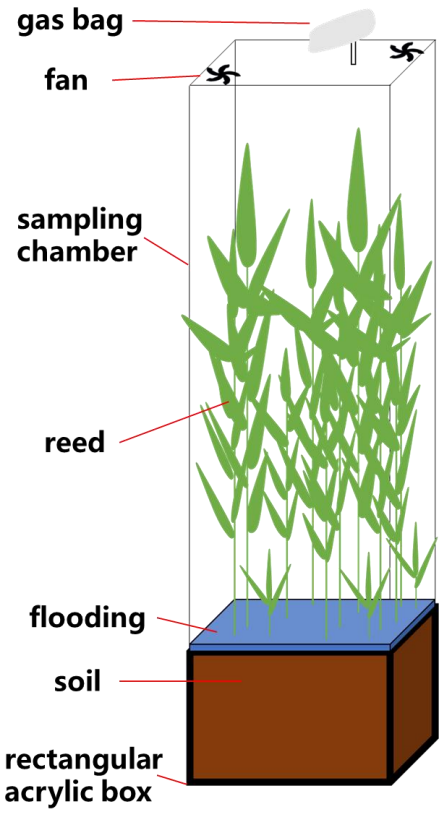

Figure S2. Construction of the flooded soil-reed system

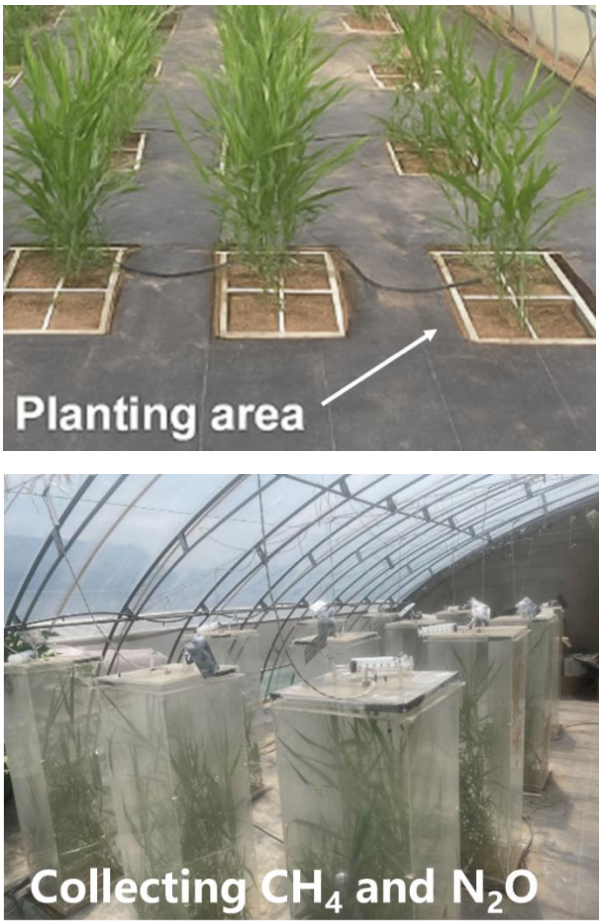

Figure S3. Photographs of the plant–soil system setup and the gas collection chambers

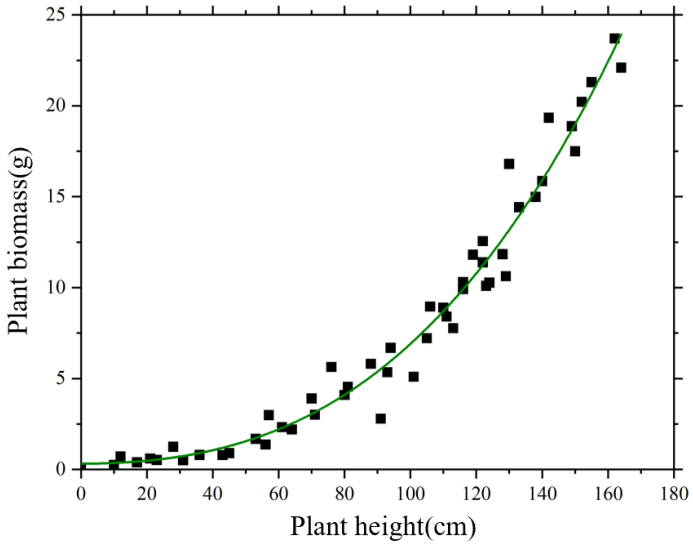

Figure S4. Conversion relationship between reed plant height and plant biomass.