Supporting Information

Zinc oxide nanoparticles cause inhibition of microbial denitrification by affecting transcriptional regulation and enzyme activity

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MATERIALS AND METHODS

Measurements of NO_3^-, NO_2^-, NO, and N_2O. This study used the spectrophotometric methods for the determinations of NO_3^- and NO_2^-. Briefly, 1 mL of sample was added in a 50-mL colorimetric tube with 49 mL of deionized (DI) water, followed by the addition of 1 mL of 1 M hydrochloric acid solution. Then, the solution was shaken thoroughly, and the absorbance of the solution and standard (sodium nitrate) was measured spectrophotometrically at 220 and 275 nm. Finally, the absorbance at 275 nm was doubled and subtracted from that at 220 nm to give the corrected nitrate absorbance using for the calculation of the nitrate concentration. For the measurement of NO_2^-, 1 mL of sample was added in a 50-mL colorimetric tube, followed by the addition of 1 mL of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthy-lethylenediamine dihydrochloride). The solution was immediately mixed by inversion and incubated at room temperature for 15 min. Then, the absorbance was determined at 540 nm, which was used to calculate the nitrite concentration. The direct microelectrode measurements of liquid-phase NO and N_2O concentrations were used in enzyme assays, and the microsensors were two-point calibrated using distilled water (zero point) and a freshly prepared 0.15 mM NO or N_2O solution according to the manufacture's instructions (Unisense, Aarhus, Denmark).

Measurements of glucose and polyhydroxybutyrate (PHB). The anthranone method was used to determine the concentration of glucose. Briefly, 5 mL of anthranone-H_2SO_4 as the color reagent was added to 1 mL of sample. The solution was boiled at 100 ^oC for 10 min, and then measured using an ultraviolet spectrophotometer (UV-1800, Shimadzu, Japan) at 620 nm. A standard linear regression was prepared using the absorbance of glucose standard solutions at 620 nm. To analyze the PHB content, the lyophilized samples were digested, methylated, and extracted with chloroform. The extracted methyl esters were analyzed using gas chromatograph (HP 4890) with a HP-5 column (length 30 m, internal

diameter 0.53 mm, film thickness 0.88 μm). Helium gas was used as the carrier gas (30 mL/min) and makeup gas. The flame ionisation detection (FID) was operated at 250 ^{o}C with an injection port temperature of 230 ^{o}C. The oven temperature was set to 80 ^{o}C for 2 min, increased at 8 ^{o}C/min to 120 ^{o}C, and then to 220 ^{o}C at 30 ^{o}C/min and held for 2 min. A calibration curve using a PHB standard (Sigma-Aldrich) was obtained according to the same procedure.

Transmission electron microscopy analysis. After exposure to 50 mg/L ZnO NPs, cells were harvested by centrifugation, washed thrice with 0.1 M PBS (pH 7.4), and fixed in the perfluorocarbon containing osmium tetroxide (1%) for 1 h. After three rinses in pure perfluorocarbon, the samples were dehydrated, embedded, sectioned, stained, and imaged with a JEM-1230 transmission electron microscope equipped with an energy-dispersive X-ray spectrometer (EDX) (JEOL, Tokyo, Japan).

Lactate dehydrogenase release assay. Cell membrane damage was estimated by the evaluation of the activity of LDH released from cells. After exposure to ZnO NPs, the culture supernatants were assayed for the LDH activity using a cytotoxicity detection kit (Roche Applied Science) according to the manufacturer's instructions. Briefly, the supernatants were seeded on a 96-well plate, followed by the addition of 50 μL of substrate mix (Roche Applied Science). After 30 min of incubation at room temperature in dark, 50 μL of stop solution (Roche Applied Science) was added to each well. Thereafter, the absorbance was recorded at 490 nm using a microplate reader (BioTek, USA).

Measurements of key trace metal ions in the absence and presence of ZnO NPs. To examine the potential effects of ZnO NPs on the concentrations of trace metal ions (especially Fe, Cu, and Mo) in the medium, a series of serum bottles that respectively contained 50 mL of the mineral medium with 0, 1, 10, and 50 mg/L ZnO NPs were shaken at 30 ^{o}C and 200 rpm. After 24 h, 5 mL of the mixture was withdrawn from each bottle, and ultrasonicated at 16000 rpm for 10 min. The supernatant was then

obtained and used for the measurements of the concentrations of Fe, Cu, and Mo ions via an ICP-MS (Agilent Technologies, USA). The serum bottle without ZnO NPs was used as the control, and the concentration of trace metal ions in other bottles was compared with that in the control.

Table S1. Summary of the clean reads mapped to the reference genomes and genes of *Paracoccus denitrificans* in the absence (Control) and presence of 50 mg/L ZnO NPs.

		Control		ZnO NPs	
		Number of reads	Percentage	Number of reads	Percentage
Total number of high-quality reads		13065246	100.00%	13708988	100.00%
Total basepairs of high-quality reads		1175872140	100.00%	1233808920	100.00%
Map to Genome	Total mapped reads	12523280	95.85%	13023029	95.00%
	Perfect matches	9776703	74.83%	9850701	71.86%
	Unique matches	11927305	91.29%	12134278	88.51%
	Multi-position matches	595975	4.56%	888751	6.48%
	Total unmapped reads	541966	4.15%	685959	5.00%
Map to Gene	Total mapped reads	6009430	46.00%	6622864	48.31%
	Perfect matches	4881975	37.37%	5171696	37.72%
	Unique matches	5770343	44.17%	6324313	46.13%
	Multi-position matches	239087	1.83%	298551	2.18%
	Total unmapped reads	7055816	54.00%	7086124	51.69%

Table S2. Key up- and down-regulated genes of *Paracoccus denitrificans* exposed to 50 mg/L ZnO NPs.

Gene ID	log_2Ratio(NPs/Control)	p-value	Gene description
NC_008688.1: c494525-493422	3.43	0	pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase)
NC_008688.1: c496534-495557	4.33	0	pyruvate dehydrogenase E1 component subunit alpha
NC_008687.1: c1026593-1025223	1.62	0	pyruvate dehydrogenase E1 component subunit beta
NC_008686.1: 16615-18417	2.59	4.58E-13	alcohol dehydrogenase (cytochrome c)
NC_008686.1: 2367955-2368989	3.70	1.35E-13	alcohol dehydrogenase, propanol-preferring
NC_008687.1: 1367233-1369002	1.40	0	polyhydroxyalkanoate synthase
NC_008688.1: 585961-587415	3.03	3.22E-14	3-hydroxybutyryl-CoA dehydrogenase
NC_008686.1: 2033477-2034652	1.23	1.64E-11	acetyl-CoA C-acetyltransferase
NC_008686.1: c877065-876043	1.92	1.55E-10	cytochrome c peroxidase
NC_008688.1: c97948-95816	1.25	2.52E-13	catalase
NC_008686.1: 1616089-1616586	1.90	0	Cu/Zn superoxide dismutase
NC_008688.1: c271791-271318	1.91	8.06E-14	4-carboxymuconolactone decarboxylase
NC_008688.1: c492587-492258	2.42	3.22E-14	4-carboxymuconolactone decarboxylase
NC_008686.1: c2409807-2408431	1.99	2.66E-15	outer membrane channel protein
NC_008686.1: c2465959-2463608	1.16	1.04E-12	type VI secretion system secreted protein VgrG
NC_008686.1: 2451018-2451506	4.19	0	type VI secretion system secreted protein Hcp
NC_008686.1: 2456373-2456846	4.54	9.33E-14	type VI secretion system protein VasD
NC_008686.1: 2458202-2459593	4.06	1.22E-05	type VI secretion system protein ImpK

NC_008686.1: c868792-867833	-1.12	1.35E-28	6-phosphofructokinase 1
NC_008687.1: 1679499-1680500	-1.26	0	6-phosphofructokinase
NC_008686.1: 1913972-1915168	-1.66	0	phosphoglycerate kinase
NC_008687.1: c784039-783440	-4.32	1.43E-23	phosphoglycerate mutase
NC_008687.1: 1213306-1214583	-1.15	1.30E-198	enolase
NC_008687.1: 700418-701539	-1.70	2.62E-76	L-lactate dehydrogenase
NC_008686.1: c2236523-2234982	-1.58	0	NADH dehydrogenase I subunit M
NC_008687.1: c199830-199462	-1.58	4.72E-08	succinate dehydrogenase iron-sulfur protein
NC_008686.1: 2305820-2307172	-2.00	0	ubiquinol-cytochrome c reductase cytochrome c1 subunit
NC_008687.1: c43895-43248	-2.42	0	F-type H^+-transporting ATPase subunit b
NC_008688.1: c387327-386548	-1.16	2.55E-15	nitrate/taurine transport system permease protein
NC_008687.1: 1296748-1297728	-3.97	0	zinc transport system substrate-binding protein
NC_008687.1: c1295404-1294622	-1.04	4.39E-14	zinc transport system permease protein
NC_008686.1: 2677055-2677759	-1.74	7.70E-31	molybdate transport system permease protein
NC_008688.1: c418851-417208	-1.29	2.51E-08	iron(III) transport system permease protein
NC_008686.1: c1335343-1334483	-4.33	0	iron complex transport system substrate binding protein
NC_008687.1: c1395057-1394356	-2.21	0	nitrate reductase 1, gamma subunit
NC_008686.1: 2507025-2508179	-2.38	0	nitrite reductase (NO-forming)
NC_008686.1: c2500374-2498986	-1.74	0	nitric oxide reductase subunit B
NC_008687.1: c1382598-1380640	-4.62	0	nitrous oxide reductase subunit Z

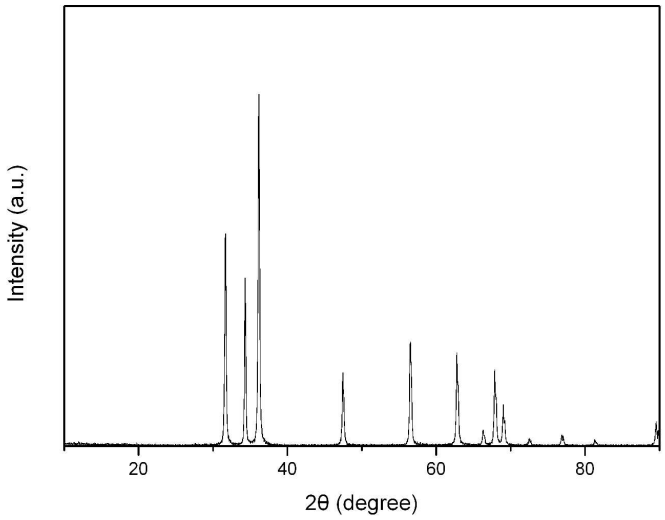

Figure S1. X-ray diffraction (XRD) pattern of ZnO NPs used in this study.

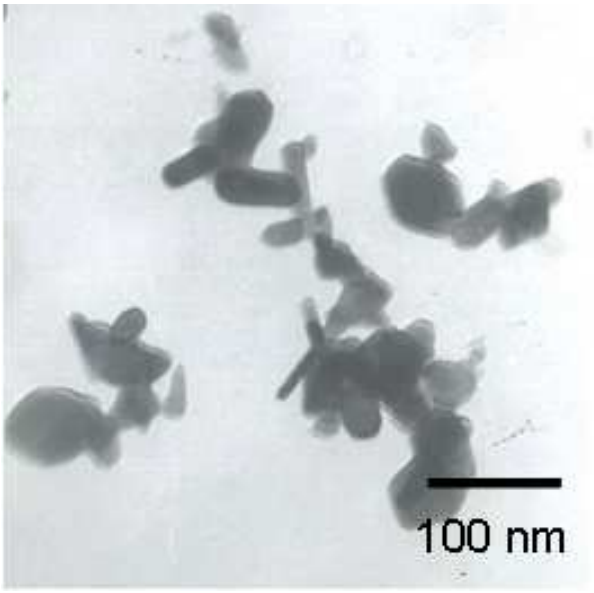

Figure S2. Transmission electron microscopy (TEM) image of ZnO NPs used in this study.

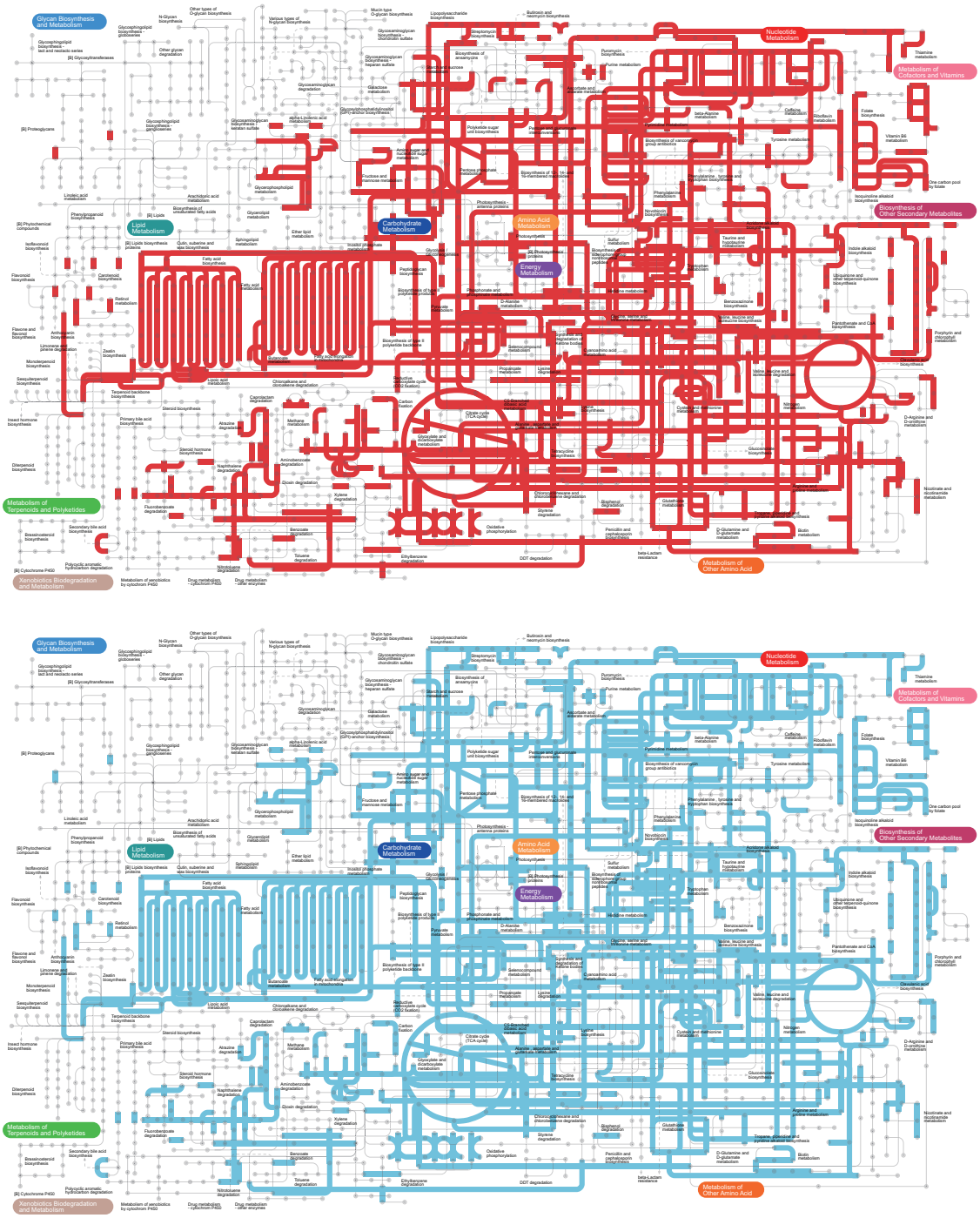

Figure S3. Projection of the global transcriptional profiling on KEGG pathways in the absence (red) and presence (blue) of 50 mg/L ZnO NPs.