Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Appendix A. Supplementary data

Sensitivity Assessment of Denitrifying Bacteria against Typical Antibiotics in Groundwater

Environmental Science Processes & Impacts

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Text. S1 Preliminary isolation of denitrifying bacteria

A total of 10 mL of mixed denitrifying bacteria, which were obtained from a long-term groundwater denitrification environment[1], was inoculated into the enrichment medium and incubated at 30°C (175 rpm) in the dark for 24 h. The enrichment fluid was serially diluted and spread onto BTB medium plates, which were incubated at 30°C for 1 to 3 d. Blue colonies were picked from the BTB medium plates, transferred to fresh BTB medium, and streaked repeatedly. Then, the enrichment medium was used to enrich the blue colonies. The turbid culture fluid was inoculated in DM with the volumetric ratio of 5%. The denitrifying bacteria were obtained by incubating at 30°C (175 rpm), and stored at -80°C for further detection.

Text. S2 The basic principle of the disk diffusion test

The disk diffusion test is suitable for qualitative assay. The basic principle of the test[2] involves placing the antibiotic-impregnated disk onto agar previously inoculated with bacteria, and producing an antibiotic concentration gradient by diffusing outward through the agar medium. The bacteria growth is suppressed and a clear zone forms around the antibiotic disk within a certain distance.

Text. S3 The basic principle of the E-test

The E-test is a quantitative assay for determining the minimal inhibitory concentration (MIC) of antibiotics against microorganisms. The test strips are impregnated with a predefined concentration gradient of an antibiotic. When the test strip is applied onto an inoculated agar surface, the preformed exponential gradient of antibiotic is immediately transferred to the agar matrix. After incubation, a symmetrical inhibition ellipse centered along the strip forms. The MIC is read directly from a scale in terms of μg·mL^{-1} at the point where the edge of the inhibition ellipse intersects the strip[3].

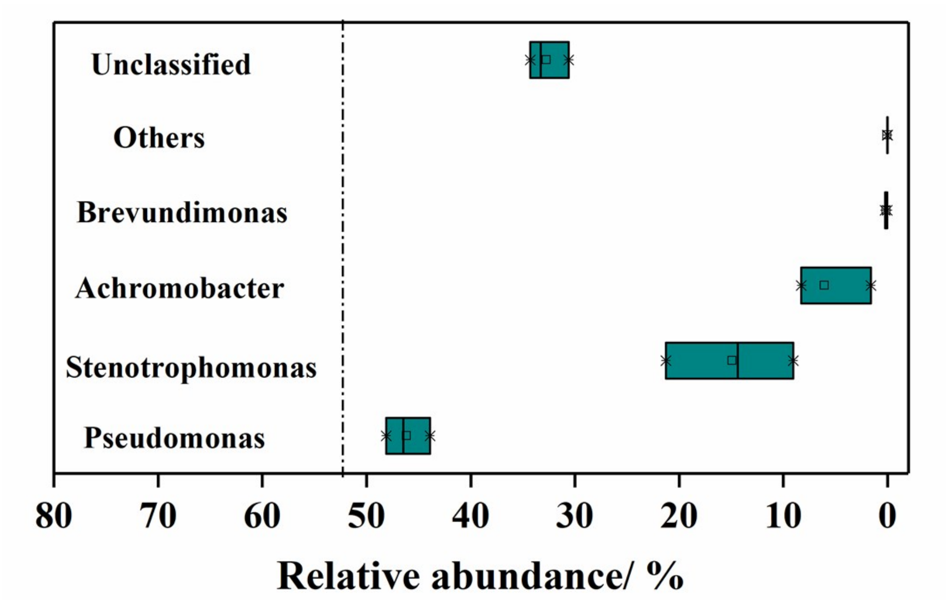

Fig. S1 Identification results of denitrifying bacteria.

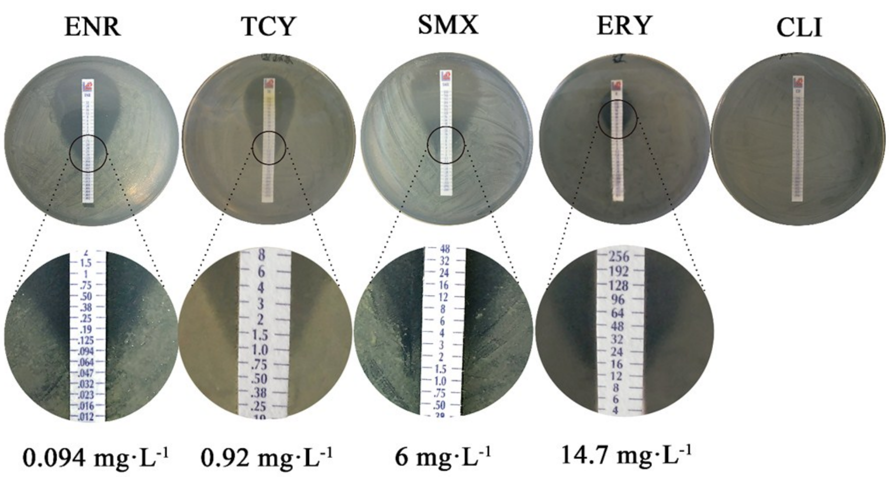

Fig. S2 The MIC of five typical antibiotics. Circled inserts show a magnified view.

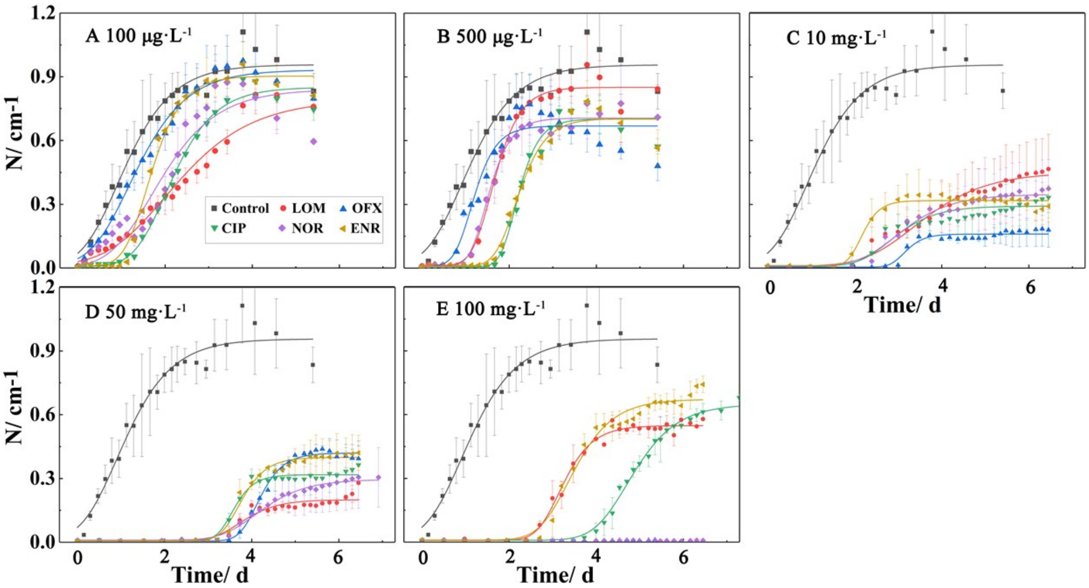

Fig. S3 The bacterial growth characteristics at FQs concentrations of 100 μg·L^{-1} (A), 500 μg·L^{-1} (B), 10 mg·L^{-1} (C), 50 mg·L^{-1} (D) , 100 mg·L^{-1} (E).

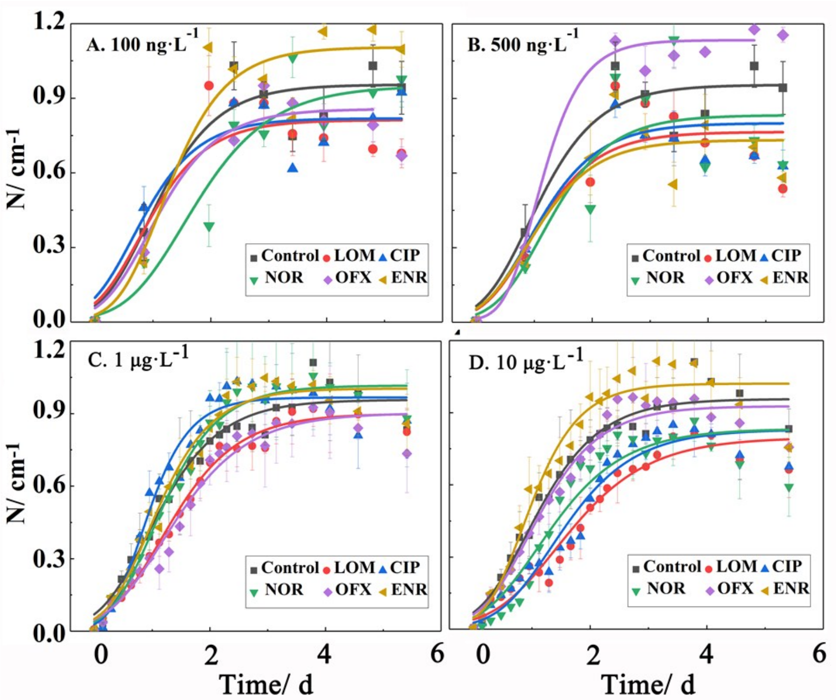

Fig. S4 The bacterial growth characteristics at FQs concentrations of 100 ng·L^{-1} (A), 500 ng·L^{-1} (B), 1 μg·L^{-1} (C), 10 μg·L^{-1} (D).

Table. S1 Occurrence of antibiotics and their concentration in groundwater of different countries

Country	Compounds	Groups	Min (ng·L^{-1})	Med (ng·L^{-1})	Max (ng·L^{-1})	Sampling location	Reference
The United States	Sulfamethoxazole	Sulfonamides	-	-	1110	A wide area of the US	[4]
	Sulfamethazine	Sulfonamides	n.d.	68	616		
	Sulfathiazole	Sulfonamides	n.d.	114	305		
	Erythromycin	Macrolides	n.d.	224.5	2380		
	Lincomycin	lincosamides	n.d.	311.5	416		
	Tetracycline	Tetracyclines	n.d.	-	5.2		
China	Sulfamethoxazole	Sulfonamides	7.2	-	9.5	Groundwater in vegetable planting area of Tianjin	[5]
	Sulfamethoxine	Sulfonamides	n.d.	-	78.3		
	Chloramphenicol	chloramphenicols	n.d.	-	28.1		
	Ciprofloxacin	Fluoroquinolones	n.d.	-	42.5		
	Lincomycin	lincosamides	n.d.	-	8.3		
	Sulfamethazine	Sulfonamides	-	128	-	Groundwater in pig farms in Guangxi	[6]
	Sulfadiazine	Sulfonamides	-	1.47	-		
	Sulfamonomethoxine	Sulfonamides	-	19	-		
	Tetracycline hydrochloride	Tetracyclines	n.d.	n.d.	48	A wide area of China	[7]
	Chlortetracycline hydrochloride	Tetracyclines	n.d.	1.4	76		
	Doxycycline	Tetracyclines	n.d.	n.d.	39		
	Oxytetracycline hydrochloride	Tetracyclines	n.d.	2.9	39		
	Ciprofloxacin hydrochloride	Fluoroquinolones	n.d.	n.d.	155		
	Ofloxacin	Fluoroquinolones	n.d.	14	80		
	Norfloxacin	Fluoroquinolones	n.d.	n.d.	503		
	Enrofloxacin	Fluoroquinolones	n.d.	1.3	49		
	Lomefloxacin hydrochloride	Fluoroquinolones	n.d.	1.0	159		
	Difloxacin hydrochloride	Fluoroquinolones	n.d.	n.d.	35		
	Sulfamerazine	Sulfonamides	n.d.	1.1	15		
	Sulfamethazine	Sulfonamides	n.d.	n.d.	49		
	Sulfisoxazole	Sulfonamides	n.d.	n.d.	8.4		
	Sulfamethoxazole	Sulfonamides	n.d.	15	250		
	Sulfamonomethoxine	Sulfonamides	n.d.	n.d.	29		
	Sulfachloropyridazine	Sulfonamides	n.d.	n.d.	117		
	Sulfathiazole	Sulfonamides	n.d.	n.d.	32		
	Trimethoprim	Sulfonamides	n.d.	9.4	40		
	Erythromycin	Macrolides	n.d.	16	143		
	Azithromycin	Macrolides	n.d.	4.8	73		
Sweden	Ciprofloxacin	Fluoroquinolones	44	555	14000	Groundwater near pharmaceutical factory	[8]
	Enoxacin	Fluoroquinolones	n.d.	715	1900		
	Enrofloxacin	Fluoroquinolones	n.d.	30	67		
	Lomefloxacin	Fluoroquinolones	n.d.	15	35		
	Norfloxacin	Fluoroquinolones	n.d.	21	31		
	Ofloxacin	Fluoroquinolones	n.d.	160	480		

Germany	Anhydro-erythromycin	Macrolides	-	-	49	Groundwater in Baden-Wurttemberg	[9]
	Sulfamethoxazole	Sulfonamides	-	-	410		
France	Sulfamethoxazole	Sulfonamides	-	3.0	-	Groundwater in the Rhône-Alpes region	[10]
	Roxithromycin	Macrolides	-	1.3	-		
Spain	tetracycline	Tetracyclines	n.d.	17.3	141	Barcelona, northeastern Spain	[11]
	Oxytetracycline	Tetracyclines	n.d.	6.52	41		
	Doxorubicin	Tetracyclines	n.d.	19.5	188		
	Chlortetracycline	Tetracyclines	n.d.	2.63	34.2		
	Azithromycin	Macrolides	31.5	257	1620		
	Clarithromycin	Macrolides	1.62	2.6	5.11		
	Spiramycin	Macrolides	n.d.	300	2980		
	Tilmicostar	Macrolides	n.d.	102	820		
	Sulfadiazine	Sulfonamides	n.d.	6.4	37.1		
	Sulfamethoxazole	Sulfonamides	n.d.	4.83	29.1		
	Ciprofloxacin	Fluoroquinolones	17.5	87.9	443		
	Enoxacin	Fluoroquinolones	11.8	75.2	323		
	Enrofloxacin	Fluoroquinolones	12.5	74.8	264		
	Norfloxacin	Fluoroquinolones	16.6	123	462		
	Ofloxacin	Fluoroquinolones	13.1	79.5	367		
	Danoxacin	Fluoroquinolones	n.d.	105	543		

n.d.: below limit of detection (non detected).

Table. S2 The maximum increment (N_m), maximum specific growth rate (μ_m), and lag time (λ) of denitrifying bacteria with exposure to 0-100 mg·L^{-1} CIP, NOR, ENR, OFX, and LOM. R^2 respects the square of the correlation coefficient of fitting curves.

Antibiotic /mg·L^{-1}		0	100 ng·L^{-1}	500 ng·L^{-1}	1 μg·L^{-1}	10 μg·L^{-1}	100 μg·L^{-1}	500 μg·L^{-1}	1 mg·L^{-1}	10 mg·L^{-1}	50 mg·L^{-1}	100 mg·L^{-1}
	N_m	0.954	0.818	0.800	0.964	0.827	0.848	0.699	0.591	0.29	0.217	0.647
CIP	μ_m	0.118	0.116	0.115	0.185	0.112	0.133	0.283	0.158	0.100	0.217	0.111
	λ	-22.24	-21.87	-19.17	-10.31	-18.91	8.21	32.2	10.94	35.1	68.78	75.09
	R^2	0.97	0.87	0.84	0.96	0.95	0.99	0.98	0.86	0.91	0.99	0.99
	N_m	0.954	0.949	0.832	1.013	0.83	0.838	0.703	0.678	0.345	0.293	0
NOR	μ_m	0.118	0.095	0.116	0.143	0.115	0.113	0.250	0.208	0.084	0.088	0
	λ	-22.24	-15.50	-14.30	-17.49	-27.64	-14.86	14.65	11.42	31.46	62.94	155
	R^2	0.97	0.90	0.71	0.98	0.92	0.90	0.99	0.98	0.98	0.99	-
	N_m	0.954	1.104	0.732	1.001	1.018	0.901	0.701	0.710	0.316	0.397	0.67
ENR	μ_m	0.118	0.134	0.115	0.124	0.146	0.170	0.185	0.152	0.221	0.193	0.122
	λ	-22.24	-13.17	-19.21	-14.47	-12.69	10.64	27.8	30.89	38.22	67.85	47.06
	R^2	0.97	0.9	0.811	0.97	0.95	0.99	0.98	0.98	0.97	0.99	0.99
	N_m	0.954	0.856	1.134	0.900	0.924	0.929	0.666	0.684	0.158	0.418	0
OFX	μ_m	0.118	0.116	0.182	0.109	0.113	0.103	0.220	0.251	0.24	0.214	0

	λ	-22.24	-19.29	-4.09	-24.98	-18.91	-17.51	7.45	14.10	60.53	78.30	128.5
	R^2	0.97	0.90	0.981	0.97	0.97	0.98	0.93	0.96	0.95	0.99	-
	N_m	0.954	0.812	0.765	0.897	0.794	0.791	0.848	0.779	0.449	0.198	0.546
LOM	μ_m	0.118	0.118	0.116	0.098	0.081	0.063	0.190	0.131	0.068	0.117	0.164
	λ	-22.24	-21.62	-18.46	-16.79	-19.17	-17.99	15.72	9.01	27.36	61.74	49.9
	R^2	0.97	0.82	0.78	0.99	0.96	0.97	0.99	0.99	0.98	0.93	0.99

References

[1]Y. Q. Lian, J. T. He, Y. Liang, B. N. He, Simulation experiments on gas change in enhanced denitrification of nitrate process by nano emulsified oil, *China Environmental Science*, 2018, **38**, 2105-2115. (in Chinese)

[2]E. A. Tendencia, Disk diffusion method. In *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment*, Aquaculture Department, Southeast Asian Fisheries Development Center, 2004, pp 13-29.

[3]C. Cayrou, D. Raoult, M. Drancourt, Broad-spectrum antibiotic resistance of planctomycetes organisms determined by Etest, *J Antimicrob Chemother*, 2010, **65**, 2119-2122.

[4] K. K. Barnes, D. W. Kolpin, E. T. Furlong, S. D. Zaugg, M. T. Meyer, L. B. Barber, A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States— Ⅰ) Groundwater, *Sci Total Environ*, 2008, **402**, 192-200.

[5] X. G. Hu, Q. X. Zhou, Y. Luo, Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China, *Environ Pollut*, 2010, **158**, 2992-2998.

[6] L. J. Zhou, G. G. Ying, S. Liu, J. L. Zhao, F. Chen, R. Q. Zhang, F. Q. Peng, Q. Q. Zhang, Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry, *J Chromatogr* A, 2012, **1244**, 123-138.

[7] Y. P. Ma, M. Li, M. M. Wu, Z. Li, X. Liu, Occurrences and regional distributions of 20 antibiotics in water bodies during groundwater recharge, *Sci Total Environ*, 2015, **518-519**, 498-506.
[8] J. Fick, H. Soederstrom, R. H. Lindberg, C. Phan, M. Tysklind, D. G. J. Larsson, Pharmaceuticals and personal care products in the environment:
Contamination of surface, ground, and drinking water from pharmaceutical production, *Environ Toxicol Chem*, 2009, **28**, 2522-2527.
[9] F. Sacher, F. T. Lange, H. J. Brauch, I. Blankenhorn, Pharmaceuticals in groundwaters analytical methods and results of a monitoring program in Baden-Wurttemberg, Germany, *J Chromatogr* A, 2001, **938**, 199-210.
[10] E. Vulliet, C. Cren-Olivé, Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption, *Environ Pollut*, 2011, **159**, 2929-2934.
[11] R. López-Serna, A. Jurado, E. Vázquez-Suñé, J. Carrera, M. Petrović, D. Barceló, Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain, *Environ Pollut*, 2013, **174**, 305-315.