

Supplementary Material

Temporal dynamics of bacterial communities and predicted nitrogen metabolism genes in a full-scale wastewater treatment plant

Xiao-Yan Fan, Jing-Feng Gao *, Kai-Ling Pan, Ding-Chang Li, Hui-Hui Dai, Xing Li

*National Engineering Laboratory for Advanced Municipal Wastewater Treatment and
Reuse Technology, Beijing University of Technology, Beijing 100124, China*

*Corresponding author: Dr. Jingfeng Gao, E-mail: gao.jingfeng@bjut.edu.cn or
gao158@gmail.com, Tel.: 0086-10-67391918; Fax: 0086-10-67391983.

Tel: +86-10-6739-2627(office); Fax: +86-10-6739-1983

E-mail: gao.jingfeng@bjut.edu.cn or gao158@gmail.com

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Table S1 Detailed information concerning variation of water quality index (WQI), operational parameters (OP) and temperature (T) during sampling period.

Samples ID	Influent(mg/L)			Effluent(mg/L)			MLSS (mg/L)	SRT (d)	HRT (h)	T (°C)	DO (mg/L)
	BOD	COD	NH ₄ ⁺ -N	BOD	COD	NH ₄ ⁺ -N					
YF.1	45	91	12	1.4	4.3	0.1	3925	15	10.1	13.5	0.3
YF.2	66	104	16	1.6	11.5	0.1	3441	16.5	10	13	0.3
YF.3	47	89	14	2.4	10.8	0.2	4480	15.3	11	15	0.5
YF.4	62	97	14	1.4	15.9	0.2	4886	15.5	10	17.5	0.2
YF.5	43	81	18	2.6	8.7	0.1	3236	15.3	10.5	21.6	0.4
YF.6	40	73	21	1.5	11.5	0.2	3152	15.6	10.5	23.8	0.3
YF.7	71	103	15	1.2	5.4	0.1	4194	15	10.7	26	0.5
YF.8	78	152	24	2.1	10.6	0.1	5293	15	10	25.5	0.5
YF.9	42	65	18	1.4	16.1	0.1	2829	15.4	10.2	23.5	0.3
YF.10	69	100	25	1.2	17.9	0.9	3634	15.5	10	20	0.2
YF.11	56	80	33	3.2	19.5	0.1	2877	15.5	10.1	17.5	0.3
YF.12	150	264	51	3.2	25.7	0.2	4318	15.3	10.5	14	0.3

Abbreviations: MLSS: mixed liquor suspended solids; SRT: sludge retention time; HRT: hydraulic retention Time; T: Temperature; DO: dissolved oxygen.

Table S2 Primers, thermal programs and standard curves of qPCR in this study.

Target prokaryote	Target gene	Sequence (5'-3') of primer pairs	Thermal program	Linear range of standard curves (copies μl^{-1})	R^2	Efficiency (%)	Reference
Total bacteria	16S rRNA	Uni1055F: ATGGCTGTCGTCAGCT 1392R: ACGGGCGGTGTGTAC	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 53°C, and 30 s at 72°C	1.38 \times (10 ² -10 ⁹)	0.998	90.0%	1
Total archaea	16S rRNA	934f: GAATTGGCGGGGAGCAC 1040r: GGCCATGCACCWCCTCTC	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 59°C, and 30 s at 72°C	1.65 \times (10 ² -10 ⁹)	0.998	107.7%	2
AOA	<i>amoA</i>	Arch-amoA26F: GACTACATMTTCTAYACWGAYTGGGC Arch-amo417R: GGKGTCA TRTATGGWGGYAAAYGTTGG	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 56°C, and 45 s at 72°C	2.88 \times (10 ² -10 ⁹)	0.999	90.5%	3
AOB	<i>amoA</i>	amoA-1F: GGGGTTTCTACTGGTGGT amoA-2R: CCCCTCKGSAAAGCCTTCTTC	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 58°C, and 30 s at 72°C	3.25 \times (10 ¹ -10 ⁸)	0.996	99.2%	4
Comammox (<i>Ca. Nitrospira inopinata</i>)	<i>amoA</i>	Nino_amoA_19F: ATAATCAAAGCCGCCAAGTTGC Nino_amoA_252R: AACGGCTGACGATAATTGACC	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 60°C, and 30 s at 72°C	6.87 \times (10 ¹ -10 ⁸)	0.999	92.1%	5
<i>Nitrospira</i>	16S rRNA	Nsr1113F: CCTGCTTTCAGTTGCTACCG Nsr1264R: GTTTGCAGCGCTTTGTACCG	10 min at 95°C, 40 cycles of 45 s at 95°C, 30s at 65°C, and 30 s at 72°C	5.72 \times (10 ¹ -10 ⁸)	0.998	95.0%	6
Denitrifying bacteria	<i>nirS</i>	nirSCd3aFm: AACGYSAAGGARACSGG nirSR3cdm: GASTTCGGRTGSGTCTTSAYGAA	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 60°C, and 30 s at 72°C	1.48 \times (10 ¹ -10 ⁸)	0.994	112.0%	7
Denitrifying bacteria	<i>nirK</i>	nirK876: ATYGGCGVCA YGGCGA nirK1040: GCCTCGATCAGRTTRTGTT	10 min at 95°C, 40 cycles of 45 s at 95°C, 30 s at 60°C, and 30 s at 72°C	5.13 \times (10 ¹ -10 ⁸)	0.996	96.0%	8

Table S3 The KOs of nitrogen cycle

KOs	Genes
Nitrogen fixation	
K02588	<i>nifH</i> ; nitrogenase iron protein NifH
K02586	<i>nifD</i> ; nitrogenase molybdenum-iron protein alpha chain
K02591	<i>nifK</i> ; nitrogenase molybdenum-iron protein beta chain
K00531	<i>anfG</i> ; nitrogenase delta subunit
Assimilatory nitrate reduction	
K00367	<i>narB</i> ; ferredoxin-nitrate reductase
K10534	<i>NR(NAR)</i> ; nitrate reductase (NAD(P)H)
K00372	<i>nasA</i> ; assimilatory nitrate reductase catalytic subunit
K00360	<i>nasB</i> ; assimilatory nitrate reductase electron transfer subunit
K00366	<i>nirA</i> ; ferredoxin-nitrite reductase
K17877	<i>NIT-6</i> ; nitrite reductase (NAD(P)H)
Dissimilatory nitrate reduction	
K00362	<i>nirB</i> ; nitrite reductase (NADH) large subunit
K00363	<i>nirD</i> ; nitrite reductase (NADH) small subunit
K03385	<i>nrfA</i> ; nitrite reductase (cytochrome c-552)
K00374*	<i>narI, narV</i> ; nitrate reductase gamma subunit
K15876	<i>nrfH</i> ; cytochrome c nitrite reductase small subunit
Denitrification	
K00370*	<i>narG, narZ, nxrA</i> ; nitrate reductase / nitrite oxidoreductase, alpha subunit
K00371*	<i>narH, narY, nxrB</i> ; nitrate reductase / nitrite oxidoreductase, beta subunit
K02567*	<i>napA</i> ; periplasmic nitrate reductase NapA
K02568*	<i>napB</i> ; cytochrome c-type protein NapB
K00368	<i>nirK</i> ; nitrite reductase (NO-forming)
K15864	<i>nirS</i> ; nitrite reductase (NO-forming) / hydroxylamine reductase
K04561	<i>norB</i> ; nitric oxide reductase subunit B
K02305	<i>norC</i> ; nitric oxide reductase subunit C
K15877	<i>CYP55</i> ; fungal nitric oxide reductase
K00376	<i>nosZ</i> ; nitrous-oxide reductase
Nitrification	
K10944	<i>pmoA-amoA</i> ; methane/ammonia monooxygenase subunit A
K10945	<i>pmoB-amoB</i> ; methane/ammonia monooxygenase subunit B
K10946	<i>pmoC-amoC</i> ; methane/ammonia monooxygenase subunit C
K10535	<i>hao</i> (hydroxylamine dehydrogenase)

Asterisks (*) indicate KOs that are classified under both the dissimilatory nitrate reduction and denitrification category in the KEGG database, but K00374 were classified as dissimilatory nitrate reduction, and the other four KOs (K00370, K00371, K02567 and K02568) were classified as denitrification for all the analyses herein.

Table S4 Raw and effective reads, plus numbers of OTUs, Good's coverage, Shannon, Chao1, ACE, and Simpson of the five Groups

Group	Sequences				3% cut off						
	Sample ID	Trimmed sequences	Effective sequences	Effective sequences proportion/ (%)	Sequence numbers	OTUs	Good coverages	ACE	Chao1	Shannon	Simpson
Group I	YF.9	41724	39233	94.03	28230	1563	96.44%	1799.31	1812.86	5.53	0.025
Group II	YF.11	33157	29308	88.39	28230	798	98.16%	959.62	987.42	4.88	0.021
Group III	YF.2	37445	35234	94.10	28230	1557	96.40%	1831.68	1845.41	5.77	0.012
Group IV	YF.1, YF.3, YF.4, YF.5,	36543±	34403±	94.11±1.05	28230	1650	96.24%	1889.26	1919.39	6.01±	0.007
	YF.6, YF.8, YF.10, YF.12	4572	4431			±58	±0.20%	±64.79	±71.40	0.11	±0.001
Group V	YF.7	35236	33331	94.59	28230	1600	96.26%	1873.87	1906.51	5.82	0.010

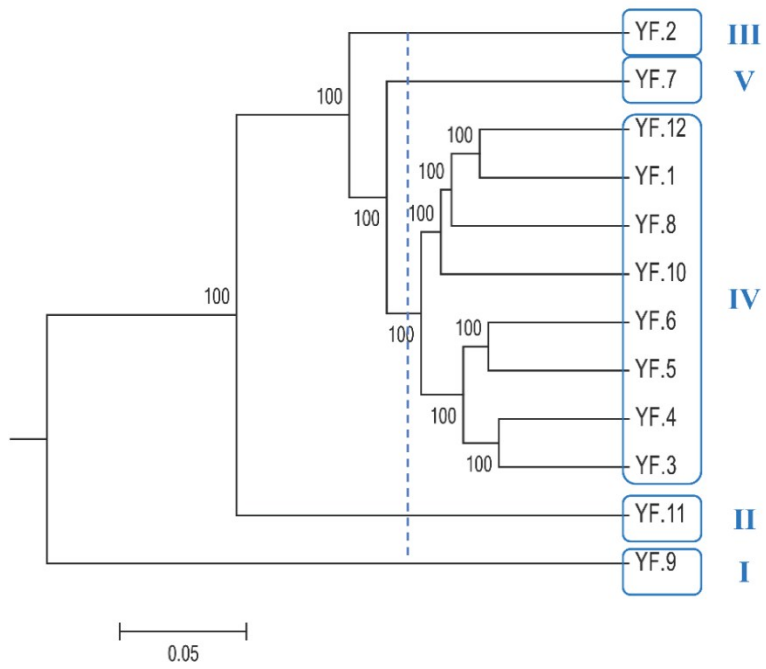


Fig. S1 Bacterial community difference across 12 activated sludge samples collected from different seasons as revealed by cluster analysis.

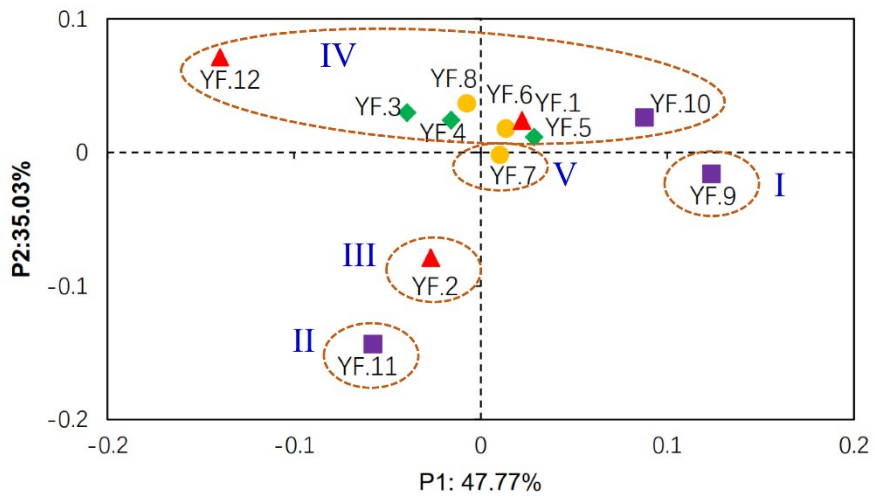


Fig. S2 Shifts in bacterial functions as revealed by PCoA.

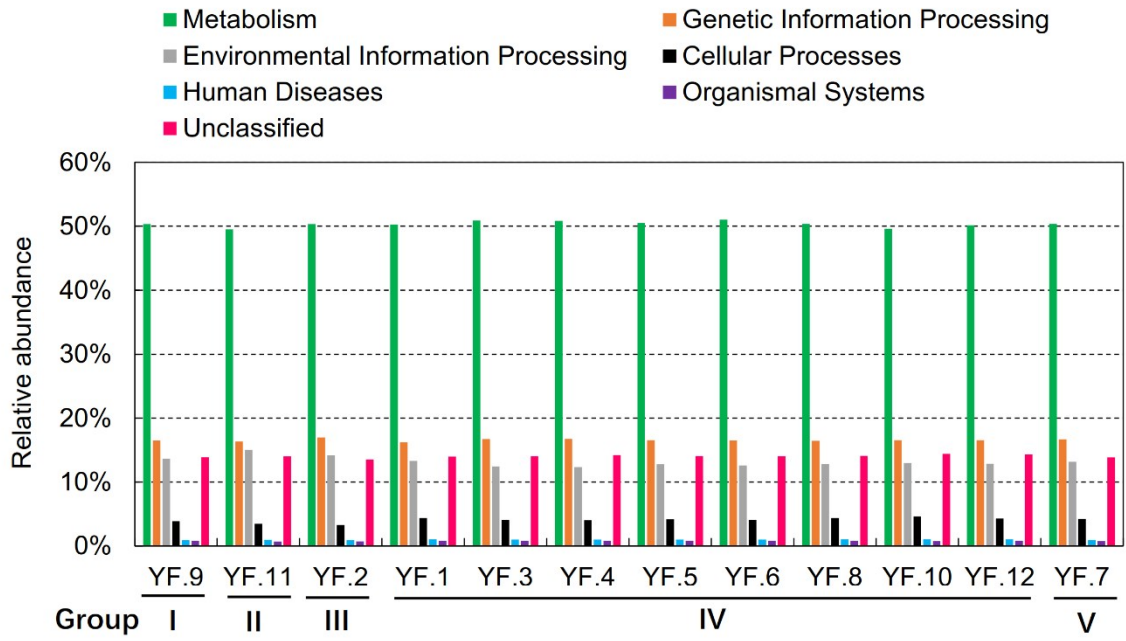


Fig. S3 Relative abundance of different bacterial functions across 12 activated sludge samples.

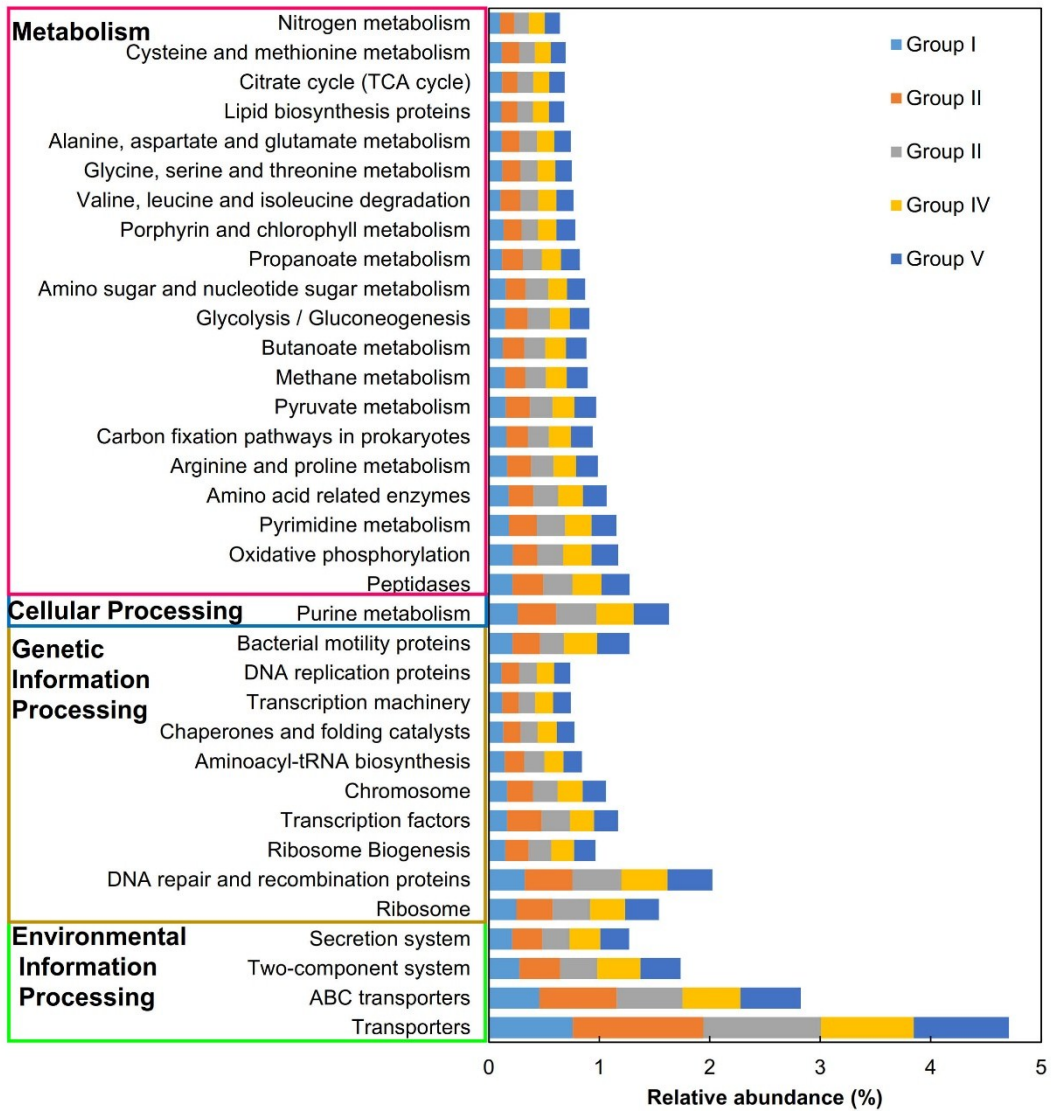


Fig. S4 Top 35 potential functions of the microbes in different Groups.

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