Supplementary Information

Nutrient removal and microbial mechanisms in constructed wetland microcosms treating high nitrate/nitrite polluted river water

Cheng Cheng [a], Huijun Xie [b], En Yang [a,c], Xuanxu Shen [a], Peng Dai [a,d] and Jian Zhang [a,1]

[a] Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan 250100, China

[b] Environmental Research Institute, Shandong University, Jinan 250100, China

[c] Rizhao Environmental Protection Bureau, Rizhao 276800, China

[d] Department of Civil and Environmental Engineering, South Dakota State University, Brookings, SD 57007, United States

[1] Corresponding author: Tel.: +86 531 8836 9518; Fax: +86 531 88364513; E-mail address: zhangjian00@sdu.edu.cn

Table S1. Primers of target genes used in qPCR analysis

Target gene	Primer	Primer sequence (5'-3')	Amplification size (bp)	Reference
Bacterial 16S rRNA	338F	ACTCCTACGGGAGGCAGCAG	180	[1]
	518R	ATTACCGCGGCTGCTGG		
amoA (ammonia monooxygenase)	amo598f	GAATATGTTCGCCTGATTG	120	[2]
	amo718r	CAAAGTACCACCATACGCAG		
narG (nitrate reductase)	1960m2f	TA(CT)GT(GC)GGGCAGGA(AG)AAACTG	100	[3]
	2050m2r	CGTAGAAGAAGCTGGTGCTGTT		
nirK (copper-containing nitrite reductase)	nirK583F	TCA TGGTGCTGCCGCGKGACGG	326	[4]
	nirK909R	GAA CTTGCCGGTKGCCCAGAC		
nirS (cd1-containing nitrite reductase)	nirScd3aF	GT(C/G)AACGT(C/G)AAGGA(A/G)AC(C/G)GG	425	[5]
	nirSR3cd	GA(C/G)TTCGG(A/G)TG(C/G)GTCTTGA		
nosZ (nitrous oxide reductase)	nosZ1527F	CGCTGTTCHTCGACAGYCA	250	[6]
	nosZ1773R	ATRTCGATCARCTGBTCGTT		

Supplementary References for Table S1

1. G. Muyzer, E. C. De Waal and A. G. Uitterlinden, *Applied and environmental microbiology*, 1993, **59**, 695-700.
2. H. M. Dionisi, A. C. Layton, G. Harms, I. R. Gregory, K. G. Robinson and G. S. Sayler, *Applied and Environmental Microbiology*, 2002, **68**, 245-253.
3. J. C. López-Gutiérrez, S. Henry, S. Hallet, F. Martin-Laurent, G. Catroux and L. Philippot, *Journal of Microbiological Methods*, 2004, **57**, 399-407.
4. T. Yan, M. W. Fields, L. Wu, Y. Zu, J. M. Tiedje and J. Zhou, *Environmental Microbiology*, 2003, **5**, 13-24.
5. P. Reassessing, *FEMS Microbiology Ecology*, 2004, **49**, 401-417.
6. D. J. Scala and L. J. Kerkhof, *FEMS Microbiology Letters*, 1998, **162**, 61-68.

Table S2. Protocols and parameters of target genes used in qPCR analysis

Target gene	Programs
Bacterial 16S rRNA	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min
amoA	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 56 °C for 45 s, and extension at 72 °C for 30 s
narG	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 58 °C for 45 s, and extension at 72 °C for 30 s
nirK	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 64 °C for 40 s, and extension at 72 °C for 30 s
nirS	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s
nosZ	Pre-heating at 50 °C for 2 min, pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 58 °C for 50 s, and extension at 72 °C for 30 s