Supplementary materials for

Comparative Metagenomic and Metatranscriptomic Analyses Reveal the Functional Species and Metabolic Characteristics of an Enriched Denitratation Community

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Text S1 Determination methods for the activities of nitrate reductase (NAR), nitrite reductase (NIR).

Sludge samples were obtained from the reactor, and washed for 3 times with 0.01 M phosphate buffer (pH 7.4). Then, the resuspended pellets were sonicated at 20 kHz and 4 °C for 5 min to break down the cell structure of bacteria in sludge. The debris was centrifuged at 12,000 *g* and 4 °C for 10 min and the crude extracts in supernatant were obtained for the enzyme activity measurement. The NAR and NIR activities were determined by using the methyl viologen as the electron donor. A volume of 300 μL crude extracts was added to start the reaction in the cuvette containing 0.01 M phosphate buffer (pH 7.4), 1mM $NaNO_3$ or 1 mM $NaNO_2$, 1mM methyl viologen and 5 mM sodium hyposulfite ($Na_2S_2O_4$) in a final volume of 2 mL. The incubation was performed at 30 ^oC for 30 min, followed by measuring the increase of nitrite in NAR activity assay or the decrease of nitrite in NIR activity assay. The specific NAR and NIR activities were expressed as mgN h^{-1} g^{-1}protein. The activities of NirS and NirK were identified by adding diethyl dithio carbamate (10 mM).[1] Protein content was determined using the Lowry method with bovine serum albumin as standard.[2]

References:

1. Li, W.; Shan, X.; Wang, Z.; Lin, X.; Li, C.; Cai, C.; Abbas, G.; Zhang, M.; Shen, L.; Hu, Z.; Zhao, H.; Zheng, P., Effect of self-alkalization on nitrite accumulation in a high-rate denitrification system: Performance, microflora and enzymatic activities. *Water Res.* **2016**, *88*, 758-765.
2. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265-275.

Table S1. Operational parameters of the reactor at different phases.

Phase	Time	Inf.NH_4^+-N (mg L^{-1})	Inf.NO_3^--N (mg L^{-1})	COD/NO_3^--N	pH	HRT (h)	Cycle	Nitrate Loading Rate (kgN m^{-3} d^{-1})
1	1 ~ 30	10	30	1.5	8.5	8.0	6	0.09
2	31 ~ 80	10	30	2.5	8.5	6.0	8 → 12	0.12 → 0.18
3	80 ~ 94	10	30	2.5	7.5	4.0	12	0.18
4	95 ~ 210	10	30 → 60	2.8	8.0	4.0	12	0.18 - 0.37
5	210 ~ 270	40	60	2.8	7.5	4.0	12	0.37

Table S2. Experimental parameters in batch assays. Phosphate buffer (20 mM) was added to serum flasks to control the pH at 7.5, 8.0 and 8.5.

Run	Sample	Nitrate (mgN L^{-1})	Nitrite (mgN L^{-1})	Acetate (mgCOD L^{-1})	pH
1	S0	60	0	150	7.5
2	S0	60	0	150	8.0
3	S0	60	0	150	8.5
4	S2	60	0	150	7.5
5	S2	60	0	150	8.0
6	S2	60	0	150	8.5
7	S3	60	0	150	7.5
8	S3	60	0	150	8.0
9	S3	60	0	150	8.5
10	S4_3	60	0	150	7.5
11	S4_3	60	0	150	8.0
12	S4_3	60	0	150	8.5
13	S4_3	0	60	150	8.0
14	S4_3	60	60	150	8.0
15	S4_3	60	0	300	8.0

Table S3. Alpha diversity indices of the eight samples.

Sample	Ace	Chao	Shannon	Simpson	Coverage
S0_1	1245.1	1274.2	5.41	0.010	0.997
S0_2	1158.3	1150.5	5.31	0.012	0.996
S1	1205.7	1213.6	5.06	0.018	0.996
S2	1014.5	1009.9	4.92	0.020	0.998
S3	807.9	828.0	4.32	0.030	0.998
S4_1	584.5	599.4	3.61	0.061	0.998
S4_2	565.3	578.1	3.78	0.060	0.998
S4_3	609.7	660.5	3.78	0.064	0.998
S5	236.7	246.1	1.72	0.43	0.999

Table S4. Sequencing, assembly and annotation statistics. Reads containing one or more unknown nucleotides, reads containing bases with quality score lower than 20, or reads with length less than 50 bp were removed.

Samples	Clean reads	Percent in raw reads (%)	Clean base(bp)	Percent in raw bases (%)	Contigs	Contigs bases(bp)	N50(bp)
S0	103,157,992	99.0	15,538,457,467	98.8	1,232,430	900,637,026	796
S4_3	99,395,412	99.0	14,984,206,676	98.8	565,667	528,062,261	1263
S4_3-feast	137,399,407	97.3	20,529,800,548	97.3	614,779	232,416,976	393
S4_3-famine	129,935,977	96.4	19,435,421,817	96.4	335,060	122,253,540	372

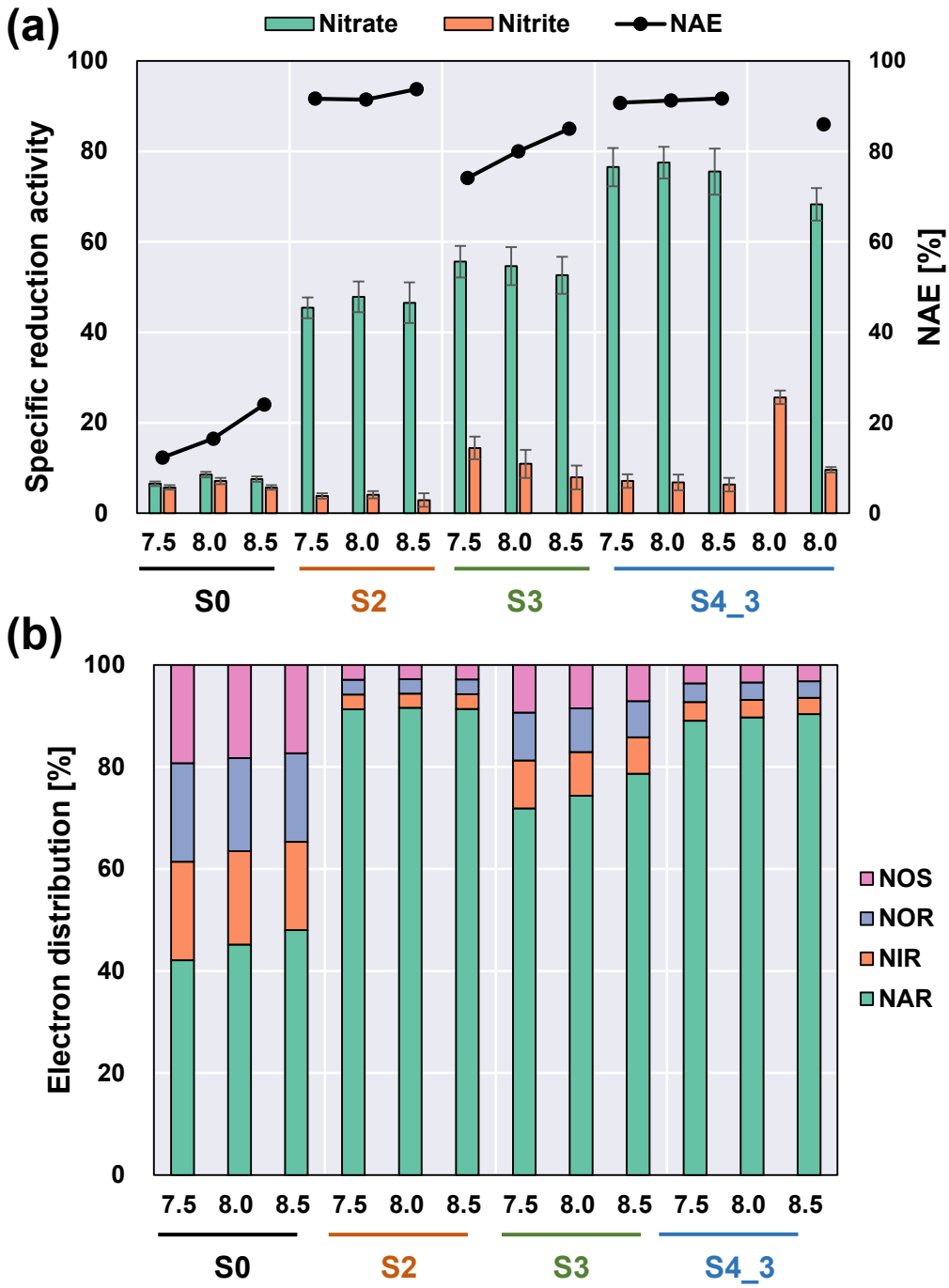

Figure S1. Effect of pH on the denitrification activity(a) and electron distribution (b) of various enrichments. NRE, nitrite accumulation efficiency, is defined as the ratio of nitrate reduction rate to nitrite reduction rate.

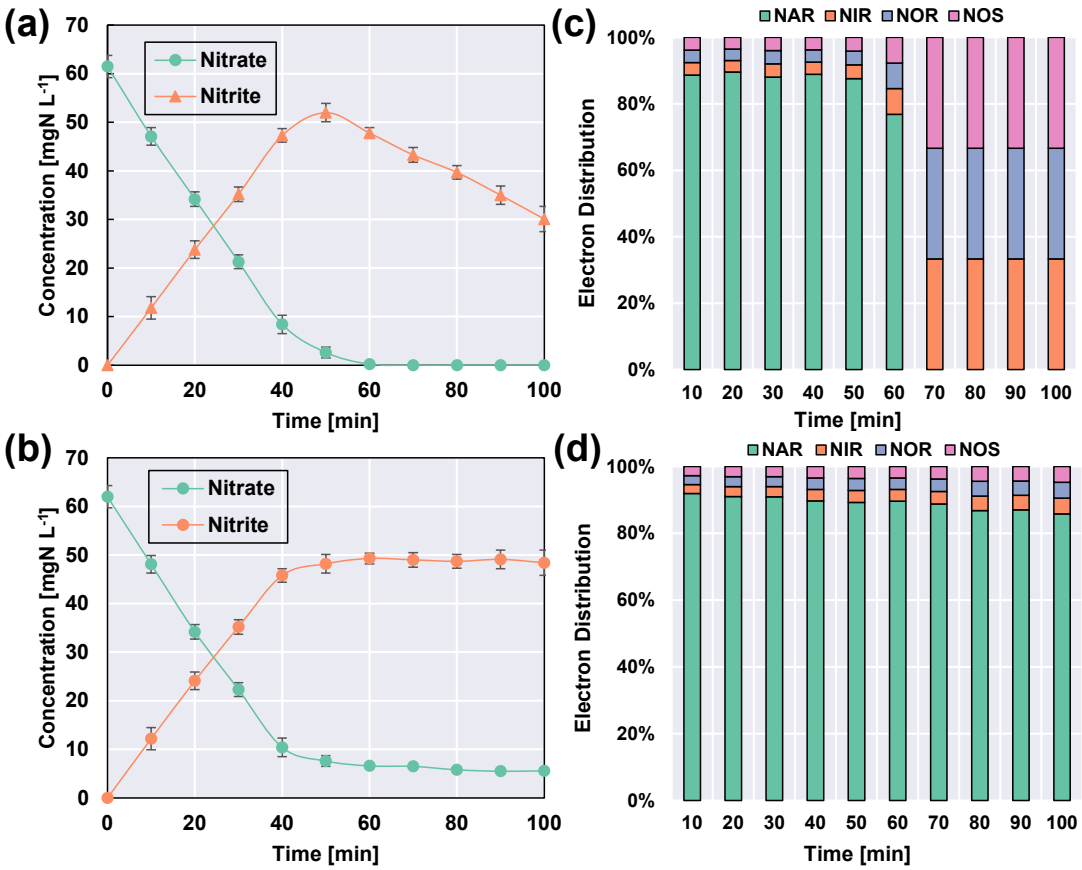

Figure S2. Time course of nitrate reduction and electron distribution of S4_3 at the C/N ratio of 5.0 (a, c) or 2.5 (b, d) when the initial pH was 8.0.

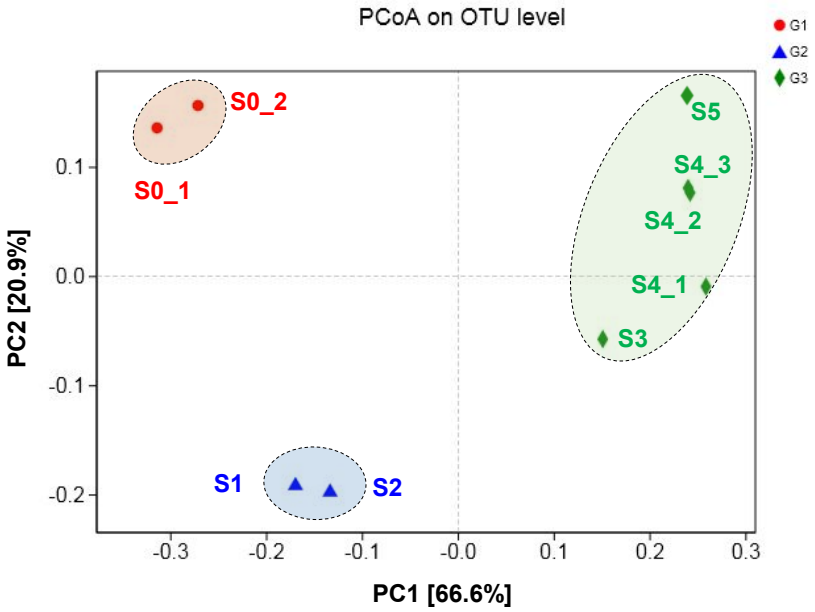

Figure S3. Principal coordinate analysis (PCoA) plot based on the Bray–Curtis distance metric illustrating the variability in samples throughout the enrichment process.

Species	Feast	Famine	Change
Flavobacterium columnare	157.8	319.9	-162.1
Sphingobacterium mizutaii	56.0	82.6	-26.6
Marivirga tractuosa	40.4	72.1	-31.7
Flavobacterium johnsoniae	42.7	60.3	-17.6
Thauera sp. MZ1T	132.5	52.2	80.3
Pontibacter actiniarum	43.4	46.8	-3.4
Niastella koreensis	23.9	24.5	-0.6
Azospira oryzae	31.3	0.0	31.3
Hymenobacter sp. DG25B	12.8	2.4	10.3
Maribacter sp. HTCC2170	12.1	13.7	-1.6

Figure S4. Taxonomic origins of nosZ transcripts (TPM: Trans Per Million) in the sample S4_3 and transcriptional dynamics in the feast and famine phases as indicated by the change of color. (Only the TOP 10 transcripts were showed.)

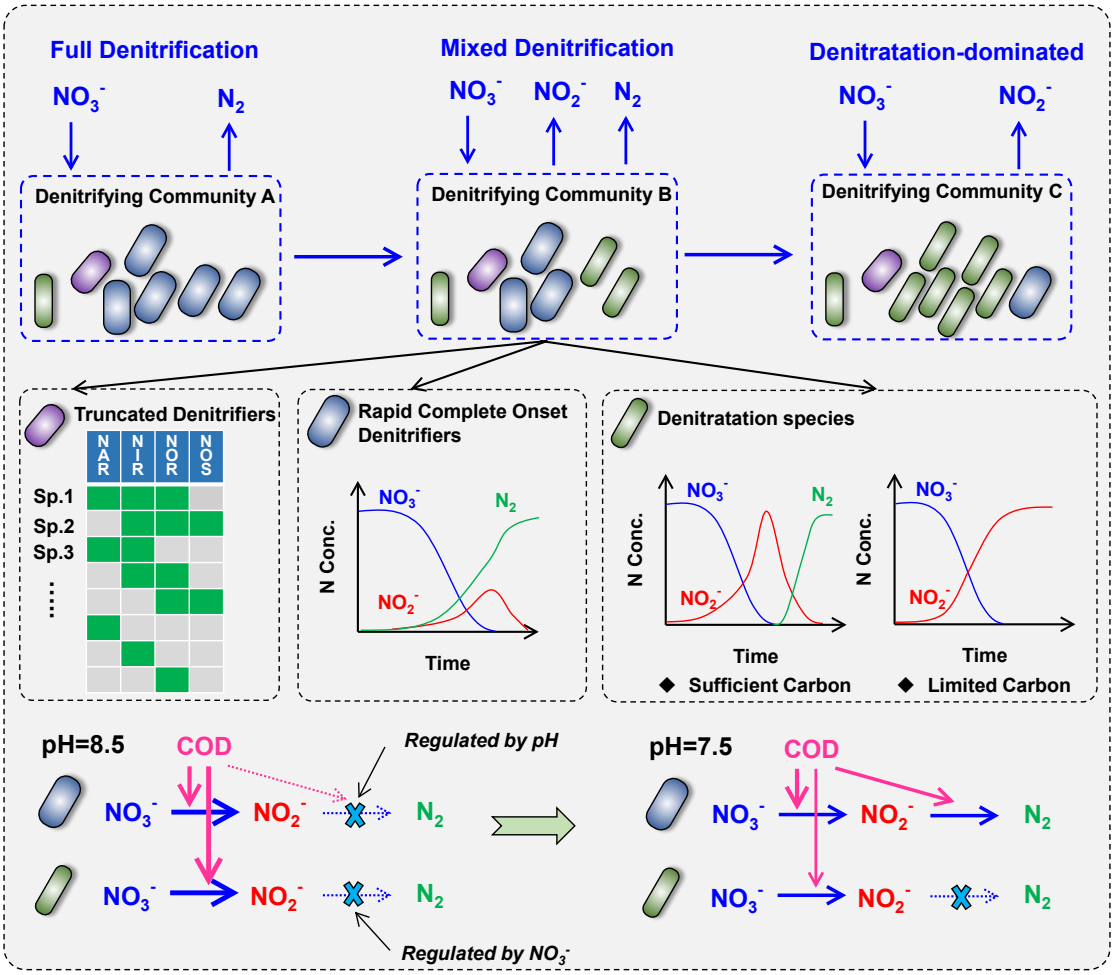

Figure S5. Proposed mechanisms for the achievement of denitratation.

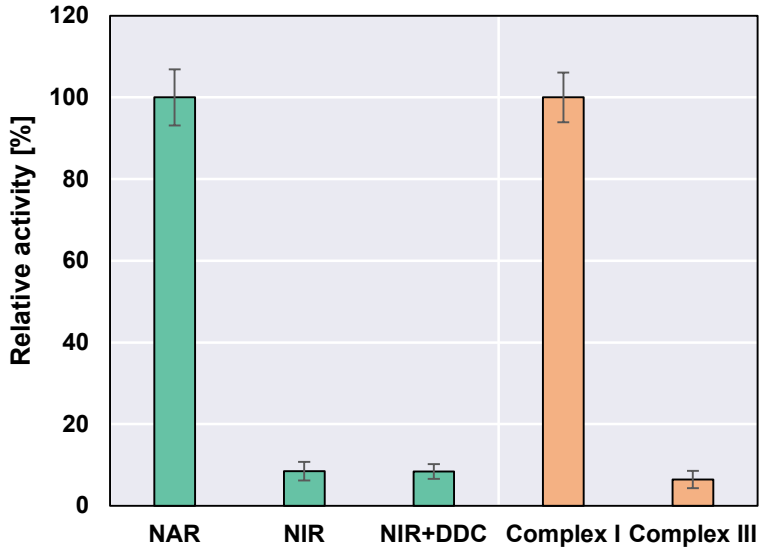

Figure S6. Relative activity of nitrate reductase (NAR), nitrite reductase (NIR) and their corresponding electron transport modules (Complex I and Complex III). The activities of NirS and NirK were identified by adding diethyl dithio carbamate (DDC).