Supporting Information

Comammox *Nitrospira* Species Dominate in an Efficient Partial Nitrification–anammox Bioreactor for Treating Ammonium at Low Loadings

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Table S1. Oligonucleotide primers used in the HOPE assay.

Primer	Target	Primer sequence (5'-3')	References
Ntspa1429	*Nitrospira* lineage I	TGGCTTGGGCGACTTCAG	[1]
Ntspa1151_M	*Nitrospira* lineage II	CCGTTCTCCTGGGCAGTCTCT	Modified from[2]
NmoCL6_M	*Nitrosomonas* lineage 6	AAGCATAAGGTCTTTCGATCCCC	Modified from[3]
NmoCL7	*Nitrosomonas* lineage 7	CTCTTTCTTTCCGACTAAAA	[3]
AMX193R	*Ca.* Jettenia	GCCCCAAACCTTTGACTC	This study
AMX598R	*Ca.* Brocadia	CGGAAGGCTTTCACAACCG	This study
1390Rm_II	Most Bacteria	CGGGCGGTGTGTRCAA	Modified from[4]

Supplementary Figure Legends

Figure S1. Time-course variation in dissolved oxygen (DO) in stage I of reactor operation. Dots represent daily averaged values from data on-line collected every 30 mins.

Figure S2. Time-course variations of functional gene copies (day 686-1062) after stage V of reactor operation.

Figure S3. Pairwise identity matrix of comammox *amoA* gene sequences (396 bp) of ASVs from the present study and their relatives. A color legend indicates the pairwise identities and the colors displayed in the matrix.

Figure S4. Variations of total ammonia concentration (NH_3 + NH_4^+) in the pre-cultivation experiment. Bottles with initial free ammonia (FA) concentration of 1.4 (A), 2.9 (B), 5.8 (C), 8.9 (D), 13.5 (E), and 26.3 mg-N/L (F). Data from triplicate bottles are presented with average and standard deviation.

Figure S1

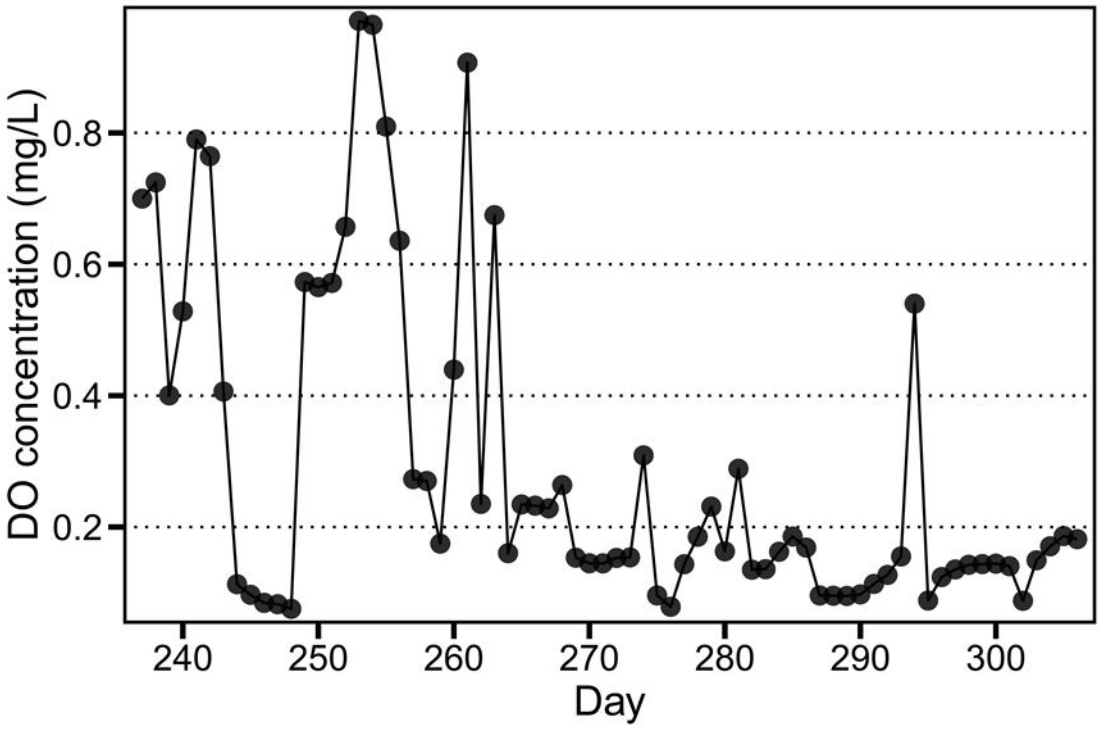

DO concentration (mg/L)
0.8
0.6
0.4
0.2
240
250
260
270
280
290
300
Day

Figure S2

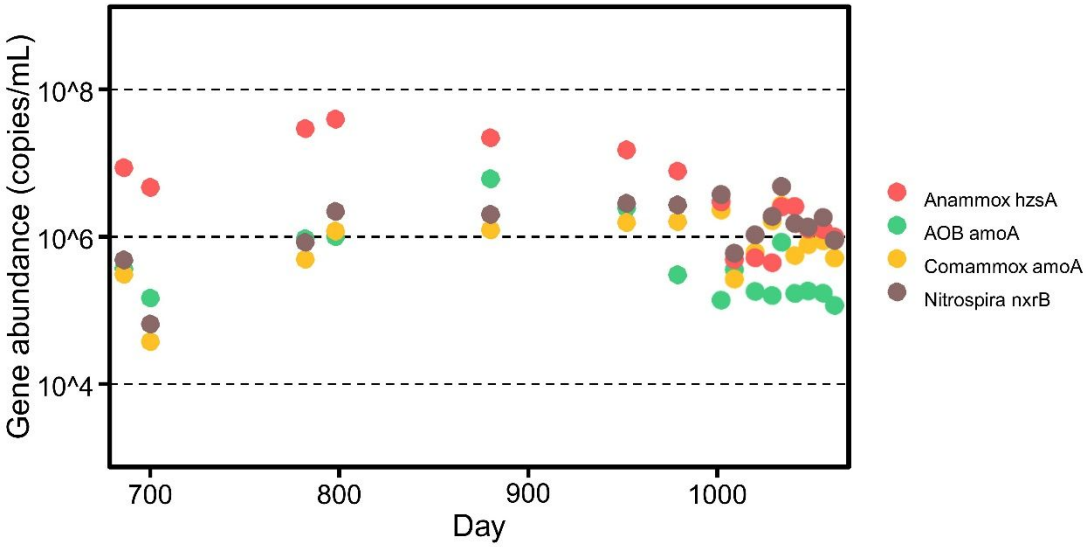

Gene abundance (copies/mL)
10^8
10^6
10^4
700
800
900
1000
Day
Anammox hzsA
AOB amoA
Comammox amoA
Nitrospira nxrB

Figure S3

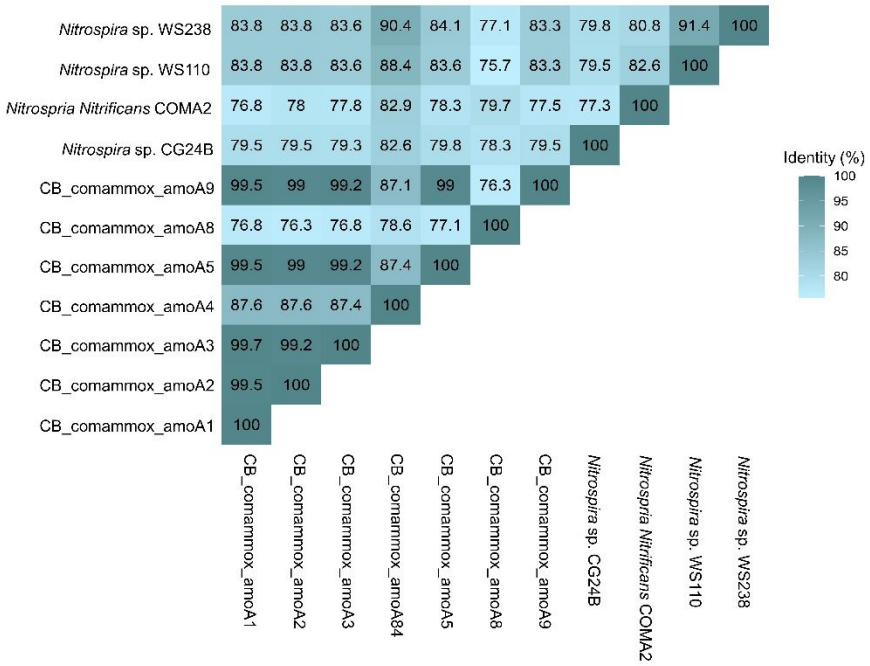
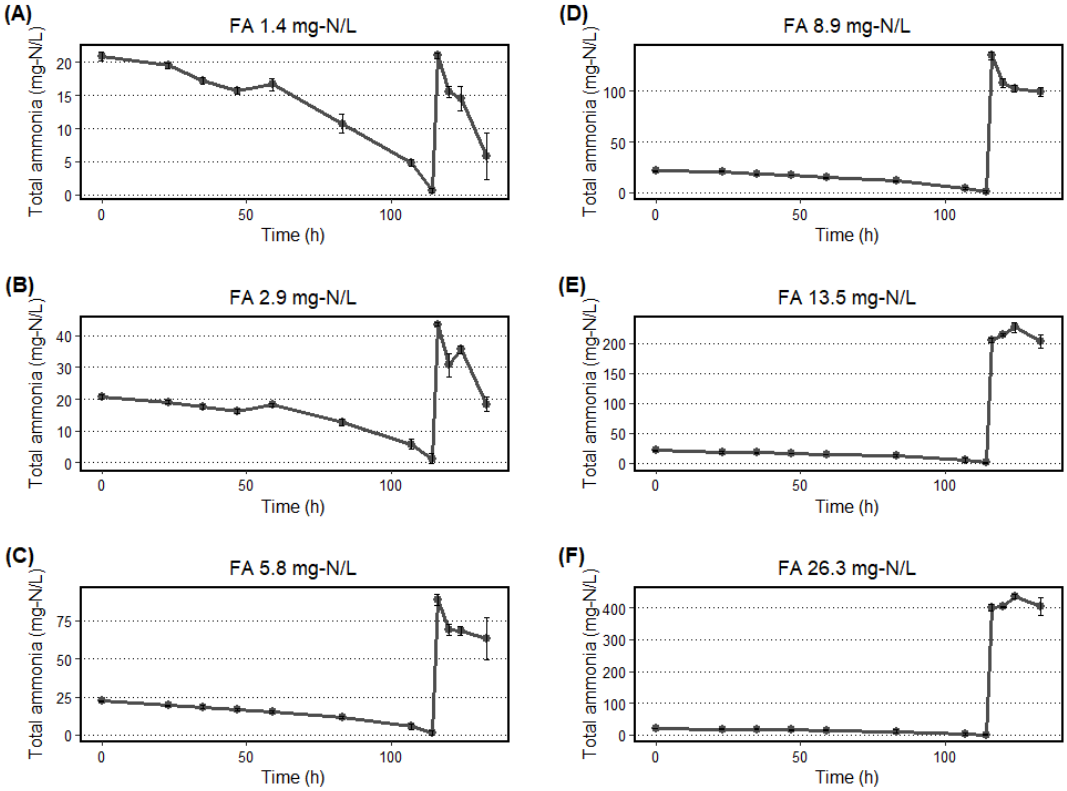

Nitrospira sp. WS238 83.8 83.8 83.6 90.4 84.1 77.1 83.3 79.8 80.8 91.4 100
Nitrospira sp. WS110 83.8 83.8 83.6 88.4 83.6 75.7 83.3 79.5 82.6 100
Nitrospria Nitrificans COMA2 76.8 78 77.8 82.9 78.3 79.7 77.5 77.3 100
Nitrospira sp. CG24B 79.5 79.5 79.3 82.6 79.8 78.3 79.5 100
CB_comammox_amoA9 99.5 99 99.2 87.1 99 76.3 100
CB_comammox_amoA8 76.8 76.3 76.8 78.6 77.1 100
CB_comammox_amoA5 99.5 99 99.2 87.4 100
CB_comammox_amoA4 87.6 87.6 87.4 100
CB_comammox_amoA3 99.7 99.2 100
CB_comammox_amoA2 99.5 100
CB_comammox_amoA1 100
CB_comammox_amoA1
CB_comammox_amoA2
CB_comammox_amoA3
CB_comammox_amoA84
CB_comammox_amoA5
CB_comammox_amoA8
CB_comammox_amoA9
Nitrospira sp. CG24B
Nitrospria Nitrificans COMA2
Nitrospira sp. WS110
Nitrospira sp. WS238
Identity (%)
100
95
90
85
80

Figure S4


(A)
FA 1.4 mg-N/L
Total ammonia (mg-N/L)
Time (h)
(B)
FA 2.9 mg-N/L
Total ammonia (mg-N/L)
Time (h)
(C)
FA 5.8 mg-N/L
Total ammonia (mg-N/L)
Time (h)
(D)
FA 8.9 mg-N/L
Total ammonia (mg-N/L)
Time (h)
(E)
FA 13.5 mg-N/L
Total ammonia (mg-N/L)
Time (h)
(F)
FA 26.3 mg-N/L
Total ammonia (mg-N/L)
Time (h)

References

1. Scarascia, G.; Cheng, H.; Harb, M.; Hong, P. Y., Application of hierarchical oligonucleotide primer extension (HOPE) to assess relative abundances of ammonia- and nitrite-oxidizing bacteria. *BMC Microbiol.* **2017,** *17* (1), 85.
2. Maixner, F.; Noguera, D. R.; Anneser, B.; Stoecker, K.; Wegl, G.; Wagner, M.; Daims, H., Nitrite concentration influences the population structure of *Nitrospira*-like bacteria. *Environ. Microbiol.* **2006,** *8* (8), 1487-1495.
3. Stephen, J. R.; Kowalchuk, G. A.; Bruns, M. A. V.; McCaig, A. E.; Phillips, C. J.; Embley, T. M.; Prosser, J. I., Analysis of beta-subgroup proteobacterial ammonia oxidizer populations in soil by denaturing gradient gel electrophoresis analysis and hierarchical phylogenetic probing. *Appl. Environ. Microbiol.* **1998,** *64* (8), 2958-2965.
4. Zheng, D.; Alm, E. W.; Stahl, D. A.; Raskin, L., Characterization of universal small-subunit rRNA hybridization probes for quantitative molecular microbial ecology studies. *Appl. Environ. Microbiol.* **1996,** *62* (12), 4504.