

Supplementary Information

Characterization of the nitrogen-transforming microbial community in the biofilms of a full-scale rotating biological contactor system treating wastewater from a fresh market building

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Table S1. Primers used in this study.

Microorganism, target gene	Primer set	Annealing temp (°C)		Reference
		PCR*	qPCR	
AOA, <i>amoA</i>	Arch- <i>amoA</i> F Arch- <i>amoA</i> R	53	53	(1)
AOB, <i>amoA</i>	<i>amoA</i> -1F <i>amoA</i> -2R	56	53	(2)
Anammox, 16S rRNA	A438f A684r	55	55	(3)
Comammox, <i>amoA</i>	<i>comamoA</i> AF <i>comamoA</i> SR	58	58	(4)
<i>Nitrospira</i> , <i>nxrB</i>	<i>nxrB</i> 169f <i>nxrB</i> 638r	56.2	56	(5)
<i>Nitrobacter</i> , <i>nxrA</i>	F1 <i>nxrA</i> R2 <i>nxrA</i>	60	55	(6)
<i>Nitrotoga</i> , 16S rRNA	Ntoga124F Ntoga1462R	63	-	(7)
DNRA, <i>nrfA</i>	<i>nrfA</i> 2aw <i>nrfA</i> R1	53	-	(8)
	F1 <i>nrfA</i> R1 <i>nrfA</i>	45	-	(9)

*Primers were added with overhang adapters for sequencing analysis

forward overhang sequences: 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus-specific sequence]

reverse overhang sequences: 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus-specific sequence]

References

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Table S2. Diversity indices of T1 and T2 samples

	T1	T2
Ace	1,319	1,477
Chao1	1,325	1,480
Shannon	8.69	8.88

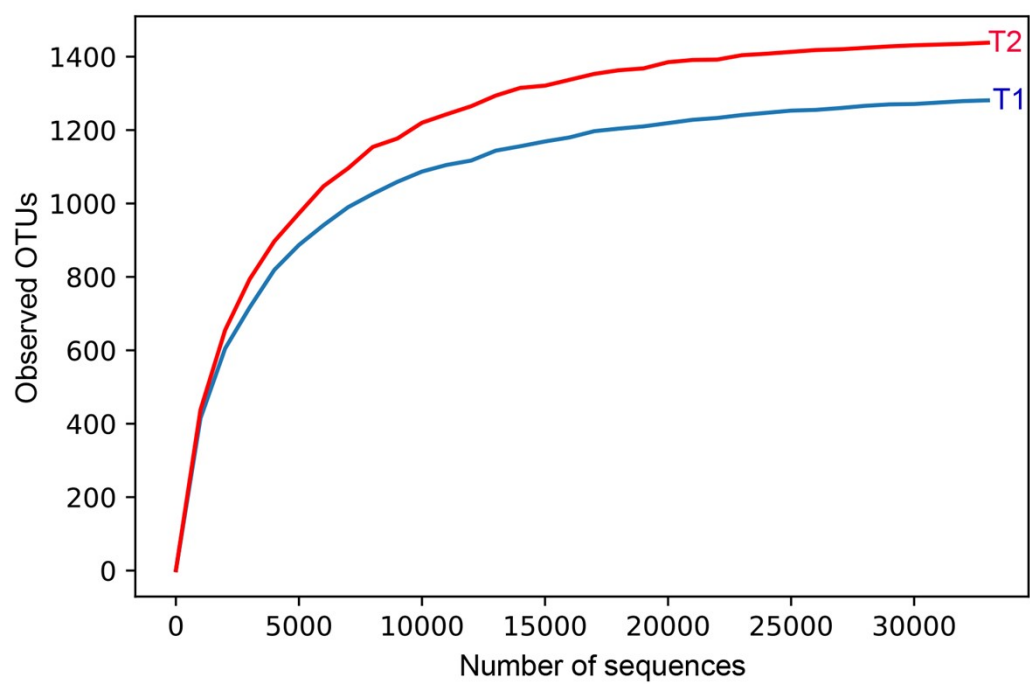


Figure S1. Rarefaction curves of T1 and T2 samples

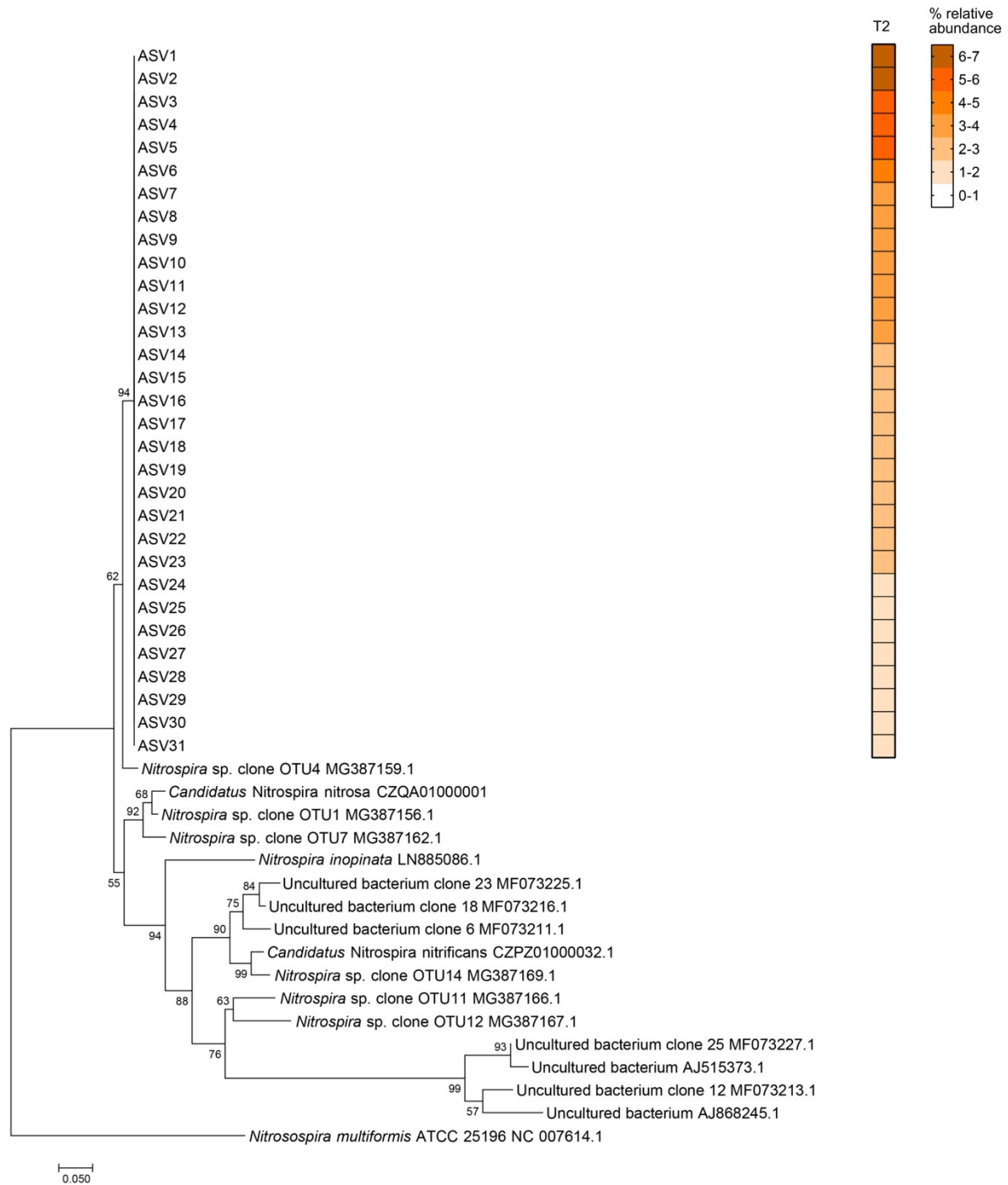


Figure S2. Neighbor-joining tree calculated based on sequences of comammox *amoA* gene fragments. The heat map on the right was generated based on the ASVs that show $\geq 1\%$ relative abundance of sequences in the sample. In total, $>94\%$ of the total sequences was included in the analysis.

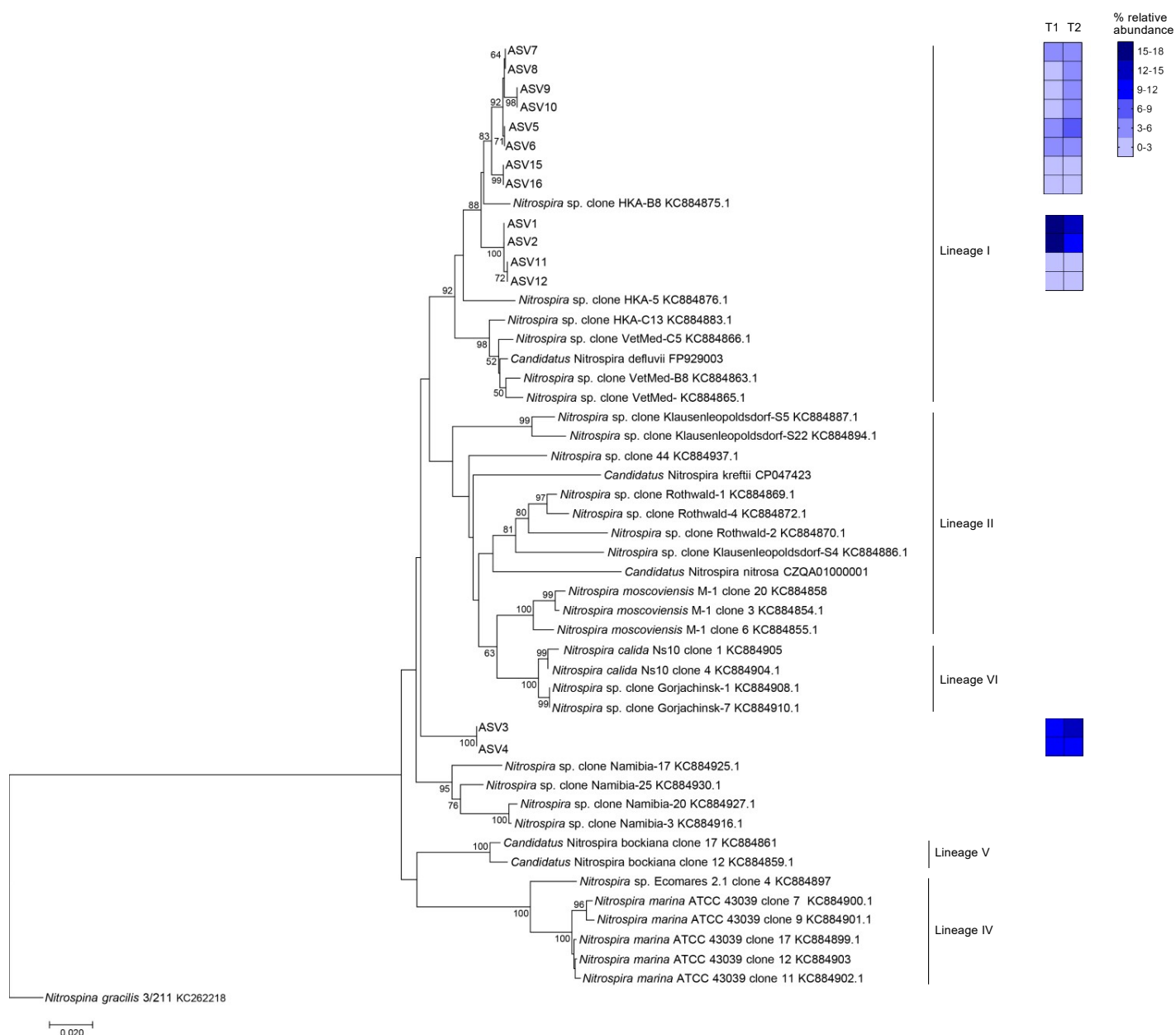


Figure S3. Neighbor-joining tree calculated based on sequences of *Nitrospira nxrB* gene fragments. The heatmap on the right was generated based on the ASVs that show $\geq 1\%$ relative abundance of sequences in each sample. In total, $>80\%$ of the total sequences were included in the analysis.