

## **Supplementary Material**

# **Evidence of Potential Anammox Activities from Rice Paddy Soils in Microaerobic and Anaerobic Conditions**

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**Table S1.** Primer sequences used in this study

Primers	Sequence 5'- 3'	Annealing temperature (°C)	References
27F	AGAGTTTGATCMTGGCTCAG	55°C	Brunk et al. (1996)
519R	GWATTACCGCGGCKGCTG	55°C	Brunk et al. (1996)
341F	CCTACGGGNGGCWGCAG	used in MiSeq	Herlemann et al. (2011)
805R	GACTACHVGGGTATCTAATCC	used in MiSeq	Herlemann et al. (2011)
amoA-1F	GGGGTTTCTACTGGTGGT	52°C	Rotthauwe et al. (1997)
amoA-2R	CCCCTCKGSAAAGCCTTC	52°C	Rotthauwe et al. (1997)
nxrB169f	TACATGTGGTGGAACA	42°C	Lücker et al. (2010)
nxrB638r	CGGTTCTGGTCRATCA	42°C	Lücker et al. (2010)
hzsA_382F	GGYGGDTGYCAGATATGGG	59°C	Harhangi et al. (2012)
hzsA_1857R	AAABGGYGAATCATARTGGC	59°C	Harhangi et al. (2012)

**Table S2.** Alpha-diversity of the microbial communities from the anaerobic batch incubation (Experiment 1)

Sample	normalized sequences	coverage	species observed (OTU)	Chao1	Shannon	inverse Simpson
BR_D01_a	20159	0.9029 ±0.0013	3728.0±19.8	6374.4 ±125.0	6.681±0.007	168.1±2.1
BR_D01_b	20159	0.9110 ±0.0015	3310.9±24.0	6091.3 ±165.0	6.333±0.011	97.3±1.7
BR_D15_a	20159	0.9188 ±0.0012	3289.5±17.6	5317.6 ±105.1	6.341±0.008	93.3±1.2
BR_D15_b	20159	0.9190 ±0.0013	3170.7±21.3	5235.9 ±119.5	6.122±0.010	72.9±1.0
BR_D56_a	20159	0.9334	3083.0	4345.1	6.448	131.6
BR_D56_b	20159	0.9208 ±0.0011	3299.9±17.4	5121.7 ±94.1	6.548±0.007	159.1±2.0

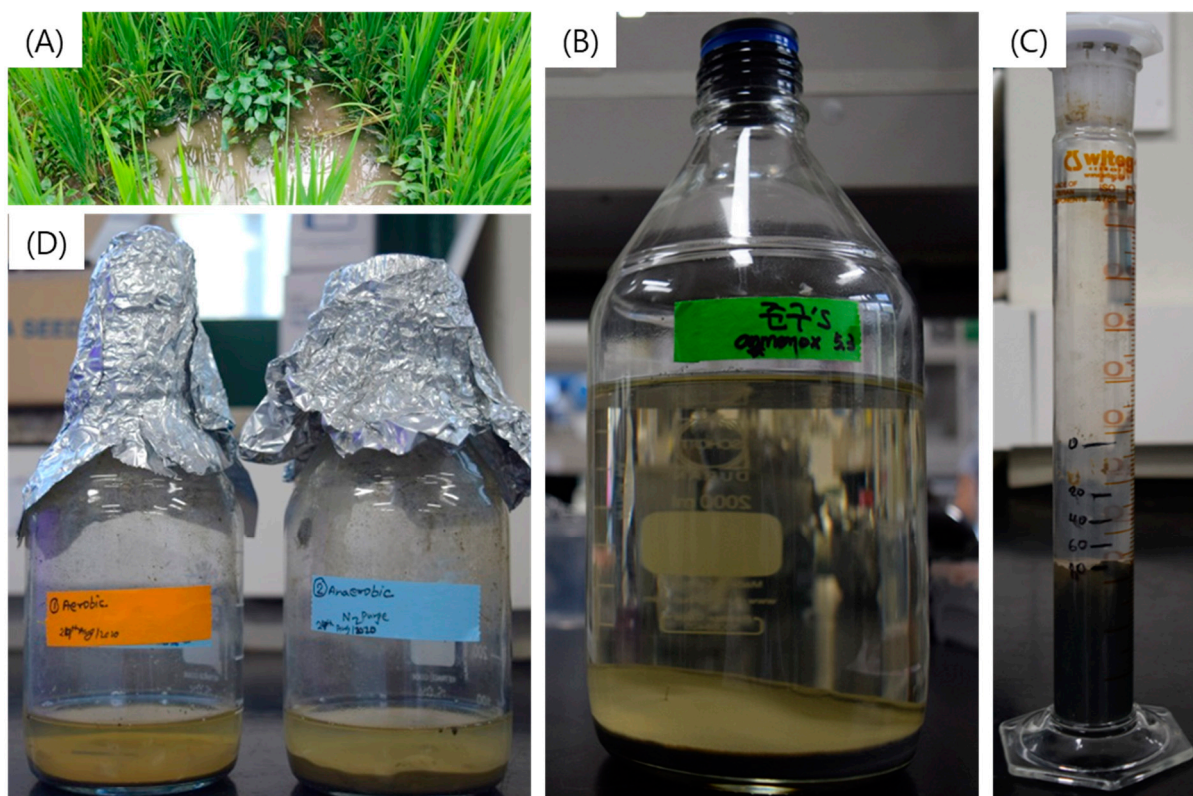
**Table S3.** Ratios of *hzsA* gene to 16S rRNA gene from the compared aerobic and anaerobic incubations (Experiment 3)

Sample	The ratio of <i>hzsA</i> to 16S rRNA gene (by mean)
Initial (AC-0)	4.019
Aerobic, after 30 d (AES-30)	5.949
Anaerobic, after 30 d (ANS-30)	5.510

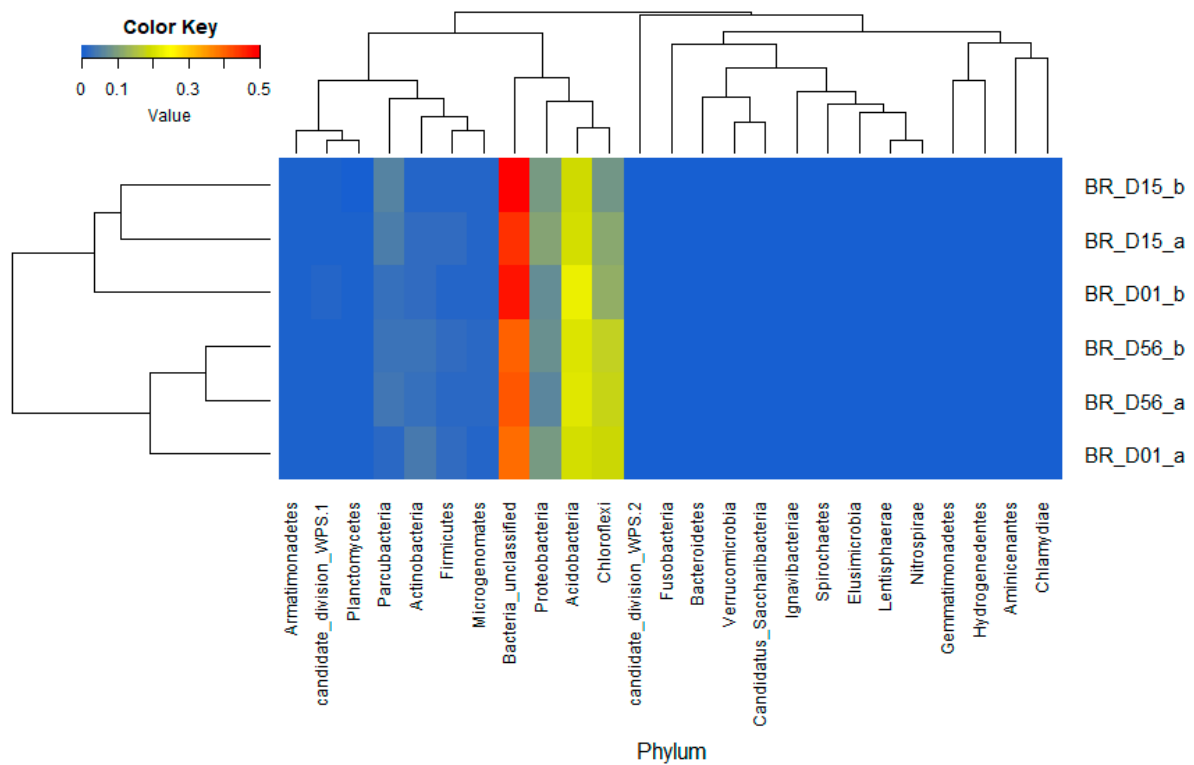
**Table S4.** Alpha-diversity of the microbial communities from the compared aerobic and anaerobic incubations (Experiment 3)

Sample	No. of sequence	Good's coverage	observed species (OTU)	Chao1	Shannon	inverse Simpson
AC_T00	12402	0.8868±0.0003	2588.2±2.7	5089±28	6.632±0.001	237.4±0.5
AES_30	12402	0.9063	2161	4344	6.364	194.0
ANS_30	12402	0.8988±0.0003	2303.8±3.0	4841±36	6.427±0.002	187.5±0.5

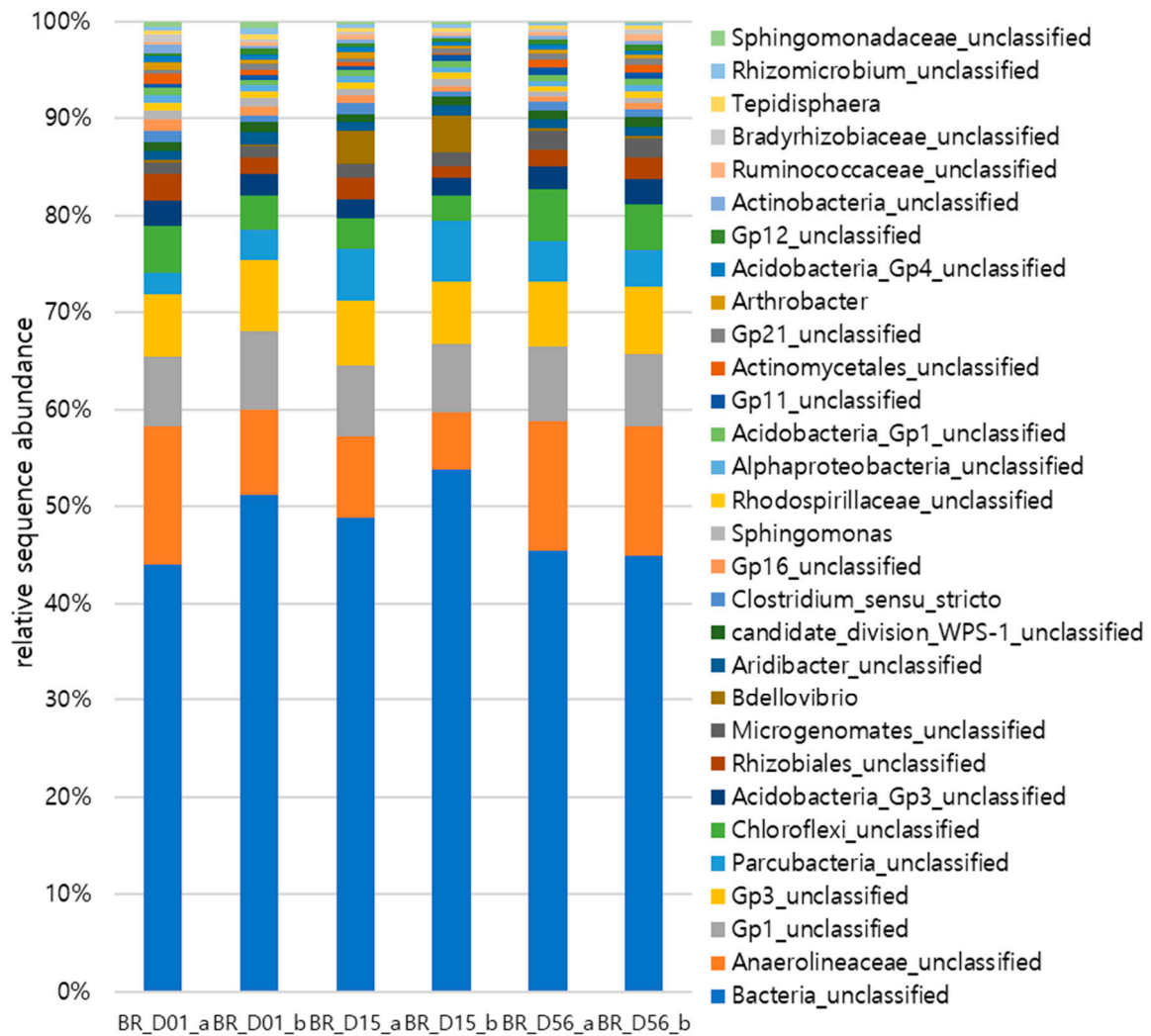
**Figure S1.** Anammox enrichment batches using the rice paddy soils from Rural Development Administration field, Jeonju, South Korea (A). (B) Experiment 1 – Anaerobic incubation, (C) Experiment 2 – Microaerobic column incubation, (D) Experiment 3 – Compared aerobic and anaerobic incubations.



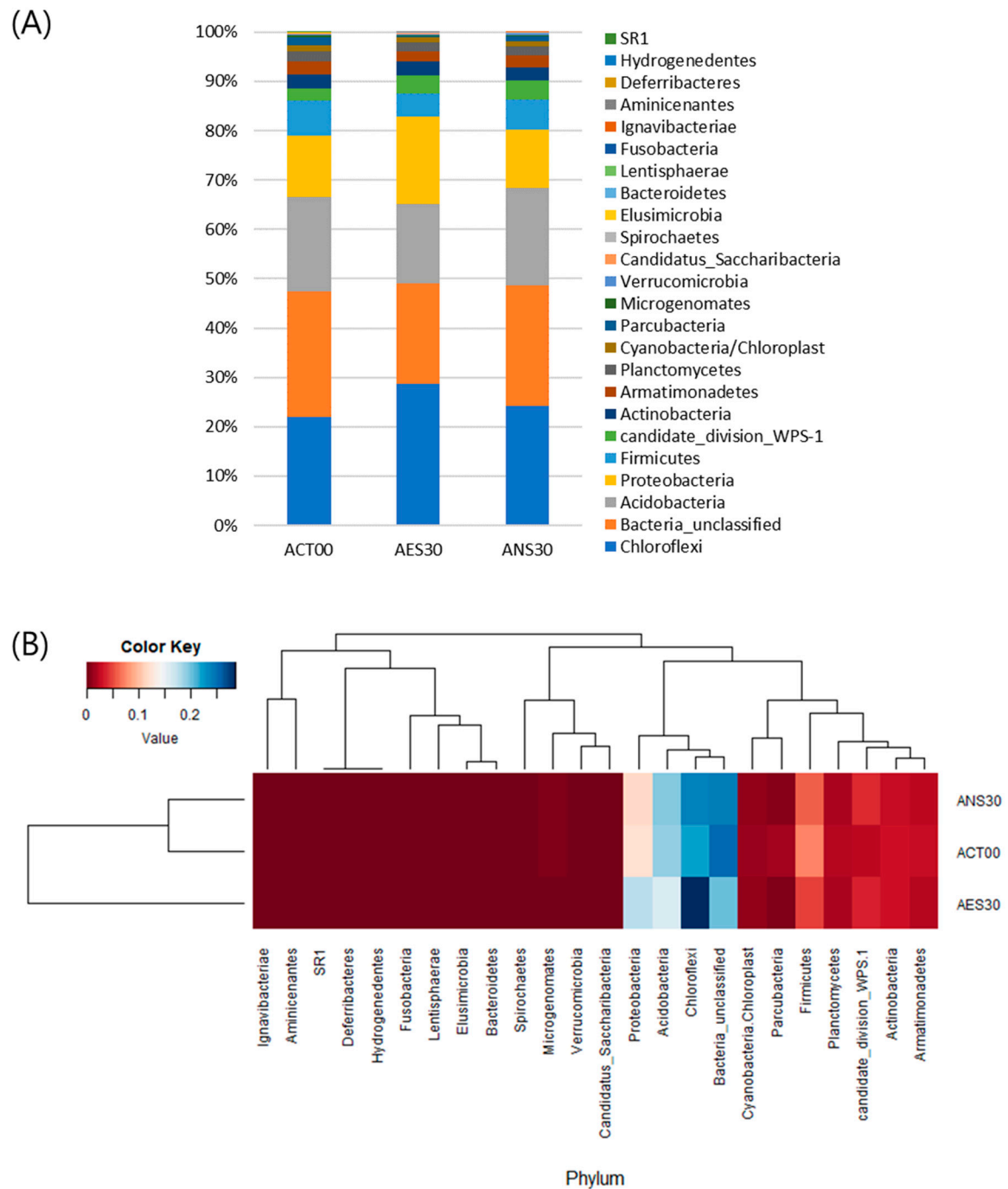
**Figure S2.** Heatmap of the sequence abundance at the phylum level for the anaerobic incubation (Experiment 1). Vertical and horizontal dendrograms based on the Bray-Curtis dissimilarity.



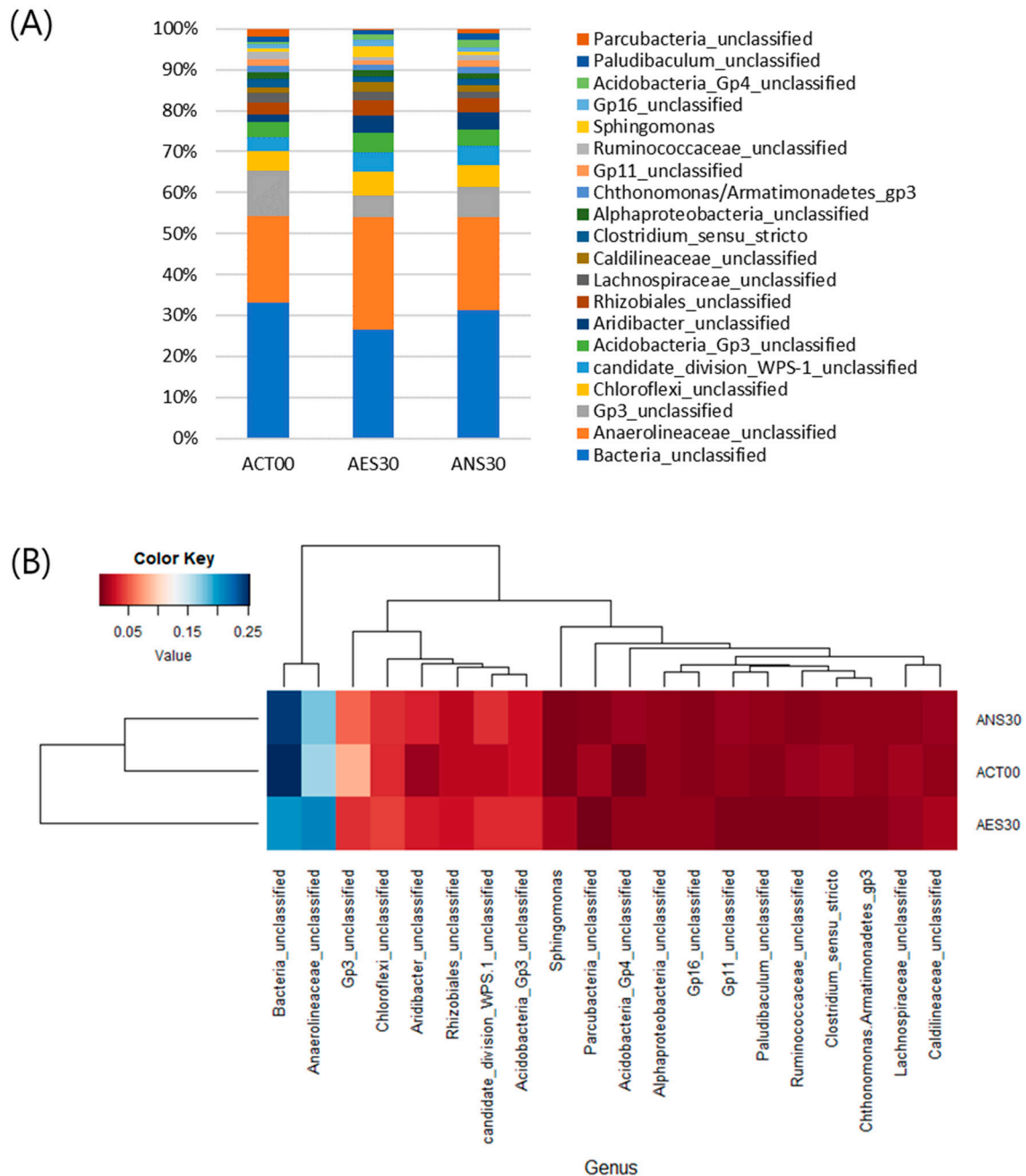
**Figure S3.** Relative sequence abundance of the top 30 genera for the anaerobic incubation (Experiment 1).



**Figure S4.** Relative sequence abundance (A) and its heatmap (B) of phyla from the compared aerobic and anaerobic incubations (Experiment 3).



**Figure S5.** Relative sequence abundance (A) and its heatmap (B) of top 20 genera from the compared aerobic and anaerobic incubations (Experiment 3).



## References

- Brunk, C.F.; Avaniss-Aghajani, E.; Brunk, C.A. A computer analysis of primer and probe hybridization potential with bacterial small-subunit rRNA sequences. *Appl. Environ. Microbiol.* **1996**, *62*, 872–879.
- Harhangi, H.R.; Le Roy, M.; van Alen, T.; Hu, B.L.; Groen, J.; Kartal, B.; Tringe, S.G.; Quan, Z.X.; Jetten, M.S.; Op den Camp, H.J. Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. *Appl. Environ. Microbiol.* **2012**, *78*, 752–758.
- Herlemann, D.P.R.; Labrenz, M.; Jürgens, K.; Bertilsson, S.; Waniek, J.J.; Andersson, A.F. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* **2011**, *5*, 1571–1579.
- Lücker, S.; Wagner, M.; Maixner, F.; Pelletier, E.; Koch, H.; Vacherie, B.; Rattei, T.; Damsté, J.S.S.; Spieck, E.; Le Paslier, D.; et al. A *Nitrospira* metagenome illuminates the physiology and evolution of globally important nitrite-oxidizing bacteria. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13479–13484.
- Rotthauwe, J.H.; Witzel, K.P.; Liesack, W. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **1997**, *63*, 4704–4712.