Supplementary Information

Transcriptional Response of the Archaeal Ammonia Oxidizer *Nitrosopumilus maritimus* to Low and Environmentally Relevant Ammonia Concentrations

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Supplementary Materials and Methods

Medium. The basal salt medium contained NaCl 26 g, $MgSO_4 \cdot 7H_2O$ 5 g, $MgCl_2 \cdot 6H_2O$ 5 g, $CaCl_2 \cdot 2H_2O$ 1.5 g, KBr 0.1 g in 1000 ml of ultra pure water. The following solutions were autoclaved separately and added to 1 liter of autoclaved basal salt medium at room temperature; 10 ml of 1 M HEPES in 0.6 M NaOH, 2 ml of 1 M sodium bicarbonate, 5 ml of 2.93 mM KH_2PO_4, 1 ml of 7.5 mM FeNa-EDTA, 1 ml of non-chelated trace element mixture (0.5 mM H_3BO_3, 0.5 mM $MnCl_2 \cdot 4H_2O$, 0.8 mM $CoCl_2 \cdot 6H_2O$, 0.1 mM $NiCl_2 \cdot 6H_2O$, 0.01 mM $CuCl_2 \cdot 2H_2O$, 0.5 mM $ZnSO_4 \cdot 7H_2O$, 0.15 mM $Na_2MoO_4 \cdot 2H_2O$ dissolved in 100 mM HCl), and 1 ml of 1 M NH_4Cl. The pH of the medium after adding the above stock solutions was 7.5.

qPCR. The DNA was extracted from the cells fixed with RNALater solution using DNeasy Blood & Tissue Kit (QIAGEN). The *amoA* genes of *N. maritimus* SCM1 in medium were quantified by real-time PCR in a LightCycler system with LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics). The primer set, CrenAmo-SCM1-F and CrenAmoAModR (Table S1), was used for the quantitative PCR. Ten-fold dilution series of genomic DNA of *N. maritimus* SCM1 was used for DNA standard for the quantitative PCR. The cycling condition was as follows: an initial denaturation at 95°C for 5 min; 35 cycles at 95°C for 10 s, 55°C for 10 s and 72°C for 13 s, followed by a melting curve analysis. The fluorescence intensity was measured at 77˚C. The amplification efficiency of quantitative PCR averaged 87.2% ($r^2 = 0.999$).

RNA standards for qRT-PCR. To generate RNA standards for qRT-PCR, DNA fragments for each RNA standard were amplified with the following PCR primer sets: CrenAmo-SCM1-F and CrenAmoA-ModR (1) for the *amoA* gene, amt1F-490 and amt1R-644 for the *amt1* gene and amt2F-956 and amt2R-811 for the *amt2*, hcd-911F-SCM1 and hcd-1267R-SCM1 for the *hcd* gene, Slayer2614F and Slayer2821R for the *slp1* gene, and SL2-2908F and SL2-3030R for the *slp2* gene (Table S1). PCR amplified DNA fragments were cloned into pCRII-TOPO (Life Technologies) with TOPO TA Cloning Dual Promoter Kit (Life Technologies). Plasmids were extracted with PureLink Quick Plasmid Miniprep Kit (Life Technologies) and RNA amplified from purified plasmids using T7 RNA Polymerase (Life Technologies). The RNA was extracted with a general acidic phenol method and treated twice with DNase (Life Technologies). The length and quality of each RNA standards was confirmed with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA), and quantified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

REFERENCE

1. **Mincer TJ, Church MJ, Trent Taylor L, Preston C, Karl DM, DeLong EF.** 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. **9:**1162-1175.

58 **Table S1** PCR primers used in this study.

primer	target gene	sequence (5' - 3')
CrenAmo-SCM1-F	*amoA* of SCM1 strain	CCAAGTAGGTAAGTTCTATAA
CrenAmoA-ModR	*amoA* of SCM1 strain	AAGCGGCCATCCATCTGTA
amt1F-490	*amt1* of SCM1 strain	GGTGGTATTGTAATTCACAC
amt1R-644	*amt1* of SCM1 strain	AGCACTACCTGCGTTAAATC
amt2F-956	*amt2* of SCM1 strain	GTAGTACACATTTCTTCAGG
amt2R-811	*amt2* of SCM1 strain	TTCACTACCTGCGTTAAAGC
hcd-911F-SCM1[a]	*hcd* of SCM1 strain	AGCTATGTTTGCAAAACAGG
hcd-1267R-SCM1	*hcd* of SCM1 strain	CTCATTCTGTTTTCCACATC
hcd-1153F-SCM[b]	*hcd* of SCM1 strain	TTAGCTCAAGATATTGCAGG
Slayer2614F	*slp1* of SCM1 strain	GTTGTTGGTTCAGCAACTAC
Slayer2821R	*slp1* of SCM1 strain	GATCTACACCAATCAACTGG
SL2-2908F	*slp2* of SCM1 strain	ACACCATATACCTACGCAGG
SL2-3030R	*slp2* of SCM1 strain	TCTAACACCTGCTGAGTCGC

[a] Primer hcd-911F-SCM1 was used only to amplify *hcd* gene fragment during the making of RNA standard.

[b] Primer hcd-1153F-SCM1 was used only for qRT-PCR analyses.

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61 **Table S2** PCR cycles for qRT-PCR.

PCR cycle	target RNA of SCM1
61˚C for 20 min, 95˚C for 30 sec, 35 cycles of 95˚C for 5 sec, 56˚C for 10 sec, and 72˚C for 13 sec, and a detection at 77˚C for 3 sec, followed by a melting curve (65 to 95˚C) with heating rate of 0.1˚C/sec.	*amoA, amt1, amt2, slp1*
61˚C for 20 min, 95˚C for 30 sec, 35 cycles of 95˚C for 5 sec, 56˚C for 10 sec, and 72˚C for 13 sec, and a detection at 75˚C for 3 sec, followed by a melting curve (65 to 95˚C) with heating rate of 0.1˚C/sec.	*hcd*
61˚C for 20 min, 95˚C for 30 sec, 35 cycles of 95˚C for 5 sec, 56˚C for 10 sec, and 72˚C for 13 sec, and a detection at 79˚C for 3 sec, followed by a melting curve (65 to 95˚C) with heating rate of 0.1˚C/sec.	*slp2*

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Fig. S1 Overview of experiment and sampling time points for batch culture, dialysis bag systems, and NH_4^+-starvation experiments.

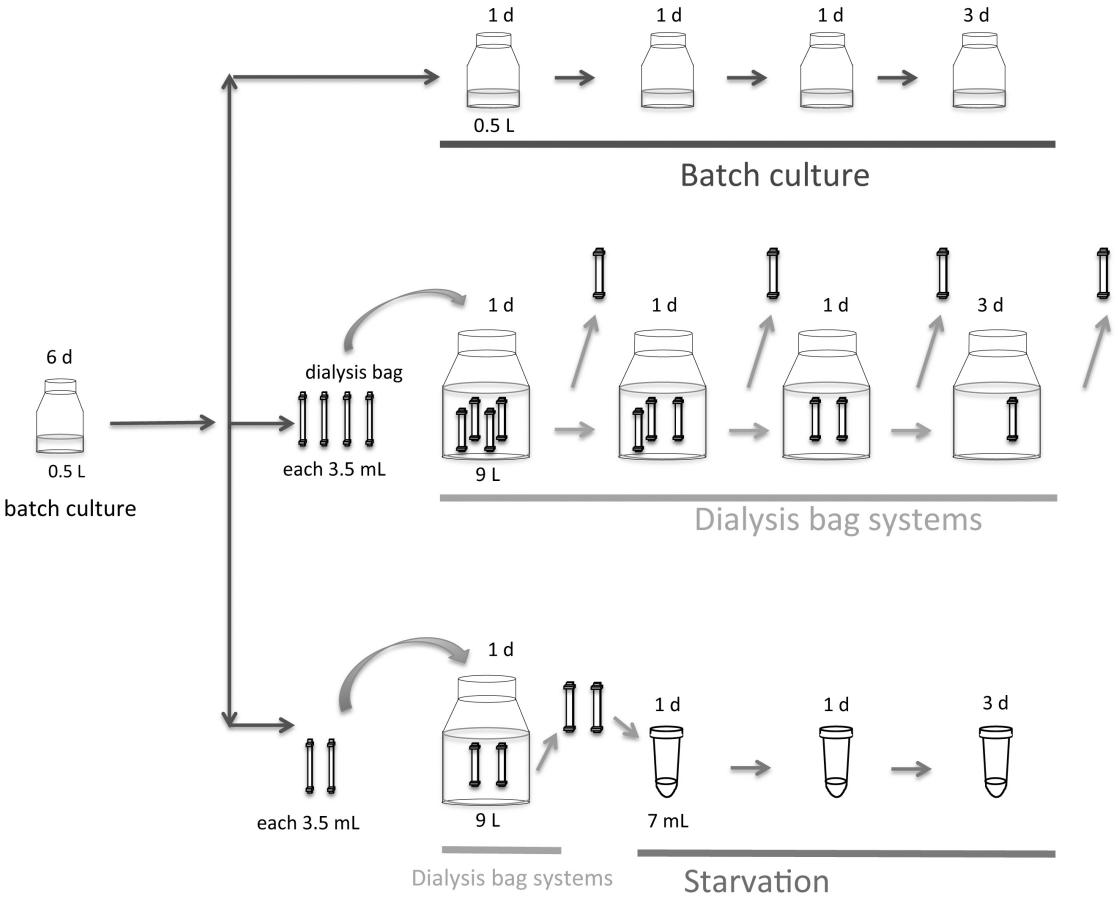

Fig. S2 Overview of experiment and sampling time points for short-term ammonia starvation and readdition studies.

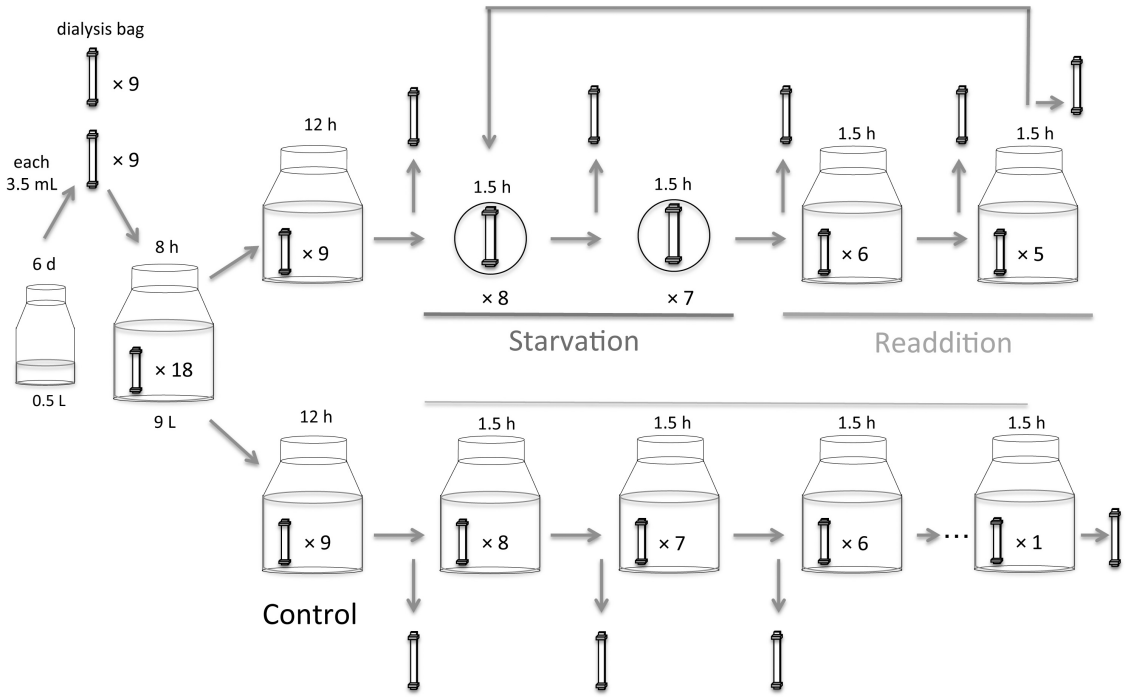

Fig. S3 Changes in concentrations of (**A**) NH_4^+ and (**B**) NO_2^- in media of *N. maritimus* SCM1 during short-term starvation and readdition experiments (open squares, inner dialysis bag; closed squares, outer dialysis bag), and continuous dialysis bag growth (open circles, inner dialysis bag; closed circles, outer dialysis bag). These concentrations were determined with each dialysis bag described in Fig. 3.