

Table S1 In situ biogeochemical parameters at the incubation experiment sites.

Site_Layer	Temperature (°C)	DO ($\mu\text{mol L}^{-1}$)	NH_4^+ ($\mu\text{mol L}^{-1}$)	NO_3^- ($\mu\text{mol L}^{-1}$)	NO_2^- ($\mu\text{mol L}^{-1}$)
P05_S	30	61.3	40.1	126.1	20.5
P05_B	30	54.7	33.3	123.5	24.5
P01_S	29	30.9	167.2	84.0	11.9
P01_B	29	30.0	166.5	82.0	11.6

S, surface; B, bottom.

1 **Table S2** Isotopic fractionation of ^{15}N during bacterial and archaeal ammonia oxidation, bacterial nitrifier-denitrification, and bacterial
 2 denitrification.

Pathway	Microorganisms	Species	Substrate	$\delta^{15}\text{N-N}_2\text{O}$	References
Ammonia oxidation	β -proteobacteria	<i>Nitrosomonas europaea</i>	NH_4^+	-68 – -60‰	Yoshida, 1988; Toyoda et al., 2017
			NH_4^+	-46.9 – -46.1‰	Sutka et al., 2006
		NH_4^+	-19.88 ± 0.39‰	Jung et al., 2014	
		<i>Nitrosomonas marina</i> C-113a	NH_4^+	-54.9 – -15.2‰ (0.5% O_2) ^a	Frame and Casciotti, 2010
			NH_4^+	-13.6 – -6.7‰ (20% O_2) ^a	
		γ -proteobacteria	<i>Methylomonas methanica</i>	NH_4^+	-39.4‰
	Archaea	CN25 (marine)	NH_4^+	6.3 – 10.2‰	Santoro et al., 2011
			NH_4^+	-13.53 ± 2.12‰	Jung et al., 2014
			NH_4^+	-16.96 ± 1.81‰	Jung et al., 2014
			NH_4^+	-16.49 ± 2.18‰	Jung et al., 2014
			NH_4^+	-15.32 ± 0.16‰	Jung et al., 2014
			NH_4^+	-12.91 ± 1.50‰	Jung et al., 2014

		CS (acid mine)	NH ₄ ⁺	-35.54±0.89‰	Jung et al., 2014
			NH ₂ OH	-38.1 – -20.4‰	Sutka et al., 2003
		<i>Nitrosomonas europaea</i>	NH ₂ OH	-5.5 – 5.1‰	Sutka et al., 2006
	β -proteobacteria		NH ₂ OH	-34.0 – -13.8‰	Yamazaki et al., 2014
		<i>Nitrospira multiformis</i>	NH ₂ OH	-3.9 – 1.7‰ (Average -0.3±2.9‰)	Sutka et al., 2006
Hydroxylamine oxidation ^b		<i>Nitrosomonas marina</i> C-113a	NH ₂ OH	-6.7‰	Frame and Casciotti, 2010
		<i>Nitrosococcus oceani</i>	NH ₂ OH	-17.9 – -5.8‰	Yamazaki et al., 2014
	γ -proteobacteria		NH ₂ OH	-0.3 – 1.7‰ (Average 0.0±1.2‰)	Sutka et al., 2003
		<i>Methylococcus capsulatus</i>	NH ₂ OH	1.3 – 5.2‰ (Average 3.4±1.9‰)	Sutka et al., 2006
		<i>Nitrosomonas marina</i> C-113a	NO ₂ ⁻	-57.6±4.1‰	Frame and Casciotti, 2010
Nitrifier-denitrification	β -proteobacteria	<i>Nitrosomonas europaea</i>	NO ₂ ⁻	-39.1 – -31.0‰ (Average -34.8±2.7‰)	Sutka et al., 2003
		<i>Nitrospira multiformis</i>	NO ₂ ⁻	-24.2 – -21.5‰ (Average -22.9±0.6‰)	Sutka et al., 2006
Denitrification	γ -proteobacteria	<i>Pseudomonas fluorescens</i>	NO ₃ ⁻	-37.2 – -14.9‰	Toyoda et al., 2005
	α -proteobacteria	<i>Paracoccus denitrificans</i>	NO ₃ ⁻	-20.0 – -7.9‰	Toyoda et al., 2005

1 ^aO₂ conditions of the incubation experiments.

- 1 ^bIsotopic fractionation for ^{15}N when using NH_4^+ as a substrate was discussed in natural environments, although the values when using NH_2OH as
- 2 a substrate are listed here.

1 **References**

- 2 Frame, C. H., and Casciotti, K. L.: Biogeochemical controls and isotopic signatures of
3 nitrous oxide production by a marine ammonia-oxidizing bacterium,
4 *Biogeosciences*, 7, 2695–2709, 2010.
- 5 Jung, M. Y., Well, R., Min, D., Gieseemann, A., Park, S. J., Kim, J. G., and Rhee, S. K.:
6 Isotopic signatures of N₂O produced by ammonia-oxidizing archaea from soils,
7 *ISME J.*, 8, 1115–1125, 2014.
- 8 Mandernack, K. W., Mills, C. T., Johnson, C. A., Rahn, T., and Kinney, C.: The $\delta^{15}\text{N}$
9 and $\delta^{18}\text{O}$ values of N₂O produced during the co-oxidation of ammonia by
10 methanotrophic bacteria, *Chem. Geol.*, 267, 96–107, 2009.
- 11 Santoro, A. E., Buchwald, C., McIlvin, M. R., and Casciotti K. L.: Isotopic Signature
12 of N₂O Produced by Marine Ammonia-Oxidizing Archaea, *Science*, 333,
13 1282–1285, 2011.
- 14 Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Gandhi, H., and Breznak, J. A.: Nitrogen
15 isotopomer site preference of N₂O produced by *Nitrosomonas europaea* and
16 *Methylococcus capsulatus* Bath, *Rapid Commun. Mass Spectrom.*, 17, 738–745,
17 2003.
- 18 Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Breznak, J. A., Gandhi, H., Pitt, A. J., and
19 Li, F.: Distinguishing Nitrous Oxide Production from Nitrification and
20 Denitrification on the Basis of Isotopomer Abundances, *Appl. Environ.*
21 *Microbiol.*, 72, 638–644, 2006.
- 22 Toyoda, S., Mutoke, H., Yamagishi, H., Yoshida, N., Tanji, Y.: Fractionation of N₂O
23 isotopomers during production by denitrifier, *Soil. Biol. Biochem.*, 37,
24 1535–1545, 2005.
- 25 Toyoda, S., Yoshida, N., and Koba, K.: Isotopocule analysis of biologically produced
26 nitrous oxide in various environments, *Mass Spectrom. Rev.*, 36, 135–160,
27 2017.
- 28 Yamazaki, T., Hozuki, T., Arai, K., Toyoda, S., Koba, K., Fujiwara, T., and Yoshida,

- 1 N.: Isotopomeric characterization of nitrous oxide produced by reaction of
2 enzymes extracted from nitrifying and denitrifying bacteria, *Biogeosciences*, 11,
3 2679–2689, 2014.
- 4 Yoshida, N.: ^{15}N -depleted N_2O as a product of nitrification, *Nature*, 335, 528–529,
5 1988.