

Supplementary Table 1, 2

Supplementary Data1, 2 and 3

Communications biology

Shotgun metagenomic analysis of the microbial communities and associated network of nitrogen metabolism genes in the Ryukyu limestone aquifer

Rio Maruyama¹, Ko Yasumoto¹, Nanami Mizusawa¹, Mariko Iijima², Mina Yasumoto-Hirose³, Akira Iguchi², Oktanius Richard Hermawan⁴, Takahiro Hosono^{5,6}, Ryogo Takada⁷, Ke-Han Song⁸, Ryuichi Shinjo^{8,9}, Shugo Watabe¹, Jun Yasumoto^{10*}

¹Kitasato University School of Marine Biosciences, 1-15-1 Kitasato, Minami, Sagamihara, Kanagawa 252-0373, Japan,

²National Institute of Advanced Industrial Science and Technology (AIST), AIST Tsukuba Central 7, 1-1-1 Higashi, Tsukuba, Ibaraki, 305-8567 Japan,

³Tropical Technology Center Ltd., 5-1 Suzaki, Uruma, Okinawa 904-2234, Japan

⁴Earth and Environmental Science, Graduate School of Science and Technology, Kumamoto University, 2-39-1, Kurokami, Kumamoto 860-8555, Japan

⁵Faculty of Advanced Science and Technology, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan

⁶International Research Organization for Advanced Science and Technology, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan

⁷Center for Strategic Research Projects, University of Ryukyus, Nishihara, Senbaru, Okinawa 903-0213, Japan

⁸Department of Physics and Earth Sciences, University of the Ryukyus, Senbaru-1, Nishihara, Okinawa 903-0213, Japan

⁹Research Institute for Humanity and Nature, 457-4 Motoyama, Kamigamo, Kita-ku, Kyoto, 603-8047, Japan

¹⁰Faculty of Agriculture, University of the Ryukyu, 1 Senbaru, Nishihara, Nakagami, Okinawa 903-0213, Japan.

*Corresponding author (yasumoto@agr.u-ryukyu.ac.jp)

Supplementary Table 1 Total number of reads in each classification at sites 1, 2 and 3 in November and December 2021 and, January 2022.

Reads	Nov21 Site1	Dec21 Site1	Jan22 Site1	Nov21 Site2	Dec21 Site2	Jan22 Site2	Nov21 Site3	Dec21 Site3	Jan22 Site3
Domain	367679	362166	363161	367264	368928	364364	362959	346508	342602
Phylum	290309	257322	277378	270614	286119	261160	241435	251530	233873
Class	226210	207982	223534	219475	226220	206638	188679	221970	211605
Order	205991	185826	199407	151037	181878	155346	153777	185834	175503
Family	157205	161903	171425	120240	148606	134062	127975	153338	150804
Genus	132104	138825	142921	86403	115490	109252	92502	119054	118923

Supplementary Table 2 Precipitation one week prior to sampling date at sites 1, 2 and 3 in November and December 2021 and January 2022. Using data from Itokazu City, Okinawa, Japan.

Sampling date	Before sampling days ago	precipitation (mm/day)
Nov.2021	7	0
	6	4
	5	5
	4	0
	3	0
	2	0
	1	0
	0	0
Dec.2021	7	1.125
	6	0
	5	0
	4	0
	3	0
	2	0
	1	37.5
	0	13
Jan.2022	7	2
	6	0
	5	36
	4	5
	3	0
	2	0
	1	19.5
	0	53

Supplementary Data 1 Percentage of microorganisms in each domain that harbor nitrogen metabolism genes were performed using data sets obtained from groundwater samples collected from Site 2 in November and December 2021 and, January 2022

Supplementary Data 2 Percentage of microorganisms in each domain that harbor nitrogen metabolism genes were performed using data sets obtained from groundwater samples collected from Site 3 in November and December 2021 and, January 2022.

Supplementary Data 3 Microorganisms in each domain that harbor nitrogen metabolism genes were performed using data sets obtained from groundwater samples collected from Site 2 in November and December 2021 and January 2022.

Supplementary Data 4 Microorganisms in each domain that harbor nitrogen metabolism genes were performed using data sets obtained from groundwater samples collected from Site 3 in November and December 2021 and January 2022.