Supplemental information for

Tracking the direct impact of rainfall on groundwater at Mt. Fuji by multiple analyses including microbial DNA

Ayumi Sugiyama[1, 2, 3], Suguru Masuda[1], Kazuyo Nagaosa[1], Maki Tsujimura[3] and Kenji Kato[1]

[1] Department of Geosciences, Graduate School of Science, Shizuoka University, Shizuoka, Japan, [2] Asano Taiseikiso Engineering Co., Ltd., [3] Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.

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Introduction

This supplemental information provides a summary of samples analysis, father method and results by bacterial gene analysis using a Denaturing Gradient Gel Electrophoresis and results of hexadiagram of groundwater and precipitation at the foot of Mt. Fuji.

Table S1. Summary of sample analysis.

	rainfall event (amount of rainfall)	SP-0m, Shibakawa, spring water, 726ma.s.l.				GW-42m, Yodoshi, groundwater, 150ma.s.l., 42m depth				Gw-550m, Aoki, groundwater, 175ma.s.l., 550m depth				R1, Go-gome, rainwater, 2,364m a.s.l.				R2, Kokuyurin, rainwater, 1,431m a.s.l.				R3, Ni-gome, rainwater, 1,081m a.s.l.				R4, Asagiri, rainwater, 850m a.s.l.				R5, Shibakawa, rainwater, 723m a.s.l.			
sampling date		E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS	E	B	DG	NGS
2012/6/15	Event1 (30mm)	○	○																																		
2012/10/18		○	○																															○	○		
2012/11/8		○	○																																		
2012/11/22		○	○																																		
2013/6/17		○	○	○										○	○											○	○			○	○			○	○		
2013/7/2						○	○	○		○	○	○														○	○										
2013/7/10		○	○	○	○																									○	○			○	○		
2013/8/6		○	○	○														○	○											○	○			○	○		
2013/8/7						○	○	○		○	○	○														○	○										
2013/8/27																		○	○							○	○			○	○			○	○		
2013/9/4		○	○	○		○	○	○		○	○	○																						○	○		
2013/9/9	Event2 (>300mm)	○	○	○														○	○							○	○			○	○			○	○		
2013/9/19			○	○	○	○	○	○		○	○	○	○					○	○	○						○	○	○		○	○			○	○	○	○
2013/10/17		○	○			○	○	○		○	○																										
2014/3/28		○	○	○		○	○	○		○	○															○	○			○	○			○	○		
2014/5/14																														○	○						
2014/5/31																														○	○						
2014/6/27		○	○	○		○	○			○	○																										
2014/6/28	Event3 (100mm)																													○	○						
2014/7/18		○	○	○		○	○			○	○							○	○							○	○			○	○			○	○		
2014/8/14		○	○	○		○	○			○	○							○	○							○	○			○	○			○	○		
2014/9/16	Event4 (>300mm)	○	○	○		○	○			○	○							○	○							○	○			○	○			○	○		
2014/10/16		○	○	○		○	○			○	○		○					○	○							○	○			○	○			○	○		
2014/10/30		○	○	○		○	○			○	○															○	○			○	○			○	○		
2014/11/20		○	○	○		○	○			○	○																										
2015/5/30													○																								

E: environmental parameters, stable isotopic analysis, Ion analysis and dissolved silica,
B: number of prokaryote(TDC), Bacteria (CARD-FISH) and Archaea (CARD-FISH),
DG: DGGE analysis,
NGS: next generation sequence analysis.

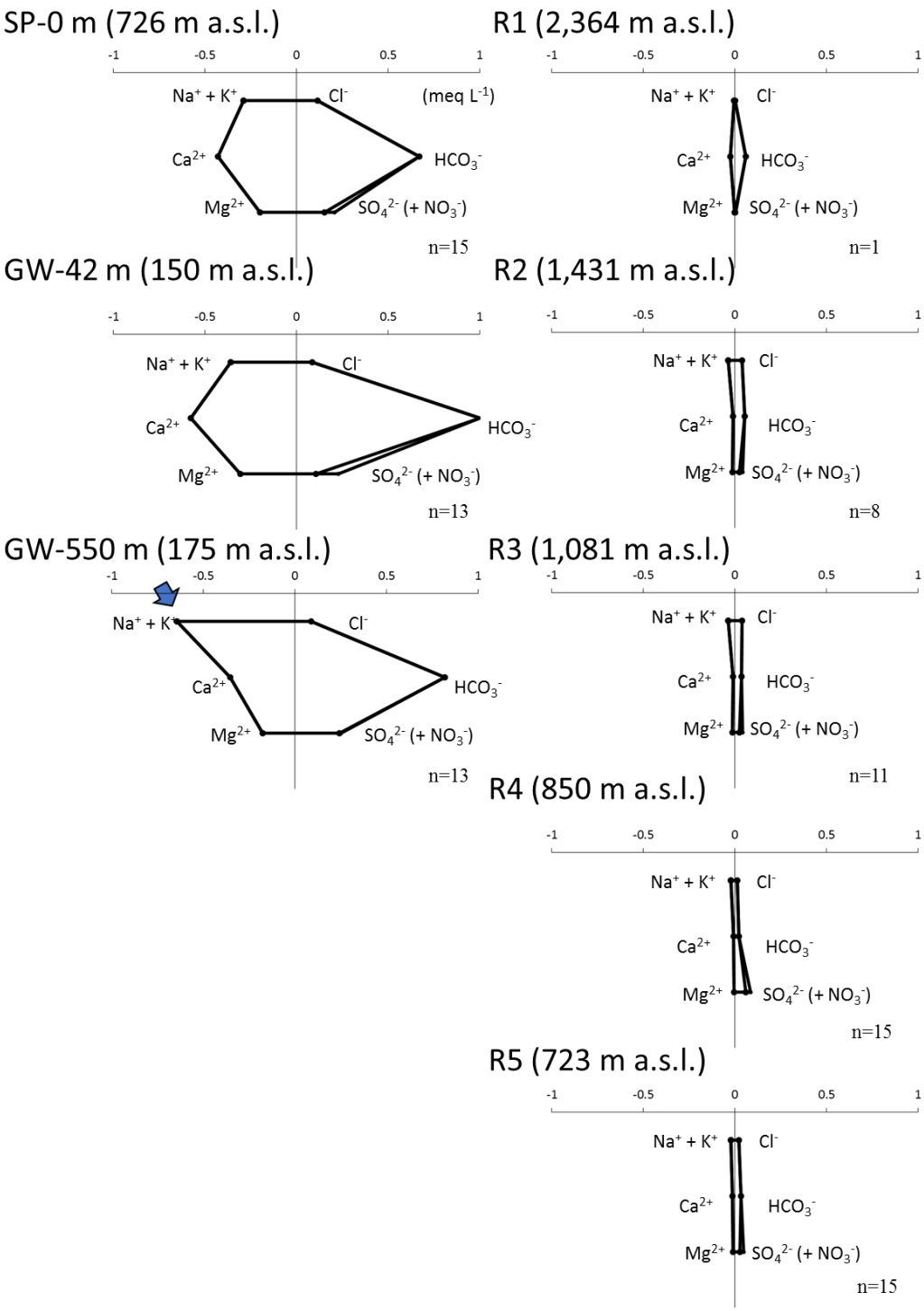

Figure S1. Hexadiagram shows major eight ions dissolved in water by their relative abundance. Anions are shown in the right side of the figure, while cations are in the left. The shape of diagram suggests characteristics of water examined. Thus that of groundwater (left panels) and precipitation (right panels). Observation period is from May 2013 to November 2014. Blue arrow indicates high concentration of Na^+ at GW-550m.

Methods for denaturing gradient gel electrophoresis (DGGE) analysis

A nested-PCR approach was employed with Bacteria-specific primers Bac27F(5′-AGA GTT TGA TCM TGG CTC AG-3′)-Uni1492R(5′-GGY TAC CTT GTT ACG ACT T-3′) (DeLong, 1992) and 341F-GC(5′- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG -3′)-534R(5′- ATT ACC GCG GCT GCT GG -3′) (Muyzer et al., 1993) or Archaea-specific primers ARC344F(5′-ACG GGG YGC AGC AGG CGC GA -3′)-ARC915R(5′- GTG CTC CCC CGC CAA TTC CT -3′) (Vetriani et al., 1999) and 344F-GC(5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GAC GGG GYG CAG CAG GCG CGA-3′)-518R(5′- ATT ACC GCG GCT GCT GG -3′) (Muyzer et al., 1993) was employed to amplify variable V3 region of bacterial and archaeal 16S rRNA gene. Primary and secondary amplification reactions were performed in a 25 μL PCR mixture consisted of 2.5 μL 10×PCR buffer (TaKaRa Bio Inc., Shiga, Japan), 250 μM dNTPs (TaKaRa Bio Inc.) 2.0 μL, 1 U of ExTaq polymerase (TaKaRa Bio Inc.) 0.125 μL, 1.5 M of each primer and 50 ng template. The products were amplified in Thermal cycler Wako WK-0232 (Wako Pure Chemical Industries Ltd.) under the following conditions: for *Bacteria*, 94°C for 5 min, then 20 cycles for primary reactions and 40 cycles for secondary reactions of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 40 sec, a final extension of 72°C for 10 min [Jiang et al., 2014], for Archaea, 94°C for 5 min, then 20 cycles for primary reactions and 40 cycles for secondary reactions of 94°C for 30 sec, 48°C for 30 sec, and 72°C for 30 sec, a final extension of 72°C for 10 min (Vetriani et al., 1999). PCR products confirmed by 2% (w/v) agarose gel electrophoresis, stained with ethidium bromide (EB), and visualized under UV light. Obtained PCR products were stored at −20°C for subsequent DGGE analysis.

DGGE was performed by Dcode Universal Mutation Detection System (BIO－RAD Laboratories Inc., CA, USA) according to Muyzer et al. (1993). A 35%-60% vertical denaturing gradient polyaclylamide gel was formed mixing 0% denaturant solution (40% Acrylamide/Bisacrylamide 5 mL, 50×TAE Buffer 250 μL, MilliQ 19.75 mL) and 100% denaturant solution (40% Acrylamide/Bisacrylamide 3 mL, 50×TAE Buffer 150 μL, formamide 6 mL, Urea 6.3 g, MilliQ 200 μL) with Model 475 gradient former. PCR products were applied directly onto polyaclylamide gel. Electrophoresis was performed at a constant voltage of 75 V and at constant temperature of 60°C for 12 hours in 0.5×TAE buffer. Following electrophoresis, the gel was stained by SYBR Gold Nucleic Acid Gel Stain (SYBR Gold 0.5 μL, Thermo Fisher Scientific Inc. 1×TAE 5 mL) for 30 min in dark and photographed with UV transillumination by AE-6932GXES-U (ATTO, Tokyo, Japan).

DNA fragments from the major DGGE bands were extracted and purified using AxyPrep PCR Clean-up kit (Axygen Scientific Inc., CA, USA) for sequencing. The sequences of PCR products were determined by the capillary DNA sequencer (CEQ8000 DNA Analysis System, Beckman Coulter Inc. CA, USA or Applied Biosystems 3730xl, Thermo Fisher Scientific Inc.). Sequences were aligned using Basic Local Alignment Search Tool (BLAST; Altschul et al., 1997) in the DNA Data Bank of Japan (DDBJ; Thompson et al., 1994).

Reference;

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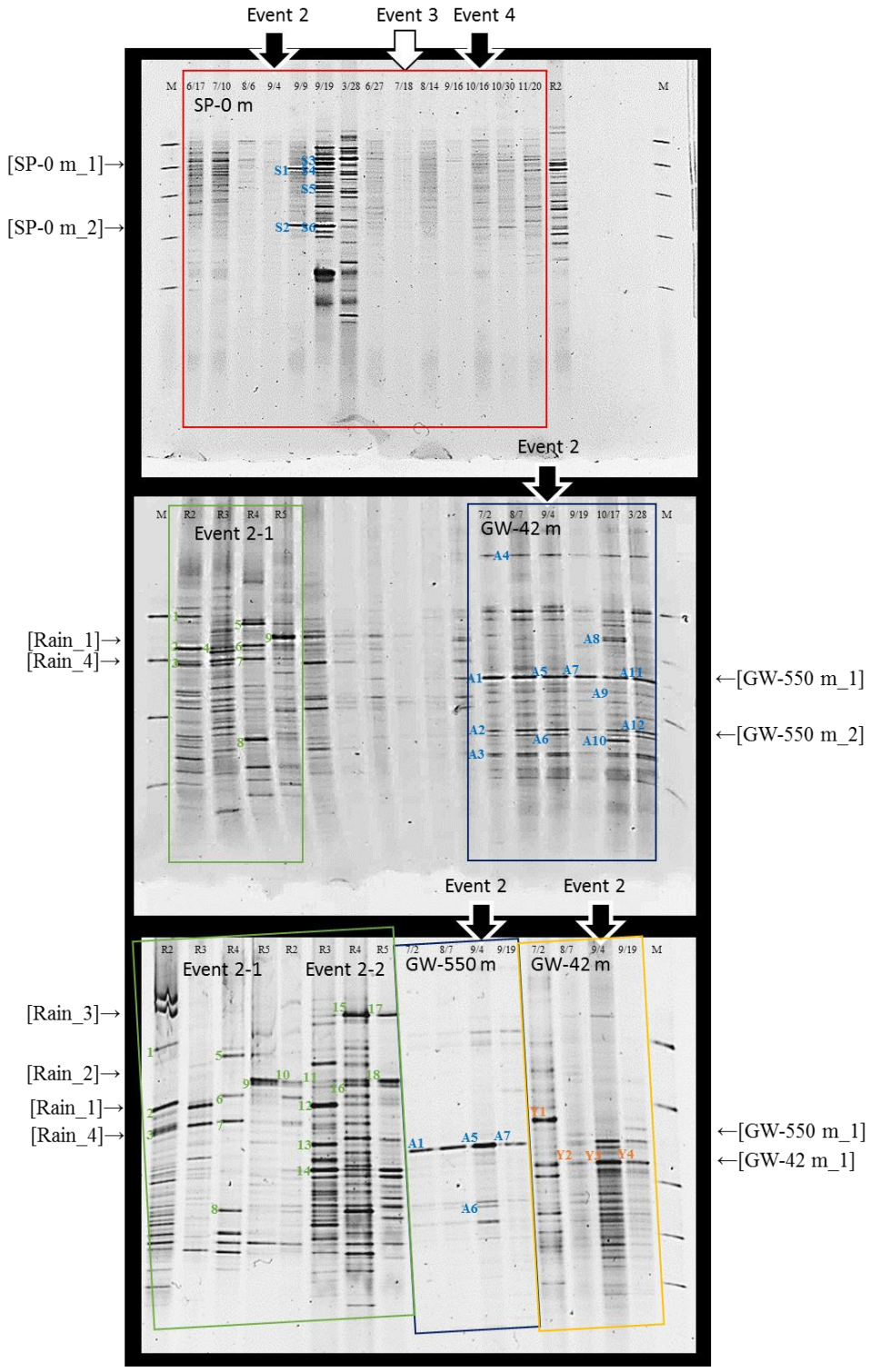

Figure S2. *Bacterial* community composition of groundwater (SP-0m, GW-42m and GW-550m) and rainwater (R2, R3, R4 and R5). Black and open arrows indicate the rainfall event; Event 2, Event 3 and Event 4. Black arrows particularly indicate the torrential rainfall.

Table S2. Similarity of sequences from DGGE bands to those of most related strains.

Band name	Band No.	Most relative strain in DDBJ		Match		Rain				Groundwater		
		Class	related species			R2	R3	R4	R5	SP-0m	GW-42m	GW-550m
Rain_1	2, 4, 6	*Sphingobacteriia*	*Mucilaginibacter* sp. 001	168/170	98%	+	+	+				
Rain_2	10, 11, 18	*Sphingobacteriia*	*Mucilaginibacter* sp. KJ029	153/155	98%	+	+	+	+			
Rain_3	15, 17	*Nostocales*	*Tolypothrix* sp. CNP3-B1-C1	68/75	90%			+	+			
Rain_4	7	*Betaproteobacteria*	*Herbaspirillum* sp. MMD15	174/174	100%			+		+		
	1	*Sphingobacteriia*	*Mucilaginibacter* sp. Aws5	164/171	95%	+						
	3	*Gammaproteobacteria*	*Pseudomonas oryzihabitans*	150/156	96%	+						
	5	*Sphingobacteriia*	*Mucilaginibacter* sp. KJ029	162/170	95%			+				
	8	*Alphaproteobacteria*	*Caulobacter* sp. JM6	149/150	99%			+				
	9	*Sphingobacteriia*	*Mucilaginibacter* sp. KJ029	112/112	100%				+			
	12	*Sphingobacteriia*	*Mucilaginibacter* sp. PAMC 26640	153/155	98%		+					
	13	*Betaproteobacteria*	*Variovorax paradoxus*	161/162	99%		+					
	14	*Alphaproteobacteria*	*Sphingomonas* sp. PXM	127/127	100%		+					
	16	*Gammaproteobacteria*	*Pseudomonas fluorescens*	48/49	97%			+				
SP-0m_1	S1, S4	*Betaproteobacteria*	*Herbaspirillum* sp. MMD15	174/177	98%			+		+		
SP-0m_2	S2, S6	*Betaproteobacteria*	*Sphaerotilus* sp. IMCC12769	176/176	100%					+		
	S3	*Betaproteobacteria*	*Oxalobacteraceae bacterium* AKB-2008-RN12	153/155	98%					+		
	S5	*Bacilli*	*Paenibacillus* sp. PAMC26516	170/175	97%					+		
GW-42m_1	Y2, Y3, Y4	*Nitrospirales*	*Nitrospiraceae bacterium* vj1_c7	94/96	97%						+	
	Y1	*Sphingobacteriia*	*Flavihumibacter* sp. 7B-231	162/162	100%						+	
GW-550m_1	A1, A5, A7, A8	*Betaproteobacteria*	*Gallionella* sp. JA52	164/172	95%							+
GW-550m_2	A2, A6, A11	*Nitrospirales*	*Nitrospiraceae bacterium* vj1_c7	159/175	90%							+
	A3	*Nitrospirales*	*Nitrospiraceae bacterium* vj1_c7	159/175	90%							+
	A4	*Nitrospirales*	*Nitrospiraceae bacterium* vj1_c7	159/168	94%							+
	A8	*Gammaproteobacteria*	*Acinetobacter* sp.	176/177	99%							+
	A9	*Alphaproteobacteria*	*Sphingomonas* sp.	150/150	100%							+
	A10	*Alphaproteobacteria*	*Brevundimonas diminuta*	149/149	100%							+

Color indicates the DGGE band were obtained from more than two samples.

Table S3. Sequences affiliated with thermophilic bacteria retrieved from deep groundwater (GW-550m).

Order	GW-550m-1	GW-550m-2	GW-550m-3
Firmicutes/Clostridia/Thermoanaerobacterales	0.00	1.25	0.00
Actinobacteria/Thermoleophilia/Gaiellales	0.21	0.61	0.27
Deinococcus-Thermus/Deinococci/Deinococcales	0.01	1.36	0.05

*Number indicates relative percentage to whole reads which were removed chimeric sequence.