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Biogeosciences Discussions

Interactive comment on "Tracking the direct impact of rainfall on groundwater at Mt. Fuji by multiple analyses including microbial DNA" *by* Ayumi Sugiyama et al.

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Reply to Anonymous Referee #2 Received and published: 7 November 2017

General comments:

In this manuscript, the authors assess the impact of heavy rainfall events on Mt Fuji groundwater using isotopic, chemical and microbiological (DNA-based) tracers. The overall study yielded interesting and relevant results both from the chemical and the microbiological sides about the hydrology and the subsurface diversity of a unique site. However, the authors are making many important assumptions based on the microbial

DNA analysis which are not necessarily true. The manuscript can be improved by nuancing the assumptions made and by the addition references on previous similar works in the introduction and the discussion sections. Besides this, the manuscript can be published in Biogeosciences.

Reply:

Thank you so much for your precise reading our manuscript and valuable suggestions. We missed to refer a couple of suggested references, which we incorporate into the text. Detailed is shown below.

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Specific comment 1:

The introduction doesn't refer enough to previous microbiology works made on similar environments, to cite a few: ex. Zhou et al., 2012, Nyyssönen et al., 2013 for somewhat similar sites; Ben Maamar et al., 2015 for using a similar approach. The authors use too much space to justify their approach and not enough for referencing literature.

Reply 1:

We agree with your suggestion and revise the text as follows;

[Original text]

To get indication on the route of groundwater we herein newly applied microbial DNA analysis focusing on heavy rainfall at the foot of Mt. Fuji located in central Japan, which is the largest Quaternary stratovolcano in Japan with a peak at 3,776 m a.s.l. At the foot of this mountain we previously found that pH of groundwater decreased from 7.29 to 7.02 a few weeks after a typhoon in August and September 2011 (total rainfall was > 800 mm) (Segawa et al., 2015) at 200 m a.s.l. (page 2 lines 6-8ïijL'

[Revised text]

Development in gene sequence of in situ microbial community enables us to discuss relation between environment and community constituents (Zhou et al. 2012, Nyyssinen et al. 2014). And, population dynamics of predominant prokaryote can be discussed with changes in environment. Concerning subsurface environment Ben Maamar et al. (2015) recently showed a good correlation between different condition of groundwater with oxygen and dominant microbial population, and suggested mixing of groundwater. We herein tried to apply microbial DNA analysis to indicate the route of groundwater focusing on heavy rainfall at the foot of Mt. Fuji located in central Japan, which is the largest Quaternary stratovolcano in Japan with a peak at 3,776 m a.s.l. (page 2 lines 6-12ïijL'

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Specific comment 2:

I didn't find any substantial justification about the choice of using a piston-flow model rather another one like the Exponential piston model, except the occurrence of Archaea in the deep groundwater. Maybe adding some comments/schema on the geometry of the aquifer can help.

Reply 2:

Thank you for the comment. We discuss the route of groundwater based on apparent age and the apparent age assumes piston flow of the groundwater. The preceding study performed in this study area applied the piston flow model (Tosaki et al., 2011). In addition, some studies on the groundwater age conducted in volcanic area applied piston flow to get apparent age (e.g., Koh et al., 2007). Thus, we discuss the influence of heavy rainfall appeared in deep groundwater by the concept of piston flow. And, to discuss the flow system isn't the aim herein. We just intend to suggest the impact of heavy rainfall on the deeper groundwater from actual increase in abundance of archaea and clear difference found in archaeal constituents. Water doesn't tell us any influence of heavy rainfall there, but particles.

[Reference]

Tosaki et al., Estimation of groundwater residence time using the 36Cl bomb pulse, Ground Water, 49, 891-902, 2011.

Koh, et al., Evidence for terrigenic SF6 in groundwater from basaltic aquifers, Jeju Island, Korea: Implications for groundwater dating, J. Hydrol., 339, 93-104, 2007.

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Specific comment 3:

Finding thermophilic microbes in environments with temperatures < 40âŮęC is very common, same for halophilic microbes that can be found in low salts environments. Halobacteriales can be found in salted lakes, oceans and also, though not in high abundance, in temperate regions soils as well as on tree leaves, same for Methanobacteriales. In addition making some assumptions on microbes physiological optima using the classification at the order level is very risky and questionable. The authors should discuss the relative ubiquity of these microorganisms in different environments and maybe should specify the genus of these Archaea in order to give more credit to their assumptions. However, I strongly encourage the authors to moderate their assumptions based on detected taxa given the very low Archeae abundances observed.

Reply 3:

Thank you for your comment on how to interpret the meaning of the findings of some certain group of bacteria. The point we insist herein is from where they came. If you find thermophilic prokaryote from cold water, which leads a question as from where do they come? Fig.5 expressed contribution of each group of archaea at the level of Order, but the original data, Haloarcula cpmprised 99.7 % of Halobacteriales and Methanothermobacter comprised 97.4 % of Methenobacteriales. Thus, we add this information into the text; And we think that an increasing in contribution of both constituents of Halobacteriales (Haloarcula) and Methenobacteriales (Methanothermobacter) is plau-

sible because they might be retrieved from deep subsurface environment as unique constituents which were just found after the heavy rainfall when the density of archaea sharply increased after the Event 2 (Fig. 3).

[Original text]

However, we observed an interesting increase in abundance of Archaea at GW-550m 2 weeks after event 2, which was supported by an apparent change in constituents of archaeal OTUs. Halobacteriales, which inhabit environments with high concentrations of sodium and Methanobacteriales, a strict anoxic methane producer, were dominant members after the torrential rainfall. (page 9 lines 16-19)

[Revised text]

However, we observed an interesting increase in abundance of Archaea at GW-550m 2 weeks after event 2, which was supported by an apparent change in constituents of archaeal OTUs. Halobacteriales comprised of Haloarcula with 99.7%, which inhabit environments with high concentrations of sodium and Methanobacteriales comprised of Methanothermobacter with 97.4%, a strict anoxic methane producer, were dominant members after the torrential rainfall. (page 9 lines 16-19)

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Specific comment 4:

In Material and Methods, in the DNA extraction section no sampling triplicates were mentioned. Did the authors assessed the biological variability of their observations? If not, the authors should justify why and how their data might be representative of their environment.

Reply 4:

We concentrated 10 L of groundwater for each analysis, and it is almost practical limit to do in situ environment for each observation (We reserved far more number of sam-

ples.). Though we did not get water with triplicate for each sample, e.g., the similarity in the first three bands of DGGE pattern obtained from before the Event-2 at SP-0m (Supplement Fig. S2 (original)) supports that the employed method might not be biased much to represent the microbial community so far examined for the groundwater.

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Specific comment 5:

It would also be nice to add any water table measurements somewhere for each sampling campaigns as It may be relevant to discuss any increase/decrease in bacterial density during rainfall events.

Reply 5:

Thank you for your suggestion. We add a figure of fluctuation of the amount of discharge of spring water at SP-0m as Figure S1. Figure S1 shows the amount of discharge observed at SP-0m did not affect the density of microbes. Sharply increased discharge observed during Event 4 correlated with just the density of Archaea in deep groundwater at GW-550m, which well located 1.2km downstream from the site of SP-0m.

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Specific comment 6:

The authors should also add the standard deviation for each total cell counts, as it helps to realize if observed increases in cells density are substantial, and gives an idea to readers of of the counting method sensitivity.

Reply 6:

We add standard deviation to Figure 3 calculated from the total density of prokaryotes and its individual contribution of Bacteria and Archaea. Bacterial density of June 17 2013 was corrected in the revised Figure 3. The previous Figure 3 (c) showed obser-

vation date incorrect (each point is shown one time ahead). This is also revised in the new Figure 3 (c).

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Specific comment 7:

most microbes in aquifers are living in an attached mode within biofilms, the authors should include a point in their discussion about how representative is a groundwater sample of the groundwater and subsurface biodiversity over time and space (specifically regarding the major attached fraction of microbes, see Flynn et al., 2008) and how it can affect their measurements.

Reply 7:

Yes, we agree on the concept that abundance of microbial particles exceeds in attached form in subsurface environment. An apparent increase in relative contribution of Methannobacteriaales and Holobacteriales shown in Fig. 5 for the deep groundwater after the heavy rainfall, thus, can be ascribable to detached from the rocks in the deeper layer, which was suggested from the chemistry. Similar estimate is applied to explain the sharp increase in Bacterial abundance in spring water at SP-0m (Fig. 3, (a)), which was mostly supplied from soil constituents through the extraction after Event 2 (page 8 line 25 – page 9 line 3). To make more clear the discussion, ï¡Ůe add a reference you suggested into the part of Discussion as follows. Thank you.

[Revised text]

Thus, not only strict anaerobic but halophilic archaea may be abundant within the deep subsurface environment of the study area, although they were not retrieved from groundwater in other examinations, likely because they were embedded in the matrix of geologic layers (Flynm et al. 2008). (page 9 lines23-26ïijL'

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Specific comment 8:

page 9 line 21, the reported Na+ concentrations are not particularly high compared to other aquifers (ex. Ben Maamar et al., 2015), specifically regarding Halobacteriales which are usually found in water saturated or nearly saturated with salt. They can live in somewhat less concentrated salt water though. Halobacteriales are mostly aerobes and they need organic material available which are usually in very low concentration in deep groundwater. The authors should add some information on the organic carbon availability in deep groundwater or maybe consider these Halobacteriales could also be introduced from soil.

Reply 8:

Yes, we just refer the concentration of Na+ obtained from the examined deep water was higher than the other shallow groundwater in the examined area. The reviewer asked the possibility to retrieved Halobacteriales from soil. But, we found Methanothermobacter, a strict anaerobe, together with them. This could suggest that the possibility to retrieve Halobacteriales from soil might not be high.

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Specific comment 9:

The paper would be improved with the addition of informations about the connectivity of the deep groundwater with surface, and if some surficial water inputs into deep groundwater are possible and in which proportions.

Reply 9:

Thank you for your suggestion. But the diversity of microbes inhabit in surface environment in particular in soil, which can be regarded as the topmost, must be very high and varies with the characteristics of soil (e.g. Katsuyama et al. 2008). Thus we simply refer to the predominant prokaryote, Burkholderia, in major (Fig.4).

[Reference]

Katsuyama et al. Denitrification activity and relevant bacteria revealed by nitrite reductase gene fragments in soil of temperate mixed forest. Microbes and Environments, 23:337-345, 2008

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Specific comment 10:

The authors are a bit overselling The use of DNA as a flowpath tracer. Despite using DNA as a tracer is useful, It has several limits. for instance, microbes in aquifers are majorly living into heterogeneous biofilms and while some biofilms can be widespread, some others might develop only very locally and in very specific conditions. Defining The original location of each microbe based on their taxonomic assignation is far from being straightforward. also, The authors should take into account that DNA can be more or less degraded according to The environmental conditions and keep in mind that The vast majority of microbes are ubiquists, The main variable being their abundance in different environments. The use of DNA as a tracer is highly informative as long as used in combination with other tracers such as isotopic and chemical tracers.

Reply 10:

We totally agree with your comment that "The use of DNA as a tracer is highly informative as long as used in combination with other tracers such as isotopic and chemical tracers." To get information on the degradation for each target prokaryote, in particular, is the subject to be studied in the next step. Thus the last sentence of Discussion is; In addition to the chemical analyses of groundwater, we showed that microbes could show the route of groundwater in the invisible subsurface environment. But, a sentence in "Conclusion" we modify as follows;

[Original text]

Here, we first indicated the route of groundwater using a next-generation sequencing

analysis of Bacteria and Archaea. (page 10 lines 21-22)

[Revised text]

Here, we first showed the possibility to chase the route of groundwater using a next-generation sequencing analysis of Bacteria and Archaea for the event of heavy rainfall. (page 10 lines 22-24)

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Specific comment 11:

At the end of the discussion, unless I misunderstood it seems the authors assume the microbial diversity should go back to its initial structure after heavy rainfall events. This might be the case for very deep groundwater which seems to be poorly impacted by heavy rainfall but not necessary true for shallow groundwater that may host very fluctuating microbial diversity and structure over time because of the rapid water flow and variable contribution of soil over time.

Reply 11:

Thank you for your comment and we mostly agree with your suggestion. As you suggested, resilience in deep archaeal community constituents was shown herein to some extent (Fig. 5). But, we think we do not have enough information about the ability of resilience in subsurface microbial community. We just discussed a possible estimate on the influence of heavy rainfall even for deep groundwater from the finding just after the heavy rainfall.

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Comments on figures 12:

Figure 4: Too many orders are represented, particularly for SP-0m-1. Please only show discussed or most relevant orders, or only depict orders representing more than 2 or 5 percents in relative abundance. Also please remove the shadow on colors.

Reply 12:

Thank you for your suggestions. We modify the legend of Figure 4 showing orders contributed exceeding 2%.

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Comments on figures 13:

Fig. S1, please add a table showing representative raw chemical concentrations for the different chemical species depicted, for comparison with other aquifers.

Reply 13:

Thank you for your suggestion. We add Table S2 showing chemical character as averages of major ions.

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Technical correction 14:

page 7 line 16: what do 384, 268 and 278 correspond to? number of orders? Please reformulate

Reply 14:

Yes, the numbers are amounts of order, so we change the sentence as follows;

[Original text]

Next-generation sequencing retrieved diversified community constituents at the level of order with 384, 268 and 278 from rainwater (R5), spring water before event 2 (SP-0m-1) and spring water after event 2 (SP-0m-2), respectively. (page 7 lines 16-17)

[Revised text]

Number of constituents retrieved by Next-generation sequencing at the level of Order was 384, 268 and 278 for rainwater (R5), spring water before event 2 (SP-0m-1) and

spring water after event 2 (SP-0m-2), respectively. (page 7 lines 16-17)

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page 9 line 4: replace "was" by "were"

Reply 14-2:

We correct the word “was” to “were”. ([Original text] page 9 line 4, [Revised text] page 9 line 5)

—

page 9 lines 4-7 this is a run-on sentence please split it into 2, and please clarify the point as this is not clear.

Reply 14-3:

We separate this sentence into 2 as followed.

[Original text]

Furthermore, sequences affiliated with thermophilic bacteria was scarcely retrieved from the samples of the examined SP-0m after event 2, which supports the assertion of enforced piston flow through a deep subsurface zone > 600 m which given temperature exceeding 40 °C, where thermophilic bacteria inhabited was not considerable. (page 9 lines 4-7)

[Revised text]

Furthermore, sequences affiliated with thermophilic bacteria were scarcely retrieved from the samples of the examined SP-0m where thermophilic bacteria inhabited was not considerable after event 2. This finding supports the assertion of enforced piston flow through a deep subsurface zone > 600 m which given temperature exceeding 40 °C. (page 9 lines 5-7)

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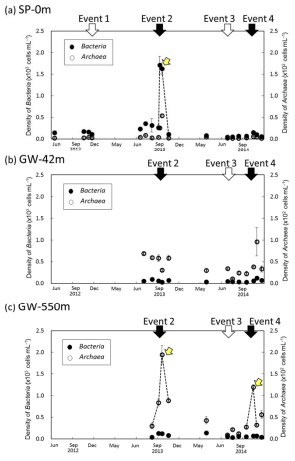

Figure 3. Changes in community structure of prokaryotes in groundwater. (a) Groundwater at SP-0m, (b) shallow groundwater at GW-42M, (c) deep groundwater at GW-550m. Black and open arrows indicate the rainfall event; Event 1, Event 2, Event 3 and Event 4. Black arrows particularly indicate the torrential rainfall. Yellow arrows indicate the signature of impact of rainfall.

Fig. 1. Figure 3

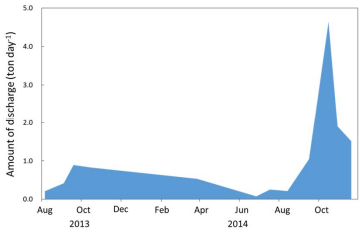

Figure S1. Fluctuation of the Amount of discharge at the spring of SP-0m.

3

Fig. 2. Figure S1

Table S2. Chemical character of spring and groundwater.

(meq L^{-1})

Site ID	n	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	SO_4^{2-}	HCO_3^-
SP-0m	15	0.258	0.032	0.200	0.429	0.115	0.057	0.151	0.670
GW-42m	13	0.313	0.046	0.308	0.578	0.086	0.122	0.104	1.002
GW-550m	13	0.631	0.015	0.177	0.353	0.088	0.007	0.243	0.818
R1	1	0.002	0.002	0.001	0.025	0.002	0.004	0.000	0.059
R2	8	0.034	0.002	0.013	0.011	0.040	0.019	0.024	0.053
R3	11	0.034	0.002	0.013	0.011	0.040	0.019	0.024	0.035
R4	15	0.011	0.012	0.006	0.008	0.013	0.026	0.058	0.021
R5	15	0.017	0.006	0.010	0.013	0.020	0.021	0.026	0.032

5

Fig. 3. Table S2

1 Introduction

Many hydrological studies explained runoff processes of groundwater affected by rainfall (e.g. Dunne and Black, 1970; McDonell et al., 1990; Beven et al., 2001; Tekleab et al., 2014). However, runoff process of groundwater affected directly by rainfall is not precisely explained. The contribution of rainfall through subsurface pass to stream water was estimated by preceding studies, but they did not address the route of groundwater until it affected streamflow.

Development in gene sequence of in situ microbial community enables us to discuss relation between environment and community constituents (Zhou *et al.* 2012, Nyyssinen *et al.* 2014). And, population dynamics of predominant prokaryote can be discussed with changes in environment. Concerning subsurface environment Ben Maamar *et al.* (2015) recently showed a good correlation between different condition of groundwater with oxygen and dominant microbial population, and they suggested mixing of groundwater. We herein tried to apply microbial DNA analysis to indicate the route of groundwater focusing on heavy rainfall at the foot of Mt. Fuji located in central Japan, which is the largest Quaternary stratovolcano in Japan with a peak at 3,776 m a.s.l. This decrease of pH was probably influenced by low pH of the rainwater (pH from 4.7 to 6.4; Watanabe et al., 2006). This rapid decrease of pH cannot be explained by piston flow transport of groundwater in which newly supplied water pushes out older water preserved in the subsurface bed (e.g., Bethke and Johnson, 2008). Considering the pH of rainfall at Mt. Fuji, the lowering of groundwater pH suggested that the newly supplied rainwater mixed directly with groundwater over a period of weeks.

In addition, our preceding microbiological study of groundwater at the foot of Mt. Fuji furnished a clue to estimate possible groundwater routes by finding thermophilic bacterial DNA in spring water, whose temperature was as low as ~10–15 °C throughout the year (Segawa et al., 2015). Thermophilic prokaryotes are optimally adapted to temperatures > 40 °C. This suggests that at least some of the groundwater source was at a depth of 600 m or greater, based on a temperature gradient of 4 °C/100 m. This depth is far below the lava layer that was taken to be a substantial pool of this groundwater (Tsuchi, 2007). Thus, microbial information can help estimate the route of groundwater.

Following the above findings, we tried to estimate the groundwater route with a focus on heavy rainfall, by tracing the signature of direct rainfall impacts. This was done using (i) stable oxygen and hydrogen isotopic analysis to track the movement of water molecules, (ii) chemical analysis of silica concentration in groundwater, which indicates its possible dilution by rainwater with low silica concentration, and (iii) microbial analysis including DNA sequencing to estimate the groundwater route, which may include a possible function of microbes in a given geological environment. Whereas stable isotopic and chemical analyses show average values of the water originated from various sources, microbes transported by groundwater suggest the route and place where they proliferated through their eco-physiological characteristics constrained by their optimal growth condition. In other word, microbial DNA brings a message of their route, though the examined water was already blended with various groundwater prior to examination. To elucidate microbial properties in the studied groundwater, we used total direct counting (TDC) of prokaryotes, catalyzed reporter deposition – fluorescence in situ hybridization (CARD-FISH), 16S rRNA gene-targeted polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and a next-

Fig. 4. Text p2

3.4 Microbial analysis of rainwater and groundwater

3.4.1 Abundance of prokaryotes

Abundance of prokaryotes of rainwater ranged from $(4.04 \pm 0.02) \times 10^4$ to $(1.67 \pm 0.08) \times 10^6$ cells mL^{-1} (n=48), which significantly exceeded that of groundwater, whose range was $(6.86 \pm 1.53) \times 10^2$ to $(1.12 \pm 0.09) \times 10^4$ cells mL^{-1} (n=45). The abundance of prokaryote in groundwater was two orders of magnitude smaller than that of rainwater. CARD-FISH revealed *Bacteria* and *Archaea* respectively comprised 20.7% to 40.0% (n=8) and 0.8% to 6.6% (n=8) of the total number of prokaryotes in rainwater. Under such ordinary low abundance of prokaryotes in groundwater, there was an apparent influence in event 2 at SP-0m. SP-0m was located ~1 km below the average altitude of the recharge zone. Abundance of *Bacteria* at SP-0m increased sharply after event 2, from 2.6×10^2 cells mL^{-1} to 1.7×10^3 cells mL^{-1}, and total abundance of prokaryotes increased from 3.21×10^3 cells mL^{-1} to 1.12×10^4 cells mL^{-1} (Fig. 3a). A similar phenomenon did not appear at GW-42m, in shallow groundwater flushed out ~12 km downstream of SP-0m (Fig. 3b). In addition, there was a very interesting increase in the abundance of *Archaea* in deep groundwater at GW-550m, 12 km downstream of SP-0m, where water was obtained from 550-m depth. The number of *Archaea* increased from 3.0×10^1 cells mL^{-1} to 1.9×10^2 cells mL^{-1} at GW-550m (Fig. 3c). A similar phenomenon was observed after event 4, though the response was somewhat weaker than in event 2.

3.4.2 Bacterial community constituents

Number of constituents retrieved by Next-generation sequencing at the level of Order was 384, 268 and 278 for rainwater (R5), spring water before event 2 (SP-0m-1) and spring water after event 2 (SP-0m-2), respectively. Five groups with orders *Burkholderiales*, *Rhizobiales*, *Sphingobacteriales*, *Pseudomonadales* and *Sphingomonadales* comprised about 90% of all constituents of the R5 community (Fig. 4). In contrast to rainwater, for spring water before event 2 (SP-0m-1), only *Burkholderiales* were major constituents among the five major groups retrieved from rainwater, with 17.3% of the entire bacterial community. The bacterial community in spring water (SP-0m-1) was more diversified than that in rainwater, R5. A clear difference in bacterial community structure appeared at SP-0m after torrential rainfall (SP-0m-2 in Fig. 4). After event 2, *Burkholderiales* became apparently dominant in groundwater at SP-0m (58.8%). Following *Burkholderiales*, *Flavobacteriales* was substantial with 8.9%, but had a contribution that did not vary before and after the event. *Rhizobiales*, *Sphingobacteriales*, *Pseudomonadales* and *Sphingomonadales*, which were the major dominant groups in rainwater following *Burkholderiales*, were apparently not retrieved from the spring water at SP-0m after event 2. In contrast, *Bdellovibrionales* and *Bacillales*, which were not a major constituent at SP-0m before that event, became dominant after the torrential rainfall.

3.4.3 Archaeal community constituents

We examined the archaeal community, focusing on deep groundwater at GW-550m, where a remarkable increase was observed in the abundance of *Archaea* after event 2. Next-generation sequencing retrieved 12 major groups of *Archaea* at the level of order for each analyzed sample. Rainwater (R5 in Fig. 5) comprised WCHD3-30 and YLA114 belonging to *Parvarchaeota*,

Fig. 5. Text p7

rainwater, *Rhizobiales*, *Sphingomonadales* and *Pseudomonadales* were not significantly retrieved from the spring water after event 2. Such extraction might increase the relative abundance of *Bdellovibrionales* including a typical soil-dweller as *Peredibacter starrii* in groundwater (Davidov and Jurkevitch, 2004), a group known to generally inhabit the soil environment (Garrity et al., 2005b).

Furthermore, sequences affiliated with thermophilic bacteria were scarcely retrieved from the samples of the examined SP-0m after event 2. This finding supports the assertion of enforced piston flow through a deep subsurface zone > 600 m which given temperature exceeding 40 °C. Viral particles have previously been used as a tracer of water movement. Hunt et al. (2014) showed preferential flow paths using this method. Viral particles only provide information on groundwater flow paths, whereas microbial analysis including DNA provides additional information on the location of origin of the microbes and the magnitude of impact of water flow on microbes extracted from geologic layers. Thus, microbial analysis can give insight into the route of groundwater through both shallow and deep environments. The latter is discussed below.

In contrast to the findings for shallow groundwater and spring water, no direct influence of torrential rainfall was detected in either the stable isotope signature or concentration of silica in deep groundwater at GW-550m (~12 km downstream of SP-0m) after event 2. Considering the difference of horizontal distance and depth from which water was sampled between GW-42m and GW-550m, the direct impact of rainfall from the observation is expected to be barely noticeable at 550 m depth.

However, we observed an interesting increase in abundance of *Archaea* at GW-550m 2 weeks after event 2, which was supported by an apparent change in constituents of archaeal OTUs. *Halobacteriales* comprised of *Haloarcula* with 99.7%, which inhabit environments with high concentrations of sodium and *Methanobacteriales* comprised of *Methanothermobacter* with 97.4%, a strict anoxic methane producer, were dominant members after the torrential rainfall. Increasing in abundance of such archaea can be supported by the finding that archaeal abundance increased with depth in both terrestrial (Kato et al., 2009) and marine (Lipp et al., 2008, Inagaki et al., 2015) subsurface environments. Deep groundwater in the study area contained high concentrations of Na^+, from 14.3 mg L^{-1} to 14.6 mg L^{-1} (n=13), while these were 5.4 mg L^{-1} to 7.8 mg L^{-1} (n=32) in groundwater at SP-0m and GW-42m (supplemental information Fig. S1). Thus, not only strict anaerobic but halophilic archaea may be abundant within the deep subsurface environment of the study area, although they were not retrieved from groundwater in other examinations, likely because they were embedded in the matrix of geologic layers (Flynn et al. 2008). An augmented flow rate caused by torrential rainfall might have extracted *Halobacteriales* and *Methanobacteriales* from the matrix of those layers into the studied groundwater (Fig. 5). In contrast to the spring water (SP-0m), some sequences affiliated with thermophilic bacteria were retrieved from the deep groundwater (GW-550m), which suggested microbes in the deep groundwater contained *in situ* populations. This suggests that strengthened piston flow caused by the heavy rain transported archaeal particles from the deep geologic layer along the groundwater route.

A possible reason why there was no apparent influence of heavy rain on microbial particles in groundwater at GW-42m, ~12 km downstream of SP-0m, may be attributable to the trapping of microbial particles by soil and lava across the flow trajectory. There was a question whether in situ population change through the growth in groundwater could be explained by their estimated growth rates. The doubling time of prokaryotes in the groundwater was estimated at 85 days, from the observed

Fig. 6. Text p9

frequency of dividing cells' to the entire population (Newell and Christian, 1981). Thus, the possibility of altered populations via growth within a few weeks may be small. Microbes observed in the groundwater may represent the original locations where they grew.

Wels et al. (1991) separated streamflow into three components, surface water, soil water and groundwater, using a two-step separation with stable isotopic ratios and silica concentration. Based on their method, an estimated 21% and 5% of water in the subsurface environment at SP-0m and GW-42m, respectively, were attributed to soil water following event 2. The effect of soil water at SP-0m was estimated to be stronger than that at GW-42m. This difference appeared in microbial abundance and constituents as well as in water molecule movement. This supports the sudden appearance of rapid flow through the shallow aquifer consisting partly of soil layers in groundwater at SP-0m. This phenomenon was driven by heavy rainfall.

In contrast, direct and rapid effects of rainwater movement into groundwater were not observed for weak rainfall, as evidenced by the results of events 1 and 3. However, a question remains as to why an impact similar to event 2 was not observed for another heavy rainfall, that of event 4. The impact of rainfall before event 4 persisted, as suggested by a stronger $\delta^{18}O$ signature, which might have veiled that impact (Fig. 2).

In addition to the chemical analyses of groundwater, we showed that microbes could show the route of groundwater in the invisible subsurface environment.

5 Conclusions

Chemical analyses using stable isotopes and dissolved ions show the properties of the groundwater mixed throughout the route it flowed. In contrast, microbial particles suggest the locations where they were incorporated in the groundwater as far as they survived. Thus, microbial analysis can provide information about the origin and route of the groundwater.

Next-generation gene sequencing provides detailed information of high resolution on the examined microbial community constituents, which was never attained by the conventional gene sequencing technique targeting small subunit ribosomal DNA. Previous conventional gene sequencing does not give quantitative information on microbial community constituents. Here, we first showed the possibility to chase the route of groundwater using a next-generation sequencing analysis of *Bacteria* and *Archaea* for the event of heavy rainfall. Bacterial abundance and community constituents showed that torrential rainfall caused rapid flow of rainwater through the shallow part of the aquifer, and archaeal abundance and constituents suggested fast and accelerated piston flow in deep groundwater within a few weeks after that rainfall. The former finding was mostly ascertained by the chemical analysis, but the latter finding was not shown by chemical analysis.

Acknowledgments. This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS KAKENHI Grant Number JP26257402) and a research fund of the River Front Research Institute. The work was done with support from a Joint Research Grant for Environmental Isotope Study of the Research Institute for

Fig. 7. Text p10