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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 #1 Received and published: 4 October 2017

Review of "Tracking the direct impact of rainfall on groundwater at Mt. Fuji by Multiple analyses including microbial DNA" by Sugiyama and others General comment: In this manuscript, the authors were trying to state that the information from microbial DNA in groundwaters was useful as a tracer to determine the contributions of runoff components. Presented data and descriptions include interesting and important information in groundwater pathways in the volcanic environments. However, there are several points have to be improved before publication in Biogeosciences.

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Comment 1 (General): For the essential part of discussions in this manuscript, it has been assumed thatthe sources of transported bacteria were mainly situated in the soil horizons, and the sources of archaea were mainly in the "geologic layer". These assumptions may be common recognitions for general microbiologists. But, I feel there is a necessity to show evidences for guaranteeing these assumptions. Or, at least the authors have to explain how these assumptions were likely in this study site.

Reply 1: Thank you for the comment on the important standpoint of microbial distribution in subsurface environment. At first, the key point in consideration of microbes as an indicator of the route of groundwater is due to their "vertical" distribution. Sift of environment from soil to rock, slightly aerobic to absolute anaerobic, and increase in temperature with 3-4 °C/100m are the great constrain to characterize microbes in subsurface environment. Soil is thus clearly characterized from beneath environment with it very high abundance (108-9 cells/g; Katsuyama et al. 2008) and some dominant species as Burkholderiales and Bdellovibrionales (Garrity et al., 2005b). In order to clear the content, we add some words as follows;

p8, L26 [Original] An apparent predominance in the bacterial community of Burkholderiales suggests incorporation of microbes from soil...

[Revised] An apparent predominance in the bacterial community of Burkholderiales with high density suggests incorporation of microbes from soil...

p9,L2 [Original] Such extraction might increase the relative abundance of Bdellovibrionales in groundwater,

[Revised] Such extraction might increase the relative abundance of Bdellovibrionales including a typical soil-dweller as Peredibacter starrii in groundwater (Davidov and Jurkevitch, 2004),

Concerning Archaea, similarly we change wording;

p.9, L 19; [Original]It has been shown that archaeal abundance increased with depth in both terrestrial (Kato et al., 2009)

[Revised] 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)

[References] 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.

Davidov and Jurkevitch, Diversity and evolution of Bdellovibrio-and-like organisms (BALOs), reclassification of Bacteriovorax starrii as Peredibacter starrii gen. nov., comb. nov., and description of the Bacteriovorax-Peredibacter clade as Bacteriovoracaceae fam. nov. Int J Syst Evol Microbiol. 54:1439-52, 2004.

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Comment 2 (General): In “Introduction”, the authors are telling: “Whereas stable isotopic and 25 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.”

If the source locations (distributions) of each microbe could specified, pathways and origins of specific water sources could be identified. If the habitat of a microbe expanded in large spatial area, specifying capability of this microbe were low. Generally, this tendency can be applied also to isotope and chemical tracers. There is another issue. Conservativeness is also important for tracers. If you need to estimate relative contributions precisely of multiple end members, all tracers have to be conservative. In this point, microbial DNA may have disadvantage, because they may proliferate not only at the source points (area), but also in the pathways toward destinations. I think that the microbial DNA is a certainly useful tracer, but it can show its high capability

being accompanied with other multiple tracers, such as isotopes and chemical tracers. The logic behind the above sentences was exaggerating the capability of microbial DNA as a tracer, if the authors cannot show the sufficient evidences or generally accepted recognitions on the characteristics of microbial DNA as a tracers (spatially specific source and conservativeness).

Reply 2: Thank you for your comment on the critical point. The great advantage of microbes as a tracer is stemmed from the fact that whether microbes which could suggest specific environment exist or not. Then, their relative abundance leads further discussion. In addition, the growth rate expressed by frequency of dividing cells (FDC in a given community) of subsurface microbes observed for groundwater and spring water in Mt. Fuji was very low (from 0.05 to 0.3 %, unpublished data) compared with surface waters (3 to 6 %). This suggests influence of proliferation of miscellaneous microbes through the pass of groundwater until examined may not alter the understanding shown here. This is shown in p9, Line32 as;

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 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.

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Individual comments:

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Comment 3 (Individual): P2, L2-5ïijŽ “Though runoff process of stream water and runoff peak response time of streams influenced by rainfall have been well studied (e.g., Hubert et al., 1969; Onda et al., 1999; Asai et al., 2001; Tekleab et al., 2014), runoff

processes of groundwater affected directly by rainfall is not precisely explained." Cited references were not always representative literatures for stating L1-2. For example, Dunne and Black (1970), Beven and Kirkby (1979), Burns et al. (2001), etc. many fundamental studies should be cited.

Dunne, T., and R. D. Black (1970), Partial area contributions to storm runoff in a small New England watershed, Water Resour. Res., 6(5), 1296– 1311, doi:10.1029/WR006i005p01296. Beven, K. J., and M. J. Kirkby (1979), A physically based, variable contributing area model of basin hydrology, Hydrol. Sci. Bull., 24(1), 43– 69.

Burns, D. A., J. J. McDonnell, R. P. Hooper, N. E. Peters, J. E. Freer, C. Kendall, and K. J. Beven (2001), Quantifying contributions to storm runoff through end-member analysis and hydrologic measurements at the Panola Mountain Research Watershed (Georgia, USA), Hydrol. Processes, 15(10), 1903– 1924, doi:10.1002/hyp.246.

The statement of this sentence was not true. Many hydrological studies explained runoff processes of groundwater affected by rainfall.

e.g. McDonnell JJ, Bonell M, Stewart MK, Pearce AJ. (1990), Deuterium variations in storm rainfall: Implications for stream hydrograph separation. Water Resources Research. 26(3):455-8.

Kendall, C. and McDonnell, JJ (1993), Effect of intrastorm isotopic heterogeneities of rainfall, soil water, and groundwater on runoff modeling. IAHS Publication, 215, 41-48.

Reply 3: Thank you for your comment on the basic references. We change the sentence and add some references accordingly.

[Original] Though runoff process of stream water and runoff peak response time of streams influenced by rainfall have been well studied (e.g., Hubert et al., 1969; Onda et al., 1999; Asai et al., 2001; Tekleab et al., 2014), runoff processes of groundwater affected directly by rainfall is not precisely explained.

[Revised] Many hydrological studies explained runoff processes of groundwater af-

fected 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.

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Comment 4 (Individual): Figure 1: Why the unit of depth in the legend panel was m-1?

Reply 4: Yes, it was mistake. We correct the word in legend panel m-1 to m.

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Comment 5 (Individual): The line 3 – 5 of the caption was not formed a complete sentence. No indication for "Shibukawa" and no mark for "SP-0m" in the map.

Reply 5: Thank you for your suggestion. We revised the map to show the site SP-0m. And the figure legend is revised as follows;

Figure 1. Study sites in western foot of Mt. Fuji. Red arrows indicate main fast flow (GETFLOWS; Kato et al., 2015 partially modified). Precipitation was sampled at R1 to R5. Groundwater was sampled at SP-0m, GW-42m and GW-550m. R1 is located at 2,364 m a.s.l., R2 is at 1,431 m a.s.l., R3 is at 1,081 m a.s.l., R4 is at 850 m a.s.l. and R5 is at 723 m a.s.l. SP-0m, spring water, shows sampling site of Shibakawa located at 726 m a.s.l. GW-42m, shallow well water obtained from 42 m, is located at Yodoshi with 150 m a.s.l. GW-550m, deep well water obtained from 550 m, is located at Aoki with 175 m a.s.l..

* Amount of precipitation for the studied area was recorded at Shiraito-no-taki Station of Japan Weather Association.

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Comment 6 (Individual): Table 1: Is it possible to show the summary of isotope measurements?

Reply 6: We add the summary of isotopic data in Table 1.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-306, 2017.

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.

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. 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-generation sequencing. Here, we first employed microbial analysis to reveal the groundwater route in the shallow and deep subsurface environment.

Fig. 1. Text_p2

E2 belonging to *Thermoplasmata*, unclassified *Euryarchaeota*, *Cenarchaeales*, unclassified MBGA (*Crenarchaeota*), and *Micrarchaeles*. WCHD3-30 and YLA114 also dominated in deep groundwater of GW-550m-1 in the non-rainy period, followed by E2 and *Cenarchaeales*. The relative proportion of each order group did not vary much after event 4 (GW-550m-3) and the non-rainy period (GW-550m-1). However, a few weeks following the torrential rainfall of event 2 (GW-550m-2), *Halobacteriales* and *Methanobacteriales* were predominant in the deep groundwater. These relative constituents to the whole community were clearly different from other results.

4 Discussion

Tracer hydrology studies of rainfall-runoff processes have revealed the mixing process of rainfall and groundwater in stream water shortly after heavy rain in a range from days to weeks (e.g., Pearce et al., 1986; McDonnell et al., 1991; Silliman and Booth, 1993; Blume et al., 2008). A sharp decrease in pH of spring water influenced by heavy rainfall, suggesting a direct effect of rainwater on groundwater, was observed at the foot of Mt. Fuji (Segawa et al., 2015). Following these studies, we investigated heavy (> 300 mm) and light (100 mm) rain at the foot of volcanic Mt. Fuji at sites SP-0m and GW-42m (shallow groundwater) and GW-550m (deep groundwater), where average recharge of rainfall and snowfall was estimated between 1,700 and 2,500 m a.s.l.

We found fast flow of groundwater caused by torrential typhoon rainfall in multiple analyses, including those of microbes. Rainwater exceeding 300 mm traversed the very shallow portion of the subsurface aquifer and appeared 2 weeks after the event at SP-0m. That site is ~1 km lower in altitude and ~5–7 km downstream horizontally from the average recharge area of the rainfall. This finding was deduced from the movement of microbial particles and of water molecules tracked by the stable isotope signature of $\delta^{18}O$, as well as measurement of dissolved silica concentration. The silica concentration in groundwater is ascribable to the extraction of silicate from soil and rock (Wels et al., 1991; Asano et al., 2003). Thus, decrease of that concentration in groundwater after torrential rain suggests that the flow of groundwater was substantially faster than usual, or a dilution of the concentration by great amounts of infiltrated water. Rapid flow of groundwater was also detected in shallow groundwater at GW-42m, which was ~600 m below the altitude of SP-0m and ~12 km downstream. This finding was associated with an increase in $\delta^{18}O$ and decline in silica concentration.

The effect of torrential rainfall was also clearly detected by a sharp increase in the abundance of *Bacteria* at site SP-0m. An apparent predominance in the bacterial community of *Burkholderiales* with high density suggests incorporation of microbes from soil through extraction by enforced flow rate, because the abundance of prokaryotes in soil is about four or five orders of magnitude greater than that of groundwater (reviewed by Whitman et al., 1998), and *Burkholderiales* is known to inhabit the soil environment (Garrity et al., 2005a). Direct incorporation of microbes from rainwater (Fig. 4, R5 and SP-0m-2) is another possibility. Additional analysis with DGGE showed that *Herbaspirillum* sp. belonging to *Burkholderiales* was retrieved from SP-0m spring water 2 weeks and 5 days after event 2 (DGGE, supplemental information Table S1). Infiltration of microbes from the soil matrix, however, seems more likely, because the second, third and fourth dominant groups of *Bacteria* in

Fig. 2. Text_P8

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 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. 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*, which inhabit environments with high concentrations of sodium and *Methanobacteriales*, 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. 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. 3. Text_P9

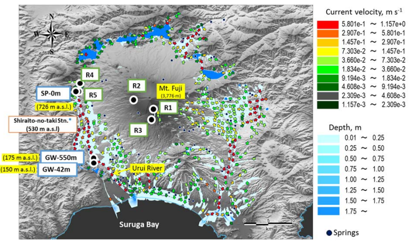

Figure 1. Study sites in western foot of Mt. Fuji. Red arrows indicate main fast flow (GETFLOWS; Kato et al., 2015 partially modified). Precipitation was sampled at R1 to R5. Groundwater was sampled at SP-0m, GW-42m and GW-550m. R1 is located at 2,364 m a.s.l., R2 is at 1,431 m a.s.l., R3 is at 1,081 m a.s.l., R4 is at 850 m a.s.l. and R5 is at 723 m a.s.l. SP-0m, spring water, shows sampling site of Shibakawa located at 726 m a.s.l. GW-42m, shallow well water obtained from 42 m, is located at Yodoshi with 150 m a.s.l. GW-550m, deep well water obtained from 550 m, is located at Aoki with 175 m a.s.l..

* Amount of precipitation for the studied area was recorded at Shiraito-no-taki Station of Japan Weather Association.

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Fig. 4. Figure1

Table 1. Study site and observed environmental parameters.

Site ID	Site name	Type of water	Altitude (m a.s.l.)	Sampling depth (m)	Observation period	Number of Investigation	Water temperature (℃)	pH	EC (μS cm-1)	Eh (Pt) (mV)	DO, degree of saturation (%)	δ18O (‰)	δD (‰)
SP-0 m	Shibakawa	Spring water	726		2012/6/15～2014/11/20	n=19	10.1～11.9	5.84～7.35	49.5～128.0	343~497	86.7～92.9	-9.38～-8.63	-61.9～-57.3
GW-42 m	Yodoshi	Groundwater	150	42	2013/7/2～2014/11/20	n=13	13.8～20.6	6.34～7.24	109.8～161.5	300~478	93.6～100.7	-9.26～-8.69	-60.7～-58.4
GW-550 m	Aoki	Groundwater	175	550*	2013/7/2～2014/11/20	n=13	13.7～19.8	6.63～8.26	122.7～142.8	246~447	73.0～89.9	-11.36～-10.81	-76.1～-74.2
R1	Go-gome	Rainwater	2,364		2013/6/17	n=1	22.6	5.30	-	-	-	-6.36	-38.6
R2	Kokuyurin	Rainwater	1,431		2013/8/6～2014/10/16	n=8	11.9～22.8	4.04～6.26	-	-	-	-13.27～-7.01	-95.2～-47.7
R3	Ni-gome	Rainwater	1,081		2013/6/17～2014/10/16	n=11	14.2～23.4	3.94～5.82	-	-	-	-11.98～-4.61	-89.1～-29.8
R4	Asagiri	Rainwater	850		2013/6/17～2014/10/16	n=15	13.0～31.9	4.12～6.14	-	-	-	-12.85～-3.42	-92.4～-13.4
R5	Shibakawa	Rainwater	723		2012/10/18～2014/10/16	n=16	10.1～30.8	4.06～6.29	-	-	-	-10.93～-5.09	-80.1～-29.6

* Measurements were conducted for the water soon after it was pumped up.

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Fig. 5. Table1