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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.

**Ayumi Sugiyama et al.**

kato.kenji@shizuoka.ac.jp

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Reply to Anonymous Referee #2 Received and published: 10 May 2016

General comments

Comment (General): The data presented in this study is interesting and gives insight into patterns of rainfall influence on groundwater. However, the text, especially the Results and Discussion parts, was hard to follow because of language issues. For example, the results paragraphs describing bacterial community diversity needs editing. The introduction needs changes (please see specific comments below). The Discussion paragraphs mix previous results with results from the actual study, and at times



it is hard to figure out which is which. Thus a better organization of the paragraphs is needed.

Reply to general comment: Thank you for your comments. Concerning "Introduction", we received similar comment and we devised it (please see the web page in the tag of Discussion, AC1: 'Reply to Anonymous Referee #1', Kenji Kato, 09 May 2016: C7-8, revised Introduction). Concerning "Discussion", we changed the wording to minimize readers' confusion.

Specific comments

Comment 1 (Specific): The introduction needs to be re-written and/or re-arranged. Some paragraphs seem like a discussion, and the relevant principal information is difficult to extract. Also it is difficult to understand what the last 2 sentences (P3-L4-L7) the introduction mean or want to show, and should probably be inserted before in the introduction.

Reply 1: We answered this comment above. On the last two sentences, if it should be required strongly we may delete it, but we thought to show the contents what we analyzed herein is not inappropriate as the analyzed subjects were different from ordinal hydrological studies.

Comment 2 (Specific): Material and Methods P3-L23: How many samples were taken? Were different time points used? Was only one groundwater sample from one site (G1?) taken for microbial diversity analysis?

Reply 2: We add a new Table to show the details of the analyzed samples as Supplementary Table 1, Table S1, moving the previous Table S1 to Table S2.

Comment 3 (Specific): P4-L2: Please explain and rephrase 'Rainwater samplers prevented form evaporation'

Reply 3: In order to minimize evaporation of the sampled rainwater we put a plastic ball in between the bottom of funnel and glass bottle. We add this explanation at the

almost last sentence of the text in 2.2 of Materials and Methods as follows; Rainwater samplers prevented from evaporation using a plastic ball in between the bottom of funnel and glass bottle were set up at 1.5 meters above ground.

Comment 4 (Specific): Results P5-L23: It is unclear to me whether the rainfall and groundwater samples for one event were taken at the same date or with an interval of a few hours or days? A delay in sample recovery from one to the other could be necessary in order to observe an influence of rainfall on the groundwater?

Reply 4: The sampling strategy is shown by a newly added table, Table S1.

Comment 5 (Specific): P7-L1: Again it might nice to have a table in the supplementary material with all the rainfall and groundwater samples that were taken, and at each date, and for what types of analyses (chemical or microbial). It is again difficult to understand which samples were used for microbial analyses compared to those used for the chemical analyses.

Reply 5: Yes, we added a new table, Table S1, as explained above.

Comment 6 (Specific): P7-L16L17: Please rephrase this sentence.

Reply 6: We revised text of P7-L16-L17 as follows; 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. (We changed name of the site from G1 to SP-0m, see the web page in the tag of Discussion, AC1: Reply 3 in C3 and revised Figure 1 in C10.)

Comment 7 (Specific): P7-L15: Is there a specific reason why the authors chose to discuss the bacterial (and archaeal) community diversity at the order level?

Reply 7: If we try to analyze the data at the lower classification level, analysis becomes very complicated with greatly increasing number of minorities. For the data obtained from next generation sequence, to analyze the gene sequence data of natural microbial community at Order level was already conducted for the river environment (Staley et



al. 2014, Staley et al. 2015, Ruiz-Gonzalez et al. 2015).

Comment 8 (Specific): P7-L28: The authors should manually check their archaeal taxonomy affiliation. In my experience the sequences affiliated with the Halobacteriales and the Parvarcheota with automated databases can give inaccurate results. It might be worth BLASTing a few OTU representative sequences to make sure their affiliations are correct.

Reply 8: Thank you for your comment. We did not employ BLAST for gene analysis, but Classifier. Though BLAST analyzes clone's similarity with those in database individually. In contrast to that, Classifier software classifies the examined OTU statistically using reference sequences. Statistically classified group is confirmed by Bootstrap value higher than 80%. Thus, repeated analysis with Classifier may not give different result in classification. We analyzed sequence data of Archaea again, and we obtained the very similar result.

Comment 9 (Specific): P8-L5: 'unique', are the authors describing their results and saying that the archaea in this specific sample are unique? Or that compared to other studies they are unique?

Reply 9: Thank you for your comment. We described the relative contribution of examined community constituents was "unique" for the sample obtained after a few weeks of torrential rainfall (G3-2 in Fig. 5). Thus, we change the sentence as follows; These relative contributions to the whole community were clearly different from other results.

Comment 10 (Specific): Discussion P8-L9: 'shortly', how much time exactly?

Reply 10: It was a few hours as was shown by the cited references.

Comment 11 (Specific): P8-L30: by 'extraction' do the authors mean 'infiltration'?

Reply 11: Thank you for your comment. We replaced the wording accordingly.

Comment 12 (Specific): P9-L3: Do the authors mean DNA-based sequences affiliated

with thermophilic bacteria?

Reply 12: Yes, we confirmed it.

Comment 13 (Specific): P9-L5: ‘viral particles were used’, is this study or in a previous study?

Reply 13: It refers the previous study done by Hunt et al. (2014).

Comment 14 (Specific): P9-L15: ‘clones’, were clone libraries constructed as well?

Reply 14: We did not employ cloning procedure in sequence. Thus, we replace the term of "clones" by "OTUs".

Comment 15 (Specific): P9-L20: ‘absolute’, do the authors mean ‘strict anaerobes’?

Reply 15: Thank you for your comment. We changed the wording accordingly.

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-78, 2016.



Tabel S1. Summary of sample analyses.

| | 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 |
| 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.

**Fig. 1.** Table S1