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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.

Anonymous Referee #2

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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.

Specific comments:

-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.

-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.

-Finding thermophilic microbes in environments with temperatures < 40°C 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.

-In Material and Methods, in the DNA extraction section no sampling triplicates were mentionned. 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.

-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.

-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.

-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.

-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.

-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.

-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.

-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.

Comments on figures:

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.

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

Technical corrections:

-page 7 line 16: what do 384, 268 and 278 correspond to? number of orders? Please reformulate -page 9 line 4: replace "was" by "were" -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.

References:

Zhou, Y., Kellermann, C. & Griebler, C. Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. FEMS Microbiol. Ecol. 81, 230–42 (2012).

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Flynn, T. M., Sanford, R. A. & Bethke, C. M. Attached and suspended microbial communities in a pristine confined aquifer. Water Resour. Res. 44, 1–7 (2008).

Ben Maamar, S. et al. Groundwater isolation governs chemistry and microbial community structure along hydrologic flowpaths. Front. Microbiol. 6, 1–13 (2015).

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