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Hydrology and
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Discussions

Interactive comment on "Coalescence of bacterial groups originating from urban runoffs and artificial infiltration systems among aquifer microbiomes" *by* Yannick Colin et al.

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Replies (Rep1) to reviewer # 1 (R1-Question#) (Louis Carles, 13 Mar 2020) (line numbers are those of the initial submission)

R1-Q1: In what extend the comparison of samples taken at different time is meaningful? How did you integrate the time-scale in your analyses and discussion?

Rep1-Q1: Here, the hypothesis was that urban aquifers fed by SIS should harbor microbiota reflecting the coalescence (community assemblages and selective sorting) of aboveground microbial communities with those of the aquifer. This hypothe-

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sis was tested by comparing representative bacterial taxonomic units defined by two genetic markers (16S rRNA and tpm genes) and observed among significant compartments of the watershed (urban runoffs, sediments / urban deposits, soils in contact with the urban runoffs), with those from aquifer samples (water and biofilms). Time scale was considered in previous reports such as Voisin et al. 2018 (doi : 10.1016/j.scitotenv.2018.05.094) and Marti et al. 2017 (10.1038/s41598-017-13594-8). From these latter analyses, core and versatile bacterial communities could be observed among the investigated system. Stochasticity was not the leading rule among the investigated system because of strong environmental constraints and the significant occurrence of particular C-sources such as polycyclic aromatic hydrocarbons (PAH) e. g. see Fig. 3 which shows a clear discrimination of community profiles according to the investigated compartment. It is to be noted that over the investigated time period, no significant urbanistic changes occurred over the observatory site. The Django-R urban watershed is part of the OTHU (Lyon Urban Waters Field Observatory) long term experimental site dedicated to urban waters (http://www.graie.org/othu/)); therefore, changes over the watershed are tracked in collaboration with the Lyon Metropolitan water services. This last point is now indicated in the Materials and Methods section.

From line 109, the following sentence was added: "... No significant urbanistic changes impacting the urban watershed were recorded during the investigated period...."

R1-Q2 : In the discussion section, the authors explain the shifts observed in microbial communities structure by emitting hypotheses linked to nutrient availability and environmental factors. However, physical-chemical data describing the different compartment of the studied site are lacking.

Rep1-Q2: These datasets were cited in the discussion and not described in the result section because they were published elsewhere. In fact, we've published several physical-chemical datasets in the frame of this collaborative long-term investigation which is addressing several issues related to urban waters and their management.

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To clarify these issues, the following sentences were added in the discussion: From line 365: “..In fact, chemical pollutants have been shown to be significantly washed-off or transported with particles during rain events e. g. El-Mufleh et al. (2014; doi.org/10.1007/s11356-013-2490-3), and some of these were found to reach aquifers fed by SISs (e. g. Pinasseau et al. 2019; doi.org/10.1016/j.scitotenv.2019.03.489). Among these pollutants, Bernardin-Souibgui et al. (2018) reported that urban sediments found in the detention basin of the experimental site were heavily polluted by polycyclic aromatic hydrocarbons (PAH). Their contents were estimated at 197±36 ng/g dw (dry weight) for light PAHs, and at 955±192 ng/g dw for heavy PAHs. PCBs were also recorded for the seven congeners of the European norm for a total of 0.2 to 2.1 mg/ kg dw (Sebastian et al., 2014). Metallic trace elements (MTE) were recorded in significant amounts, with Cu being found at about 280 mg / kg dw, Pb at about 200 mg / kg dw, Zn at about 1600 mg, and Cd at about 5 mg / kg dw (Sebastian et al., 2014). MTE, PCBs and PAHs were also recorded in the soils of the infiltration basin at similar ranges e. g. in average, at 0.26 mg PCBs / kg dw, and at more than 940 ng/g dw for PAHs (Winiarski, 2014; temis.documentation.developpement-durable.gouv.fr/docs/Temis/0081/Temis-0081850/21970_A.pdf; Winiarski et al. 2006; doi.org/10.1007/s10040-006-0073-9). These sediments and soils were also found contaminated by dioxins at about 36 ng / g dw (Winiarski, 2014), and by 4-nonylphenols and bisphenol A, at concentrations varying from 6 ng/g dw to 3400 ng/g dw (Wiest et al., 2018; doi.org/10.1016/j.envpol.2018.09.138). MTE and non-polar HAPs found among SISs are unlikely to reach groundwaters. To illustrate, Pb and Cd were not recorded at depths below 1.5 m into the non-saturated zone of SISs (Winiarski et al. 2006). In contrast, polar organic pollutants were found to be transferred into aquifers as shown for some pesticides and pharmaceuticals (Pinasseau et al. 2019). These chemical contaminants represent potential energy- and carbon-sources for micro-organisms, and can also be detrimental to the growth of some organisms. They can thus have significant impacts on the biology of the contaminated soils and sediments of SISs. Functional inferences from the . . .”

From line 372: "…Twice as much dissolved organic carbons were detected among aquifer waters of the experimental site downstream the SIS (1.93 mg/L ± 0.77) than upstream (0.88 mg/L ± 0.27) (Mermillod-Blondin et al., 2015; Winiarski, 2015)…"

R1-Q3: What would be the effect of the species coming from surface environments on the functioning of the microbial communities in the aquifer?

Rep1-Q3 : Consequences of these changes are multi-factorial.

From line 437, we've clarified these issues by modifying the text below – new sentences are in red.

"… The long term incidence of allochthonous bacteria on the integrity of aquifer microbiota remains to be investigated. Free-living bacterial communities are not likely to be much impaired but those developing as biofilms on inert surfaces might be. Microbial biofilms are key structures in the transformation processes of several elements and nutrients. They often display much higher cell densities than free-living populations (Crump and Baross, 1996; Crump et al., 1998; van Loosdrecht et al., 1990). Here, we have demonstrated that runoff and SIS bacterial taxa can colonize solid matrices of a deep aquifer. These modified communities could (i) alter geochemical processes which can indirectly impact other groundwater inhabitants e. g. the amphipod Niphargus rhenorhodanensis and other taxa presented in Foulquier et al. (2011; doi.org/10.1007/s10021-011-9484-0), or (ii) directly impact these inhabitants by inducing a modification of their microbial contents, and potentially of their behavior. The stygofauna feed on bacteria, and is well known to be significantly colonized by bacteria (e. g. Smith et al., 2016; doi.10.1038/srep32738). The next step in these studies will be to investigate whether native aquifer biofilm communities can resist to repeated invasions by opportunistic r-strategists, and if these allochthonous bacteria will impact the ecological health of the stygofauna. "

R1-Q4 : L91-94. I agree with this sentence. However, one should also take into account the fact that the transition between free-living organisms and biofilm is taxadependant. Meaning that the biofilm is not 100% reflecting the diversity of free-living microorganisms living in the aquifer. This is confirmed by H' values (AQ_bio < AQ_wat) in Figure 2B.

Rep2-Q4 : OK, we've tried to clarify this issue by adding the following sentence: From L94: "Clay bead biofilms were found to capture the most abundant aquifer taxa, and taxa that could not be detected from grab samples. Although, it is to be noted that some taxa are not likely to efficiently colonize clay beads over short time periods."

R1-Q5 : L204-205. How could you explain the higher diversity in IB compared to WS and DB?

Rep2-Q5: we've added the following sentences to explain this difference. From L354: "…It is to be noted that soils of the infiltration basin showed higher bacterial diversity than those of the sediments of the detention basin and runoffs. This is most likely related to a development of plant-associated bacteria in this compartment. Indeed, the infiltration basin was covered by several plant species of Magnoliophyta like Rumex sp. which can disseminate rapidly through rhizomes (Bedell et al., 2013; doi.org/10.2166/wst.2013.526) and generate multiple ecological niches for bacteria."

R1-Q6 : Figure 3. It would be useful to add the eigenvalues for both axes.

Rep2-Q6: The proportion of the eigenvalue to the sum of all eigenvalues was calculated in order to estimate the importance of the axes in discriminating the samples from each other (PCoA1: 25.0% and PCoA2: 17.6%). These values will be indicated.

From L699, the following sentence was added: "The proportion of the eigenvalue per axis over the sum of all eigenvalues was 25.0% for PCoA1 and 17.6% for PCoA2."

R1-Q7 : L213-215 and Figure S2. Dissimilarities instead of similarities?

Rep2-Q7: we've computed similarities (100 - % of dissimilarities) to facilitate the appreciation of differences between samples

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R1-Q8 :Technical corrections L226. Remove “(Table 2)”. Table 1 should be Table 2, and inversely. Please make the correction in the text, accordingly. L305. Add “species” after “Some”. Rep2-Q8: all these problems were considered and fixed

Please also note the supplement to this comment:
https://www.hydrol-earth-syst-sci-discuss.net/hess-2020-39/hess-2020-39-AC1-supplement.pdf

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