Hydrol. Earth Syst. Sci. Discuss.,
https://doi.org/10.5194/hess-2020-39-RC2, 2020

Hydrology and Earth System Sciences
Open Access
Discussions

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

Anonymous Referee #2

Received and published: 16 March 2020

The article entitled "Coalescence of bacterial groups originating from urban runoffs and artificial infiltration systems among aquifer microbiomes" presents the results of a study monitoring the microbial community assemblage though a stormwater infiltration system. The main findings of the study surrounded the contribution of each of the sources (the runoff and natural aquifer) to the combined system. An additional tracking of the biodegradation-related tpm gene allowed for finer species level resolution of the structure and shifts in the microbial community. As stormwater infiltration systems are becoming more commonly applied, readers will be interested in understanding their community assemblage.

However, the study requires additional information and revisions to clarify the presented investigation. Recommended edits and improvements to enhance the presented study are presented within three categories: Major, Minor, and Grammar revisions. The nine major revisions recommended in this review emphasize required improvements on the presentation of the analytical methods, bioinformatic approaches, and streamlining the discussion. The minor and grammar portions present less critical revisions.

Major

Maj1. The presentation of the sequencing process employed is inadequate. The current text highlights that the sequences were run on a Illumina MiSeq, without providing additional details. First, the study does not mention how the nucleic acids are extracted from the samples, checked for quality, stored, and shipped to the facility. These points must be clarified. Second, the study must clarify within section 2.2 several key points with respect to the sequencing protocol: (1) a citation for the primers used to target the 16S rRNA gene, (2) the protocol followed by the laboratory must be unambiguously indicated or referenced (TruSeq, Nextera, etc.), (3) the target length of the sequences, and (4) whether the sequence reads were paired-end or single. The current presentation does not follow the MINSEQ guidelines for the required information for sequencing studies. Third, the presented study does not mention either positive mock community or negative comparison controls (and how those samples are incorporated into the analyses to remove contaminating sequences). The authors must present these controls.

Maj2. The results of the sequencing campaign additionally requires a more comprehensive presentation. L193-194 presents the total sequencing reads, but must present the average and range of reads per sample. A supplemental table must be provided with the raw and processed sequencing counts for each sample.

Maj3. Additionally, to explore quantitatively the mixing ratios and why certain communities are providing more biomass, the actual concentration of the community within these compartments should be mentioned or addressed as to why these measurements were neglected.

Maj4. The bioinformatic processing pipeline requires additional information. First, the approach presented divides the 16S rRNA amplicons into 97% OTUs. However, current best practices recommends utilizing the amplicon sequencing variants (ASV) approach (Knight et al., 2018). The authors should either update their approach to the ASV methodology or provide a concise defense as to why they selected the OTU approach. Second, a rarefaction analysis is presented to subsample the dataset at 20,624 sequences. This approach has been recently called into question for more directed comparisons (McMurdie and Holmes 2014). The authors should present a concise defense as to why rarefaction was employed. To bolster this defense, Figure S1 should display the rarefaction curve for the raw data, not the previously subsampled 20,624 dataset (this comment connects with Maj2 in the need to present additional information).

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., ... & Melnik, A. V. (2018). Best practices for analysing microbiomes. Nature Reviews Microbiology, 16(7), 410-422.

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. PLoS computational biology, 10(4).

Maj5. In the SourceTracker default code, the rarefied sample is then rarefied further to 1000. This procedure should be repeated to draw those 1000 reads from the full dataset, not the previously rarefied data.

Maj6. L319-337 presents a great overview of the study that is more appropriate for the abstract rather than the discussion. This section should be removed in its entirety.

Maj7. Throughout the text, the presence of a specific 16S rRNA transcript often is utilized to state the presence of a specific function within the community, notably within

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the abstract (e.g., L25, L27). Whereas the 16S taxonomical assignment is a good indicator that a specific function is likely encoded on the metagenome of the community, the linkage is not directly shown through the 16S survey and must be caveated by “likely”, “putative”, or “predicted to be”. This is recognized more consistently within the discussion of the results, but must be maintained throughout the text to recognize that the assignment provided by FAPROTAX is a hypothesis.

Maj8. The authors commendably provided the raw data as publicly available datasets through EBI. Additionally, the authors should provide all code utilized to process these data as a part of the supplemental materials to allow future readers to reconstruct the presented results.

Maj9. The authors are encouraged to focus on improving the English language and grammar associated with the presented article. A non-exhaustive list of suggested grammar improvements is provided in the final section of this review, but additional editing services are recommended to enhance the clarity and accuracy of the text.

Minor

Min1. The bulk physical and chemical properties of the sampling sites should be presented or directly cited such as pH, temperature, electroconductivity etc. Additionally, please replace “for which physico-chemical and biological monitorings have been implemented” with “that records both physico-chemical and biological properties.”

Min2. L34 – Please clarify what is meant by “DNA imprints allocated”

Min3. L70-75 – Please provide citations in support of these claims.

Min4. L78-L79 – Replace “The tested hypotheses were that” with “Two hypotheses were tested:”. Because these statements are presenting the underlying hypotheses (supported or rejected), all qualifiers for the verbs must be removed. Therefore, remove L78 “should” and L79 “could also”. L79 – Replace “but” with “, and”. Similarly with L88-90, please replace “was likely to be” with “will be”

Min5. L291-307 – The long list of species mapped to the Pseudomonas genera is difficult to interpret in the currently presented form. Please condense this section for readability.

Min6. Throughout the text, ensure that a comma appears after Latin abbreviations such as i.e., and e.g.,

Min7. Figure 1, please italicize the names of the phyla.

Grammar

L23 – Please add “basins” after “detention”. Currently, this sentence presents a broken list of items.

L24 – Please replace “made up” with “comprised”

L27 – Please add a comma before “but a higher”

L28 – Please replace “a tracking” with “the tracking”

L29 – Please replace “including the” with “including”, remove “among these communities”, and replace “the Pseudomonas” with “Pseudomonas”

L31 – Please replace “respectively, of” with “respectively, in”

L32 – Please remove the comma before “, and waters” and add “to be” after “found”

L34 – Please add a comma before “but only”

L36 – Please add “in” after “than”

L48 – Please replace “during” with “over”

L53 – Please replace “towards” with “capturing” and remove the comma after “metals)”

L59-60 – Please add “both the” before “water transit”, replace “, but also the biology” with “ and biological properties”, replace “cover and root” with “cover, root”, replace “worms” with “worm population”, and add “composition” after microbiota.

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L76 – Please replace "Here, the" with "This", replace "explored" with "explores", and remove the commas around ", with a thick vadose zone (> 10 m),"

L83 – Please replace "It" with "The site"

L84-85 – Please replace "It has an average vadose thickness of" with "The average vadose thickness of the site is"

L86-88 – Please remove "large", replace "built" with "recorded", remove ", in order", add "the" before "bacterial community", add "the" before "top", replace "among" with "into", and add "the" before "biofilm".

L92 – Please replace "among" with "within" and replace "while" with ", whereas"

L99 – Please remove "To go deeper into these inferences," and replace "were built" with "were then assembled"

L101 – Please remove the comma after "level," and replace ", and allowed gaining further insights on" with "to explore with a higher resolution"

L103 – Please replace "with" with "within"

L108 – Please replace "about" with "approximately"

L110 – Please add "a" before "part"

L112 – Please remove the comma before ", built"

L113-114 – Please replace "development of a plant cover" with "plant cover development

L116 – Please replace "deeply" with "previously" and check the format requirements for citations.

L118 – Please replace "were considered for this" with "are investigated within"

L120-122 – Please replace "have been" with "were", add an "a" before "50", and remove “of the DB”

L122 – Please remove the comma before the “and”

L124 – Please replace “had been” with “were”

L126 – Please remove the comma before “at a”

L127 – Please replace “have been” with “were”

L130 – Please replace “had been” with “was”

L132 – Please add “subsequently” before “discarded”

L133 – Please replace “using clay” with “from clay”

L134 – Please replace “the same piezometers as those for the aquifer water samplings” with “the piezometers described above” and delete the subsequent sentence whereas including the citation just after (n = 6 samples).

L137 – Please revise this title to be more informative, such as “Generation and sequencing of the DNA amplicons”

L139 – Please replace “with Illumina MiSeq technology” with “on an Illumina MiSeq”

L165 – Please capitalize “BLAST”

L166 – Please remove “in order”

L168 – Please replace “carried out” with “performed”

L178 – Please remove “down”

L179 – Please remove “, in order”

L185 – Pease remove the comma before “, with”

L199 – Please replace “superior” with “greater than”

L205 – Please replace “of detention” with “withdrawn from the detention”

L207 – Please add a “the” before “clay” and replace “for” with “over”

L212 – Please replace of “to be made of” with “to contain”

L213 – Please replace “a same” with “the same”

L214 – Please replace “while” with “whereas the”

L216 – Please replace “found” with “to be found”

L226 – Please replace “while” with “whereas”

L233 – Please replace “much to” with “substantially to the” and remove “Content of”

L236 – Please replace “even though” with “although”

L238 – Please remove “In order”

L244 – Please add “and” before “Nitratireductor.”

L245 – Please replace “while” with “, and”

L260 – Please replace “the SIS” with “of the SIS”

L261 – Please add “and” before “Flavobacterium”

L262 – Please replace “while” with “whereas”

L264 – Please add “and” before “Meganema”

L265 – Please replace “found: with “found to be”

L276 – Please replace “It is to be noted that” with “Notably,”

L277 – Please replace “part” with “representative”

L280 – Please replace “deeper” with “further”

L287 – Please remove “the”; additionally, because the data is already given as Table

S6, I recommend removing the exact percentages from this paragraph.

L295 – Please add a comma before “but”

L296 – Please add a comma before “but”

L316 – Please replace “while” with “when”

L345 – Please remove the comma before “, and”

L347 – Please replace “that can also enhance” with “enhances”

L348 – Please remove “Nevertheless” and replace “has induced” with “induces”

L350 – Please replace “that” with “than”

L352 – Please replace “the SIS” with “of the SIS”

L353 – Please replace “the SIS” with “of the SIS”

L360 – Please replace “the SIS” with “of the SIS”

L385 – Please add a comma before “, and significant”

L389 – Please replace “while” with “whereas”

L391 – Please replace “to likely” with “likely”

L396 – Please replace “the SIS” with “of the SIS”

L398 – Please remove the comma before “, and was”

L400 – Please add a comma before “but a few”

L404 – Please replace “in order to go deeper into” with “to explore further”

L414 – Please remove the comma before “, and can”

L418 – Please replace “It would thus be part of the r-strategists that could get opportunistically established” with “Therefore, an r-strategist would likely establish opportunistically”

L422 – Please remove “However,”

L425 – Please add “to be” after “shown”

L429 – Please remove the comma before “, and yield”

L431 - Please add “to be” after “found”

L432 – Please replace “at degrading” with “to degrade”

L436 – Please use an alternative term to “germcatchers”

L437 – Please remove “down”

L439-440 – Please improve the wording of the sentence beginning with “Free-living”

L443 – Please remove “to these”

L687 – Please replace “runoffs” with “runoff”

L688-689 – Please remove the commas after “(A),” and “(B),”

L700 – Remove “down the aquifer”

Table 1. Capitalize “Downstream SIS”; in the caption, replace “in (A),”, “in (B),” and “in (C),” with “(A)”, “(B)”, and “(C)”, respectively. Remove all occurrences of “is indicated”

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