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Hydrology and Earth System Sciences
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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.

Yannick Colin et al.

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Replies (R2) to reviewer # 2 (major (Maj#) comments) (anonymous, 16 Mar 2020) (line numbers are those of the initial submission)

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.

Maj1a : 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.

R2-maj1a : The following sentences were added to clarify these issues.

From L138: "About 600 mg of sediments or soils, or up to 5 L of aquifer or runoff water samples filtered using 0.22 μm polycarbonate filters, were used per DNA extraction. Total DNAs were extracted from soils/sediments or filters using the FastDNA SPIN$^{®}$ Kit for Soil (MP Biomedicals, Carlsbad, France). For clay bead biofilms, microbial cells were detached by shaking at 2500 rpm for 2 min in 10 mL of 0.8 % NaCl. These suspensions were then filtered and their DNA content was extracted as indicated above. Blank samples were performed during these extractions for both the soils/sediments or filtered cells. DNAs were quantified using a nanodrop UV-Vis Spectrophotometer. Blank DNA extracts showed values below the detection limit. DNA extracts were visualized after electrophoresis at 6V/cm using a TBE buffer (89 mM Tri-borate, 89 mM boric acid, 2 mM EDTA, (pH 8.0)) through a 0.8% (w/v) agarose gel, and DNA staining with 0.4 mg.mL-1 ethidium bromide. A Gel Doc XR+ System (Bio-Rad, France) was used to observe the stained DNA, and confirm their relative quantities (between 20-120 ng/μl; median value around 40 ng/μL) and qualities. DNAs were kept at -80°C, and shipped on ice within 24h to the DNA sequencing services when appropriate.

Quantitative PCR assays were performed on the DNA extracts to estimate their relative content in 16S rRNA gene copies. These assays were performed on a Bio-Rad CFX96 realtime PCR instrument with Bio-Rad CFX Manager software, version 3.0 (Marnes-la-Coquette, France). The 16S rRNA gene primers 338F and 518R described by Park and Crowley (2006) were used, together with the Brilliant II SYBR green low ROX qPCR master mix for SYBR Green qPCR. Melting T° was 60°C. Linearized plasmid DNAs containing a 16S rRNA gene were used as standards, and obtained from Marti et al. (2017). Presence of inhibitors in the DNA extracts was checked by spiking known amount of plasmid harboring int2 (107 copies of plasmid per μL) in the PCR mix. Number of cycles needed to get a PCR signal was compared with wells where only plasmid DNA harboring int2 was added to the qPCR mix. When a high number of cycles was needed to observe a signal, a 5- or 10-fold dilution of the DNA extract was done, and another round of tests was performed to confirm the absence of PCR inhibitions. Each assay was triplicated on distinct DNA extracts, and technical triplicates were performed. The 16S rRNA gene qPCR datasets are presented in Figure S1. These assays confirmed the high number of bacterial cells per compartment (Figure S1 and Table S2): (1) soils from the infiltration basin (IB) had a median content of 1.32 x 1011 16S rRNA gene copies per g dry weight; (2) sediments from the detention basin (DB) of 1.83 x 1011 16S rRNA gene copies per g dry weight, (3) the runoff waters (WS) had a median content of 4.75 x 108 16S rRNA gene copies per mL, (4) the aquifer waters (AQ_wat) of 3.10 x 106 16S rRNA gene copies per mL, and (5) the aquifer clay bead biofilms showed 1.35 x 107 16S rRNA gene copies per cm2."

Maj1b : 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.

R2-maj1b : After the text added for comment R2-maj1a, the following sentences were added to clarify the Maj1b issues:

Sequencing of V5-V6 16S rRNA gene (rrs) PCR products were performed by MrDNA DNA sequencing services (Shallowater, Texas, USA) on an Illumina Miseq. The PCR products were generated using DNA primers 799F (barcode + ACCMGGATTAGATACCCKG) and 1193R (CRTCCMCACCTTCCTC) reported by Beckers et al. (2016). PCR amplifications were performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) using the following temperature cycles: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, with a final elongation step at 72 °C for 5 min. PCR products and blank control samples were verified using a 2% agarose gel and following the electrophoretic procedure described above. PCR products obtained from field samples showed sizes around 430 bp but blanks did not show detectable

and quantifiable PCR products. Dual-index adapters were ligated to the PCR fragments using the TruSeq® DNA Library Prep Kit which also involved quality controls of the ligation step (Illumina, Paris, France). Illumina Miseq DNA sequencings of the PCR products were paired-end, and set up to obtain around 40K reads per sample. The tpm DNA libraries were also sequenced by the Illumina MiSeq V3 technology but by the Biofidal DNA sequencing services (Vaulx-en-Velin, France). PCR products were generated using the following mix of degenerated PCR primers: ILMN-PTCF2 (5'- P5 adapter tag + universal primer + GTGCCGYTRTGYGGCAAGA-'3), ILMN-PTCF2m (5'- P5 adapter tag + universal primer + GTGCCCYTRTGYGGCAAGT-'3), ILMN-PTCR2 (5'- P7 adapter tag + universal primer + ATCAKYGCGGCGCGGTCRTA-'3), and ILMN-PTCR2m (5'- P7 adapter tag + universal primer + ATGAGBGCTGCCCTGTCRTA-'3) targeting conserved regions defined by FavreâĂŘBonté et al. (2005). The universal primer was 5'-AGATGTGTATAAGAGACAG-'3. The P5 adapter tag was : 5'-TCGTCGGCAGCGTC-'3. The P7 adapter tag was : 5'- GTCTCGTGGGCTCGG-'3. PCR reactions were performed using the 5X Hot BIOAmp® master mix (Biofidal, France) containing 12,5 mM MgCl2, and 10% DMSO and 50 ng sample DNA final concentrations. PCR cycles were as follow: (1) a hot start at 94°C for 5 min, (2) 35 cycles consisting of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, and (3) a ïňĄnal extension of 5 min at 72°C. The mix had two carefully optimized enzymes, the HOT FIREPol® DNA polymerase and a proofreading polymerase. This enzyme blend has both 5'→ 3' exonuclease and 3'→ 5' proofreading activities. This mix exhibits an increased fidelity (up to five fold) compared to a regular Taq polymerase. PCR products and blank control samples were verified using a 2% agarose gel and following the electrophoretic procedure described above. PCR products obtained from field samples showed sizes around 320 bp but blanks did not show detectable and quantifiable PCR products. Index and Illumina P5 or P7 DNA sequences were added by Biofidal through a PCR procedure using the same Hot BIOAmp® master mix and the above temperatures, but limited to 15 PCR cycles. Indexed P5/P7 tagged PCR products were purified using the SPRIselect procedure (Beckman Coulter, Roissy, France). PCR products and blank control samples were verified using the QIAxcel DNA kit (Qiagen, France), and band sizes around 400 bp were observed but not in the blank samples. Quantification of PCR products by the picogreen approach using the Quantifluor dsDNA kit (Promega, France) and a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, France) was performed, and showed low values among the blanks which were at the limit of detection (around 0,07 ng/μl). Still, tpm harboring bacteria being in low number among a bacterial community (about 2-3%), these controls were run during the Miseq DNA sequencing of the PCR products. Illumina Miseq DNA sequencings of the tpm PCR products were paired-end, and set up to obtain around 40K reads per sample. Blank samples generated low numbers of tpm reads (blank 1 = 24 reads; blank 2 = 3 reads, blank 4 = 1028 reads, and blank 5 = 1 read), and these have been listed in Table S3. These reads mainly belonged to unknown species (86%). However, reads from P. fluorescens (from OTUs not found in the field samples), P. xanthomarina (17 reads over all blanks) and P. fragi (n=3 reads over all blanks) were recovered but did not have any impact on the coalescence analysis.

Maj1c : 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.

R2-maj1c : As indicated above in replies “R2-maj1a” and R2-maj1b, several blanks and lab controls were performed all over the investigations. Blanks were run during the DNA extractions, and did not yield detectable contaminant DNAs. Furthermore, the 16S rRNA gene qPCR datasets (Table S2) confirmed that high bacterial numbers were found among each compartment investigated in this study as indicated in reply “R2-maj1a”. In fact, blanks were performed during the 799F - 1193R PCR amplifications of the V5-V6 16S rRNA gene regions, and DNA yields were found below the detection limit (<0,05 ng/μl). Any contaminant DNA would thus be highly diluted and not expected to have major incidence on this 16S rRNA gene-based meta-barcoding community

coalescence analysis. However, it is to be noted that the bacterial tpm community being expected to be in lower number per sample, blank samples for the tpm meta-barcoding sequencing scheme were sequenced. As indicated in “R2-maj1b”, low number of tpm reads were obtained and their matching OTUs were listed in Table S3. These reads did not match tpm OTUs transferred from the above ground environments down into the aquifer.

To further clarify these issues, the following sentences were added:

From L294: It is to be noted that blank samples sequenced during the tpm meta-barcoding assay revealed 23 Pseudomonas OTUs coming from the DNA extraction kit or generated during the PCR product Illumina sequencing process (Table S3). Only OTU00573 was found in high number (867 reads) but this contaminant did not have an impact on the coalescence analysis because of its absence in the below ground datasets. Other contaminant OTUs did not represent more than 10 times the ones observed in the field samples for identical OTUs, a criterium used to distinguish significant contaminants (Lukasik et al., 2017; doi.org/10.1111/mec.14140). In fact, only seven OTUs found among the blanks matched OTUs recovered from the environmental samples, and only two of these could be related to well-defined species i. e. P. xanthomarina (17 reads among all blanks) and P. fragi (three reads among all blanks). These reads matched a single OTU over eleven allocated to P. xanthomarina in the environmental samples, and one OTU over 52 for P. fragi.

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.

R2-maj2 : These features are now indicated in Table S2, and cited in the text. From Line 193, the following sentence was added: “The analysis of the 16S rRNA V5-V6 gene libraries yielded 2,124,272 high-quality sequences distributed across 103 samples, as described in Table S2.

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.

R2-maj3 : The 16S rRNA gene qPCR datasets are now shown in Figure S1 and Table S2. They confirmed a lower number of bacterial cells among the aquifer than the runoff waters.

From L343, the following sentence was added: "...These results were confirmed by qPCR estimations of 16S rRNA gene copies per compartment. These values were much lower in the aquifer waters than the runoffs."

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).

maj4a : The authors should either update their approach to the ASV methodology or provide a concise defense as to why they selected the OTU approach.

maj4b : 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).

R2-maj4a and 4b : Figure S1 was replaced by Figure S2 which is now showing both the OTU rarefaction curves before and after having performed a sub-sampling

at 20,624 reads per sample. OTUs were defined at a 97% identity cut-off to collapse reads into groups that reduce the incidence of sequencing errors on the dataset as suggested by several authors including Eren et al. (2013; PLOS ONE 8, doi : 10.1371/journal.pone.0066643), and Johnson et al. (2019; Nat. Commun. 10:5029, doi: 10.1038/s41467-019-13036-1).

It is to be noted that the original paper by Knights et al. (2011) describing the development of the SourceTracker made use of OTU contingency tables built with a 97% identity cut-off. This was also the case of the paper describing a "reliability" test for the source tracker inferences (Henry et al., 2016; https://doi.org/10.1016/j.watres.2016.02.029). Looking at recently published papers on the SourceTracker, one can find that most research groups have maintained a use of OTU-based contingency tables e. g. O'Dea et al. (2019, https://doi.org/10.1016/j.watres.2019.114967); Han et al. (2020, https://doi.org/10.1016/j.watres.2020.115469), Chen et al. (2019, https://doi.org/10.1038/s41598-019-42548-5), Bi et al. (2019, doi:10.1111/1462-2920.14614), and so on. Still, we confirm that a few papers have used the ASV approach to build their contingency tables for the SourceTracker and for other purposes e. g. Karstens et al. 2019, https:// doi.org/10.1128/mSystems.00290-19, and Caruso et al., 2019; https:// doi.org/10.1128/mSystems.00163-18. We recognize that the ASV approach is reliable to identify conserved ASV among datasets showing variable number of reads. However, the ASV approach also has its weaknesses. For our actual application of the SourceTracker, and according to other papers, the OTU-based contingency table was thus kept for our downstream analyses. Nevertheless, we've now cited articles on ASV in order to make sure that future readers of this paper will be aware of this approach, and might consider using it for the SourceTracker analyses.

The sub-sampling performed at 20,624 reads allowed to reduce the incidence of the variable number of reads obtained per sample. An uneven sequencing depth (ranging from 6,062 to 181,207 reads per sample) was recorded, and found to be related to technical DNA sequencing problems. In fact, the qPCR datasets on 16S rRNA gene copies supported this conclusion. No correlation was observed between the 16S rRNA gene copy numbers (biomass) and the number of reads obtained per sample (see Table S2). In this context, we've decided to sub-sample our dataset to compensate for these discrepancies. In our opinion, sub-sampling datasets remain a good standardization technique to mitigate sample library size artifacts, especially for very unequal library sizes between groups. In accordance with this, our sub-sampled dataset (20,624 reads per sample) led to a very good separation of samples according to their origin (i.e. WS, DB, IB, AQ_wat and AQ_bio) (see Fig. 3).

From 155, the following sentences were added to clarify these issues: Variability in the number of cleaned reads per sample was observed but not correlated with variations in the number of 16S rRNA gene sequences (Table S2). These variations were thus considered to be due to the DNA sequencing process. Therefore, a sub-sampled dataset (20,624 reads per sample; with exclusion of samples with total reads below this threshold) was used to mitigate the artifact of sample library sizes. Operational Taxonomic Units (OTUs) were defined using a 97% identity cut-off as recommended by several authors in order to collapse sequences into groups that reduce the incidence of sequence errors on the datasets (e. g., Eren et al. 2013; and Johnson et al. 2019). It is to be noted that amplicon sequence variants (ASV) could also be used to build contingency tables (e. g., Callahan et al. 2016; Karstens et al. 2019). However, exact sequence variants can generate uncertainties when using 16S rRNA gene sequences because of variations among species and strains due to the presence of multiple copies per genome (Johnson et al. 2019). Figure S2 shows the OTU rarefaction curves for the full and the sub-sampled datasets. This sub-sampled dataset was used for all downstream analyses except those of the SourceTracker Bayesian approach.

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.

R2-maj5 : We agree with this comment. Analyses were thus re-run using the cleaned but not re-sampled 16S rRNA gene reads, and the matching OTU contingency table (the one used to build Figure S2a). We then used the default SourceTracker code, including a sub-sampling of 1,000 reads as recommended by Henry et al. (2016). This analysis was run 3 times, and the coefficient of variation (i.e. Relative Standard Deviation) was used as a gauge to evaluate confidence on the computed values as suggested by Henry et al. (2016) and McCarthy et al. (2017). Table 1 was modified according to these computings.

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.

R2-maj6 : This paragraph was deleted but a few sentences kept to facilitate the understanding of the discussion

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 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.

R2-maj7 : Ok, this was clarified over the text.

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.

R2-maj8 : From L149, the following sentences were added so that future readers can reproduce the results generated in this work : All paired-end MiSeq reads were processed using Mothur 1.40.4 by following a standard operation protocol (SOP) for MiSeq-based microbial community analysis (Schloss et al., 2009; Kozich et al.(2013), so-called MiSeq SOP available at http://www.mothur.org/wiki/MiSeq_SOP. Due to the large number of sequences to be processed, the cluster.split command was used to assign sequences to OTUs.

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.

R2-maj9 : we did a complete grammar review and rewrote some sentences to clarify certain formulations.

Minor Min1. The bulk physical and chemical properties of the sampling sites should be presented or directly cited such as pH, temperature, electroconductivity etc.

reply : fixed; the most significant chemical datasets are now indicated in the paper from L365; and a selection of papers was cited so that readers can complete their knowledge of the investigated sites through analysis of these papers which present pH, electrical conductivity, soil properties, and many other datasets. See replies to reviewer 1 for this issue.

Additionally, please replace “for which physico-chemical and biological monitorings have been implemented” with “that records both physico-chemical and biological properties.”

reply : fixed accordingly

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

reply : was changed for “Some tpm sequence types of . . .”

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

reply : fixed

Min4. L78-L79 – Replace “The tested hypotheses were that” with “Two hypotheses were tested:”.

reply : fixed

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”.

reply : fixed accordingly

Similarly with L88-90, please replace “was likely to be” with “will be”

reply : fixed accordingly

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.

reply : we’ve tried to simplify this text but citing all these species is important for specialists; several of these species had never been described in these environmental contexts or in Europe

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

reply : fixed accordingly

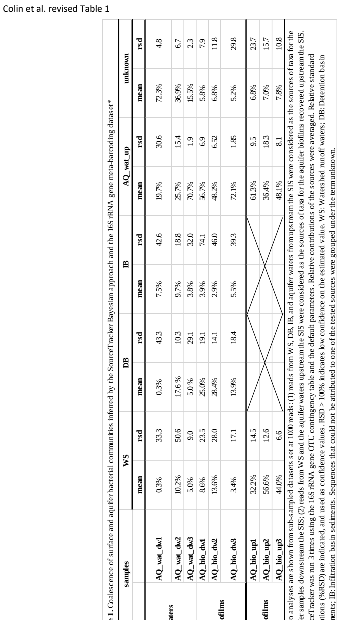
Min7. Figure 1, please italicize the names of the phyla.

reply : fixed accordingly

Grammar / reply: all grammar issues raised by this reviewer 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-AC2-supplement.pdf

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



Colin et al. revised Table 1

Table 1. Coalescence of surface and aquifer bacterial communities inferred by the SourceTracker Bayesian approach and the 16S rRNA gene meta-barcoding dataset*

samples		WS		DB		IB		AQ_wat_up		unknown	
		mean	rsd	mean	rsd	mean	rsd	mean	rsd	mean	rsd
1 - waters	AQ_wat_dw1	0.3%	33.3	0.3%	43.3	7.5%	42.6	19.7%	30.6	72.3%	4.8
	AQ_wat_dw2	10.2%	50.6	17.6 %	10.3	9.7%	18.8	25.7%	15.4	36.9%	6.7
	AQ_wat_dw3	5.0%	9.0	5.0 %	29.1	3.8%	32.0	70.7%	1.9	15.5%	2.3
1 - biofilms	AQ_bio_dw1	8.6%	23.5	25.0%	19.1	3.9%	74.1	56.7%	6.9	5.8%	7.9
	AQ_bio_dw2	13.6%	28.0	28.4%	14.1	2.9%	46.0	48.2%	6.52	6.8%	11.8
	AQ_bio_dw3	3.4%	17.1	13.9%	18.4	5.5%	39.3	72.1%	1.85	5.2%	29.8
2 - biofilms	AQ_bio_up1	32.2%	14.5					61.3%	9.5	6.8%	23.7
	AQ_bio_up2	56.6%	12.6					36.4%	18.3	7.0%	15.7
	AQ_bio_up3	44.0%	6.6					48.1%	8.1	7.8%	10.8

* Two analyses are shown from sub-sampled datasets set at 1000 reads: (1) reads from WS, DB, IB, and aquifer waters from upstream the SIS were considered as the sources of taxa for the aquifer samples downstream the SIS; (2) reads from WS and the aquifer waters upstream the SIS were considered as the sources of taxa for the aquifer biofilms recovered upstream the SIS. SourceTracker was run 3 times using the 16S rRNA gene OTU contingency table and the default parameters. Relative contributions of the sources were averaged. Relative standard deviations (%RSD) are indicated, and used as confidence values. RSD > 100% indicates low confidence on the estimated value. WS: Watershed runoff waters; DB: Detention basin sediments; IB: Infiltration basin sediments. Sequences that could not be attributed to one of the tested sources were grouped under the term unknown.

Fig. 1. revised Table 1

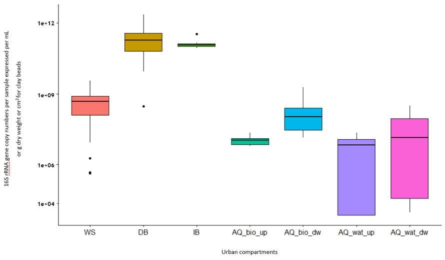


Figure S1. Boxplot representation of the 16S rRNA gene copy numbers measured by quantitative PCR per DNA extracts of runoff waters (WS), sediments from the detention basin (DB), soils from the infiltration basin (IB), aquifer waters (AQ_waters) or aquifer clay beads biofilms (AQ_bio). Values were expressed per g of dry weight soil or sediment, or per mL, or per surface for the clay bead biofilms.

Fig. 2. new Suppl. Fig S1

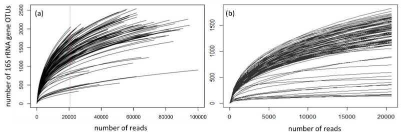


Figure S2. Rarefaction curves showing the relation between the number of V5-V6 16S rRNA (*rrs*) gene reads analyzed and OTU numbers per compartment of the Mi-plaine watershed of Chassieu (France). (a) without sub-sampling and (b) with a sub-sampling performed at 20,624 reads per sample.

Fig. 3. new Suppl. Fig S2

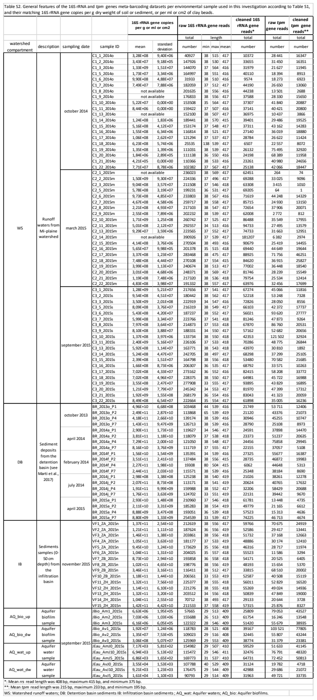


Table S2. General features of the 16S rRNA and *tpm* genes meta-barcoding datasets per environmental sample used in this investigation according to Table S1, and their matching 16S rRNA gene copies per g dry weight of soil or sediment, or per ml or cm2 of clay beads.

				16S rRNA gene copies per g or ml or cm2		raw 16S rRNA gene reads				cleaned 16S rRNA gene reads*	raw *tpm* gene reads	cleaned *tpm* gene reads**
						total	length			total	total	total
watershed compartment	description	sampling date	sample ID	mean	standard deviation	number	min	max	mean	number	number	number
WS	Runoff waters from Mi-plaine watershed	october 2014	C1_1_2014o	3,28E+08	9,40E+06	40927	38	515	417	10372	28 441	16347
			C1_2_2014o	3,43E+07	9,18E+05	147926	38	530	417	33655	31 450	16351
			C1_3_2014o	1,33E+09	1,51E+07	144070	37	564	416	31979	21 627	11945
			C1_5_2014o	1,73E+07	3,34E+06	164997	38	551	416	40110	18 394	8913
			C1_6_2014o	9,90E+08	4,88E+07	31933	38	510	416	9574	18 273	6923
			C1_7_2014o	7,49E+07	7,88E+06	182059	37	512	417	44190	26 650	13060
			C1_8_2014o	not available		201635	38	556	416	44238	13 501	2688
			C1_9_2014o	not available		176833	38	556	417	37588	28 330	15650
			C1_10_2014o	5,22E+07	0,00E+00	153508	35	564	417	37307	41 840	20887
			C1_11_2014o	8,44E+06	0,00E+00	159422	37	507	416	37141	40 421	20800
			C1_13_2014o	not available		152100	38	507	417	36975	10 437	3866
			C1_14_2014o	1,24E+08	1,83E+06	189441	38	570	415	39401	29 486	19525
			C1_15_2014o	5,16E+08	4,12E+07	153174	37	548	417	37311	43 162	14283
			C1_16_2014o	1,55E+08	6,34E+06	116814	38	521	417	27140	36 019	18880
			C1_17_2014o	1,06E+08	2,62E+07	121294	37	537	417	28784	26 622	11424
			C1_18_2014o	6,23E+08	5,74E+06	25535	138	539	417	6507	22 557	8072
			C1_19_2014o	1,35E+08	1,39E+06	111031	38	539	417	26132	75 495	32920
			C1_20_2014o	1,84E+06	2,89E+05	111138	36	550	416	24198	68 389	11958
			C1_21_2014o	4,21E+05	0,00E+00	110366	38	533	416	23261	40 980	24656
			C1_22_2014o	7,71E+07	8,78E+06	102382	37	567	417	25138	42 066	18447
		march 2015	C2_1_2015m	not available		236023	38	569	417	62451	264	74
			C2_2_2015m	1,50E+09	9,30E+07	224336	37	496	417	69288	33 025	9096
			C2_3_2015m	9,04E+08	3,57E+07	211508	37	546	418	63308	3 415	1010
			C2_5_2015m	5,78E+08	3,19E+07	199231	36	531	417	69205	64	1
			C2_6_2015m	9,73E+08	4,85E+07	233803	38	567	416	71619	44 248	14329
			C2_7_2015m	4,67E+08	4,58E+06	259717	38	558	417	85715	24 930	13150
			C2_8_2015m	2,21E+08	4,93E+07	217103	38	547	417	72654	37 906	20071
			C2_9_2015m	2,55E+08	7,89E+06	202232	38	539	417	62008	2 772	812
			C2_10_2015m	1,71E+09	1,25E+08	260742	37	525	417	86488	35 549	17955
			C2_11_2015m	5,01E+08	2,12E+07	292557	34	513	416	94733	27 495	13579
			C2_13_2015m	9,29E+07	3,59E+06	223565	37	552	417	74733	31 663	12951
			C2_14_2015m	not available		390926	37	539	417	181207	6 382	2974
			C2_15_2015m	4,14E+08	3,76E+06	270504	38	493	416	90679	25 419	14455
			C2_16_2015m	5,65E+07	9,98E+05	201378	35	515	418	69440	44 649	19644
			C2_17_2015m	3,37E+08	1,23E+07	283468	38	475	417	88925	71 756	46251
			C2_18_2015m	7,48E+08	4,44E+07	270108	37	554	415	84620	36 915	25827
			C2_19_2015m	3,99E+08	1,13E+07	240674	38	513	417	77002	36 448	18540
			C2_20_2015m	3,01E+08	4,68E+06	248371	38	569	417	81746	28 239	15549
			C2_21_2015m	1,19E+08	7,48E+06	217320	38	536	418	79754	25 534	12414
			C2_22_2015m	4,83E+08	3,98E+07	191332	38	557	417	63976	32 456	17699
		september 2015	C3_1_2015s	1,28E+09	5,21E+07	217656	37	541	417	67274	45 066	11816
			C3_2_2015s	9,54E+08	4,51E+07	180442	38	562	417	52218	53 248	7328
			C3_3_2015s	3,50E+09	2,01E+08	222919	34	547	416	72826	28 050	8556
			C3_5_2015s	8,09E+08	3,76E+07	216319	38	549	417	66103	42 372	17737
			C3_6_2015s	5,43E+08	4,20E+07	187237	38	552	417	56021	93 620	27777
			C3_7_2015s	5,99E+08	3,34E+07	223766	37	541	418	81246	47 873	9264
			C3_8_2015s	7,97E+08	3,64E+07	214873	37	553	418	67870	86 760	20531
			C3_9_2015s	8,10E+08	3,88E+07	188331	34	550	417	57162	52 682	20656
			C3_10_2015s	1,37E+08	3,97E+06	133734	38	550	418	42353	121 502	32924
			C3_11_2015s	2,40E+09	5,16E+07	226106	37	533	418	70286	48 775	26844
			C3_13_2015s	5,92E+08	1,14E+07	163771	38	543	418	43970	30 810	1892
			C3_14_2015s	5,24E+08	4,47E+07	242705	38	497	417	68298	37 299	25105
			C3_15_2015s	2,39E+08	1,51E+07	164798	38	556	418	53480	70 582	21685
			C3_16_2015s	1,66E+08	8,73E+06	206307	36	535	417	68792	33 571	10263
			C3_17_2015s	7,02E+08	4,33E+07	273162	36	552	416	82415	58 208	33772
			C3_18_2015s	7,02E+08	4,96E+07	228375	38	550	417	64981	45 722	16988
			C3_19_2015s	3,55E+08	2,47E+07	277908	33	555	417	93895	43 829	16895
			C3_20_2015s	1,21E+09	7,79E+07	245342	34	553	417	81970	47 399	17312
			C3_21_2015s	1,92E+09	1,55E+08	268179	36	554	416	83043	41 323	20059
			C3_22_2015s	8,49E+08	6,54E+07	222664	35	554	417	61898	35 005	16236
DB	Sediment deposits from the detention basin (see Marti et al., 2017)	october 2013	BR_2013o_P1	4,96E+10	6,68E+08	103468	44	539	416	21749	53 711	12406
			BR_2013o_P2	2,49E+11	2,87E+10	113868	65	539	419	21120	43376	21073
			BR_2013o_P4	4,18E+11	2,66E+10	139174	38	539	416	30946	45255	10747
			BR_2013o_P7	1,43E+11	9,47E+09	126713	38	539	416	28790	25108	8973
		april 2014	BR_2014a_P1	2,90E+11	1,73E+10	119627	34	546	417	24591	37898	14470
			BR_2014a_P2	3,81E+11	1,18E+10	118079	37	538	418	23373	51237	20635
			BR_2014a_P4	7,29E+11	2,00E+10	121050	38	548	417	24456	75858	29945
			BR_2014a_P7	8,16E+10	3,76E+09	111719	37	555	417	22155	37057	5108
		february 2014	BR_2014f_P1	1,56E+10	1,54E+09	135391	34	539	416	27325	55677	16387
			BR_2014f_P2	3,51E+11	2,41E+10	137484	38	556	415	28735	46872	19983
			BR_2014f_P4	2,27E+11	1,98E+10	19308	80	504	415	6062	44648	5313
			BR_2014f_P7	2,44E+11	2,03E+10	115571	38	539	416	25348	38184	8690
		july 2014	BR_2014j_P1	2,98E+08	1,38E+08	125238	38	540	419	21026	38261	12278
			BR_2014j_P2	2,07E+11	8,73E+08	113171	38	541	419	20624	40765	17632
			BR_2014j_P4	1,91E+11	9,99E+09	159988	38	552	417	32206	58429	20688
			BR_2014j_P7	1,76E+11	3,63E+09	124702	33	551	419	22131	39442	9670
		april 2015	BR_2015a_P1	2,93E+10	1,48E+08	210960	37	546	418	61781	13 448	4735
			BR_2015a_P2	2,11E+10	3,31E+09	185283	38	554	419	49779	21 165	6612
			BR_2015a_P4	8,88E+09	3,47E+08	193051	36	539	418	57523	15 313	4636
			BR_2015a_P7	8,80E+09	1,33E+09	254539	33	539	417	74225	46 713	4674
IB	Sediments samples (0-50 cm depth) from the infiltration basin	november 2015	VF1_ZA_2015n	1,37E+11	2,54E+10	212619	38	556	417	59766	70 675	24919
			VF2_ZA_2015n	1,21E+11	1,11E+10	187624	36	556	419	52586	29 417	13441
			VF3_ZA_2015n	1,46E+11	1,38E+10	203861	38	556	418	51732	37 168	12663
			VF4_ZA_2015n	1,05E+11	3,63E+10	181177	37	533	419	48886	30 174	12410
			VF5_ZA_2015n	9,45E+10	1,24E+10	173629	35	556	418	46316	28 717	11974
			VF6_ZA_2015n	1,04E+11	1,31E+10	204025	35	557	418	55523	11 586	3294
			VF7_ZB_2015n	8,73E+10	2,94E+10	193858	36	558	418	54171	20 525	6405
			VF8_ZB_2015n	1,02E+11	4,65E+10	198776	38	556	419	48193	15 654	5370
			VF9_ZB_2015n	3,46E+11	3,16E+11	116411	38	512	417	33815	68 510	20002
			VF10_ZB_2015n	1,18E+11	1,44E+10	206561	33	553	419	52587	40 508	15119
			VF11_ZH_2015n	1,32E+11	7,56E+10	225377	38	555	418	56011	52 829	16520
			VF12_ZH_2015n	1,14E+11	6,10E+10	221276	38	555	418	55269	49 024	14936
			VF13_ZH_2015n	1,32E+11	3,20E+10	203512	34	556	418	50839	47 849	19000
			VF14_ZH_2015n	1,04E+11	2,55E+10	70712	38	493	417	29133	20 644	3728
			VF15_ZH_2015n	1,42E+11	4,42E+10	211533	37	558	419	57315	25 876	8327
AQ_bio_up	Aquifer biofilm sample	september 2015	JBio_Am1_2015s	6,63E+06	1,95E+05	57665	29	513	409	25809	79 053	43527
			JBio_Am2_2015s	7,03E+06	1,03E+06	155688	26	513	409	61754	16 246	13548
			JBio_Am3_2015s	6,16E+06	6,05E+05	122322	28	546	409	51420	55 679	38935
AQ_bio_dw	Aquifer biofilm sample		JBio_Av1_2015s	1,92E+07	1,24E+06	118783	28	521	408	39167	103 521	77805
			JBio_Av2_2015s	1,35E+07	7,53E+05	109023	29	516	408	32445	55 807	43244
			JBio_Av3_2015s	3,06E+08	5,07E+07	125969	29	553	409	38774	31 379	23381
AQ_wat_up	Aquifer water sample		JEau_AmJ0_2015s	7,17E+03	5,81E+02	154982	29	507	410	59529	51 633	41145
			JEau_AmJ10_2015s	6,94E+03	5,13E+02	115472	29	546	411	32476	76 791	48320
			JEau_AmJ5_2015s	6,86E+03	1,65E+02	169773	31	537	410	64731	72 452	50813
AQ_wat_dw	Aquifer water sample		JEau_AvJ0_2015s	3,55E+04	1,53E+03	107788	40	529	409	31124	19 782	4718
			JEau_AvJ10_2015s	9,21E+03	1,23E+03	176475	29	544	409	42988	29 686	21072
			JEau_AvJ5_2015s	1,61E+04	1,10E+03	90793	29	514	409	31963	49 721	33735

*: Mean *rrs* read length was 408 bp, maximum 415 bp, and minimum 375 bp.

**: Mean *tpm* read length was 215 bp, maximum 233 bp, and minimum 195 bp.

WS: Watershed runoff waters; DB: Detention basin sediments IB: Infiltration basin sediments ; AQ_wat: Aquifer waters; AQ_bio: Aquifer biofilms.

Fig. 4. new Suppl. Table S2

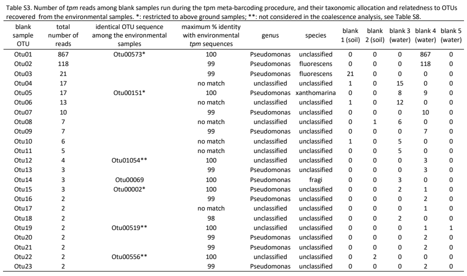


Table S3. Number of *tpm* reads among blank samples run during the tpm meta-barcoding procedure, and their taxonomic allocation and relatedness to OTUs recovered from the environmental samples. *: restricted to above ground samples; **: not considered in the coalescence analysis, see Table S8.

blank sample OTU	total number of reads	identical OTU sequence among the environmental samples	maximum % identity with environmental *tpm* sequences	genus	species	blank 1 (soil)	blank 2 (soil)	blank 3 (water)	blank 4 (water)	blank 5 (water)
Otu01	867	Otu00573*	100	Pseudomonas	unclassified	0	0	0	867	0
Otu02	118		99	Pseudomonas	fluorescens	0	0	0	118	0
Otu03	21		99	Pseudomonas	fluorescens	21	0	0	0	0
Otu04	17		no match	unclassified	unclassified	1	0	15	0	0
Otu05	17	Otu00151*	100	Pseudomonas	xanthomarina	0	0	8	9	0
Otu06	13		no match	unclassified	unclassified	1	0	12	0	0
Otu07	10		99	Pseudomonas	unclassified	0	0	0	10	0
Otu08	7		no match	unclassified	unclassified	0	1	6	0	0
Otu09	7		99	Pseudomonas	unclassified	0	0	0	7	0
Otu10	6		no match	unclassified	unclassified	1	0	5	0	0
Otu11	5		no match	unclassified	unclassified	0	0	5	0	0
Otu12	4	Otu01054**	100	unclassified	unclassified	0	0	0	3	0
Otu13	3		99	Pseudomonas	unclassified	0	0	0	3	0
Otu14	3	Otu00069	100	Pseudomonas	fragi	0	0	3	0	0
Otu15	3	Otu00002*	100	Pseudomonas	unclassified	0	0	2	1	0
Otu16	2		99	Pseudomonas	unclassified	0	0	0	2	0
Otu17	2		no match	unclassified	unclassified	0	0	0	1	0
Otu18	2		98	unclassified	unclassified	0	0	2	0	0
Otu19	2	Otu00519**	100	unclassified	unclassified	0	0	0	1	1
Otu20	2		99	Pseudomonas	unclassified	0	0	0	2	0
Otu21	2		99	Pseudomonas	unclassified	0	0	0	2	0
Otu22	2	Otu00556**	100	unclassified	unclassified	0	2	0	0	0
Otu23	2		99	Pseudomonas	unclassified	0	0	0	2	0

Fig. 5. new Suppl. Table S3