https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

Coalescence of bacterial groups originating from urban runoffs and artificial infiltration systems among aquifer microbiomes

Yannick Colin[1‡], Rayan Bouchali[1], Laurence Marjolet[1], Romain Marti[1], Florian Vautrin[1,2], Jérémy Voisin[1,2], Emilie Bourgeois[1], Veronica Rodriguez-Nava[1], Didier Blaha[1], Thierry Winiarski[2], Florian Mermillod-Blondin[2] and Benoit Cournoyer[1]

[1]University of Lyon, UMR Ecologie Microbienne Lyon (LEM), Research Team "Bacterial Opportunistic Pathogens and Environment", University Lyon 1, CNRS 5557, INRA 1418, VetAgro Sup, 69680 Marcy L'Etoile, France.

[2]University of Lyon, UMR Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés (LEHNA), Université Lyon 1, CNRS 5023, ENTPE, 69622 Villeurbanne, France.

‡ Present address : Normandie Université, UNIROUEN, UNICAEN, UMR CNRS 6143, Morphodynamique Continentale et Côtière, 76000 Rouen, France

Running title: Y. Colin et al.: Urban runoff bacteria among recharged aquifer

Keywords: Stormwater infiltration; Microbial contamination; Aquifer; Source-tracking; Biofilms

Correspondence : yannick.colin@univ-rouen.fr / benoit.cournoyer@vetagro-sup.fr

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

Abstract. The invasion of aquifer microbial communities by aboveground micro-organisms, a phenomenon known as community coalescence, is likely to be exacerbated in groundwaters fed by stormwater infiltration systems (SIS). Here, the incidence of this increased connectivity with upslope soils and impermeabilized surfaces was assessed through a meta-analysis of 16S rRNA gene libraries. Specifically, free-living and attached aquifer bacteria (i.e., water and biofilm samples) were characterized upstream and downstream a SIS, and compared with bacterial communities from watershed runoffs, detention and infiltration basins. A significant bacterial transfer was observed, with aquifer bacterial biofilms being largely made up of taxa occurring in aboveground sediments and urban runoffs (44 to 67% of the total reads). This coalesced biofilm community was rich in hydrocarbon degraders such as *Sphingobium* and *Nocardia.* The bacterial community of the downstream SIS aquifer waters showed similar coalescence with aboveground taxa (26.7-66.5%) but a higher number of taxa involved in the N- and S-cycles was observed. A DNA marker named *tpm* enabled a tracking of bacterial species from 24 genera including the *Pseudomonas*, *Aeromonas* and *Xanthomonas* among these communities. Reads related to the *Pseudomonas* were allocated to 50 species, of which 16 were found in the aquifer samples. *P. umsongensis* and *P. chengduensis* were inferred to be in higher proportions among the *tpm*-harboring bacteria, respectively, of the aquifer biofilms, and waters. Several of these aquifer species were found involved in denitrification but also hydrocarbon degradation (*P. aeruginosa*, *P. putida*, and *P. fluorescens*). Reads related to *Aeromonas* were allocated to 11 species but only those from *A. caviae* were recovered in the aquifer samples. DNA imprints allocated to the *X. axonopodis* phytopathogen were recorded in higher proportions among the *tpm*-harboring bacteria of the aquifer waters than aboveground samples. A coalescence of microbial communities from an urban watershed with those of an aquifer was thus observed, and recent aquifer biofilms were found dominated by runoff opportunistic taxa able to use urban C-sources from aboveground compartments.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions

1 Introduction

Urbanization exerts multiple pressures on natural habitats and particularly on aquatic environments (Konrad and Booth, 2005; McGrane, 2016; Mejía and Moglen, 2009). The densification of urban areas, combined with the conversion of agricultural and natural lands into urban land-use, led to the replacement of vegetation and open fields by impervious urban structures (*i.e.* roads, rooftops, side-walks and parking lots) (Barnes et al., 2001). These impervious structures reduce the infiltration capacity of soils. They also exacerbate the speed and volume of stormwater runoff that favor soil erosion, flooding events, and affect adversely natural groundwater recharge processes (Booth, 1991; Shuster et al., 2005). Due to these consequences, stormwater infiltration systems (SIS) or managed aquifer recharged systems (MAR) have been developed during the last decades, and are gaining more interest in developed countries (Pitt et al., 1999). Such practices reduce direct stormwater discharges to surface waters and alleviate water shortages (Barba et al., 2019; Dillon et al., 2008; Marsalek and Chocat, 2002). However, stormwater represents a major source of nonpoint pollution, and its infiltration into the ground may have adverse ecological and sanitary impacts (Chong et al., 2013; Pitt et al., 1999; Vezzaro and Mikkelsen, 2012).

The vadose zone of a SIS can act as a natural filter towards pollutants (hydrocarbons and heavy metals), and micro-organisms washed-off by runoffs (e. g. Murphy and Ginn, 2000; Tedoldi et al., 2016). Nevertheless, the effectiveness of SIS in preventing the migration of contaminants towards aquifers is not always optimal (Borchardt *et al.*, 2007; Lapworth *et al.*, 2012; Arnaud *et al.*, 2015; Voisin et al., 2018). The filtering properties of SIS are influenced by various abiotic factors such as the nature of the media (rocks, sand and other soil elements), the physical properties (e. g. granulometry, hydrophobicity index, organization), and the runoff water flow velocity (Lassabatere et al., 2006; Winiarski et al., 2013). These constraints will impact water transit time from the top layers to the aquifer, but also the biology of these systems including the plant cover and root systems, worms and microbiota (Barba et al., 2019; Bedell et al., 2013; Crites, 1985; Pigneret et al., 2016). The thickness of the vadose zone was found to be one of the key parameters explaining chemical transfers such as phosphate and organic-carbon sources (Voisin et al., 2018). The situation is much less clear regarding the microbiological communities that flow through these systems (e. g. Barba et al., 2019; Voisin et al., 2018).

According to the microbial community coalescence concept conceptualized by Tikhonov, (2016) and adapted to riverine networks by Mansour et al. (2018), 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. Indeed, during rain events, microbial communities will be re-suspended through runoff-driven surface erosion processes, favoring detachment of micro-organisms from plant litter, wastes, soil, and other particles. These re-suspended communities will merge and generate novel assemblages. The resulting community will initially match the relative contributions of the various sub-watersheds to the overall microbiological complexity of the assemblages. The prevailing ecological constraints among the downward systems will then gradually drive this coalescence towards the most fit community structures. These resulting communities might be highly efficient at degrading urban pollutants trapped among a SIS but could also disturb the ecological equilibria of the connected and more sensitive systems like those of deep aquifers.

Here, the study explored the impact of a SIS, with a thick vadose zone (> 10 m), on the coalescence of urban runoff microbial communities in a connected aquifer. The tested hypotheses were that (1) highly specialized taxa (often termed K-strategists e. g. Vadstein et al., 2018) of an aquifer should outcompete the intrusive community members of aboveground taxa but (2) nutrient inputs from runoffs and pollutants could also drive changes among

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions

these communities and favour environmental opportunists (often termed r-strategists e. g. Vadstein et al., 2018). The targeted SIS is part of a long-term experimental site (http://www.graie.org/portail/dispositifsderecherche/othu/) for which physico-chemical and biological monitorings have been implemented. It is connected to the eastern aquifer of Lyon (France) which is fed by three low hydraulic conductivity corridors (10^{-5}–10^{-8} m s^{-1}) separated by moraine hills (Foulquier et al., 2010). It has an average vadose zone thickness of 15 m, and the delay between a rainfall event and the impact on the aquifer waters was estimated at 86±11h (Voisin et al., 2018). A large DNA meta-barcoding dataset was built for this site, in order to investigate bacterial community coalescence from top compartments among the connected aquifer waters but also biofilm communities developing on inert surfaces. This investigation was built on the hypothesis that a less significant microbial community coalescence was likely to be observed among aquifer water samples than biofilms. This is supported by previous reports which suggested the occurrence of transient free-living bacteria among aquifers acting as a traveling seed bank (Griebler et al., 2014). More precisely, water grab samples were found to give access to snapshots of the diversity found among an aquifer (Voisin et al., 2018) while aquifer biofilms developing on artificial surfaces (clay beads) have been shown to be more integrative and informative of the groundwater microbiological quality (Mermillod-Blondin et al., 2019). Clay bead biofilms were found to capture the most abundant aquifer taxa, and taxa that could not be detected from grab samples. A field based investigation was thus performed to further explore the relative contributions of a set of sources such as runoffs and urban soils on the observed biofilm assemblages recovered from an aquifer. A Bayesian methodology, named SourceTracker (Knights et al., 2011), was used to investigate community coalescence from 16S rRNA gene –based DNA meta-barcoding datasets. To go deeper into these inferences, complementary datasets were built from an additional DNA marker named *tpm* (encoding EC:2.1.1.67 which catalyzes the methylation of thiopurine drugs) (Favre-Bonté et al., 2005). This genetic marker enables finer taxonomic allocations down to the species level, and allowed gaining further insights on the coalescence of a set of waterborne bacterial species and sub-species, including plant and human pathogens, with the aquifer microbial community.

2 Material and Methods

2.1 Experimental site

The Chassieu urban catchment is located in the suburbs of Lyon (France). It has a surface of 185 ha and hosts mainly industrial and commercial activities (*i.e.* wholesaling, recovery and waste management, metal surface treatment, car wash and repair services). The imperviousness coefficient of the catchment area is about 75 %. Stormwater and dry weather flows from industrial activities are drained by a network separated from the sewer. This network transfers waters into the Django-R SIS, which is part of the OTHU long term experimental observatory dedicated to urban waters (http://www.graie.org/othu/). This SIS contains an open and dry detention basin (DB) (32,000 m^3), built on a concrete slab, with edges impermeabilized by a thick plastic lining. This DB allows a settling of coarse and medium size particles, resulting in sedimentary deposits which favor development of a plant cover. The DB water content is delivered within 24h into an infiltration basin (IB) (61,000 m^3), which favors the recharge of the connected aquifer (AQ). This infiltration basin had a vadose zone of about 11 m during the experiments, and its geology, hydrology, ecology and pollution levels have been deeply investigated e. g. Barraud et al. (2002); Le Coustumer and Barraud (2007).

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions

The Chassieu watershed, the Django-R SIS, and the Lyon aquifer were considered for this study (Figure 1, Table S1). Watershed runoff waters (hereafter WS) have been collected from sampling points spread over the catchment (21 sub-watersheds over three sampling periods, n=64 samples). Sediments from the detention basin (hereafter DB) have been recovered from 50 cm^2 area covering the full sediment column down to the concrete slab of the DB (n=20 samples). These sediments (or urban soils) often had an herbaceous plant cover, and were sampled in four areas defined according to the hydrological forces prevailing in the basin (e. g. Marti et al., 2017; Sébastian et al., 2014). Infiltration basin soil samples (hereafter IB) had been collected from 3 main zones (the area receiving the inflow waters, the bottom area of the basin, and an upper zone of the basin exposed to inflow waters only during strong rain events) (n=5 samples per zone), at a 0-10 cm depth covering a surface of 50 cm^2. The aquifer samples have been recovered from piezometers located upstream (up, in a zone of the aquifer not influenced by water recharge) and downstream (dw, in a zone of the aquifer influenced by water recharge) of the SIS of the Django-R site at a depth of 2 m below the water table (e. g. Barraud et al., 2002; Voisin et al., 2018) (Fig. 1). Groundwater samplings (n=6; named AQ_wat) had been performed with an immerged pump, used at a pumping rate of 6–8 L/min (PP36 inox, SDEC, Reignac-sur-Indre, France), and previously cleaned with 70% ethanol. The first 50 L were used to rinse the sampling equipment and discarded. The following 6 L were used for the microbiological analyses. The biofilm samples (AQ_bio) from the aquifer were recovered using clay beads incubated in the aquifer over 10 days using the same piezometers as those used for the aquifer water samplings (n=6 samples). Clay beads were used as physical matrix to sample groundwater biofilms according to Voisin et al. (2016).

2.2 PCR products DNA sequencings

Sequencing of the V5-V6 16S rRNA gene (*rrs*) PCR products were performed by the MrDNA company (Shallowater, TX, USA) with Illumina MiSeq technology and using the primers set 799F-1193R. The *tpm* DNA libraries were generated using the following mix of degenerated primers: ILMN-PTCF2 (GTGCCGYTRTGYGGCAAGA), ILMN-PTCR2 (ATCAKYGCGGCGCGGTCRTA), ILMN-PTCF2m (GTGCCCYTRTGYGGCAAGT), and ILMN-PTCR2m (ATGAGBGCTGCCCTGTCRTA) as suggested by Favre-Bonté et al. (2005). PCR reactions were performed under the following conditions: (1) a hot start at 94°C for 3 min, (2) 35 cycles consisting of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, and (3) a final extension of 5 min at 72°C. The PCR products were sequenced by Biofidal (Vaulx-en-Velin, France) using the Illumina MiSeq technology. The 16S rRNA and *tpm* gene sequences are available at the European Nucleotide Archive (https://www.ebi.ac.uk/ena).

2.3 Bioinformatic analyses

All MiSeq sequences were processed using Mothur (v.1.40.4) (Schloss et al., 2009) following the standard operating procedure developed by Kozich *et al.*(2013). For the 16S rRNA (*rrs*) gene sequences, reads were filtered for length (>300bp), quality score (mean, ≥25), number of ambiguous bases (=0), and length of homopolymer runs (<8) using the trim.seqs script in Mothur, and singletons were discarded. The 16S rRNA gene sequences passing these quality criteria were aligned to the SILVA reference alignment template (release 128) and an 80% bootstrap P-value threshold was used for taxonomic assignments. Chimeric sequences were identified using the chimera.uchime command and removed. To avoid any biases related to sequencing depth, a subsampling-based normalization was applied (20,624 sequences per sample) and the normalized dataset was used for all downstream

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

analyses. Operational Taxonomic Units (OTUs) were defined using a 97% identity cut-off. FAPROTAX (Louca et al., 2016) functional inferences were performed on the MACADAM Explore web site (http://macadam.toulouse.inra.fr/) according to Le Boulch et al. (2019). For the *tpm* gene sequences, chimeric sequences, primers, barcodes were removed, and the dataset was limited to sequences of a minimum length of 210 bp (average length=215 bp). The number of sequences was then normalized between the samples (4,636 sequences per sample) and Operational Taxonomic Units (OTUs) were defined with a 100% identity cut-off. The "BD_TPM_Mar18_v1.unique_770seq" database (http://www.graie.org/othu/donnees) was used to classify the sequences using the "Wang" text-based Bayesian classifier (Wang et al., 2007) and a P-bootstrap value above 80%. Local Blast analyses were performed on the "BD_TPM_Mar18_v1.unique_770seq" database using the NCBI BLASTX program in order to check the quality of the taxonomic affiliations.

2.4 Statistical analyses

All statistical analyses were carried out in R (v.3.5.1). For the 16S rRNA gene sequences, alpha-diversity estimates were computed using the function "rarefy" from the 'Vegan' package (Oksanen et al., 2015). Richness (S_{obs}) was computed as the number of observed OTUs in each sample. The diversity within each individual sample was estimated using the non-parametric Shannon index. To estimate whether the origin of the samples influenced the alpha-diversity, an ANOVA with Tukey's post-hoc tests was performed for each index. Shared and unique OTUs were depicted in Venn-diagrams with the "limma" package (Ritchie et al., 2015). Concerning the beta-diversity between samples, a neighbor-joining tree was constructed with a maximum-likelihood approximation method using FastTree (Price et al., 2009). Weighted UniFrac distances were calculated for all pairwise OTU patterns according to Lozupone et al. (2011). Based on the distance matrices, Principal Coordinates Analysis (PCoA) (Anderson and Willis, 2003) were used to determine changes in the bacterial community structure from the watershed down to the aquifer. Permutation tests of distances (PERMANOVA) (Anderson, 2001) were performed using the "vegan" package (Oksanen et al., 2015), in order to establish the significance of the observed groupings.

2.5 Bacterial community coalescence analyses

The SourceTracker computer package (Knights et al., 2011) was used to investigate community coalescence. SourceTracker is a Bayesian approach built to estimate the most probable proportion of user-defined "source" OTU in a given "sink" community. In the present analysis, various scenarios of community coalescence were investigated such as the coalescence of bacterial taxa from the watershed runoff waters and sediments from the detention and infiltration basins, with those of the downstream SIS aquifer water samples or of recent biofilms developing on clay beads incubated in the aquifer. SourceTracker was run with the default parameters (rarefaction depth 1000, burn-in 100, restart 10) to identify sources explaining the OTU patterns observed among the aquifer samples (waters and clay bead biofilms, n=12). Alpha values were tuned using cross-validation (alpha 1= 0.001 and alpha 2= 1). The relative standard deviation (RSD) based on three runs was used as a gauge to evaluate confidence on the computed values (Henry et al., 2016; McCarthy et al., 2017).

3. Results

3.1 16S rRNA V5-V6 gene sequences distribution biases and profilings

The analysis of the 16S rRNA V5-V6 gene libraries yielded 2,124,272 high-quality sequences distributed across 103 samples. Subsampling-based normalization was applied (20,624 reads per sample) and sequences were

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

distributed into 10,231 16S rRNA gene OTUs at a 97 % threshold. The rarefaction curves indicated that the sequencing depth was sufficient to cover bacterial diversity (Figure S1). At all sampling sites, bacterial communities were dominated by Proteobacteria, Bacteroidetes and Actinobacteria (WS=95.1% of total reads, DB=84.3%; IB=71.4%; AQ_bio=98.8% and AQ_wat=58.6%), but 10 other phyla with relative abundances superior to 0.5% were also detected (Figure 2A and Table S2). Alpha-diversity estimates showed that aquifer samples harbored a microbiome with a significantly lower richness (AQ_bio: S_{obs}=278 OTUs ± 106 and AQ_wat: S_{obs}=490 OTUs ± 333) and a less diverse bacterial community (AQ_bio: H'=2.9 ± 0.3 and AQ_wat: H'=4.3 ± 0.7) than the ones of the upper compartments ($S_{obs\text{-}WS}$=1,288 OTUs ± 232; $S_{obs\text{-}DB}$=1,566 OTUs ± 245, $S_{obs\text{-}IB}$=1,503 OTUs ± 177 and H'_{WS}=5.0 ± 0.5; H'_{DB}=5.4 ± 0.5, H'_{IB}=5.7 ± 0.4) (ANOVA, $p<0.001$) (Figure 2B and Table S3). Among the surface samples, a greater diversity was observed among the soil samples from the infiltration basin than from samples of watershed runoff waters and sediments of detention basin (ANOVA, $p<0.05$). In the aquifer, water grab samples were more diverse and showed higher 16S rRNA gene OTU contents than biofilms recovered from clay beads incubated for a 10-day period (ANOVA, $p<0.05$) (Figure 2B and Table S3).

The structure of bacterial communities inferred from V5-V6 16S rRNA gene sequences changed markedly along the watershed down the aquifer. A PCoA ordination of the OTU profiles based on weighted Unifrac distances revealed that samples clustered according to their compartment of origin (*i.e.* WS, DB, IB, AQ_bio and AQ_wat) (Figure 3). These changes in community structures between compartments were supported by PERMANOVA statistical tests ($F=20.7$, $P<0.001$). Bacterial communities per compartment were found to be made of core and flexible (defined as not conserved between all sampling periods) bacterial taxa. Within a same compartment, similarities between bacterial community profiles ranged from 64.9% (AQ_wat) to 82.0% (IB), while similarities across compartments ranged from 47.8% (DB vs AQ_bio) to 65.9% (DB vs IB) (Figure S2). Bacterial community profiles of the aquifer waters were found closer to the ones of the detention basin deposits (57.5%) and soils of the infiltration basin (61.4%) than those of the aquifer biofilms (47.8 and 49.2%, respectively). However, more than 89% of the 16S rRNA gene OTUs (n=8,284) identified above the aquifer (WS, DB and IB) were not detected in groundwater samples (AQ_bio and AQ_wat) (Figure S3). This large group of OTUs was made of minor taxa which accounted for 37.1%, 44.3% and 47.3% of the total reads recovered from the WS, DB and IB samples, respectively.

3.2 Coalescence of surface and aquifer bacterial communities

A SourceTracker analysis was performed to estimate the coalescence of V5-V6 16S rRNA gene OTUs from the watershed and SIS down into the aquifer waters and biofilm bacterial communities. This analysis indicated significant coalescence between the bacterial communities of the runoffs, the soils of the SIS, and the aquifer samples. The aquifer water microbial community upstream the SIS was found to explain between 0.02%-12.6% of the downstream water microbial community (Table 2), while OTUs from the runoff waters were found to explain 23 to 59% of the observed patterns (Table 2). OTUs from the infiltration basin explained 0.8-3.8% of the observed diversity among the SIS impacted aquifer community, and, those of the detention basin, between 0.02 and 9% of the community. The aquifer biofilm bacterial communities were also found to be assemblages of communities from the surface environments. The origin of more than 90% of the SIS impacted aquifer biofilms could be explained. Main sources were the runoff waters (33%), the sediments of the detention basin (20%), and the upstream aquifer waters (39%) (Table 2). Soils from the infiltration basin did not appear to have contributed much to taxa recovered from these aquifer biofilms (<4%) (Table 2). Content of the aquifer biofilms recovered upstream the SIS showed similar origins with a high proportion related to those observed among the runoff waters

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

(64%) and the aquifer waters (30%). This was not considered surprising because runoff infiltration can occur in several sites upstream of the SIS (even though no direct relation with other SIS were made).

3.3. 16S rRNA gene inferred bacterial taxa undergoing coalescence in the aquifer

In order to identify the bacterial taxa involved in the coalescence process, OTUs of the 16S rRNA gene dataset were allocated to taxonomic groups using the SILVA reference alignment template. These taxonomic allocations indicated that (1) 14 genera were only recorded in the aquifer samples, (2) 421 genera were only recorded in the upper surface compartments of the watershed, and (3) 219 were recorded among aboveground and aquifer compartments (Table S4). The following bacterial genera were exclusively associated to the aquifer bacterial communities: *Turicella, Fritschea, Metachlamydia, Macrococcus, Anaerococcus, Finegoldia, Abiotrophia, Dialister, Leptospirillum, Omnitrophus, Campylobacter, Sulfurimonas, Haemophilus, Nitratireductor*. These bacterial genera were recovered from all water samples while 5 were also detected in biofilms (Table S4). These genera were associated to 926 16S rRNA gene OTUs that accounted for 48.0% and 1.8% of total reads recovered from aquifer waters and aquifer biofilms developing on clay beads, respectively. FAPROTAX functional inferences indicated some of these genera to be host-associated such as *Fritschea, Metachlamydia, Finegoldia, Campylobacter* and *Haemophilus*, with the latter two being well-known to contain potential pathogens. *Campylobacter* and *Sulfurimonas* cells have also been associated with nitrogen and sulfur respiration processes, and *Leptospirillum* with nitrification.

Regarding the bacterial taxa of the aboveground communities matching those of the aquifer samples, a total of 1,021 16S rRNA gene OTUs was found to be shared between these compartments (Table 1 and Figure S3). These OTUs consisted of abundant taxa as they accounted for 9.7-39.4% of the total reads for the samples recovered from the surface compartments, and for 33.6-83.4% and 95.0-99.4% of the total reads of the water and biofilm aquifer samples, respectively. The β- and γ-proteobacteria dominated this group. It is noteworthy that aquifer samples collected upstream of the SIS shared less OTUs with the surface compartments (125 OTUs ± 41) than samples under the influence of the infiltration system (332 OTUs ± 85) (Table 1). The shared OTUs between aquifer samples and the upper compartments represented a higher fraction of bacterial communities in samples recovered downstream the SIS (81.3% ± 22.8 of total reads) compared to those collected upstream (68.9% ± 30.9 of total reads) (Table 1). Reads from *Pseudomonas, Nitrospira, Neisseria, Streptococcus, Flavobacterium* were the most abundant (>1%) of the shared OTUs recovered in the aquifer water samples, while those allocated to *Pseudomonas, Duganella, Massilia, Nocardia, Flavobacterium, Aquabacterium, Novosphingobium, Sphingobium, Perlucidibaca, Meganema* were the most abundant (>1%) among the aquifer biofilms (Table S4). Most of these aquifer water taxa (except *Streptococcus*) were found involved in denitrification or nitrification as inferred from FAPROTAX. The biofilm taxa were more often associated with hydrocarbon degradation (*Novosphingobium, Sphingobium,* and *Nocardia)* by FAPROTAX. Several of these biofilm bacterial genera were also found to be likely containing potential human pathogens (*Duganella, Massilia, Nocardia*, and *Aquabacterium*) by FAPROTAX (and published clinical records). A set of 14 potentially hazardous bacterial genera was selected from Table S4, and used to illustrate the coalescence of bacterial taxa among the aquifer samples on Fig. 4. The 16S rRNA gene reads from *Flavobacterium* prevailed in all upper compartments (WS=6.9% of total reads, DB=13.4% and IB=8.3%) and were in significant numbers among the connected aquifer (AQ_wat = 1.1% and AQ_bio = 3.1%) (Figure 4B and Table S4C). *Pseudomonas* 16S rRNA gene reads were in relatively lower numbers

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions

in the upper compartments (WS = 0.4% of total reads, DB = 0.4% and IB < 0.05%) but increased in the aquifer samples (AQ_wat = 8.4% and AQ_bio = 35.5%) (Figure 4B and Table S4). Similar trends were observed for *Nocardia* and *Neisseria* OTUs (Figure 4B). It is to be noted that OTUs exclusively recovered from the upper compartments were mainly part of the *Gemmatimonas* (0.2-1.6% of total reads), *Geodermatophilus* (0.1-1.8%) and *Roseomonas* (0.1-1.0%) (Table S4).

3.4 Coalescence of *Pseudomonas* and other *tpm*-harboring bacterial species

DNA sequences from *tpm* PCR products generated according to Favre-Bonté et al. (2005) allowed a deeper analysis of the bacterial species undergoing a coalescence with the aquifer microbiome. A total of 19,129 *tpm* OTUs was identified among the samples (from datasets re-sampled to reach 4,636 reads per sample). As expected, these *tpm* reads were mainly assigned to the *Proteobacteria* (WS = 91.7% of total reads, DB = 86.5% ; IB = 76.3% ; AQ_wat = 82.9% and AQ_wat = 85.0%), but some reads could also be attributed to the *Bacteroidetes*, *Nitrospirae* and *Cyanobacteria* (Table S5). These taxonomic allocations allowed the identification of 24 bacterial genera and 91 species whose distributions are summarized in Tables S6 and S7. The *tpm* sequences were mainly allocated to the *Pseudomonas* (WS = 35.5% of total reads, DB = 27.2% ; IB = 7.3% ; AQ_wat = 51.4% and AQ_bio = 47.6%), *Aeromonas* (WS = 0.8% of total reads, DB = 2.7%; IB <0.05%; AQ_wat = 0.07% and AQ_bio < 0.05%), *Xanthomonas* (WS = 4.4% of total reads, DB <0.05%; IB =1.3%; AQ_wat = 8.3% and AQ_bio < 0.05%), *Herbaspirillum* (WS = 10.74% of total reads) and *Nitrosomonas* (DB = 4.4% of total reads; IB = 0.23%) (Table S6). Reads related to *Pseudomonas* were allocated to 50 species, including pollutant-degraders (*P. pseudoalcaligenes, P. aeruginosa, P. fragi, P. alcaligenes, P. putida* and *P. fluorescens*), phytopathogens (*P. syringae, P. viridiflava, P. stutzeri,* and *P. marginalis*) and human opportunistic pathogens (*P. aeruginosa, P. putida, P. stutzeri, P. mendocina, S. acidaminiphila*) (Table S7). Reads related to the *Aeromonas* were attributed to 11 species but only reads allocated to *A. caviae* could be recovered from the aquifer and aboveground compartments (Table S7). Reads related to the *Xanthomonas* were allocated to 9 species but only those allocated to the *X. axonopodis/campestris* complex and *X. cannabis* species were recovered from the aquifer and upper compartments (Table S7). Regarding the *Pseudomonas*, *tpm* reads allocated to *P. jessenii*, *P. chlororaphis*, and *P. resinovorans* were restricted to the aquifer samples. Reads allocated to *P. aeruginosa*, *P. anguilliseptica*, *P. chengduensis*, *P. extremaustralis*, *P. fluorescens*, *P. fragi*, *P. gessardii*, *P. koreensis*, *P. pseudoalcaligenes*, *P. putida*, *P. stutzeri*, *P. umsongensis*, and *P. viridiflava*, were recovered from the aquifer and upper compartments (Table S7). FAPROTAX analysis indicated that a significant number of the species detected in the aquifer can be involved in denitrification (*P. aeruginosa, P. fluorescens, P. putida, P. stutzeri, S. acidaminiphila, X. autotrophicus, P. chlororaphis*) or nitrification (*Nitrospira defluvii, Nitrosomonas oligotropha*) but also in hydrocarbon degradation (*P. aeruginosa, P. fluorescens, P. putida*). Some were also suggested by FAPROTAX to be human pathogens or invertebrate parasites (e. g. *P. chlororaphis*). These functional inferences were in line with those obtained with the 16S rRNA gene dataset.

The *tpm* OTUs (representative of infra-specific complexes) shared between the upper compartments and the aquifer (Table 3 and Table S8) were allocated to 14 species and 5 genera (Table 3). Four of these OTUs led to higher relative numbers of reads in the aquifer samples, in the following decreasing order: *P. umsongensis* (Otu00005) > *P. chengduensis* (Otu00024) > *X. axonopodis/campestris* (Otu00019 & Otu00878) > *P. stutzeri* (Otu00119 & Otu10066). These co-occurrences of OTUs between aboveground and aquifer samples support the

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

hypothesis of significant coalescence between these bacterial communities. The other OTUs showed higher number of reads among the top compartments. The OTU allocated to *X. cannabis* showed the highest relative number of reads of this group among runoff waters. The distribution pattern of this OTU suggested a relative decline while moving down the aquifer. The *P. aeruginosa* Otu00066 was recovered in the runoff waters, and biofilms developing on clay beads incubated in the aquifer.

4. Discussion

Urban microbial communities mobilized by runoffs will merge, after migration through a vadose zone, with aquifer communities. This coalescence will lead to novel microbial assemblages through selective species sorting. SIS are significantly contributing at the recharge of aquifers by runoff waters. They can receive large volumes of runoff waters that will contain significant amount of chemical pollutants but also microbial assemblages representative of the connected urban biomes. Here, the incidence of a SIS on the microbial assemblages observed among an aquifer was investigated. The structure and fate of such assemblages remain poorly investigated but must be better understood to assess the environmental and health risks related to stormwater infiltration practices (Abu-Ashour et al., 1994; Powelson et al., 1993; Redman et al., 2001). The tested hypotheses were that (1) highly specialized K-strategists of an aquifer should outcompete the intrusive community members of aboveground systems but (2) nutrient inputs from runoffs and pollutants could also drive changes among these communities and favour some environmental opportunists or r-strategists which are growing fast when significant energy sources are available. The genetic structure of coalesced aquifer communities should be representative of these trade-offs. Here, DNA meta-barcoding datasets were thus used to estimate the proportion of communities from sediments of a detention basin, soils of an infiltration basin, and runoff waters from a watershed that have merged with communities of an aquifer. Furthermore, taxonomic and functional inferences were performed in order to assess changes among the aquifer bacterial functional groups. A genetic marker named *tpm* was used to track species and particular sequence types of the *Pseudomonas*, *Aeromonas*, *Xanthomonas*, and a few other genera, from runoffs down into the SIS impacted aquifer. These trackings demonstrated the successful coalescence of some species like *P. umsongensis*, *P. chengduensis*, *X. axonopodis/campestris* and *P. stutzeri*.

Estimation of alpha-diversity indices from the 16S rRNA bacterial community profilings indicated that groundwater samples (*i.e.* waters and biofilms) harbored a less diverse microbiome than those of the top compartments (*i.e.* WS, DB, IB). A 2 to 5-fold reduction in bacterial richness was observed from the surface compartments down into the aquifer. This result suggests that a large proportion of bacterial taxa carried by stormwater runoffs or thriving in the detention/infiltration basins were retained and/or eliminated by the vadose zone filtration process. In fact, more than 89% of the 16S rRNA gene OTUs in the top compartments were not detected in the underground samples. This is in agreement with previous works which have shown that immobilization of micro-organisms through porous media are high in the top soil layers, and triggered by mechanical straining, sedimentation and adsorption (Kristian Stevik et al., 2004; Krone et al., 1958). Moreover, particles that accumulate as water passes through the soil can form a mat that can also enhance this straining process (Krone et al., 1958). Nevertheless, despite this filtering effect, infiltration has induced significant changes in the diversity of groundwater bacterial communities. Both water and biofilm aquifer samples recovered downstream the SIS had higher bacterial richness that those collected upstream. These diversity changes were found related to a coalescence of bacterial taxa from the top compartments with the aquifer microbial communities.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

Indeed, downstream the SIS, aquifer water samples shared more OTUs (up to 47%) with those of the runoff waters than those upstream the SIS. Furthermore, aquifer biofilms downstream the SIS were heavily colonized by OTUs (90% of the datasets) from the top compartments.

The SourceTracker Bayesian probabilistic approach based on 16S rRNA gene meta-barcoding datasets (Knights et al., 2011) was applied to refine our understanding of the coalescence of microbial communities from aboveground environments down into an aquifer. These inferences revealed variable levels of coalescence in the SIS recharged aquifer depending upon the investigated sink *i.e.* waters or biofilms developing on clay beads incubated in the aquifer. Bacterial community structures of the groundwater samples (upstream and downstream the SIS) were significantly built from aboveground communities (*e. g.* those from runoff waters). However, the origin of a high proportion of the diversity observed among the aquifer waters downstream the SIS remained undefined. This is likely related to the emergence of novel biomes among the vadose zone of a SIS fed with urban waters and pollutants. These biomes would have emerged from the build-up of novel biotopes during the construction and functioning of the SIS. The prevailing environmental constraints and pollutants would then have favored minor taxa (not detectable by meta-DNA barcoding approaches) from the aboveground compartments. It is to be noted that functional inferences from the knowledge on bacterial genera suggested an occurrence of several aquifer taxa involved in the nitrogen and sulfur cycles. *Campylobacter*, *Flavobacterium*, *Pseudomonas*, *Sulfurimonas* cells have been associated with nitrogen and sulfur respiration processes, and *Nitrospira* and *Leptospirillum* with nitrification. The oligotrophic nature of the aquifer waters (concentrations of biodegradable dissolved organic carbon < 0.5 mg/L, Mermillod-Blondin et al., 2015) is thus likely to have induced a significant selective sorting of microbial taxa among the merged community. Most abundant above ground taxa often require high energy (organic carbon) and nutrient levels to proliferate (Cho and Kim, 2000; Griebler and Lueders, 2009).

Similarly, a large part of the bacterial taxa identified from aquifer biofilms was attributed to aboveground sources by the SourceTracker approach. Indeed, watershed runoff waters and detention basin deposits were found to have significantly contributed to the build-up of the observed biofilm community structures. Aquifer waters collected upstream the SIS were also major contributors (11-46%) of taxa for these biofilm assemblages. These biofilms showed a high content of 16S rRNA gene sequences belonging to the *β*- and *γ-proteobacteria.* According to the ecological concept of r/K selection, these proteobacteria are often considered as r-strategists, able to respond quickly to environmental fluctuations, and colonize more efficiently newly exposed surfaces than other groups of bacteria (Araya et al., 2003; Fierer et al., 2007; Lladó and Baldrian, 2017; Manz et al., 1999; Pohlon et al., 2010). Moreover, because they tend to concentrate nutrients (Flemming et al., 2016), biofilms are likely to favor the survival of opportunistic bacterial cells capable of exploiting spatially and temporally variable carbon and nutrient sources. Here, taxa recovered from aquifer biofilms were previously recorded to have the ability to use hydrocarbons as carbon- and energy sources e. g. *Nocardia*, *Pseudomonas*, *Sphingobium*, and *Novosphingobium*. SIS and urban runoffs are well known to be highly polluted by such molecules (e. g., Marti et al., 2017) and significant organic matter enrichments were detected in aquifers downstream to SISs (e. g. Mermillod-Blondin et al., 2015). The r/K selection ecological concept thus seems to apply to the community assemblages observed in this work. K-strategists would be the specialists described above which can perform well at densities close to the carrying capacity of the system, while the r-strategists would be environmental opportunists taking advantage of the newly available surfaces offered by the clay beads and the co-occurrence of aboveground C-sources.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

Taxonomic allocations of the 16S rRNA OTUs suggested the aquifer waters and biofilms to likely harbor opportunistic human, plant and animal pathogens of the genus *Finegoldia*, *Campylobacter*, *Haemophilus*, *Duganella*, *Massilia*, *Nocardia*, *Aquabacterium*, *Flavobacterium*, *Pseudomonas*, *Streptococcus*, and *Aeromonas*. Among these, the most striking results were the observed enrichment of 16S rRNA gene reads allocated to the *Nocardia* (about 4% of total reads) and *Pseudomonas* (about 35% of total reads) in the biofilms recovered from clay beads incubated downstream the SIS. *Nocardia* and *Pseudomonas* 16S rRNA gene sequences were in much lower relative proportions in the aboveground compartments. The genus *Pseudomonas* was previously found to be abundant under low flow conditions, and was often associated with biofilm formation (Douterelo et al., 2013). Moreover, pseudomonads are well-known for their ability at using hydrocarbons as energy and C-sources. Regarding the *Nocardia* cells, there is a poor knowledge of their ecology but a few reports indicated a tropism for hydrocarbon polluted urban soils and sediments (e. g., Bernardin-Souibgui et al., 2018; Sébastian et al., 2014). There was no additional approach to further investigate the molecular ecology of *Nocardia* cells found among the investigated urban watershed. However, a *tpm* meta-barcoding analytical scheme could be applied on DNA extracts investigated in this study in order to go deeper into the taxonomic allocations of the pseudomonads and some other *tpm*-harboring genera. The applied *tpm* meta-barcoding approach allowed an investigation of the coalescence of about 90 species among the investigated watershed including 50 species of *Pseudomonas*, 11 species allocated to the *Aeromonas*, and some additional species allocated to the *Nitrospira*, *Nitrosomonas*, *Stenotrophomonas*, *Xanthobacter*, and *Xanthomonas*. A single *Aeromonas* species, *A. caviae*, was recorded among the above- and under-ground environments. More than 10 *Pseudomonas* species thriving in the recharged aquifer were detected among the aboveground compartments. *P. umsongensis* and *P. chengduensis tpm* OTUs were detected aboveground, and represented a significant fraction of the *tpm*-harboring bacteria retrieved from the aquifer samples. These two species were initially isolated from farm soil and landfill leachates (Kwon et al., 2003; Tao et al., 2014), further supporting the hypothesis that such soil-associated bacteria can be transferred from runoffs down to natural hydrosystems, and can merge with aquifer communities. Regarding the *Pseudomonas* species that may pose health threats to humans, a *tpm* OTU affiliated to *P. aeruginosa* was found to be shared between the surface compartments and the biofilm *tpm* community developing on clay beads incubated downstream the SIS. *P. aeruginosa* thus had the properties allowing an opportunistic development among the aquifer. This species is known for its metabolic versatility and ability to thrive on hydrocarbons. It would thus be part of the r-strategists that could get opportunistically established in aquifer biofilm communities impacted by urban pollutants. Apart from *P. aeruginosa*, the species *P. putida* and *P. stutzeri*, frequently detected in soils and wastewater treatment plants (*e.g.* Igbinosa et al., 2012; Luczkiewicz et al., 2015; Miyahara et al., 2010), were also recovered along the watershed and aquifer. However, although these two species were identified in human infections (Fernández et al., 2015; Noble and Overman, 1994), information about their virulence remains scarce. These species are therefore considered to be of less concern than *P. aeruginosa* and *A. caviae*, another opportunistic infectious agent (Antonelli et al., 2016). *P. putida* isolates have been shown involved in hydrocarbon degradation, and *P. stutzeri* to play part in the N-cycle either through denitrification or nitrogen-fixation.

5 Conclusions

The knowledge gained from the present study demonstrated that coalescence of microbial communities from an urban watershed with those of an aquifer can occur, and yield novel assemblages. Specialized bacterial

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020


Hydrology and Earth System Sciences
Discussions

communities of aquifer waters were slightly re-shuffled by aboveground communities. However, the assemblages observed among recent aquifer biofilms were found dominated by opportunistic r-strategists coming from aboveground compartments, and often associated with the ability at degrading hydrocarbons e. g. the pseudomonads, *Nocardia* and *Novosphingobium* cells. The aquifer of the investigated site was found, for the first time, to be specifically colonized by species like *P. jessenii*, *P. chlororaphis*, and *P. resinovorans* but also undesirable human opportunistic pathogens such as *P. aeruginosa* and *A. caviae*. Artificial clay beads incubated in the aquifer through piezometers appeared highly efficient germcatchers to evaluate the ability of a SIS at preventing transfer of undesirable r-strategists down to an aquifer. The long term incidence of allochthonous bacteria on the integrity of aquifer microbiota remains to be investigated. Free-living 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. The next step is now to investigate whether native aquifer biofilm communities can resist to these repeated invasions by opportunistic r-strategists.

Data availability. The 16S rRNA gene sequences are available at the European Nucleotide Archive (https://www.ebi.ac.uk/ena) using the following accession numbers: PRJEB33510 (IB), PRJEB21348 (DB), PRJEB29925 (AQ), and PRJEB33507 (WS), and the *tpm* gene sequences using the PRJEB33622 accession number.

Supplement. The supplementary materials related to this article is available online at: https://doi.org/

Author contribution. BC coordinated the work. YC and BC designed the experiments. YC, VRN, TW, FMB, RB, LM, RM, FV, EB, DB, JV, and BC performed the experiments and contributed at the analysis of the datasets. YC and BC prepared the manuscript with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions

Acknowledgments. This work was partly funded by l'Agence Nationale de la Recherche through the ANR-16-CE32-0006 (FROG) and ANR-17-CE04-0010 (Infiltron) projects, by Labex IMU (Intelligence des Mondes Urbains), the Greater-Lyon Urban Community, the French national research program for environmental and occupational health of ANSES under the terms of project "Iouqmer" EST 2016/1/120, the School of Integrated Watershed Sciences H2O'LYON (ANR 17-EURE-0018), "Investissements d'avenir" program), the MITI CNRS project named Urbamic, and the Urban School of Lyon (ANR-17-CONV-0004). Authors thank the OTHU network for technical assistance and financial supports.

Edited by:
Reviewed by:

References

Abu-Ashour, J., Joy, D.M., Lee, H., Whiteley, H.R., Zelin, S.: Transport of microorganisms through soil. Water Air Soil Poll., 75, 141–158, https://doi.org/10.1007/BF01100406, 1994.

Anderson, M.J.: A new method for non-parametric multivariate analysis of variance. Austral. Ecol., 26, 32–46, https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x, 2001.

Anderson, M.J., Willis, T.J.: Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology, 84, 511–525, https://www.jstor.org/stable/3107905, 2003.

Antonelli, A., D'Andrea, M.M., Montagnani, C., Bartolesi, A.M., Di Pilato, V., Fiorini, P., Torricelli, F., Galli, L., Rossolini, G.M.: Newborn bacteraemia caused by an *Aeromonas caviae* producing the VIM-1 and SHV-12 β-lactamases, encoded by a transferable plasmid. J. Antimicrob. Chemother., 71, 272–274, https://doi: 10.1093/jac/dkv304, 2016.

Araya, R., Tani, K., Takagi, T., Yamaguchi, N., Nasu, M.: Bacterial activity and community composition in stream water and biofilm from an urban river determined by fluorescent in situ hybridization and DGGE analysis. FEMS Microbiol. Ecol., 43, 111–119, https://doi: 10.1111/j.1574-6941.2003.tb01050.x, 2003.

Arnaud, E., Best, A., Parker, B.L., Aravena, R., Dunfield, K.: Transport of *Escherichia coli* through a thick vadose zone. J. Environ. Qual., 44, 1424, https://doi:10.2134/jeq2015.02.0067, 2015.

Barba, C., Folch, A., Gaju, N., Sanchez-Vila, X., Carrasquilla, M., Grau-Martínez, A., Martínez-Alonso, M.: Microbial community changes induced by managed aquifer recharge activities: linking hydrogeological and biological processes. Hydrol. Earth Syst. Sci., 23, 139–154. https://doi.org/10.5194/hess-23-139-2019, 2019.

Barnes, K.B., Iii, J.M.M., Roberge, M.C.: Impervious surfaces and the quality of natural and built environments, Department of Geography and Environmental Planning. Towson University, 2001.

Barraud, S., Gibert, J., Winiarski, T., Bertrand Krajewski, J.-L.: Implementation of a monitoring system to measure impact of stormwater runoff infiltration. Water Sci. Technol., 45, 203-210, https://iwaponline.com/wst/article-pdf/45/3/203/425178/203.pdf, 2002.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

493 Bedell, J.-P., Mourier, B., Provot, J., Winiarski, T.: Influences on the establishment and dominance of vegetation
494 in stormwater infiltration basins. Water Sci. Technol., 68, 2576–2583, https://doi.org/10.2166/wst.2013.526,
495 2013.

496 Bernardin-Souibgui, C., Barraud, S., Bourgeois, E., Aubin, J.-B., Becouze-Lareure, C., Wiest, L., Marjolet, L.,
497 Colinon, C., Lipeme Kouyi, G., Cournoyer, B., Blaha, D.: Incidence of hydrological, chemical, and physical
498 constraints on bacterial pathogens, *Nocardia* cells, and fecal indicator bacteria trapped in an urban stormwater
499 detention basin in Chassieu, France. Environ. Sci. Poll. Res., 25, 24860–24881,
500 https://doi.org/10.1007/s11356-018-1994-2, 2018.

501 Booth, D.B.: Urbanization and the natural drainage system-impacts, solutions, and prognoses. The Northwest
502 Environ. J., 7, 93–118, 1991.

503 Borchardt, M.A., Bradbury, K.R., Gotkowitz, M.B., Cherry, J.A., Parker, B.L.: Human enteric viruses in
504 groundwater from a confined bedrock aquifer. Environ. Sci. Technol., 41, 6606–6612,
505 https://doi.org/10.1021/es071110+, 2007.

506 Cho, J.-C., Kim, S.-J.: Increase in bacterial community diversity in subsurface aquifers receiving livestock
507 wastewater input. Appl. Environ. Microbiol., 66, 956–965, https://doi: 10.1128/aem.66.3.956-965.2000, 2000.

508 Chong, M.N., Sidhu, J., Aryal, R., Tang, J., Gernjak, W., Escher, B., Toze, S.: Urban stormwater harvesting and
509 reuse: a probe into the chemical, toxicology and microbiological contaminants in water quality. Environ.
510 Monit. Assess., 185, 6645–6652. https://doi.org/10.1007/s10661-012-3053-7, 2013.

511 Crites, R.W.: Micropollutant removal in rapid infiltration, in: Artificial Recharge of Groundwater. Butterworth
512 Publishers, Boston, Massachusetts, pp. 579–608, 1985.

513 Crump, B., Baross, J.: Particle-attached bacteria and heterotrophic plankton associated with the Columbia River
514 estuarine turbidity maxima. Mar. Ecol. Prog. Ser., 138, 265–273, 1996.

515 Crump, B.C., Simenstad, C.A., Baross, J.A.: Particle-attached bacteria dominate the Columbia River estuary.
516 Aquat. Microb. Ecol., 14, 7–18, 1998.

517 Dillon, P., Page, D., Vanderzalm, J., Pavelic, P., Toze, S., Bekele, E., Sidhu, J., Prommer, H., Higginson, S., Regel,
518 R., Rinck-Pfeiffer, S., Purdie, M., Pitman, C., Wintgens, T.: A critical evaluation of combined engineered and
519 aquifer treatment systems in water recycling. Water Sci. Technol., 57, 753–762.
520 https://doi.org/10.2166/wst.2008.168, 2008.

521 Douterelo, I., Sharpe, R.L., Boxall, J.B.: Influence of hydraulic regimes on bacterial community structure and
522 composition in an experimental drinking water distribution system. Water Res., 47, 503–516. https://doi.org/
523 10.1016/j.watres.2012.09.053, 2013.

524 Favre-Bonté, S., Ranjard, L., Colinon, C., Prigent-Combaret, C., Nazaret, S., Cournoyer, B.: Freshwater selenium-
525 methylating bacterial thiopurine methyltransferases: diversity and molecular phylogeny. Environ. Microbiol.
526 7, 153–164, https://doi.org/10.1111/j.1462-2920.2004.00670.x, 2005.

Fernández, M., Porcel, M., de la Torre, J., Molina-Henares, M.A., Daddaoua, A., Llamas, M.A., Roca, A., Carriel, V., Garzón, I., Ramos, J.L., Alaminos, M., Duque, E.: Analysis of the pathogenic potential of nosocomial *Pseudomonas putida* strains. Front Microbiol. 6:871, https://doi.org/10.3389/fmicb.2015.00871, 2015.

Fierer, N., Bradford, M.A., Jackson, R.B.: Toward an ecological classification of soil bacteria. Ecology, 88, 1354–1364. https://doi.org/10.1890/05-1839, 2007.

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S.: Biofilms: an emergent form of bacterial life. Nat. Rev. Microbiol., 14, 563–575, https://doi.org/10.1038/nrmicro.2016.94, 2016.

Foulquier, A., Malard, F., Mermillod-Blondin, F., Datry, T., Simon, L., Montuelle, B., Gibert, J.: Vertical change in dissolved organic carbon and oxygen at the water table region of an aquifer recharged with stormwater: biological uptake or mixing? Biogeochem., 99, 31–47, https://doi.org/10.1007/s10533-009-9388-7, 2010.

Griebler, C., Lueders, T.: Microbial biodiversity in groundwater ecosystems. Freshwater Biol., 54, 649–677, https://doi.org/10.1111/j.1365-2427.2008.02013.x, 2009.

Griebler, C., Malard, F., Lefébure, T.: Current developments in groundwater ecology—From biodiversity to ecosystem function and services. Current Opinion Biotechnol., 27, 159–167, https://doi.org/10.1016/j.copbio.2014.01.018, 2014.

Henry, R., Schang, C., Coutts, S., Kolotelo, P., Prosser, T., Crosbie, N., Grant, T., Cottam, D., O'Brien, P., Deletic, A.: Into the deep: evaluation of SourceTracker for assessment of faecal contamination of coastal waters. Water Res., 93, 242–253, https://doi.org/10.1016/j.watres.2016.02.029, 2016.

Igbinosa, I.H., Nwodo, U.U., Sosa, A., Tom, M., Okoh, A.I.: Commensal *Pseudomonas* species isolated from wastewater and freshwater milieus in the Eastern Cape Province, South Africa, as reservoir of antibiotic resistant determinants. Int. J. Environ. Res. Public Health, 9, 2537–2549, https://doi.org/10.3390/ijerph9072537, 2012.

Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., Kelley, S.T.: Bayesian community-wide culture-independent microbial source tracking. Nat. Methods, 8, 761, https://doi.org/10.1038/nmeth.1650, 2011.

Konrad, C.P., Booth, D.B.: Hydrologic changes in urban streams and their ecological significance, in: American Fisheries Society Symposium, 47, 157-177, 2005.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D.: Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol., 79, 5112–5120, https://doi.org/10.1128/AEM.01043-13, 2013.

Kristian Stevik, T., Kari Aa, Ausland, G., Fredrik Hanssen, J.: Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Res., 38, 1355–1367, https://doi.org/10.1016/j.watres.2003.12.024, 2004.

Krone, R.B., Orlob, G.T., Hodgkinson, C.: Movement of coliform bacteria through porous media. Sewage Ind. Wastes, 30, 1–13, 1958.

563 Kwon, S.W., Kim, J.S., Park, I.C., Yoon, S.H., Park, D.H., Lim, C.K., Go, S.J., *Pseudomonas koreensis* sp. nov.,
564 *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in
565 Korea. Int. J. Syst. Evol. Microbiol., 53, 21–27, https://doi.org/10.1099/ijs.0.02326-0, 2003.

566 Lapworth, D.J., Baran, N., Stuart, M.E., Ward, R.S.: Emerging organic contaminants in groundwater: A review of
567 sources, fate and occurrence. Environ. Pollut. 163, 287–303, https://doi.org/10.1016/j.envpol.2011.12.034,
568 2012.

569 Lassabatere, L., Angulo-Jaramillo, R., Soria Ugalde, J.M., Cuenca, R., Braud, I., Haverkamp, R.: Beerkan
570 estimation of soil transfer parameters through infiltration experiments—BEST. Soil Sci. Soc. Am. J., 70, 521–
571 532, https://doi.org/10.2136/sssaj2005.0026, 2006.

572 Le Boulch, M., Déhais, P., Combes, S., Pascal, G.: The MACADAM database: a MetAboliC pAthways DAtabase
573 for Microbial taxonomic groups for mining potential metabolic capacities of archaeal and bacterial taxonomic
574 groups. Database 2019, https://doi.org/10.1093/database/baz049, 2019.

575 Le Coustumer, S., Barraud, S.: Long-term hydraulic and pollution retention performance of infiltration systems.
576 Water Sci. Technol., 55, 235–243, https://doi.org/10.2166/wst.2007.114, 2007.

577 Lladó, S., Baldrian, P.: Community-level physiological profiling analyses show potential to identify the
578 copiotrophic bacteria present in soil environments. PLoS One, 12: e0171638,
579 https://doi.org/10.1371/journal.pone.0171638, 2017.

580 Louca, S., Parfrey, L.W., Doebeli, M.: Decoupling function and taxonomy in the global ocean microbiome.
581 Science, 353, 1272–1277, https://doi.org/10.1126/science.aaf4507, 2016.

582 Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R.: UniFrac: an effective distance metric for
583 microbial community comparison. ISME J., 5, 169–172, https://doi.org/10.1038/ismej.2010.133, 2011.

584 Luczkiewicz, A., Kotlarska, E., Artichowicz, W., Tarasewicz, K., Fudala-Ksiazek, S.: Antimicrobial resistance of
585 *Pseudomonas* spp. isolated from wastewater and wastewater-impacted marine coastal zone. Environ. Sci.
586 Pollut. Res., 22, 19823–19834, https://doi.org/10.1007/s11356-015-5098-y, 2015.

587 Mansour, I., Heppell, C.M., Ryo, M., Rillig, M.C.: Application of the microbial community coalescence concept
588 to riverine networks: Riverine microbial community coalescence. Biol. Rev., 93, 1832–1845,
589 https://doi.org/10.1111/brv.12422, 2018.

590 Manz, W., Wendt-Potthoff, K., Neu, T.R., Szewzyk, U., Lawrence, J.R.: Phylogenetic composition, spatial
591 structure, and dynamics of lotic bacterial biofilms investigated by fluorescent in situ hybridization and confocal
592 laser scanning microscopy. Microb. Ecol., 37, 225–237, https://doi.org/10.1007/s002489900148, 1999.

593 Marsalek, J., Chocat, B.: International report: Stormwater management. Water Sci. Technol. 46, 1–17, 2002.

594 Marti, R., Bécouze-Lareure, C., Ribun, S., Marjolet, L., Bernardin Souibgui, C., Aubin, J.-B., Lipeme Kouyi, G.,
595 Wiest, L., Blaha, D., Cournoyer, B.: Bacteriome genetic structures of urban deposits are indicative of their
596 origin and impacted by chemical pollutants. Sci. Rep., 7: 13219, https://doi.org/10.1038/s41598-017-13594-8,
597 2017.

598 McCarthy, D.T., Jovanovic, D., Lintern, A., Teakle, I., Barnes, M., Deletic, A., Coleman, R., Rooney, G., Prosser,
599 T., Coutts, S.: Source tracking using microbial community fingerprints: method comparison with
600 hydrodynamic modelling. Water Res., 109, 253–265, https://doi.org/10.1016/j.watres.2016.11.043, 2017.

601 McGrane, S.J.: Impacts of urbanisation on hydrological and water quality dynamics, and urban water management:
602 a review. Hydrol. Sci. J., 61, 2295–2311, https://doi.org/10.1080/02626667.2015.1128084, 2016.

603 Mejía, A.I., Moglen, G.E.: Spatial patterns of urban development from optimization of flood peaks and
604 imperviousness-based measures. J. Hydrol. Eng., 14, 416–424, https://doi.org/10.1061/(ASCE)1084-
605 0699(2009)14:4(416), 2009.

606 Mermillod-Blondin, F., Simon, L., Maazouzi, C., Foulquier, A., Delolme, C., Marmonier, P.: Dynamics of
607 dissolved organic carbon (DOC) through stormwater basins designed for groundwater recharge in urban area:
608 assessment of retention efficiency. Water Res., 81, 27–37, https://doi.org/10.1016/j.watres.2015.05.031, 2015.

609 Mermillod-Blondin, F., Voisin, J., Marjolet, L., Marmonier, P., Cournoyer, B.: Clay beads as artificial trapping
610 matrices for monitoring bacterial distribution among urban stormwater infiltration systems and their connected
611 aquifers. Environ. Monit. Assess., 191, 58, https://doi.org/10.1007/s10661-019-7190-0, 2019.

612 Miyahara, M., Kim, S.-W., Fushinobu, S., Takaki, K., Yamada, T., Watanabe, A., Miyauchi, K., Endo, G., Wakagi,
613 T., Shoun, H.: Potential of aerobic denitrification by *Pseudomonas stutzeri* TR2 to reduce nitrous oxide
614 emissions from wastewater treatment plants. Appl. Environ. Microbiol., 76, 4619–4625,
615 https://doi.org/10.1128/AEM.01983-09, 2010.

616 Murphy, E.M., Ginn, T.R.: Modeling microbial processes in porous media. Hydrogeol. J., 8, 142–158,
617 https://doi.org/10.1007/s100409900043, 2000.

618 Noble, R.C., Overman, S.B.: *Pseudomonas stutzeri* infection. A review of hospital isolates and a review of the
619 literature. Diagn. Microbiol. Infect. Dis., 19, 51–56, https://doi.org/10.1016/0732-8893(94)90051-5, 1994.

620 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P.,
621 Stevens, M.H.H., Wagner, H.: vegan: Community Ecology Package. R package version 2.3–0. 2015, 2015.

622 Pigneret, M., Mermillod-Blondin, F., Volatier, L., Romestaing, C., Maire, E., Adrien, J., Guillard, L., Roussel, D.,
623 Hervant, F.: Urban pollution of sediments: Impact on the physiology and burrowing activity of tubificid worms
624 and consequences on biogeochemical processes. Sci. Total Environ., 568, 196–207,
625 https://doi.org/10.1016/j.scitotenv.2016.05.174, 2016.

626 Pitt, R., Clark, S., Field, R.: Groundwater contamination potential from stormwater infiltration practices. Urban
627 Water, 1, 217–236, https://doi.org/10.1016/S1462-0758(99)00014-X, 1999.

628 Pohlon, E., Marxsen, J., Küsel, K.: Pioneering bacterial and algal communities and potential extracellular enzyme
629 activities of stream biofilms. FEMS Microbiol. Ecol., 71, 364–373, https://doi.org/10.1111/j.1574-
630 6941.2009.00817.x, 2010.

631 Powelson, D.K., Gerba, C.P., Yahya, M.T.: Virus transport and removal in wastewater during aquifer recharge.
632 Water Res., 27, 583–590, https://doi.org/10.1016/0043-1354(93)90167-G, 1993.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

633 Price, M.N., Dehal, P.S., Arkin, A.P.: FastTree: computing large minimum evolution trees with profiles instead of
634 a distance matrix. Mol. Biol. Evol., 26, 1641–1650, https://doi.org/10.1093/molbev/msp077, 2009.

635 Redman, J.A., Grant, S.B., Olson, T.M., Estes, M.K.: Pathogen filtration, heterogeneity, and the potable reuse of
636 wastewater. Environ. Sci. Technol., 35, 1798–1805, https://doi.org/10.1021/es0010960, 2001.

637 Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., Smyth, G.K.: limma powers differential
638 expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res., 43, e47–e47,
639 https://doi.org/10.1093/nar/gkv007, 2015.

640 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley,
641 B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Horn, D.J.V., Weber, C.F.:
642 Introducing mothur: open-source, platform-independent, community-supported software for describing and
643 comparing microbial communities. Appl. Environ. Microbiol., 75, 7537–7541,
644 https://doi.org/10.1128/AEM.01541-09, 2009.

645 Sébastian, C., Barraud, S., Ribun, S., Zoropogui, A., Blaha, D., Becouze-Lareure, C., Kouyi, G.L., Cournoyer, B.:
646 Accumulated sediments in a detention basin: chemical and microbial hazard assessment linked to hydrological
647 processes. Environ. Sci. Pollut. Res., 21, 5367–5378, https://doi.org/10.1007/s11356-013-2397-z, 2014.

648 Shuster, W.D., Bonta, J., Thurston, H., Warnemuende, E., Smith, D.R.: Impacts of impervious surface on
649 watershed hydrology: A review. Urban Water J., 2, 263–275, https://doi.org/10.1080/15730620500386529,
650 2005.

651 Tao, Y., Zhou, Y., He, X., Hu, X., Li, D.: *Pseudomonas chengduensis* sp. nov., isolated from landfill leachate. Int.
652 J. Syst. Evol. Microbiol., 64, 95–100, https://doi.org/10.1099/ijs.0.050294-0, 2014.

653 Tedoldi, D., Chebbo, G., Pierlot, D., Kovacs, Y., Gromaire, M.-C.: Impact of runoff infiltration on contaminant
654 accumulation and transport in the soil/filter media of sustainable urban drainage systems: A literature review.
655 Sci. Total Environ., 569–570, 904–926, https://doi.org/10.1016/j.scitotenv.2016.04.215, 2016.

656 Tikhonov, M.: Community-level cohesion without cooperation. eLife, 5. https://doi.org/10.7554/eLife.15747,
657 2016.

658 Vadstein, O., Attramadal, K.J.K., Bakke, I., Olsen, Y.: K-Selection as Microbial Community Management
659 Strategy: A Method for Improved Viability of Larvae in Aquaculture. Front. Microbiol., 9:2730,
660 https://doi.org/10.3389/fmicb.2018.02730, 2018.

661 van Loosdrecht, M.C., Lyklema, J., Norde, W., Zehnder, A.J.: Influence of interfaces on microbial activity.
662 Microbiol. Rev., 54, 75–87, 1990.

663 Vezzaro, L., Mikkelsen, P.S.: Application of global sensitivity analysis and uncertainty quantification in dynamic
664 modelling of micropollutants in stormwater runoff. Environ. Modell. Softw., 27, 40–51,
665 https://doi.org/10.1016/j.envsoft.2011.09.012, 2012.

666 Voisin, J., Cournoyer, B., Mermillod-Blondin, F.: Assessment of artificial substrates for evaluating groundwater
667 microbial quality. Ecol. Indicators, 71, 577–586, https://doi.org/10.1016/j.ecolind.2016.07.035, 2016.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

668 Voisin, J., Cournoyer, B., Vienney, A., Mermillod-Blondin, F.: Aquifer recharge with stormwater runoff in urban
669 areas: Influence of vadose zone thickness on nutrient and bacterial transfers from the surface of infiltration
670 basins to groundwater. Sci. Total Environ., 637–638, 1496–1507,
671 https://doi.org/10.1016/j.scitotenv.2018.05.094, 2018.

672 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R.: Naive Bayesian classifier for rapid assignment of rRNA
673 sequences into the new bacterial taxonomy. Appl. Environ. Microbiol., 73, 5261–5267,
674 https://doi.org/10.1128/AEM.00062-07, 2007.

675 Winiarski, T., Lassabatere, L., Angulo-Jaramillo, R., Goutaland, D.: Characterization of the heterogeneous flow
676 and pollutant transfer in the unsaturated zone in the fluvio-glacial deposit. Procedia Environ. Sci., 19, 955–
677 964, https://doi.org/10.1016/j.proenv.2013.06.105, 2013.

678

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

679

Table 1. Aquifer 16S rRNA gene (*rrs*) OTUs detected in the upper compartments of the investigated watershed and SIS*.

	Upstream SIS						
	AQ_bio_up1	**AQ_bio_up2**	**AQ_bio_up3**	**AQ_wat_up1**	**AQ_wat_up2**	**AQ_wat_up3**
(A) Number of aquifer *rrs* OTUs shared with the upper compartments	185/220	110/160	118/173	93/143	80/164	165/464
(B) Relative abundance of the shared *rrs* OTUs in the aquifer (in %)	99.4	95.0	96.4	43.8	45.4	33.6
(C) Relative abundance of the shared *rrs* OTUs in the upper compartments (in %)	24.9	15.5	15.8	9.7	9.8	11.3
	downstream SIS					
	AQ_bio_dw1	**AQ_bio_dw2**	**AQ_bio_dw3**	**AQ_wat_dw1**	**AQ_wat_dw2**	**AQ_wat_dw3**
(A) Number of aquifer *rrs* OTUs shared with the upper compartments	340/403	308/353	321/362	203/523	357/594	468/1052
(B) Relative abundance of the shared *rrs* OTUs in the aquifer (in %)	99.4	99.4	99.6	52.2	83.4	53.7
(C) Relative abundance of the shared *rrs* OTUs in the upper compartments (in %)	29.7	30.7	39.4	12.5	32.0	24.2

*in (A), the number of aquifer *rrs* OTUs found in the upper compartments (WS, DB, IB) was computed per aquifer sample recovered upstream (up) or downstream (dw) the SIS (see Fig. 1 for the sampling design), after a re-sampling of the reads set at 20,624 per sample; in (B), the relative abundance of these shared OTUs per aquifer sample is indicated; in (C), the relative abundance of these shared aquifer OTUs among the upper compartments is indicated. AQ_wat: Aquifer waters; AQ_bio: Aquifer clay beads biofilms; up: upstream the SIS, dw: downstream the SIS.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

680

Table 2. 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	22.82%	9.67	0.02%	94.37	3.83%	10.71	0.02%	96.25	73.31%	2.49
	AQ_wat_dw2	58.94%	6.03	6.26%	10.74	1.27%	20.32	12.64%	21.27	20.89%	20.27
	AQ_wat_dw3	25.49%	7.06	9.07%	10.47	0.81%	24.58	3.83%	31.01	60.81%	2.06
1 - biofilms	AQ_bio_dw1	24.04%	13.55	19.95%	8.47	0.17%	107.45	46.37%	4.71	9.14%	4.72
	AQ_bio_dw2	29.44%	18.54	17.28%	9.91	0.16%	94.43	44.71%	7.05	8.40%	6.77
	AQ_bio_dw3	44.66%	8.39	22.22%	15.6	0.37%	105.66	25.90%	4.21	6.84%	13.55
2 - biofilms	AQ_bio_up1	51.18%	0.98					46.35%	1.14	2.47%	1.12
	AQ_bio_up2	81.11%	0.23					10.93%	4.68	7.95%	4.19
	AQ_bio_up3	60.31%	0.74					32.30%	1.32	7.39%	4.31

* Two analyses are shown: (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.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

681

Table 3. Relative distribution of *tpm* reads per OTU (mean ± sd) shared between the upper compartments and the aquifer, and that were allocated to well-defined species.[1]

Genus	Species	OTU code[2]	WS	DB	IB	AQ_Wat_up	AQ_Bio_up	AQ_Wat_dw	AQ_Bio_dw
Nitrosomonas	*oligotropha*	Otu00035	nd	1.5 ± 3.40	0.15 ± 0.30	nd	+	+	nd
Pseudomonas	*aeruginosa*	Otu00066	0.42 ± 1.13	nd	+	nd	nd	nd	0.17 ± 0.30
Pseudomonas	*chengduensis*	Otu00024	nd	+	+	20.43 ± 35.39	nd	+	nd
Pseudomonas	*extremaustralis*	Otu04178	nd	+	nd	nd	nd	+	nd
Pseudomonas	*fragi*	Otu00197	0.61 ± 4.05	nd	nd	nd	nd	+	nd
Pseudomonas	*pseudoalcaligenes*	Otu00197	0.07 ± 0.38	+	nd	+	nd	nd	nd
Pseudomonas	*putida*	Otu00800	+	+	nd	nd	nd	+	nd
Pseudomonas	*stutzeri*	Otu00119 & Otu10066	0.06 ± 0.33	nd	+	3.06 ± 5.29	nd	nd	+
Pseudomonas	*umsongensis*	Otu00005	+	+	nd	0.41 ± 0.71	17.79 ± 20.11	5.34 ± 8.58	11.71 ± 13.17
Pseudomonas	*viridiflava*	Otu00204	0.06 ± 0.31	nd	0.3 ± 1.09	nd	nd	0.07 ± 0.12	nd
Stenotrophomonas	*acidaminiphila*	Otu00072 & Otu01119	0.09 ± 0.42	0.29 ± 0.91	0.06 ± 0.22	nd	nd	+	nd
Xanthobacter	*autotrophicus*	Otu00501	+	+	nd	nd	nd	0.06 ± 0.11	+
Xanthomonas	*axonopodis/campestris*	Otu00019 & Otu00878	0.25 ± 0.75	nd	1.24 ± 2.07	16.04 ± 27.78	nd	nd	+
Xanthomonas	*cannabis*	Otu00004	3.74 ± 9.47	nd	nd	nd	+	+	+

[1] All reads from *tpm* OTUs shared between the upper compartments and the aquifer were used to compute the relative abundances.
[2] *tpm* sequences of the OTUs are shown in Table S8. WS: Watershed runoff waters; DB: Detention basin deposits; IB: soil of the infiltration basin; AQ_water: Aquifer waters; AQ_bio: Aquifer biofilms. + : OTUs with a relative abundance < 0.05%, nd : not detected.

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions

682 **Figure captions**

683 **Figure 1.** Scheme illustrating the stormwater runoff path from the industrial watershed (WS) towards the
684 stormwater infiltration system (SIS) used in this study. The urban watershed is located in Chassieu (France). The
685 SIS is made of a detention basin (DB) and an infiltration basin (IB), and is connected to the Lyon 200 km^2 east
686 aquifer (AQ). (© Google)

687 **Figure 2.** General features of the V5-V6 16S rRNA gene meta-barcoding DNA sequences obtained from runoffs,
688 SIS, and aquifer samples. See Fig. 1 for a description of the experimental design. The main bacterial phyla (A),
689 and alpha diversity indices (B), are shown per sampled compartment. Bacterial diversity was estimated using the
690 Shannon index. One-way ANOVA with multiple Tukey post hoc tests were performed to investigate the
691 differences between compartments. Different letter codes indicate significant differences ($p<0.05$). WS, runoff
692 waters from the watershed; DB: sediments from the detention basin; IB: soils from the infiltration basin;
693 AQ_water: Aquifer waters; AQ_bio: Aquifer clay beads biofilms.

694 **Figure 3.** PCoA analysis of weighted UniFrac dissimilarities between the V5-V6 16S rRNA gene OTU profiles
695 of the watershed runoff waters (WS), urban sediments and soils from the connected detention (DB) and infiltration
696 (IB) basins receiving the runoffs, and waters (AQ_water) and biofilms (AQ_bio) from the connected aquifer. See
697 Fig. 1 for a description of the experimental site. Ellipses are representative of the variance observed (standard
698 error) between the ordinations of a group of samples. PERMANOVA tests confirmed the significance ($p < 0.001$)
699 of the groupings.

700 **Figure 4.** Relative numbers of potentially pathogenic bacterial genera along the watershed down the aquifer. The
701 abundance (rel. abund.) of bacterial genera exclusively detected in upper compartments (A) or both in upper
702 compartments and aquifer (B) are presented. Size of bubbles is proportional to the relative abundance (in %) of
703 each bacterial genus per sampled compartment. WS, runoff waters from the watershed; DB: sediments from the
704 detention basin; IB: sediments from the infiltration basin; AQ_water: Aquifer waters; AQ_bio: Aquifer clay beads
705 biofilms.

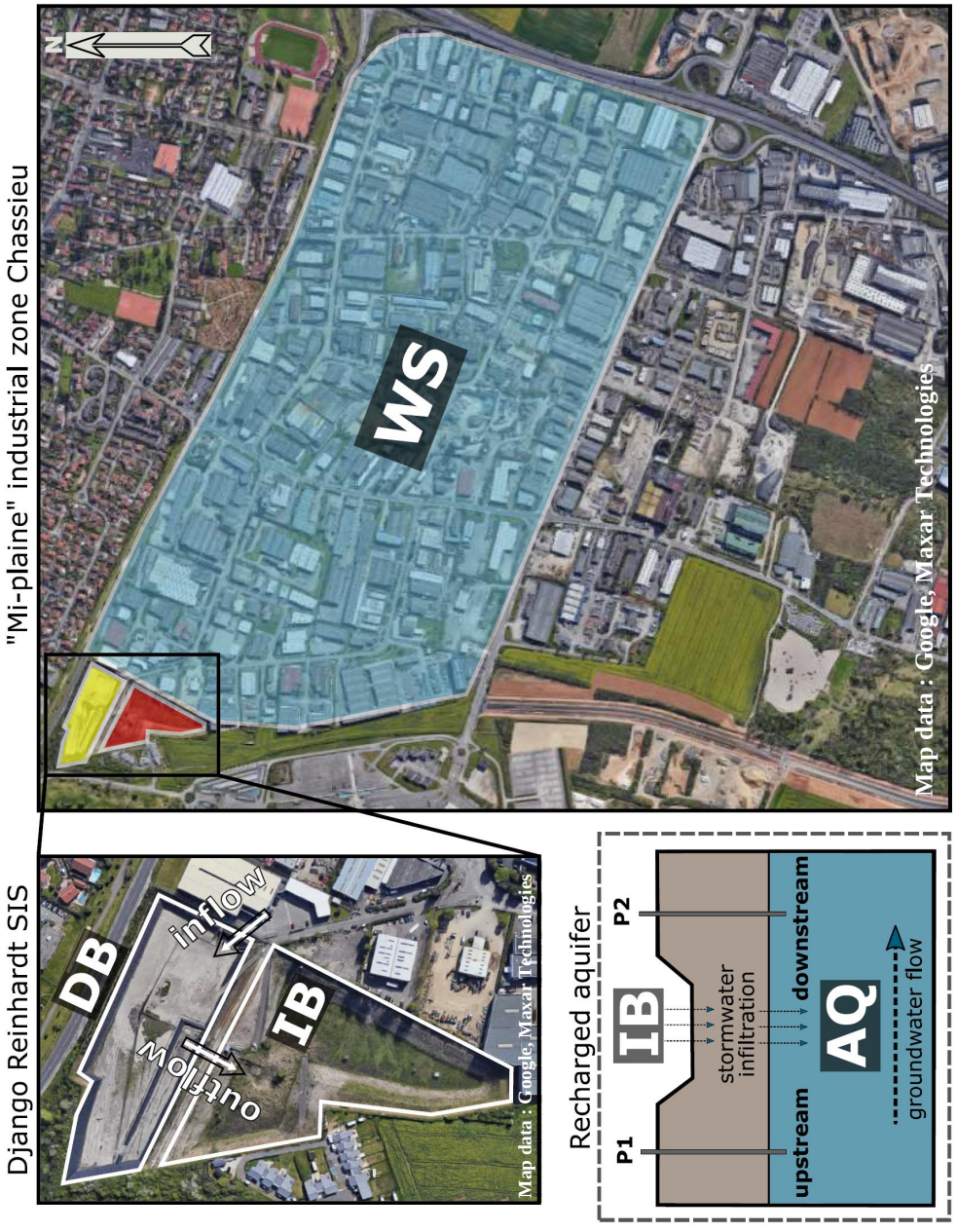
706

707

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

708 Fig. 1 - Colin et al. hess-2020-39



709

710

711

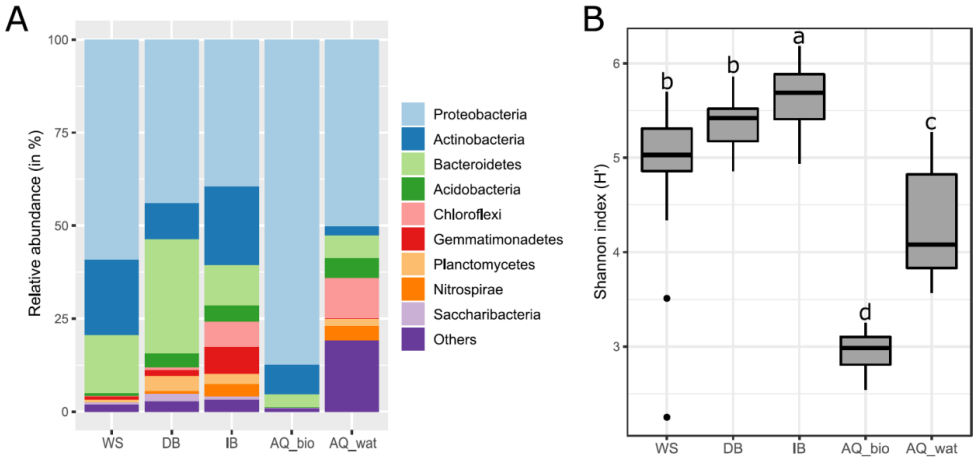
712

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

713 Fig. 2 - Colin et al. hess-2020-39

714

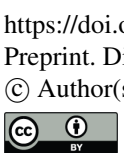


715

716

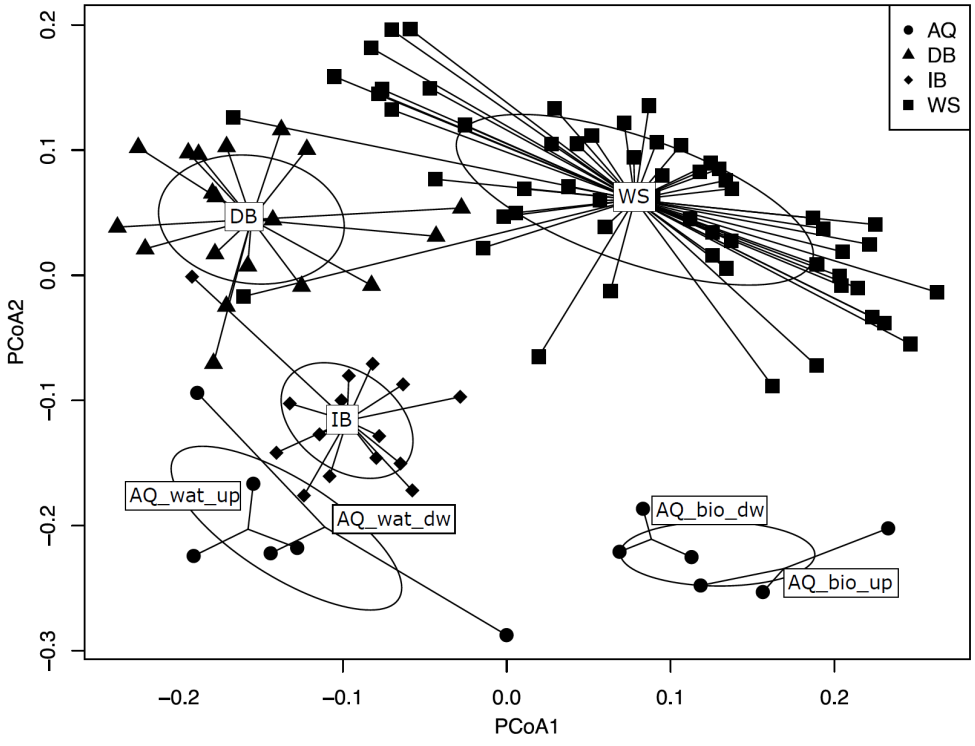
717

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020



Hydrology and Earth System Sciences
Discussions
Open Access
EGU

718 Fig. 3 - Colin et al. hess-2020-39



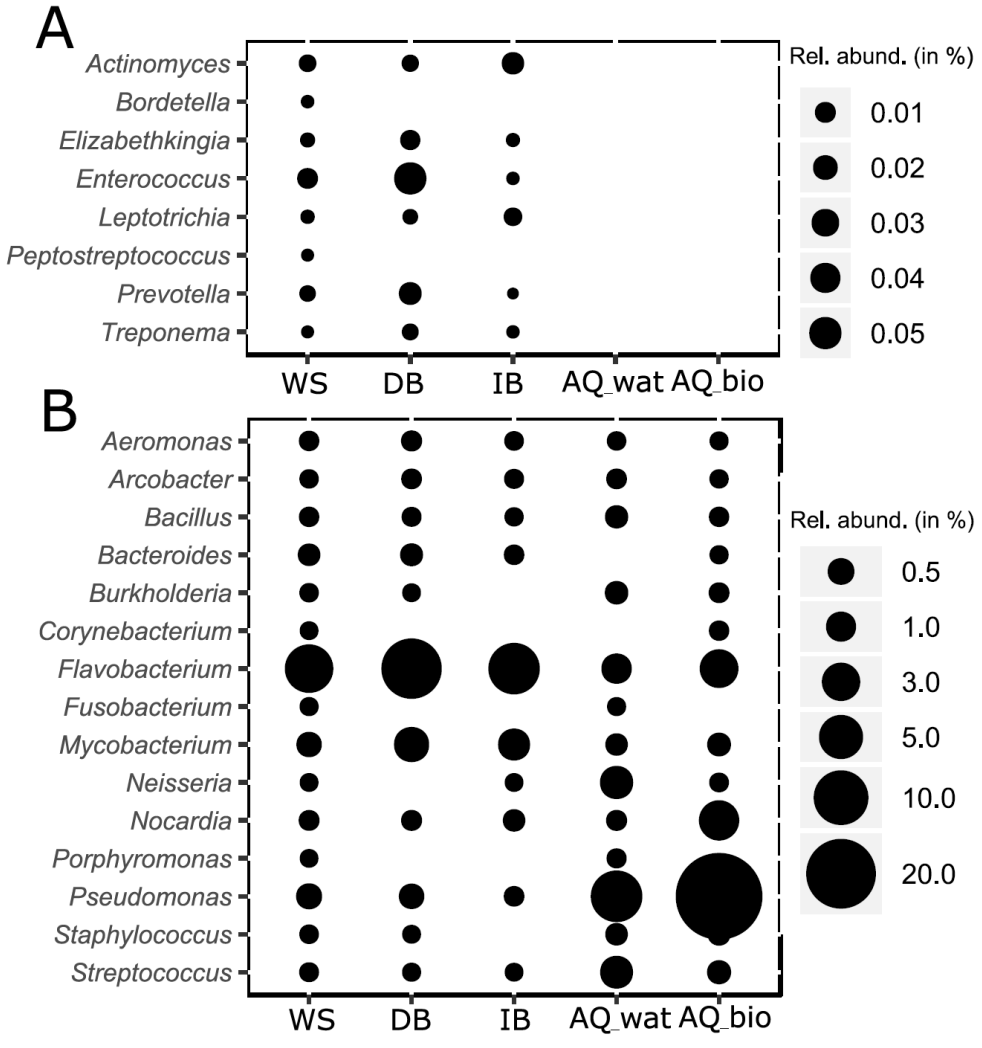
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733

https://doi.org/10.5194/hess-2020-39
Preprint. Discussion started: 17 February 2020

Hydrology and Earth System Sciences
Discussions
Open Access
EGU

734 Fig. 4 - Colin et al. hess-2020-39

735



736