Significant transfer of bacteria from runoff to groundwater impacted by alternative stormwater management techniques

Transfert significatif de bactéries des eaux de ruissellement vers les nappes impactées par les techniques alternatives de gestion des eaux pluviales

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RÉSUMÉ

Les processus d'infiltration des eaux pluviales présentent un intérêt croissant dans les pays développés, car ils contribuent à réduire les phénomènes d'inondation et d'érosion, et permettent de préserver les réserves en eau. Cependant, le ruissellement des eaux pluviales en milieu urbain remobilise divers contaminants dont des microorganismes pouvant présenter un risque pour la santé humaine. Pour évaluer l'incidence des transferts bactériens sur la qualité des eaux souterraines, nous avons effectué une méta-analyse de banques de séquences codant l'ARN ribosomique 16S issus du système d'infiltration des eaux pluviales d'une zone industrielle (Chassieu, France). Les jeux de données analysés englobent plus de 100 échantillons, comprenant des eaux de ruissellement, des dépôts du bassin de rétention, des sols du bassin d'infiltration ainsi que des eaux et biofilms prélevés dans l'aquifère connecté. Une approche Bayésienne permettant le suivi des sources bactériennes avec ces données a révélé que les communautés bactériennes libres dans l'aquifère étaient peu ou modérément affectées par l'infiltration des eaux pluviales. En revanche, les eaux de ruissellement du bassin versant et les dépôts du bassin de rétention ont été identifiés comme les principaux contributeurs de bactéries formant des biofilms à la surface de billes d'argile incubées dans les eaux souterraines. Des genres bactériens potentiellement pathogènes ont été détectés à la fois en surface et dans l'aquifère.

ABSTRACT

Stormwater infiltration processes are of increasing interest in developed countries as they reduce urban floods and soil erosion, and alleviate water shortages. However, while flowing over impervious urban surfaces, stormwaters remobilize diverse contaminants and become a source of nonpoint pollution. Among urban contaminants, microbial pathogens reaching underground waters are one of the most serious threats to human health. To gain insights on the incidence of bacterial transfers on the quality of underground waters, a meta-analysis of bacterial 16S rRNA gene libraries from a model stormwater infiltration system (SIS) of an industrial area (Chassieu, France) was performed. The DNA datasets came from more than 100 samples, including watershed runoff, detention basin deposits, infiltration basin soils as well as waters and biofilms taken from the connected aquifer. A Bayesian bacterial source tracking approach using such DNA datasets revealed that free-living bacterial communities in groundwater were slightly affected by bacteria from the upper parts of the SIS and the watershed. In contrast, bacteria from runoffs and detention basin deposits were found to be the main groups developing as biofilms over clay beads exposed to groundwaters. Potentially pathogenic bacterial genera found in the surface samples were detected in the aquifer samples.

MOTS CLÉS

Bacterial contaminations; Opportunistic pathogens; Stormwater infiltration system

1 INTRODUCTION

The densification and widening of urban areas lead to the replacement of natural lands by impervious structures such as roads, rooftops or parking lots. The reduced infiltration capacity of soils exacerbates the speed and volume of stormwater runoff favoring soil erosion, flooding events and affecting adversely groundwater recharge processes (Shuster et al., 2005). Due to economical and ecological considerations, water resource management tools based on infiltration processes are becoming increasingly popular as they help reduce stormwater discharges to surface waters and participate to groundwater recharge. However, stormwater represents a major source of nonpoint pollution for groundwater, as it flows over impermeable lands and collect hazards related to urban and industrial activities (i.e. chemicals, oil and grease, dirt, salt, sediment, fertilizers and fecal wastes). Among urban contaminants, microbial pathogens reaching recharged waters are one of the most serious threats to human health. Fecal indicator microorganisms and pathogenic bacteria were previously reported in aquifers, even for stormwater infiltration systems (SIS) with thick unsaturated zones (> 10 m) (Li et al., 2015). The transfer and survival of microbial contaminants was suggested to depend on a multitude of factors (land management practices, hydrodynamic forces, physical and chemical soils parameters, water geochemistry, predation and microbial antagonisms) making difficult to predict their fate around and through water management systems. Hence, additional studies are still needed on alternative stormwater management systems to evaluate their ability at preventing microbial transfers which can impact the ecology of underground waters or contribute at the dissemination of hazardous micro-organisms for human health. Here, we present a study evaluating the ability of a well-established SIS, with a thick unsaturated zone (13 m), the Django-Rheinhardt (DjR) experimental site, at preventing bacterial transfers to the connected aquifer.

2 MATERIALS ET METHODS

2.1 Study site

The Chassieu stormwater catchment is located in the suburbs of Lyon (France). It has a surface of 185 ha and host mainly industries. Stormwater and dry weather flows from industrial activities are drained by a network (separated from that of sewers) down to the Django Reinhardt experimental site (Figure 1). This area contains an open and dry retention basin (DB) allowing the settling of coarse and medium size particles in the form of sedimentary deposits. Water is then discharged to a second basin (IB) located above an aquifer (AQ) to facilitate stormwater infiltration. This infiltration basin is instrumented by the Field Observatory in Water Management (OTHU).
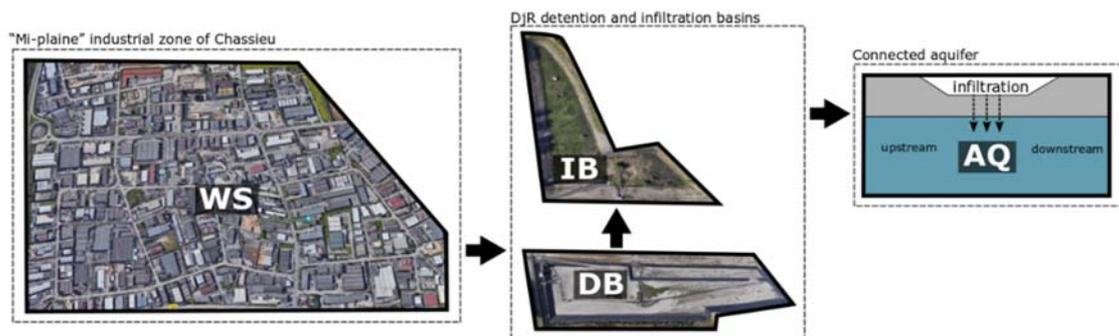

Figure 1. Stormwater infiltration system in the Chassieu industrial area (Lyon, France). WS: Watershed runoff waters; DB: Detention basin deposits; IB: soil of the Infiltration basin and AQ: Aquifer.

2.2 Characterization of bacterial communities

Bacterial communities present in watershed runoff waters (WS), detention basin deposits (DB), infiltration basin soils (IB) and the connected aquifer (AQ) were characterized using a 16S rRNA gene meta-barcoding approach. In the aquifer, bacterial diversity analyses were performed from underground water samples (AQ_wat) and biofilms developing over clay beads (AQ_bio) that could be recovered from piezometers, as indicated in Mermillod-Blondin et al. (in review). The datasets analyzed were partially recovered from previous investigations (DB: Marti et al., 2017; AQ_wat: Voisin et al., 2018 and AQ_bio: Mermillod-Blondin et al. (in review), and were completed with novel 16S rRNA gene libraries performed on IB and WS samples. Previous investigations had not considered the entire path of urban waters from the industrial watershed towards the SIS-impacted underground waters. After filtering, quality control and chimera removal (see Marti et al. (2017) for the procedures used), 16S rRNA gene

sequences were aligned and operational taxonomic units (OTUs) were defined at < 3% identity. To avoid any biases associated with different numbers of sequences among the samples, random subsampling was performed (20,624 reads per sample). The source tracker program (v0.9.1), a Bayesian approach, was used for estimating the proportions of 16S rRNA gene OTUs of surface samples (soils, sediments, WS waters) among the connected aquifer (Knights et al. 2011). All surface samples (WS, DB and IB) were considered as "sources", while water and biofilm samples taken from the aquifer (AQ_wat and AQ_bio) were designated as "sinks". SourceTracker was run with the default settings, and dirichlet hyperparameters were tuned using cross-validation (alpha1=0.001 and alpha2=1). Relative standard deviations (%RSD) were calculated for improving overall predictive confidence and only results showing %RSD <100 were considered.

3   RESULTS & DISCUSSION

3.1   Total bacterial community composition and diversity

The analysis of V5-V6 *rrs* gene libraries yielded 2,124,272 high-quality sequences that were allocated into 10,231 OTUs. Whatever the compartment considered, 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%) (Figure 2A). At the OTU level, the structure of bacterial communities was found to change markedly across the studied water path, with samples being clustered according to their compartment of origin (PermANOVA; F=20.7, p<0.001). Overall, aquifer samples harbored a microbiome that was significantly less rich and less diverse in V5-V6 *rrs* OTUs than those from the upper compartments (ANOVA, p<0.001) (Figure 2B). The decline of bacterial diversity in aquifer is probably related to the limited carbon and nutrient levels favoring bacterial communities adapted to oligotrophic conditions. Moreover, significant differences were further observed in the aquifer, where the bacterial composition and alpha-diversity varied significantly depending upon the sample type. Specifically, biofilms (AQ_bio) were found less diverse than groundwater samples (AQ_wat) and were largely dominated by Proteobacterial taxa known to adhere efficiently to surfaces (Figures 2A and 2B).
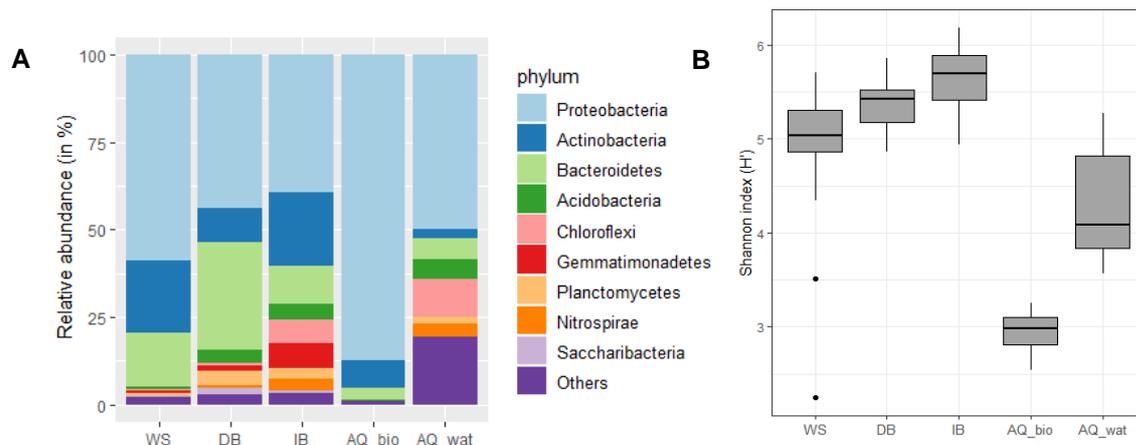

Figure 2. Changes in relative abundance of the main bacterial phyla (A) and alpha diversity (Shannon index) (B) in the different compartments of the studied water paths. WS: Watershed runoff waters; DB: Detention basin deposits; IB: soil of the Infiltration basin; AQ_bio: Aquifer biofilms and AQ_wat: Aquifer waters.

3.2   Bacterial taxa shared between top and underground compartments

Based on the SourceTracker analysis, only a low impact of bacterial V5-V6 *rrs* OTUs from stormwater infiltration was evidenced on the free-living bacterial communities in the aquifer. Nevertheless, water samples collected downstream of the infiltration basin logically tended to be more affected by the OTUs stormwater inputs (Table 1). In contrast, noticeable levels of OTUs from WS and DB samples were consistently detected in groundwater-induced biofilms. OTUs from watershed runoff waters and detention basin deposits represented on average 56.8-67.8% and 16.9-34.3% of the bacterial communities of groundwater biofilms, respectively (Table 1). An increased contribution of OTUs from detention basin deposits was evidenced in biofilms collected downstream of the basin in an infiltration zone of the aquifer impacted by stormwater infiltration (34.3%) compared to biofilms collected upstream in the aquifer (16.9%). Microbes absorbed on solid surfaces being considered to be dominant in groundwater systems in terms of biomass and activity (Iribar et al., 2008), allochthonous bacteria occurring in biofilms are therefore likely to have a significant impact on the groundwater ecosystem.

Table 1. Bacterial SourceTracker evaluation of contamination sources in water and biofilm samples from the aquifer but collected upstream or downstream of SIS.

Aquifer samples		% Source detected			
		WS	DB	IB	Others
Waters	upstream	0.7%	0.6%	1.0%	**97.3%**
	downstream	18.4%	9.9%	6.4%	**65.1%**
Biofilms	upstream	**67.8%**	16.9%	1.9%	**13.7%**
	downstream	**56.8%**	**34.3%**	1.0%	**8.3%**

A detailed analysis of the meta-barcoding datasets revealed that surface and aquifer samples had in common 1,022 OTUs. Interestingly, 2 of the most abundant bacterial genera shared between the surface and underground samples, namely *Flavobacterium* and *Pseudomonas*, contain taxa that may pose a risk to human health (Bernardet & Nakagawa, 2006; Mena & Gerba, 2009). *Flavobacterium* prevailed in the surface samples (6.9-13.4%) and was also observed in the aquifer (AQ_bio=3.12% and AQ_wat=1.1%). Reads assigned to *Pseudomonas* were in a lower proportion in the upper compartments (0.03-0.4%) than the aquifer samples (AQ_bio=35.5% and AQ_wat=8.4%).

4   CONCLUSION

Although the Django Reinhardt basin is characterized by a thick unsaturated zone serving as a natural filter for stormwater infiltration, a large fraction of the bacterial communities occurring in watershed runoff water and the detention basin were detected in the connected aquifer. The bacterial taxa likely transferred to the connected groundwater were apparently highly efficient colonizers of solid surfaces. They were found to dominate biofilms developing on clay beads introduced into the aquifer through the use of piezometers. Potentially pathogenic bacterial genera were recorded among the samples. These particular taxa will need to be further characterized from a taxonomic and functional point of view to determine their impact on the groundwater ecosystem and to assess their virulence.

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