Altering the discharge regime of a combined-sewer overflow induced a significant drop in allochthonous bacterial taxa in the microbial communities of the receiving stream: the big benefit of a small change.

La modification du régime de surverse d'un déversoir d'orage a entraîné une baisse significative des taxons bactériens allochtones dans les communautés microbiennes du cours d'eau récepteur : le grand bénéfice d'un petit changement.

Adrien C.M. POZZI [a,b,f], Stéphanie PETIT [a,b], Laurence MARJOLET [a,b], Benjamin YOUENOU [a,b], Evelyne BORGES [a,b], Françoise MAURIN [a,b], Philippe NAMOUR [c], Oldrich NAVRATIL [a,d], Laurent SCHMITT [e], Flora BRANGER [c], Pascal BREIL [c], Benoit COURNOYER [a,b,g].

[a] UNIVERSITE DE LYON ; [b] UMR ECOLOGIE MICROBIENNE LYON, CNRS 5557, INRAE 1418, Univ. Lyon 1, VetAgro Sup, Marcy L'Etoile ; [c] UR RIVERLY, INRAE Centre Lyon-Grenoble Auvergne-Rhône-Alpes, Villeurbanne ; [d] UMR ENVIRONNEMENT VILLE SOCIETE, CNRS 5600, Univ. Lyon 2, Lyon ; [e] UMR LABORATOIRE IMAGE VILLE ENVIRONNEMENT, CNRS 7362, ENGEES, Univ. Strasbourg ; [f] adrien.meynier_pozzi@vetagro-sup.fr ; [g] benoit.cournoyer@vetagro-sup.fr.

RÉSUMÉ

Les déversoirs d'orage (DO) connectés aux réseaux unitaires rejettent dans les rivières un mélange d'eaux usées et de ruissellement afin d'éviter une montée en charge des réseaux et les dégâts associés lors de forts évènements pluvieux. Suite à la directive européenne 91/271/CEE, ces rejets sont limités par des réglementations nationales dont l'impact écologique positif reste à démontrer. Une étude de terrain de long terme a été conduite pour comparer la qualité (i) des eaux superficielles, et des sédiments (ii) benthiques et (iii) hyporhéiques de tronçons de rivière, avant et après la mise en conformité d'un DO. Un jeu de données multidisciplinaire (hydrologie, physico-chimie, polluants urbains, bactéries indicatrices de contaminations fécales et communautés de bactéries *tpm*+) a été analysé. Outre la démonstration qu'un petit changement apporté au DO a entraîné de grands changements dans les régimes de surverse et une baisse significative des apports, cette étude a mis en évidence des espèces bactériennes dont les patrons de distribution dans les sédiments correspondent aux apports provenant du DO, et a conduit à proposer certaines espèces comme sentinelles écologiques pour l'évaluation de l'impact des DO. Les résultats de ce travail sont destinés simultanément à faire progresser la compréhension fondamentale de l'écologie microbienne des rivières, et à guider les gestionnaires dans l'évaluation de l'efficacité des changements de régime de surverse des DO sur l'état écologique des cours d'eau récepteurs.

ABSTRACT

To protect wastewater treatment plants from excessive water inflow during rainy weather, combined-sewer overflows (CSO) discharge an untreated mixture of storm water runoffs and wastewater in rivers. Following the 91/271/EEC European Directive, these discharges are now limited by national regulations whose positive ecological outcome remains to be demonstrated. A long-term interdisciplinary field study was designed to compare freshwaters, benthic and hyporheic sediments from river reaches with similar geo-morphological units but located upstream or downstream a CSO release point, either before or after the CSO was made compliant. A multidisciplinary dataset (hydrology, physical-chemistry, urban pollutants, fecal indicator bacteria, and *tpm*-harboring bacterial communities) was analyzed. Besides the demonstration that a small change made to a CSO resulted in big changes in overflow regimes and in a significant drop of inputs, this study highlighted bacterial species whose distribution patterns in sediments matched inputs coming from the CSO waters and led to the proposal of some species as ecological sentinels for the assessment of CSO impact. The outcomes of this work are simultaneously to advance the fundamental understanding of the microbial ecology of rivers, and to guide facility managers in the assessment of benefits related to changes in CSO regimes.

KEY WORDS

Monitoring of combined sewer overflows (CSOs), scientific evaluation of public policies, microbial communities indicative of the ecological status of watercourses, urban wet weather flows (UWWF) and pollutants.

1 INTRODUCTION

1.1 Combined sewer overflows in intermittent rivers: why it matters

About half of the world's continental surface waters are categorized as intermittent or non-perennial (IRES, Intermittent Rivers and Ephemeral Streams). IRES are often headwaters of watersheds of larger and perennial watercourses providing important ecosystem services. They are inhabited by a large diversity of organisms, including microbes. IRES are highly fluctuating environments with high and low waters closely related to seasonal climate variations and rainfall patterns. Extreme or repeated rainfall can lead to quick runoff water transfers into the stream, together with microbes and pollutants collected on surfaces exposed to precipitation, thus posing a threat to the stream ecology (Navratil *et al.,* 2020).

Combined-sewer overflows (CSOs) represent a special case of discharges to IRES. First, because they discharge an untreated mixture of stormwater runoffs and wastewater in the receiving waters, both comprising somewhat overlapping microbes and pollutants associated with human activities in the watershed. Second, because the large water volumes delivered in a short time period during high rain events could have a high ecological impact at low flow, when discharges can contribute to up to 100% of the waters flowing in an IRES, which makes them more sensitive to overflows than perennial rivers with steadier dilution capacity.

1.2 The challenge of assessing changes made to combined sewer overflows

Despite their impact on the receiving waters, CSOs are common. In order to mitigate their effect, the 91/271/EEC European Directive was adopted and enforced by the European state members (e.g. guidelines of 2015/07/21 and of 2020/07/31 for the French Republic). These guidelines require (i) a limitation of CSO discharges to less than 5% of the wastewaters collected by a municipality sewer system; or (ii) that each CSO should release waters for less than 20 days per year (no matter the number and volume of the overflows per day).

Compliance is being achieved by technical modifications of CSOs. However, their positive ecological outcome on the receiving waters remains to be demonstrated. A challenge in the assessment of these modifications would be to distinguish the impact of waters delivered by a CSO (likely spreading fecal bacteria) from those of runoff waters coming from the roads or other routes (also contaminated by fecal bacteria; Marti *et al.,* 2017), especially during heavy rainfall events when runoff waters can represent the main component of CSO waters.

1.3 Experimental setup and hypotheses

To address these issues, a long-term field study was designed using the Chaudanne stream, an IRES impacted by CSOs and connected to the Yzeron river, a tributary of the Rhône river. This study allowed a comparison of surface waters as well as benthic and hyporheic sediments from river reaches with similar geo-morphological units but located upstream and downstream a CSO. Using a community coalescence approach that considers communities as the best-fit assemblages of communities from all the main sources feeding a river system, we were able to subtract the effect of direct CSO discharges from those of the stormwater runoffs discharged upstream the CSO.

The leading hypothesis was that reducing CSO events and volumes would lead to a significant reduction of chemicals, nutrients and biological inputs from the combined sewer that, in turn, would trigger a reassortment of bacterial taxa through a decrease in opportunistic colonizers coming from the sewer system among the river bacterial communities. The secondary hypotheses were that the expected reduction in the CSO impact would be (i) greater after a discharge at low waters than at high waters, based on the dilution power of the stream, (ii) greater in riffles and runs than in pools (based on Namour et al., 2015), and (iii) best detected in sediments than in running water, based on the difference in lability between the two.

2 MATERIALS AND METHODS

2.1 Study site

The Chaudanne stream (G5, under the Rosgen classification for natural rivers) is 4.7 km long and drains a small catchment of 3.5 km^2 which is a long term ecological research site part of the Urban Water Field Observatory of Lyon (OTHU). The bedrock geology consists of gneiss and the land use is mixed with urban areas (36.6 %), grasslands (32.7 %), farming (22.1%), and forests (8.5%). The climate is temperate (Cfb, under the Köppen–Geiger climate classification) with Mediterranean and continental influences producing periods without precipitation interspersed with heavy thunderstorms in Summer, and high flow in Winter. Around 2.3 km from its source, a first CSO outlet crosses its channel at a location called “la Barge”, Grézieu-la-Varenne (France) where the study was conducted.

2.2 Data acquisition

Rainfall, river water height, and flow of CSO, were continuously monitored over a 14-year period covering the two CSO regimes investigated in this study, before and after the CSO weir crest was raised by 15 cm. Samples were taken at 3 distinct periods (no CSO at low waters, CSO at either low or high waters), in 3 geomorphological units (run, riffle, pool) and from 3 river compartments (freshwater, benthic or hyporheic sediments), in order to identify when and where the CSO impact and its expected reduction are greatest. At each sampling point and following standards for water & sediments, an interdisciplinary framework helped gather a multi-dimensional dataset depicting the river hydrology, physical chemistry, pollutants (MTE, PAH), nutrients, fecal indicator bacteria (FIB), and *tpm*-harboring bacterial community.

2.3 Data analyses

To assess the diversity and composition of bacterial communities while at the same time track organisms down the species level or below, we used a state-of-the-art metabarcoding method which targets the bacterial *tpm* gene encoding a detoxifying enzyme. Community coalescence between allochthonous and riverine *tpm*-harboring bacteria was assessed by estimating the relative contributions of upstream river and CSO "source" communities to a given "target" community downstream the CSO by an accurate probabilistic method able to handle taxa from an unknown source (*FEAST*). Reliable indicator taxa and environmental thresholds (evidence of community impacted by overflows) were identified (*Indicspecies* and *TITAN2*). Co-occurrences between bacterial indicators and other CSO inputs were also investigated.

3 RESULTS AND DISCUSSION

3.1 Changes in CSO regimes triggered by the raise of the weir crest

Making the CSO compliant has resulted in: (i) a decrease in the annual frequency of CSO overflow events with fewer than 20 days per year, as well as a decrease in the annual volume discharged to the river; (ii) a change in seasonality, with overflows now primarily concentrated in the Summer time when these events previously occurred throughout the year; and (iii) a change in the distribution of discharge volume and duration, with both an increase in average volume and duration (small discharges no longer occur), and a decrease in peak volume and duration (CSO waters at the beginning and end of each event are directed towards the unitary sewer instead of the river, the raised weir crest acting as a buffer).

3.2 Contaminant bacteria from the CSO as indicator of CSO impact

After the CSO was modified, (i) overflows still increased bacterial richness in downstream sediments, but no longer evenness (CSOs now introducing fewer new taxa that can coalesce with the river bacterial communities, and not changing the abundance of taxa already present upstream), and (ii) the contribution of CSO communities to downstream ones significantly decreased (ii.a) in benthic and hyporheic sediments; (ii.b) when no CSO was recorded within a month; and (ii.c) in run and riffle geomorphic units. Hundreds of *tpm*-harboring bacteria were detected as contaminants from the CSO, *Pseudomonas* and *Aeromonas* being the most frequent genera. The distribution of some of their species match those of other CSO inputs.

4 CONCLUSION AND FUTURE DIRECTIONS

A high-resolution *tpm* meta-barcoding dataset was generated together with environmental variables describing the hydrology, physical-chemistry, nutrients, urban pollutants and FIBs at the study site. Original analyses such as community coalescence and identification of reliable indicator taxa demonstrated the occurrence of CSO bacteria in the community assemblages of sediments downstream a CSO, and led to the proposal of several species as ecological sentinels for the assessment of benefits induced by decreasing CSO events. Their relevance will be evaluated on another site equipped with the newly developed DSM-flux (Device for Stormwater and combined sewer flows Monitoring), a technology allowing the interception of particles associated with chemical and microbiological contaminants. This work paves the way for future modeling and predicting of the impact of CSO compliance on watercourses using bacterial indicators.

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