

Microbial fingerprint for source tracking in surface water management

The quality of Dutch surface water is under pressure, partly due to sewage overflows, and effluent discharges from wastewater treatment plants. These can potentially contain faecal or other harmful micro-organisms. Is DNA fingerprinting suitable for identifying microbial contaminants, and detecting the location of the source?

Because of sewage overflows and discharges of effluent from wastewater treatment plants, surface water managers face changes in the quality of the surface water. From a management perspective, it is important to know precisely where these discharges originate from. Water managers (municipalities and water boards) have the following questions in this respect:

1. Is the impact of sewage overflows on the surface water recognisable and is it traceable to a specific overflow?
2. Is the discharge of effluent from wastewater treatment plants traceable, and if so, up to how far from the source?
3. Is leakage to the groundwater from a wastewater treatment plant's aeration tank or from the effluent traceable, and up to what distance?
4. Is the origin of surface water that discharges into other surface waters (e.g. polder water in flood storage basins) traceable?

Each type of water (surface water, effluent from wastewater treatment plants, sewage, groundwater) has its own specific microbial composition, or unique microbial 'fingerprint'. The quality of the effluent from wastewater treatment plants varies over time, however (Emissions Register; 2019), as does the frequency and extent of sewage overflows. This study aims to establish whether the microbial fingerprint can be used to answer the questions above.

Study design and method

To determine a microbial fingerprint, a marker gene in the DNA of all micro-organisms in the water is mapped. This is done using NGS (*Next Generation Sequencing, NGS*), a technique that is able to decipher the code of very many different DNA molecules simultaneously.

For this study, samples were taken of the influent and effluent from five wastewater treatment plants and of surface water (32 locations). For question 3, samples were also taken from the wastewater treatment plant aeration tank and four groundwater monitoring wells. The samples were taken over the periods of June-November 2017 and March-September 2018. DNA was isolated from the samples to determine the microbial fingerprint. This was done in two stages: specific DNA sequences were

determined with NGS, and then with bioinformatics software (Schloss et al., 2009 and Andersen et al., 2018) to establish the associated microbial fingerprint. Finally, the *Source Tracker* tool (Knights et al., 2011) was used to determine the source type of the microbial fingerprints.

Determining microbial fingerprints

For each location (wastewater treatment plant), an average was taken of the results of the samples analysed. Figure 1 shows the averages for the most common microbes for, respectively, the effluent and the influent (sewage) from the wastewater treatment plant. The effluent has a recognisable profile with characteristic microbial groups such as *Saccharimonadales*, *Neisseriaceae* and *Fodinicola*. The fingerprint of effluent appears to differ for each wastewater treatment plant, more than the profile of sewage.

Wastewater treatment plant influent (sewage) has a very typical microbial community, comprising both faecal micro-organisms (originating from the intestines of humans and animals) and bacteria that mainly grow in the sewage. Characteristically, these include *Arcobacter*, *Acinetobacter*, *Aeromonas* and *Trichococcus*.

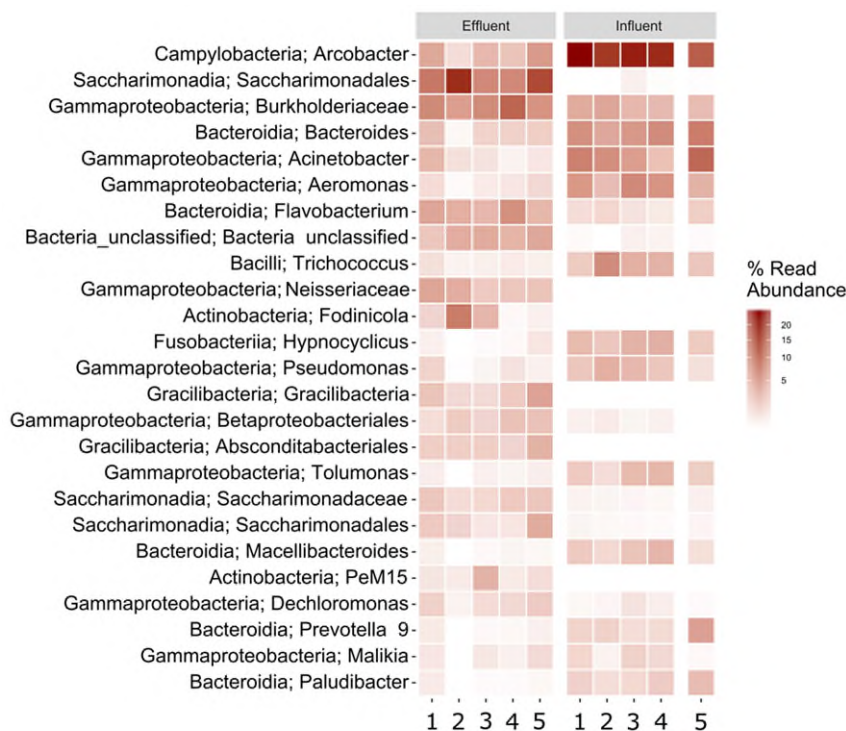


Figure 1: The 'microbial fingerprints' of wastewater treatment plant-effluent and influent. The 25 most common microbial genera can be seen in both sample types. The occurrence (abundance) is expressed as an average relative abundance (% read abundance) per sample type (average of the various times samples were taken) at the sample sites 1 to 7.

Were the questions answered?

Question 1: Tracing sewage overflow

Its highly specific profile makes sewage from overflows traceable in the receiving surface water. From the microbial fingerprint, it is thus possible to determine to what extent a body of surface water is impacted by overflows. Furthermore, we not only see the same specific and stable image of sewage in the Netherlands, but also in the United States, Australia, Brazil, China and Spain, for example (McLellan et al., 2019). Wastewater is therefore probably traceable everywhere by means of these indicator bacteria.

The flip side is that wastewater from different locations and different points in time is so similar that it was impossible to determine precisely which overflow the micro-organisms found originated from if several overflows are active in the area. If a sample had been taken at a point very close to an overflow, this could have been possible, and the overflow frequency could possibly have been determined.

Question 2: Tracing of effluent from wastewater treatment plants

To determine the traceability of effluent in surface water, a total of 32 samples were taken at different times of the year from five points in the receiving surface water (Stream 1) and in the water into which Stream 1 discharges (Stream 2). Figure 2 shows the sample points.

The surface water samples from immediately before and after the wastewater treatment plant discharge point were compared with the effluent using the *Source Tracker* tool. This tool is able to quantify to what extent the microbial population of a given water sample is influenced by that of another. This is also shown in figure 2.

It can be seen that the microbial population of sample site 1 is 41% under the influence of the population from wastewater treatment plant effluent. It was not determined whether this is effluent from the (downstream) nearby wastewater treatment plant, or effluent from other wastewater treatment plants. Sample site 3 (just after the wastewater treatment plant) contains a higher percentage of wastewater treatment plant effluent (70%). Furthermore, at different sampling times, sample site 1 was far more greatly influenced by wastewater treatment plant effluent. The composition of the effluent (sample point 2) also varies over time; the microbial profile of the effluent and the proportion of it in surface water thus varies over the seasons.

Sample site 4 is located in a larger body of receiving surface water (Stream 2), upstream of the point where stream 1 discharges, and there is virtually no influence from the wastewater treatment plant effluent. Downstream, at sample site 5, the effect is clearer; far more limited than at sample site 3, but still perceptible.

It can be concluded that the microbial fingerprint presents opportunities to indicate the influence of wastewater treatment plant effluent on surface water quality, but reducing it to a specific wastewater treatment plant

appears more difficult.

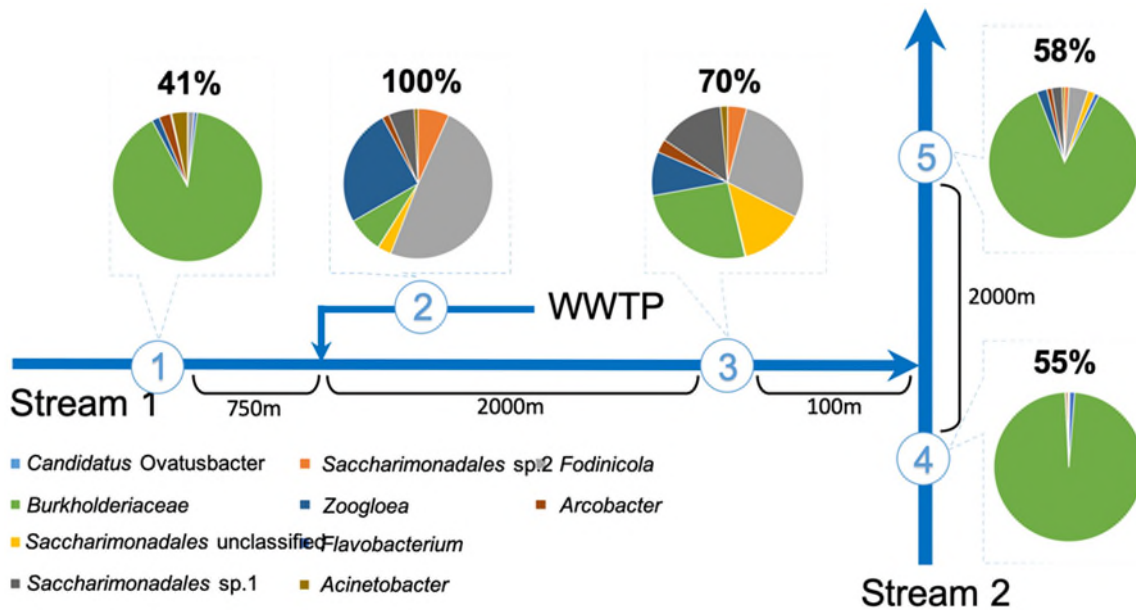


Figure 2: Microbial fingerprint of the measurement sites 1 to 5. Situation: wastewater treatment plant (WWTP) discharges effluent into Stream 1, which discharges into Stream 2. The pie charts show the relative proportion in the samples (calculated in %, using Source Tracker) of some characteristic bacterial genera associated with the fingerprint of wastewater treatment plant effluent.

Question 3: Is leakage to groundwater traceable?

A further application of NGS is in determining possible leakage from a wastewater treatment plant into the groundwater. To this end, samples were taken throughout the year from a) the groundwater around a wastewater treatment plant, b) wastewater treatment plant influent, activated sludge from the aeration tank and effluent and c) the receiving surface water.

The microbial profile of influent, effluent and the aeration tank was traceable in the groundwater (monitoring wells). This indicates leakage. It was notable that chemical analyses (NH_4 and CZV) carried out earlier showed no traces of leakage. Examination using the microbial fingerprint may therefore be interesting in detecting leakages from the wastewater treatment plant.

Question 4: Are mixed surface waters traceable?

The measurements in the surface water systems examined show that the fingerprint of a water system has specific characteristics; these vary over time, however. To obtain a precise picture of the effect of the outflow of one surface water flow into another water flow, an accurate fingerprint is needed; to do this, it is necessary to take measurements from both flows over time (over the seasons).

Follow-on research and applications

This study clearly shows that the microbial fingerprint, together with the *Source Tracker*, can in principle be used to trace the origin and relative influence of water types.

The task now is to determine how this method can be applied in practice to the research and monitoring of (waste) water management. Among other things, our results need to be validated by comparing them with the results from traditional methods of determining water quality, such as *E. coli* and *enterococcal* colony counts.

A further interesting aspect for follow-on research is to look at the extent to which quantitative data (the *volume* of 'different' water flowing in) can be derived from qualitative data (the type and relative volume of a given microbial population in a water type). A follow-on phase could then compare flow data and nutrient loads from water system analyses with the results from the microbial fingerprint.

Another question is: how does a population of micro-organisms from wastewater treatment plant effluent develop in the time following discharge into surface water?

The microbial fingerprint could also help to answer the question of what is the optimal microbiological composition of ecologically healthy water, and whether it complies with the European Water Framework Directive.

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Summary

Wastewater overflows, leakages of wastewater and the discharge of effluent from wastewater treatment plants have a major influence on surface water quality. The task now is therefore to have the capacity to effectively monitor the influence of sewage and wastewater treatment plant effluent on groundwater and surface water. Wastewater and wastewater treatment plant effluent have characteristic microbial fingerprints, which fluctuate little over time compared with surface water and groundwater. This study demonstrates that this fingerprint can be used to effectively trace sewage and wastewater treatment plant effluent in groundwater and -surface water.

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