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Changes in the planktonic microbial community during residence in a surface flow constructed wetland used for tertiary wastewater treatment

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HIGHLIGHTS

- Treated wastewater contains a distinct unnatural, highly active bacterial community.
- Constructed wetlands decrease bacterial metabolic activity and functional diversity.
- The bacterial community composition changed especially in reed bed systems.
- Outflowing planktonic bacterial community closely resembles natural ditch communities.
- Constructed wetlands can protect surface waters from anthropogenic bacteria.

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ABSTRACT

Suspended particles are a major constituent of municipal wastewater and generally contain high levels of bacteria, including human pathogens. Discharge of these particles of anthropogenic nature can have profound effects on receiving aquatic ecosystems and mitigation of these effects requires additional polishing of treated municipal wastewater. Previously it was shown that surface flow constructed wetlands are effective in improving water quality by reducing the numbers of fecal indicator organisms. However, fecal indicator organisms represent only a minor fraction of the total planktonic bacterial community and knowledge on the effects of these constructed wetlands on the composition and functioning of the entire planktonic bacterial community is limited. The aim of this descriptive study was therefore to identify changes in the planktonic bacterial community during residence of secondary treated municipal wastewater in a full-scale surface flow constructed wetland.
To this purpose water samples were taken in which the bacterial community composition and functioning were analyzed using FISH, DGGE and BIOLOG. Surprisingly, the bacterial abundance at the inflow of the constructed wetland was relatively low compared with more natural surface waters.
However, the inflowing bacterial community showed high metabolic activity and functional diversity. During residence in the surface flow constructed wetland the bacterial abundance doubled, but decreased in metabolic activity and functional diversity. Shifts in the community composition indicate that these changes are related to turn-over of the bacterial community. The planktonic bacterial community in the effluent of the constructed wetland closely resembled natural bacterial communities in urban and agricultural ditches. Based on these observations we conclude that constructed wetlands are capable to mitigate possible impacts of the particle load in treated wastewaters by transforming the anthropological bacterial community to a bacterial community resembling more "natural" surface waters.

1. Introduction

Discharge of municipal wastewater is a major source of anthropological impacts on aquatic ecosystems and therefore it is common to treat municipal wastewater before discharge. Treatment of raw municipal wastewater by wastewater treatment plants (WWTPs) strongly improves water quality, but treated municipal wastewater generally still contains high levels of nutrients, organic matter and high densities of heterotrophic bacteria originating from the WWTP (Tchobanoglous et al., 2004; Seviour and Nielsen, 2010). The discharge of treated wastewater can therefore still have a profound impact on receiving surface waters (Seviour and Nielsen, 2010; Holeton et al., 2011) and mitigation of this impact requires additional polishing of treated municipal wastewater. To this purpose, several techniques are available, but for the polishing of treated municipal wastewater surface flow constructed wetlands (CWs) are often favored (Kadlec and Wallace, 2008). Surface

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flow CWs improve the water quality by reducing nutrient concentrations, decreasing the numbers of fecal indicator organisms and improving the oxygen regime (Sundaravadivel and Vigneswaran, 2001; Vymazal, 2005; Kadlec and Wallace, 2008; van den Boomen and Kampf, 2012).

The removal of particulate matter from the water is another purification process occurring in these systems, but is only profound in CWs receiving relative high concentrations of suspended particles while CWs receiving low concentrations of suspended particles are seemingly ineffective in removing suspended particles (Ghermandi et al., 2007; van den Boomen and Kampf, 2012). However, all studies on particle dynamics in CW analyze bulk concentrations of suspended matter only, while suspended particles consist of particles of highly variable type and origin (Wotton, 1994) and includes not only dead organic and inorganic particles, but also zooplankton, phytoplankton and bacterioplankton. Especially bacteria are regarded as important players in many biological processes and biochemical cycles in aquatic ecosystems (Kalff, 2002) and the anthropogenic nature of the bacterial community in treated municipal wastewater may therefore influence the functioning of receiving aquatic ecosystems. It is expected that residence in the different functional compartments of a CW could strongly change the nature and composition of the planktonic bacterial community. However, besides detailed observations on the removal of bacterial fecal indicator organisms (Kadlec and Wallace, 2008; Vymazal, 2005; Reinoso et al., 2008; Molleda et al., 2008; Karim et al., 2004; Ghermandi et al., 2007; Diaz et al., 2010), knowledge about changes in the planktonic bacterial community during residence in CWs is virtually nonexisting. Therefore the aim of this descriptive study was to identify changes in the planktonic bacterial community during residence in a surface flow CW.

To meet this aim, we set up a sampling campaign in a full scale surface flow CW consisting of unvegetated ponds and reed beds (*Phragmites australis*), that receives secondarily treated municipal wastewater with low concentrations of suspended particles (3.6 mg L^{-1}; Mulling et al., 2013; van den Boomen and Kampf, 2012; Ghermandi et al., 2007). Water samples were taken at five points in the CW and structural and functional changes in the bacterial community were described using FISH, DGGE and BIOLOG. In addition, we analyzed water samples from six different types of surface waters for comparisons between the bacterial communities in the CW and more natural bacterial communities.

2. Material and methods

2.1. Site descriptions and sampling

This study was conducted at seven locations in The Netherlands (Fig. 1). The main location was a full scale surface flow constructed wetland (CW) located in Grou that was built in 2006 and receives a constant hydraulic loading of 1200 m^3 day^{-1} of secondary treated wastewater (mainly from municipal sources). After inflow into the CW, the treated wastewater flows through three unvegetated ponds connectedin series, then through four parallel reed beds and a collection pond after which the water is pumped into receiving surface water (Fig. 1). The ponds are open water systems without vegetation with an average depth of 1.35 m, a volume between 360 and 440 m^3 each and a combined hydraulic retention time (HRT) of 17.9 h (Fig. 1). The reed beds are covered with *P. australis* and have an average water depth of 40 cm, an approximate volume of 443 m^3, and each receive a hydraulic loading of $\pm$300 m^3 day^{-1} with an average HRT of 23.6 h. The total HRT of the CW is 41.5 h (Mulling et al., 2013).

Samples were taken in June 2010 as grab samples 10–20 cm below the water surface at five different locations in the CW: at the in- and outflow of the unvegetated ponds and at the outflow of the reed beds (further referred to as PONDS-IN; PONDS-OUT; REED-BEDS-OUT respectively) and in the middle of these compartments (further referred to as PONDS and REED-BEDS respectively) (Fig. 1). The six surface waters, which served as reference sites, were chosen to reflect a wide range of possible surface waters used for the discharge of treated wastewater. In this way the reference sites were used to indicate the direction of change in the bacterial community in comparison with different types of receiving surface waters. Locations could be described as an artificial fen created by sand excavation and fed by rain and groundwater, an agricultural ditch next to grassland with dairy cows, an urban ditch located next to an apartment building in Amstelveen (The Netherlands), an excavated recreational peat lake, an urban river flowing into the city of Amsterdam and a canal in the center of Amsterdam (Fig. 1).

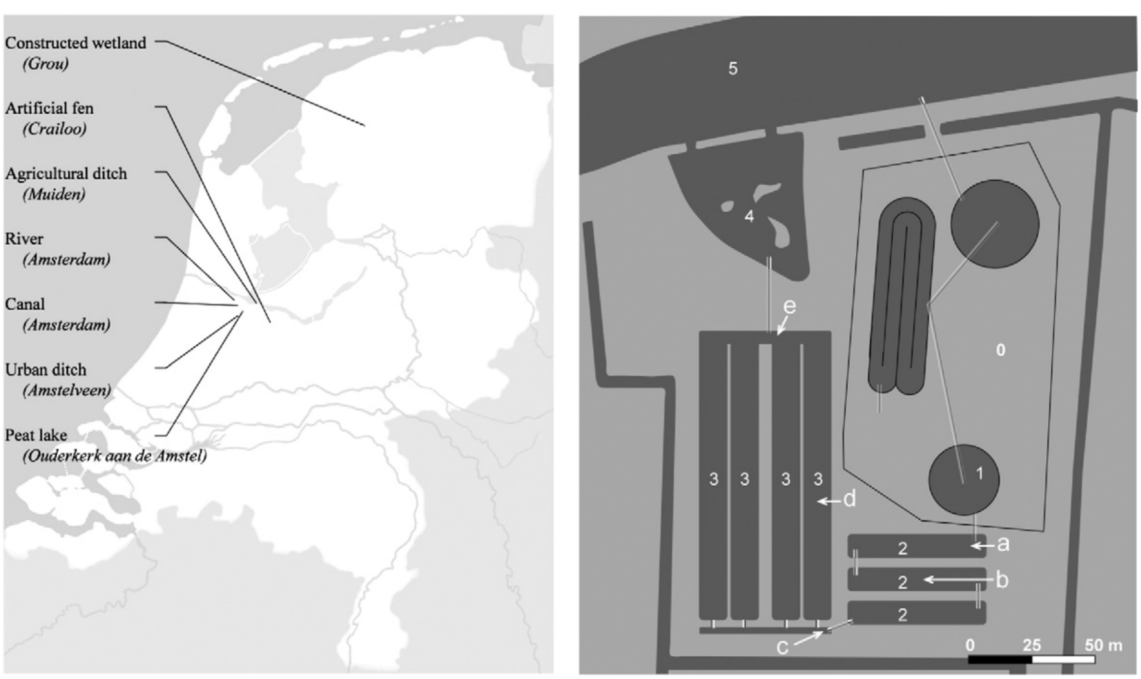

Fig. 1. Left: Sampling locations in the Netherlands. Right: Map of the WWTP in Grou, the Netherlands (0) with a sedimentation tank (1) which discharges treated municipal wastewater into a CW consisting of unvegetated ponds (2), reed beds (3) and an ecological buffer zone (4) which is in open connection the receiving channel (5). Sampling points were located at: PONDS-IN (a), PONDS (b), PONDS-OUT (c), REED-BEDS (d), and REED-BEDS-OUT (e).