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Effect of reclaimed water effluent on bacterial community structure in the *Typha angustifolia* L. rhizosphere soil of urbanized riverside wetland, China

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ABSTRACT

In order to evaluate the impact of reclaimed water on the ecology of bacterial communities in the *Typha angustifolia* L. rhizosphere soil, bacterial community structure was investigated using a combination of terminal restriction fragment length polymorphism and 16S rRNA gene clone library. The results revealed significant spatial variation of bacterial communities along the river from upstream and downstream. For example, a higher relative abundance of γ-Proteobacteria, Firmicutes, Chloroflexi and a lower proportion of β-Proteobacteria and ε-Proteobacteria was detected at the downstream site compared to the upstream site. Additionally, with an increase of the reclaimed water interference intensity, the rhizosphere bacterial community showed a decrease in taxon richness, evenness and diversity. The relative abundance of bacteria closely related to the resistant of heavy-metal was markedly increased, while the bacteria related for carbon/nitrogen/phosphorus/sulfur cycling wasn't strikingly changed. Besides that, the pathogenic bacteria markedly increased in the downstream rhizosphere soil since reclaimed water supplement, while the possible plant growth-promoting rhizobacteria obviously reduced in the downstream sediment. Together these data suggest cause and effect between reclaimed water input into the wetland, shift in bacterial communities through habitat change, and alteration of capacity for biogeochemical cycling of contaminants.

Introduction

Centralized reclaimed water treatment plants (RWTPs) are one of the most common systems for the treatment of domestic wastewater in highly urbanized areas with high population densities in Beijing, China. Therefore, RWTPs effluent represents a significant component of the supplement water of river ecosystems in Beijing. Currently, the use of reclaimed water in Beijing amounts to 2.3×10^8 m^3, 1.5×10^8 m^3 (65.2%) of which are used by lakes and rivers. However, numerous studies have documented that reclaimed water is rich in nitrogen, phosphorus and other nutrients; also, as a result of the slow stream flow of urban rivers, their long update cycle, and the single body of water ecosystem structure, the water column potentially has a higher risk of eutrophication and temporary oxygen deficits (Zhou et al.,

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2006; Meng et al., 2011). In addition, domestic wastewater is normally anthropogenic, such as municipal and hospital producing wastewater, which contains residual Pharmaceutical and Personal Care Products (PPCPs), and biologically active metabolites. These contaminants can mix into the natural water cycle through reclaimed water supplement (Yu et al., 2011; Tong and Wei, 2012; Knapp et al., 2012). Reclaimed water was one of the chlorine sources, which could produce organic pollutant, substitution reactions, disinfection byproducts, chloroform, carbon tetrachloride, *etc*. Thus, reclaimed water is an important source of water transmitted antibiotics and cross resistance genes from disinfection byproduct, which result in higher potential risk to human health (Dong et al., 2010).

The quality of reclaimed water affects the functions and biological processes of receiving river directly (Wassen et al., 1992), particularly when it enters waterways during periods of low natural flow. The urban artificial wetland, considered as a natural alternative to technical methods of wastewater treatment, which can effectively remove nitrogen, phosphorus, heavy metals oxide, various organic substances and pathogens, and reduce BOD and TSS content in water (Thurston et al., 2001; Zhao et al., 2007). Thus, urban artificial wetlands have gradually been widely used in improving water quality in urban lake and river landscapes (Cui et al., 2011). Many studies had pointed out that the interaction matrix, aquatic plants and microorganisms in the wetland system was the main mechanisms of effluent purification (Toyama et al., 2009). Among them, microorganisms play an imperative role in the process of purification, especially the plant rhizosphere bacteria. Wetland plants had a special ability called the "rhizosphere effect," which allows microbes in the plant rhizosphere to enhance the carrying capacity of constructed wetlands (Xiang et al., 2004). The rhizosphere bacterial communities propagate at high speed, with high abundances and strong metabolizing abilities (Feng et al., 2012), which play a very important role in the processes of removing, fixing and conversion of nitrogen, phosphorus and other organic/inorganic matters, including heavy metal removal, *etc*. (Jing and Yang, 2004; Zhang et al., 2007; Nicomrat et al., 2008; Li, 2012). Therefore, rhizosphere bacterial communities are the main force involved in the degradation of pollutants and play an important role in maintaining the ecological balance and achieving ecological purification of wetland systems. Characteristics of microbial biomass, activity and community composition in constructed wetlands can be directly affected by hydraulic conditions, wastewater properties, including substrate and nutrient quality and availability, filter material or soil type, plants, and other environmental factors (Truu et al., 2009). Therefore, wetland plant rhizosphere microbial characteristics can sensitively reflect the status of plant and water quality, which are considered ideal indicators of aquatic ecosystems and have been extensively used to assess the degree of toxicity imposed by various pollutants. To the best of our knowledge, most studies have focused on the impacts of wastewater treatment plants (WWTPs) effluent on bacterial communities in the water column and sediment of receiving river (Wakelin et al., 2008; Drury et al., 2013). But few studies have investigated wetland plant rhizosphere bacteria community under reclaimed water disturbance condition.

We aimed to characterize the effect of RWTPs effluent on the structure of bacterial communities in the rhizosphere bacterial community of *Typha angustifolia* L. in urbanized riverside wetland in our study. A combination of terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA library technique was used to investigate the diversity, abundance and function of bacterial community in *T. angustifolia* rhizosphere samples collected from near the reclaimed water outfall, 300 m upstream the RWTP outfall and 2000 m downstream the RWTP outfall. The two methods exhibit different properties in the analysis of community structure. T-RFLP which is widely applied to the fields of biodiversity analysis and comparison of microbial community has crucial theoretical meaning and practical applied value (Wang et al., 2012). However, it is not applicable to describe community composition because of some shortcomings. For example, phylogenetic identification is problematic because some terminal restriction fragments (T-RTs) can't match the corresponding species or genus of bacteria from database (Marsh, 1999). Conversely, the 16S rRNA clone library method is not suitable for analyses of community diversity since the limitation of conversion efficiency. However, for community composition analysis, the 16S rRNA clone library method, which has the highest resolution ability, appears to be suitable (Guo et al., 2015). Narrowleaf cattail (*T. angustifolia*) is one of the most common plants in constructed wetlands used for wastewater treatment. Earlier studies have shown that cattails' endophytic bacteria can assimilate nutrients (Whitacre, 2012), heavy metals (Demirezend and Aksoy, 2004) and phytoremediate eutrophic water bodies (Cristina et al., 2009) in the constructed wetland or semi-natural treatment wetland. But no study has investigated the effect of reclaimed water on the rhizosphere bacterial community of *T. angustifolia* in urbanized riverside wetland. Date from this study can provide better understanding of the interactions between reclaimed water variables and complex bacterial communities in wetland systems, as well as useful information of indigenous populations with potential application to reclaimed water purification. It also can provide a scientific reference to maintain the ecological balance and construct the high-efficiency wetland purification system.

1. Materials and methods

1.1. *Description of study area*

The Beijing Yongding River artificial wetland was 5.24-km-long, which geographically begins in the town of Sanjiadian, and extends to Mayu Village, located in Mentougou District. This artificial wetland mainly uses reclaimed water as a supplemental water source to sustain the water inflow in the wetland culverts. The mean water depth in the study area is 1.5–2.5 m. Water storage capacity is 1.6×10^6 m^3. The average annual rainfall of whole watershed is about 556–560 mm, and rainfall mainly concentrates in 6–9 months. To form the wetland landscape, a dam of Sanjiadian reservoir was set up in the upstream of the drainage outlet. Two major reclaimed water outfalls (Zhongmensi ditch outfall and Gaojing canal outfall) are located in approximately 4000 m downstream of the reservoir