Spatiotemporal and species variations in prokaryotic communities associated with sediments from surface-flow constructed wetlands for treating swine wastewater

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HIGHLIGHTS

- Three-stage surface-flow constructed wetlands (SFCWs) treatment was effectively.
- *Proteobacteria* dominated sediments from SFCWs for treating swine wastewater.
- Prokaryotic communities in sediments varied greatly with location and season.
- Swine wastewater strength markedly influenced community structure in sediments.

GRAPHICAL ABSTRACT
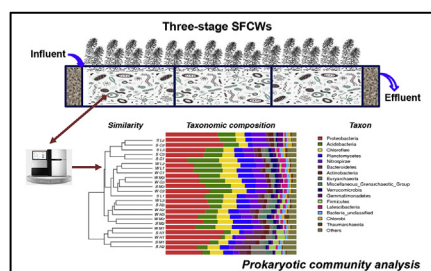
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ABSTRACT

Microorganisms are the main mechanisms of pollutants removals in constructed wetlands (CWs) used for wastewater treatment. However, the different biological processes and variations of prokaryotic community in CWs remain poorly understood. In this study, we applied a high-throughput sequencing technique to investigate the prokaryotic communities associated with sediments from pilot-scale surface-flow constructed wetlands (SFCWs) treating swine wastewater (SW) of varying strengths. Our results revealed that highly diverse prokaryotic communities were present in the SFCWs, with *Proteobacteria* (16.44–44.44%), *Acidobacteria* (3.25–24.40%), and *Chloroflexi* (5.77–14.43%) being the major phyla, and *Nitrospira* (4.14–12.02%), the most dominant genus. The prokaryotic communities in the sediments varied greatly with location and season, which markedly altered the microenvironmental conditions. Principal co-ordinates analysis indicated that SW strength significantly influenced the community structure in sediments of the SFCWs, and canonical correspondence analysis illustrated that the shifts in prokaryotic communities were strongly related to NO_3^-–N and TN in winter; and in summer with NH_4^+–N, NO_3^-–N, NO_2^-–N, TN, TP, SOM, and pH. In conclusion, the use of high-throughput

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sequencing greatly enhanced our understanding of prokaryotic communities with different functional groups in SFCWs.

1. Introduction

The rapid expansion of the livestock industry has resulted in the generation of large volumes of swine wastewater (SW), which contains high concentrations of pollutants, including suspended solids, organic material, and nutrients (Lee et al., 2004). In rural areas of China, SW has been identified as a major source of diffuse pollution, causing various environmental problems, e.g., eutrophication. To lessen such environmental impact, the wastewater must be treated prior to its discharge. Various methods have been attempted to treat SW, including activated sludge treatment plants, oxidation ditches, and rotating biological contactors (Girard et al., 2011; Guo et al., 2013; Tang et al., 2008). However, the implementation of aerobic processes is restricted by high costs of construction and operation, especially in rural areas. Cost-effective constructed wetlands (CWs) may be feasible in this case, and surface-flow wetland systems (SFCWs) are usually used for SW treatment to avoid clogging (Harrington and Scholz, 2010).

The extent of wastewater treatment in CWs depends on the wetland design, microbial community, and types of plants grown therein (Ibekwe et al., 2003). Most studies confirm that microbial processes are the major mechanisms for pollutant removal (accounting for 60—90%) from wastewater in SFCWs (Kang et al., 1998; Sirivedhin and Gray, 2006; Tao et al., 2007), and the majority (81—95%) of microbial biomass in a SFCW is located in the sediments (Truu et al., 2009). Different microbial communities have been shown to contribute to different biochemical reactions, leading to the removal of chemical pollutants in CWs (Scholz and Lee, 2005). For example, the mineralization of organic pollutants is mainly carried out by microbes both in aerobic and anaerobic conditions (Huang et al., 2008; Cheng et al., 2016). Nitrogen (N) removal from wastewater in CWs is achieved mainly by microbial activities associated with nitrification, denitrification, and anaerobic ammonium oxidation (anammox) (Wallace and Austin, 2008). Microbes may also play an important role in phosphorous (P) removal as mineralizers of organic P via biological mineralization (release of mineral P during organic matter degradation) and biochemical mineralization (release of mineral P through enzymatic hydrolysis by extracellular enzymes) (Oehl et al., 2004). The identification of microbial community structure can aid in our understanding of biological processes in CWs, which holds great potential for facilitating proper wetland design and maintenance (Adrados et al., 2014). The observed variation in effluent quality is known to be caused by shifts in microbial populations and/or their activity (Faulwetter et al., 2013; Yang et al., 2010). Therefore, an understanding of the community structure of microbes involved in biogeochemical processes, their location and distribution within the CWs, and the extent of seasonal population shifts could increase the likelihood of successfully constructing a treatment wetland having a high efficiency of water purification.

Currently, some molecular techniques such as fluorescent *in situ* hybridization, denaturing gradient gel electrophoresis, terminal-restriction fragment length polymorphism, and clone libraries have been used to study microbial diversity in wastewater treatment wetlands (Bouali et al., 2013; Dong and Reddy, 2010; Ibekwe et al., 2007; Truu et al., 2009). However, these methods all have certain biases and thus may have failed to capture a complete picture of the microbial communities (Lee et al., 2012). Moreover, the diversity of microorganisms involved in pollutant removal is expected to be high in CWs treating SW; therefore, a more comprehensive survey is required. The development of the high-throughput 16S rRNA amplicon sequencing technique has revolutionized the study of microbial diversity by traditional techniques because it can provide a sufficient number of sequences of adequate length to enable extrapolations that estimate bacterial diversity on the basis of its two components: richness and evenness or equitability (Acosta-Martínez et al., 2008). The recent use of this molecular tool has provided detailed information about the microbial community structure in different ecosystems, including agricultural soils, activated sludge, constructed wetland, and freshwater sediments (Fykse et al., 2016; Guan et al., 2015; Inceoğlu et al., 2011; Lai et al., 2016; Wang et al., 2012). However, the different biological processes of microbial communities in CWs remain unclear (Bouali et al., 2014), and the removal of pollutants in SFCWs treating SW seems to be a very complicated process, being affected by spatio-temporal variations in response to various environmental factors.

Consequently, the objective of this study was to characterize the prokaryotic (bacterial and archaeal) community composition in sediments from pilot-scale SFCWs receiving different strengths of SW using high-throughput sequencing. We compared the prokaryotic community structures in the sediment of wastewater of different strengths (differences associated with different seasons and locations) to understand the spatiotemporal variation and impact of environmental conditions on the communities.

2. Materials and methods

2.1. Constructed wetlands

Pilot-scale SFCWs located in Changsha Research Station for Agricultural and Environmental Monitoring, Changsha, Hunan Province, China (28°30′ N, 113°18′ E), had been constructed in March 2014 for SW treatment. The area is characterized by a subtropical climate, with average annual air temperature of 17.5 °C and annual precipitation of 1330 mm. The SFCWs were divided by two low-grade weirs into three identical units (length, 5 m; width, 2 m; water depth, 0.2 m), named S1, S2, and S3, according to wastewater flow direction from the inlet to the outlet. The three-stage SFCWs were planted with *Myriophyllum aquaticum* and were exposed to SW of three different strengths: (1) high-strength (H; undiluted SW, TN concentration of about 500 mg L^{-1}); (2) medium-strength (M; SW diluted 2:1 with fresh water, TN concentration of about 300 mg L^{-1}); and (3) low-strength (L; SW diluted 1:2 with fresh water, TN concentration of about 100 mg L^{-1}). An additional SFCW with fresh-water inflow was set as the control treatment (C). The characteristics of SW in the storage tank were as follows: 380—650 mg L^{-1} total nitrogen (TN), 220—550 mg L^{-1} NH_4^+—N, 0.15—0.88 mg L^{-1} NO_3^--N, 500—1200 mg L^{-1} chemical oxygen demand (COD), and 80—150 mg L^{-1} total phosphorus (TP). The detailed schematic diagram and operation of the wetland system has been detailed in our earlier publication (Chen et al., 2017).