Characteristics of microbial community indicate anthropogenic impact on the sediments along the Yangtze Estuary and its coastal area, China

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HIGHLIGHTS

- Microbial community was characterized in sediments of the Yangtze Estuary.
- Microbial communities varied spatially, especially for Euryarchaeota.
- Euryarchaeota were mainly composed of methanogens.
- Anthropogenic nutrients and metals regulated methanogen and methanotroph distribution.
- Methanogens and methanotrophs could be potential bio-indicators to human activities.

GRAPHICAL ABSTRACT
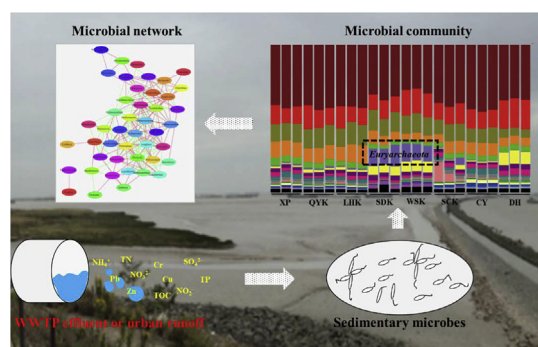
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ABSTRACT

In the contaminated coastal sediments, variations of microbial community can reflect the impact of anthropogenic activities. The identification, evaluation and monitoring of the potential bio-indicator species and bio-marker communities are vital for the ecological studies in sedimentary environments. Based on the high-throughput sequencing, the microbial communities were characterized in the sediments along the Yangtze Estuary and its coastal area. The results showed that the structure and composition of microbial communities varied greatly among different sampling sites at the phyla level, especially for *Euryarchaeota*. Metabolic pathway and quantitative PCR analyses suggested that the methane metabolism-related microbes were mainly included in the phylum of *Euryarchaeota*. Elevated abundances of methane metabolism-related microbes were found at Shidongkou (SDK) and Wusongkou (WSK), where microbes were seriously impacted by the wastewater treatment plant (WWTP) effluent and urban runoff. By comparing with the *Euryarchaeota* in WWTP sludge, the relatively high abundance of *Euryarchaeota* in sediment at SDK may be mainly related to the massive growth of indigenous species, promoted by anthropogenic nutrients. Moreover, redundancy discriminant analysis and correlation analysis revealed that methanogens and methanotrophs mainly respond to the nutrients and metals, such as total organic carbon, total phosphorus, total nitrogen, SO_4^{2-}, NO_2^-, NH_4^+, Cr, and Zn, which were often related to human activities. Network analyses showed that the species related to the metabolism of methane may play a vital role in the interassociation among different microbial communities. Therefore, methanogens,

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methanotrophs and their community compositions could be considered as potential bio-indicator species and biomarker communities, indicating anthropogenic activities in the sediments along the Yangtze Estuary and its coastal area.

1. Introduction

Estuarine ecosystems are the ecotone between river and marine environment, and usually support the development of the neighbouring cities. They also receive amounts of pollutants from land-based sources of cities via river runoffs and sewage outfalls. In estuarine environments, the fine-grained and organic rich sediments act as reservoirs for pollutants (Ducrotoy et al., 2010), such as nutrients (Liu et al., 2003), heavy metals (Lin et al., 2002), antibiotics (Shi et al., 2014), polycyclic aromatic hydrocarbons (Liu et al., 2001), pesticides (Liu et al., 2008), and antibiotic resistance genes (Guo et al., 2018a). Owing to their persistence and continuous discharge into the environment, these contaminants pose a potential risk to the sedimentary biota, especially for microbial communities in the estuarine systems (Wang et al., 2016; Guo et al., 2018b). To adapt to different kinds of habitats, microorganisms often form some specific community structures to cope with the various environmental stresses (Feng et al., 2009; Campbell and Kirchman, 2013). Therefore, the characterization of the microbial communities in contaminated sediments can provide opportunities to identify potential bio-indicator species and biomarker communities responding to specific contaminants (Ducrotoy et al., 2010).

Microbial communities play a vital role in the biogeochemical cycles in estuarine ecosystems (Piccini and Garcíaalonso, 2015, Hou et al., 2013), and the occurrence of these cyclic processes are often related to some obligated microbial-community compositions (Lyautey et al., 2005; Despland et al., 2012; Guo et al., 2018b), such as anaerobic ammonium oxidation bacteria and denitrifiers to remove nitrogen (Thamdrup and Dalsgaard, 2002; Hou et al., 2013; Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014), sulphate-reducing prokaryotes to fix sulphate (Muyzer and Stams, 2008; He et al., 2015), and methanogens to degrade organic matters (Conrad, 2007; She et al., 2016). Moreover, to a certain extent, the changes in dominant bacterial groups and their interactions with other bacterial communities usually affect the structure, function and stability of microbial ecosystems (Shannon et al., 2003). In addition, these changes in contaminated environments are usually caused by human activities either directly or indirectly. For example, polycyclic aromatic hydrocarbons contamination can regulate the composition of the bacterial communities (Tian et al., 2008); heavy metals can shift the benthic estuarine microbial communities and their metabolic functions (Echavarri-Bravo et al., 2015), and antibiotics can change the richness and diversity of antibiotic resistance bacterial communities (Fernandes et al., 2015). Thus, understanding the composition and dominant groups of bacterial communities as well as monitoring their variations, especially in sedimentary environments are essential for environmental and ecological studies.

The Yangtze Estuary is located at the mouth of the Yangtze River into the East China Sea, and it may be one of the most heavily polluted regions because large amounts of nutrients from the Yangtze River and lots of domestic sewage from the Yangtze River delta urban belt are discharged into the estuarine and intertidal environment (Zheng et al., 2016). Several studies on the functional bacterial communities have been conducted in recent years (Hou et al., 2013; Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014; Zheng et al., 2016). However, research on the whole bacterial community and their response to environment factors is limited (Guo et al., 2018b). Thus, the specific objectives of the present study were as follows: (1) to explore the microbial community structures and compositions in the sediments along the Yangtze Estuary and its coastal area using the 16S rRNA gene-based Illumina Miseq sequencing method; (2) to find some potential biomarkers of microbial communities related to the specific contaminants from human activities by metabolic function prediction in KEGG and real-time quantitative PCR (qPCR); and (3) to reveal the possible environmental factors influencing the abundance, diversity and composition of the microbial communities.

2. Materials and methods

2.1. Sample collection

Eight sites along the Yangtze Estuary and its coastal area were selected for sediment sampling (Fig. S1). The Xupu (XP) and Donghainongchang (DH) are situated at the intertidal flat where it is influenced by the water of the Yangtze River and the tide of the East China Sea. Four sites, including Qiyakou (QYK), Liuhekou (LHK), Sanchakou (SCK), Wusongkou (WSK) and Chaoyangnongchang (CY), are at the junction of the Yangtze River and urban river, which are mainly affected by urban river runoff and the estuary tide. The Shidongkou (SDK) site is located at the downstream of a wastewater treatment plant (WWTP), and this place often receives large amounts of domestic sewage and sludge. In July 2016, three sediment samples (0–5 cm deep) at each site were collected using sterile shovels, and the activated sludge was collected from the WWTP, which is located at the upstream of the SDK site. All of the samples were immediately pooled into sterile plastic bags, subsequently stored and transported to the laboratory on ice. After homogenizing the samples, one part of the sediment samples and the sludge samples were stored in sterile tubes at −20 °C for DNA extraction, and the remaining sediment samples were stored at 4 °C for the physiochemical properties analysis.

2.2. Physicochemical properties analyses

The measured environmental parameters, including salinity, pH, grain size, total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN), SO_4^{2-}, NH_4^+, NO_2^-, NO_3^- and heavy metals (Cr, Cu, Zn and Pb), are listed in Table 1. Salinity and pH of sediments were determined with a portable instrument (HQ 40d, HACH, USA), after fresh sediments were mixed with CO_2-free Milli-Q water at a ratio (sediment/water) of 1:2.5 (Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014). The LS 13 320 Laser grain sizer (Beckman Coulter, USA) and the TOC automatic analyzer (SSM-5000A, Shimadzu, Japan) were used to determine the sediment grain size and TOC concentration, respectively. TP was analyzed using the Mo-Sb colorimetric method with the UV-4802 UV/Vis spectrophotometer (Unico, China), after digestion with H_2SO_4 and $HClO_4$ (Hou et al., 2013). TN was measured using a CN thermal combustion furnace analyzer (Elementar analyzer vario Max CN, Germany), after samples were leached with a 1 M HCl solution (Li et al., 2014). SO_4^{2-}, NH_4^+, NO_2^- and NO_3^- levels were determined using an auto discrete analyzer (EasyChem Plus, Systea, Italy) with methods of Method EASY-SO4-01 rev 0, EASY-NH3-02 rev 0, Method EASY-Nitrate-354.1-01 rev 1 and Method EASY-Nitrate-353.2 rev 0, respectively (Hou et al., 2007). Heavy metals (Cr, Cu, Zn and Pb) were extracted according to the sediment environmental quality standard method in China (GB15618-1995) and measured using an inductively coupled plasma mass spectrometer (ICP-MS, PerkinElmer NexION 350D, USA).