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# Water level fluctuations influence microbial communities and mercury methylation in soils in the Three Gorges Reservoir, China

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## ABSTRACT

Reservoirs tend to have enhanced methylmercury (MeHg) concentrations compared to natural lakes and rivers, and water level fluctuations can promote MeHg production. Until now, little research has been conducted on the effects of microorganisms in soils for the formation of MeHg during different drying and flooding alternating conditions in the Three Gorges Reservoir (TGR). This study aimed to understand how water level fluctuations affect soil microbial composition and mercury concentrations, and if such microbial variations are related to Hg methylation. The results showed that MeHg concentrations and the ratios of MeHg to THg (MeHg%) in soils were higher in the seasonally drying and flooding alternating areas (DFAs, 175–155 m) than those in the non-inundated (NIAs, >175 m) and inundated areas (IAs, <145 m). However, MeHg% in all samples was less than 1%, indicating that the Hg methylation activity in the soils of the TGR was under a low level. 454 high-throughput sequencing of 16S rRNA gene amplicons showed that soil bacterial abundance and diversity were relatively higher in DFA compared to those in NIA and IA, and microbial community composition varied in these three areas. At the family level, those groups in *Deltaproteobacteria* and *Methanomicrobia* that might have many Hg methylators were also showed a higher relative abundance in DFA, which might be the reason for the higher MeHg production in these areas. Overall, our results suggested that seasonally water level fluctuations can enhance the microbial abundance and diversity, as well as MeHg production in the TGR.

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## Introduction

Mercury (Hg) is recognized as a global pollutant with severe adverse effects on human beings and ecosystem health because of its bioaccumulation in the food web (Liu et al.,

2011; Ma et al., 2015). All forms of mercury are toxic, especially its organic forms including methylmercury (MeHg), which is a neurotoxin to human beings (Clarkson and Magos, 2006). The transformation of inorganic mercury to MeHg primarily occurs in sediment, soil, periphytic biofilms, or at the sediment–water

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interface (SWI) through the processes that are mainly mediated by microorganisms (Hamelin et al., 2015; Raposo et al., 2008). Microbial communities hence play a vital role in controlling Hg toxicity and mobility in the environment by directly or indirectly promoting MeHg synthesis and degradation. The identification of microbial populations involved in mercury methylation is critical for monitoring the response of public and ecosystem health to the presence of mercury (Lin et al., 2012).

Microbial Hg methylation was firstly confirmed by Jensen and Jernelöv (1969), who observed that bottom sediments from freshwater aquaria transformed inorganic Hg to MeHg, while the sterilized sediments did not (Jensen and Jernelöv, 1969). Subsequently, a large number of field and laboratory researches have demonstrated the important roles of sulfate reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour et al., 2011; Gilmour et al., 1992; King et al., 2000), iron-reducing bacteria (FeRB) (Fleming et al., 2006; Jeffra K. Schaefer and Morel, 2009; Kerin et al., 2006), and methanogens (Hamelin et al., 2011; Yu et al., 2013) in Hg methylation in diverse environments. Recently, with the discovery of two key methylation gene clusters, *hgcA* and *hgcB* (Parks et al., 2013), Hg methylating organisms were found distributed in at least four populations, including *Deltaproteobacteria*, *Firmicutes*, *Chloroflexi*, and *Euryarchaeota* (Bae et al., 2014; Liu et al., 2014; Parks et al., 2013), which provide us a new insight in mercury methylation.

Reservoirs have been shown to play an important role in activation, methylation, and bioaccumulation of mercury, which is related to the degree of water level fluctuations (Eckley et al., 2015; Feng, 2011). Compared to natural lakes and rivers, reservoirs especially the newly created ones tend to have enhanced MeHg concentrations due to the increased organic matters, which can enhance the microbial activity (Eckley et al., 2015; Hall et al., 2005a). The Three Gorges Reservoir (TGR) of China is an extremely large reservoir in the world, with fluctuating water levels between 145 m above the sea level (a.s.l.) in summer and 175 m a.s.l. in winter. Taking the varying water levels into account, it has a flooded area ranging from 350 to 632 km<sup>2</sup>, representing the seasonal water level fluctuation zones (WLFZs) (Ye et al., 2013). The original soils in the current WLFZ were affected by human activities for hundreds of years, such as fertilization and irrigation, accompanied by the deposition of various pollutants including mercury (Xu et al., 1999). With repeated alternating submergence and exposure of the soil in the WLFZ since the construction of the TGR in 2009, the spatial variability of heavy metals, soil redox potential, physicochemical properties, and soil microbial community structure in the WLFZ have been significantly periodically altered (Chen et al., 2013; Li et al., 2013; Xiang et al., 2014; Xiao et al., 2011; Ye et al., 2011), which made the WLFZ became a distinctive ecosystem from other aquatic environments. Until now, great efforts have been made to investigate the geochemical cycling of mercury in the WLFZ of the TGR. However, much of these researches were conducted under simulated conditions and mainly focused on the abiotic factors on the migration and transformation of mercury, for instance, the influence of dominant plants (Liang et al., 2016; Zhang et al., 2014), dissolved oxygen (Wang et al., 2015), low-molecular acid

in root exudates (Qin et al., 2015; You et al., 2016), and sulfur (Liu et al., 2017a) on the cycling of mercury. Little is known about how microbial communities respond to the water level fluctuations and if such response related to mercury in terms of Hg methylation in the WLFZ of the TGR.

The overarching objective of our study was to identify the areas of enhanced MeHg production within reservoir soils and to determine the possible factors that drive this trend. Specifically, we tested the hypothesis that seasonally water level fluctuations result in increased MeHg production in the soil of the TGR, which was mainly influenced by certain specific microorganisms. A previous study conducted at this area found a significant positive correlation between *dsrB* and *hgcA* abundance and MeHg concentrations, and the *hgcA* sequences were confirmed to have three clades including *Deltaproteobacteria*, *Methanogen*, and *Clostridia* groups (Du et al., 2016). However, that research was only focused on the Hg-methylating organisms in the sediments (<155 m) and non-inundated areas (>175 m), which did not reflect the response of Hg methylation to water level fluctuations precisely because the area of 155 m is not a permanent flooding area. In this current study, we expanded on these findings by including the areas along the reservoir shoreline that underwent seasonally water level fluctuations, permanently drying or flooding hydrological histories. In addition, high-throughput 454 pyrosequencing, which can simultaneously synthesize and sequence DNA fragments effectively and significantly improve the detection of non-dominant microbial communities, was employed to investigate the microbial community structure in the soils of the TGR (Sogin et al., 2006; Wei et al., 2015).

## 1. Materials and methods

### 1.1. Field description and sampling

The sampling station was located along the north bank of the TGR in the Yangtze River, in Xinzheng Village, Shibaozhai, Zhongxian County (108°12'3"E and 30°24'36"N, Fig. 1), Chongqing, Southwest China. The primary soil type in this area is purple soil, and the yearly precipitation and the annual average temperature are around 1200 mm and 18.2°C, respectively. With the barren and agricultural lands (planted with corns by the local farmers from March to August) as the major land types in this area, soils in the barren land at each altitude were chosen to be the monitor objects avoiding the human disturbance. Soil samples in the WLFZ were collected according to the fluctuation of water levels of the TGR (from 175 to 145 m; Appendix A Fig. S1; <http://www.ctg.com.cn/inc/sqsk.php>), as the flooded areas would decrease with the drop in water level and the flooded period of soil also could change with different water levels, which can thus affect soil microbial amount or microbial community structure. Thus, corresponding soils were collected at: (1) non-inundated areas (NIAs), which never inundated by the river water of the TGR, including >175 m; (2) drying and flooding alternating areas (DFAs), which underwent seasonally drying and flooding alternating histories every year. For this area, all sampling sites are inundated by the river water of the TGR from October

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