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Bacterial, archaeal, and fungal community responses to acid mine drainage-laden pollution in a rice paddy soil ecosystem

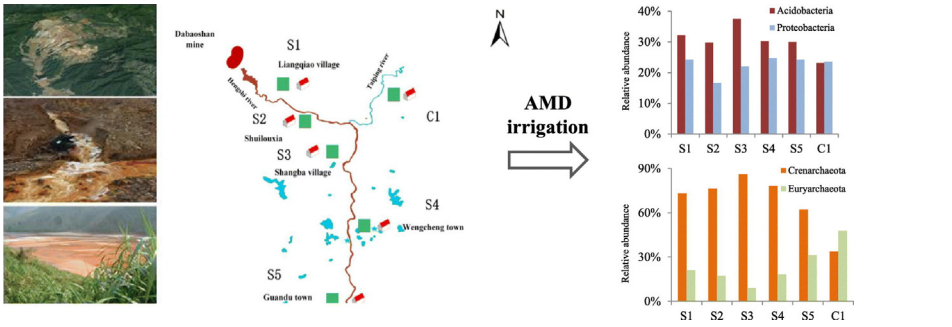
Han Wang [a], Yufei Zeng [a], Chuling Guo [a,b,*], Yanping Bao [a,c], Guining Lu [a,b], John R. Reinfelder [c], Zhi Dang [a,b,*]

[a] School of Environment and Energy, South China University of Technology, Guangzhou 510006, PR China
[b] The Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, South China University of Technology, Guangzhou 510006, PR China
[c] Department of Environmental Sciences, Rutgers University, New Brunswick, NJ 08901, USA

HIGHLIGHTS

- The microbial communities of the AMD-affected paddy soil were investigated.
- The *Acidobacteria*, *Crenarchaeota* were significantly accumulated by AMD irrigation.
- The energy metabolic processes have well adapted to tolerate AMD contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

Lacking sufficient clean water, the paddy soils along the Hengshi River have suffered from long-term acid mine drainage (AMD) contamination. The impacted cropland is too heavily contaminated to grow food safely. The microbial communities inhabiting the environment play pivotal roles in the crop growth, health, and ecological services. In this study, the bacterial, archaeal, and fungal communities in the impacted paddy soil were examined using high-throughput Illumina MiSeq sequencing. The results showed that AMD irrigation considerably enriched the bacterial phylum *Acidobacteria* and the archaeal phylum *Crenarchaeota*, while the fungal community was more stable. The abundances of *Acidobacteria* and *Crenarchaeota* were significantly positively correlated with the AMD-related environmental factors of pH and heavy metals (Cu, Pb, and Zn). In the most contaminated samples, communities were dominated by the bacteria *Candidatus Solibacter* and *Candidatus Koribacter* from the *Acidobacteria* family. Functional gene profile analysis demonstrated that the energy metabolic processes of the microbial communities, especially C/N related pathways, have adjusted and are well-adapted to tolerating AMD contamination. The present study described the structural and functional differentiation of microbial communities in the rice paddy soil under AMD irrigation. The results are useful for the development of bioremediation strategies using native microbes in the cleanup and biorestoration of AMD-contaminated agriculture soil.

1. Introduction

AMD (acid mine drainage) is a specific biogeochemical condition originating from the oxidation of ores, which has become a global environmental and health threat (Johnson, 2003). Long-term irrigation of

* Corresponding authors at: School of Environment and Energy, South China University of Technology, Guangzhou 510006, PR China.
E-mail addresses: clguo@scut.edu.cn (C. Guo), chzdang@scut.edu.cn (Z. Dang).

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cultivated land with AMD has inevitably ratcheted up soil acidity and resulted in the enrichment of toxic metals and sulfate from contaminated rivers, thereby reducing the large scale of arable land and causing the appearance of environmental disaster zones, such as the Shangba "cancer village" in the downstream of Hengshi River (Zhuang et al., 2009; Kuang et al., 2013; Larson, 2014; W. Sun et al., 2015). Various remediation strategies have been developed to clean up the contaminated farmland. Biological treatment strategies that utilize microorganisms are considered to be one of the most economical and environmentally friendly methods (Haritash and Kaushik, 2009; Fuchs et al., 2011). Therefore, understanding the indigenous microbial structure and function is helpful to target or isolate the potential microbial strains or consortium to fit in future bioremediation strategy.

Microbial activities play a critical role in nutrient elements cycles and soil functions. It has been observed that when the external environment changed harshly, such as mining, fertilization or PAH contamination, the microbial community structure would perform a specific response (Insam et al., 1991; Johnson et al., 2005; Epelde et al., 2015). For instance, Lauber et al. (2009) found that the pH parameter was a significant portion of the variability associated with observed changes in the soil microbial community. Recently, the bacterial community composition of a paddy soil irrigated with AMD was reported with particular attention to Fe and S metabolizers. A large proportion of Fe- and S-metabolizing bacteria were detected in AMD-affected area, The abundance of the Fe- and S-metabolizing bacteria slightly higher than the other non-AMD-affected paddy soil (M. Sun et al., 2015, W. Sun et al., 2015; Wang et al., 2016). However, archaea and fungi also deeply participated in the nutrition cycle, especially in relation to carbon and nitrogen metabolism (Könneke et al., 2005; Kemnitz et al., 2007), which was co-operated with bacteria and played a major role in soil function. To date, a comprehensive and detailed survey into the structures of bacterial, archaeal and fungal communities in AMD-contaminated paddy soils has not been conducted to date.

For this purpose, we have examined the soil microbial communities of several rice paddies under acid mine drainage irrigation in Daobaoshan mining area. The mines in this area generate massive amounts of drainage throughout the year with a stable low pH and high concentration of sulfate (Chen et al., 2015). The Dabaoshan areas were reliant on rice crops, and the Hengshi River was always used as the irrigation water for several decades. Since mining began in the 1970s, the rice paddy soil along the Hengshi River has suffered from long-term (over the decades) AMD contamination (Larson, 2014). Therefore, the geochemical analysis of contaminant metals and their variation along the stream was first characterized. Furthermore, a comprehensive survey of the microbial communities' variations, including bacterial, archaeal, and fungal components, in typical paddy soils was performed using high-throughput sequencing on the Illumina MiSeq platform. Finally, whether the predicted microbial metabolic profiles varied among AMD irrigated areas and the reference area was tested. Rice paddy soil along the Taiping River, a tributary of the Hengshi River, was used as a control site.

The results of this study may be useful for the future development of bioremediation strategies using native microbes to mitigate potential increases in environmental metal concentrations from mining operations. This study is one of the first to investigate the microbial community structure and gene content of microorganisms from a naturally mineralized area prior to the initiation of mining operations and therefore represents a microbial investigation that can support natural resource extraction processes.

2. Materials and methods

2.1. Study site and sample collection

The study was carried out at farmlands in the Hengshi River basin in the Dabaoshan mining area in northern Guangdong Province, China (24°34′28″N, 113°43′42″E). This mining area, which was active since the 1970s, has a subtropical, humid monsoon climate. The average annual temperature is 20 °C, and rainfall is 1350–1750 mm. Before entering the Wengjiang River, the Hengshi River is joined by several uncontaminated tributaries. The Taiping River is the largest tributary with a flow of 0.76 to 8.92 $m^3 s^{-1}$. The famous cancer village, Shangba, is located along the Hengshi River and is approximately 15 km downstream from the AMD containment dam (S3) (Fig. 1).

During the mining process of metal sulfide minerals, large volumes of AMD containing high concentrations of contaminant metals and sulfate are generated and discharged into the surrounding environments; the physicochemical property characteristics of AMD are presented in Table S1. To investigate the effects of varying extents of AMD pollution, six different rice paddies were sampled in winter (January 2015, dry climate) (Fig. 1). Sites S1 and S2 were located in the upstream of the Hengshi River, while sites S4 and S5 were selected as the downstream sites. Sites S1 and S2 were irrigated by AMD in the low flow season. Typically, S3 was a famous village with heavy pollution, and AMD was used as the irrigation water at all times in this site. Site C1 was selected as the reference site and was located at the Taiping River basin immediately upstream of S3. At each site, soil samples of each paddy soil were collected from five randomly selected locations by shovel, and approximately 50–100 g wet weight of surface soil (0–20 cm) per sample was sealed into a polythene bag. All of the samples were immediately frozen at −20 °C until analysis.

2.2. Physicochemical analysis

Soil samples were freeze-dried for 48 h and sifted through a 0.15-mm mesh. Soil pH was determined with pH meter at a dry soil: distilled water ratio of 2:5. Soil organic matter (OM) was measured using the $K_2Cr_2O_7$ oxidation – reduction titration method (Walkley and Black, 1934). To measure sulfate concentrations in soil, dry soil samples were mixed with distilled water at a ratio of 1:5. After 4 h, suspensions were centrifuged at 2200 $\times g$ for 10 min, and the supernatant was filtrated through a 0.45-μm filter membrane. Soil sulfate concentrations were determined by ion chromatography (DIONEX ICS-1500, Sunnyvale, CA, USA). The heavy metals in soils were digested by microwave and later analyzed by atomic absorption spectrometry (Z-2000, Hitachi).

2.3. Molecular analysis of microbial communities

Total soil DNA was extracted from a 0.25 g soil sample using a MoBio PowerSoil DNA Isolation Kit (Mobio Laboratory, Carlsbad, CA, USA) following the manufacturer's instructions. DNA quality assessment and quantification was conducted by using a spectrophotometric analysis on a NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA) and electrophoresis on a 1% (weight/volume) agarose gel.

The DNA of bacterial, archaeal, and fungal microorganisms were amplified from each soil sample for Illumina sequencing analysis. The bacterial 16S rRNA gene was amplified using the forward primer F515 and the reverse primer R806 (Caporaso et al., 2012). For the archaeal 16S rRNA gene, the primer pair was 349F/806R (Takai and Horikoshi, 2000). The fungal 18S rRNA gene amplification was performed using the forward primer nu-SSU-0817F and the reverse primer nu-SSU-1196R (Borneman and Hartin, 2000). Amplifications were using the following cycling conditions: 2 min hot start protocol at 98 °C, followed by 25 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension for 10 min at 72 °C. The Illumina MiSeq platform (paired-end 250-bp mode) was performed at the Guangzhou Magigen Biotechnology Co., Ltd. All of the pyrosequencing datasets have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (accession number SRP119045).