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Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing

Honghong Guo[a,b], Mubasher Nasir[a,b], Jialong Lv[a,b,*], Yunchao Dai[a,b], Jiakai Gao[a,b]

[a] College of Natural Resources and Environment, Northwest A & F University, Yangling, Shaanxi 712100, China
[b] Key Laboratory of Plant Nutrition and Agri-environment in Northwest China, Ministry of Agriculture, China

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ABSTRACT

To improve the understanding of bacterial community in heavy metals contaminated soils, we studied the effects of environmental factors on the bacterial community structure in contaminated fields located in Shaanxi Province of China. Our results showed that microbial community structure varied among sites, and it was significantly affected by soil environmental factors such as pH, soil organic matter (SOM), Cd, Pb and Zn. In addition, Spearman's rank-order correlation indicated heavy metal sensitive (*Ralstonia, Gemmatimona, Rhodanobacter* and *Mizugakiibacter*) and tolerant (*unidentified-Nitrospiraceae, Blastocatella* and *unidentified-Acidobacteria*) microbial groups. Our findings are crucial to understanding microbial diversity in heavy metal polluted soils of China and can be used to evaluate microbial communities for scientific applications such as bioremediation projects.

1. Introduction

Steady economic development in China threatens soil ecosystems due to excessive mining activities (Shu et al., 2003). China produces more than 265.4 million tons of mining waste including Cd, As and other toxic substances (Li, 2006). Once these toxins invade agricultural soils, they affect food production and safety, posing a significant threat to human health via incorporation into the food chain (Williams et al., 2009; Li et al., 2014a; Zhuang et al., 2013; Liu et al., 2013). Zhang et al. (2010) found that grain yield was diminished by more than 1.0×10^7 t due to heavy metal pollution, resulting in a direct loss up to 20 billion RMB. Approximately 20% of arable land in China has been contaminated by heavy metals, and the amount is expected to increase over the next few decades (Li et al., 2014b).

The total concentration of heavy metals in soil is a weak index for indicating the actual concentrations that microorganisms are exposed to in soil solutions (Giller et al., 2009). According to environmental quality standards, the toxicity of heavy metals to soil microorganisms is directly related to heavy metal bioavailability (Wang et al., 2007a; Kot and Namieśnik, 2000). However, data regarding the relationship between soil microbial community structure and heavy metal bioavailability are limited (Wang et al., 2007a).

Microbial community structure can play a role in the community's ability to achieve different functions, but also can resist environmental interference (Torsvik and Øvreås, 2002). Generally, heavy metal pollution reduces microbial diversity in soil because microorganisms that are sensitive to heavy metal toxicity will abruptly decline in abundance or even become extinct, whereas species that can tolerate high concentrations of heavy metals slowly become predominant (Klug and Reddy, 1984; Giller et al., 1998). Changes in microbial community structure may seriously affect the ability of soil microbes to degrade organic matter, leading to decreased soil fertility (Giller et al., 1998).

Studies of tailings reclamation have reported that the diversity and stability of vegetation on reclaimed land are closely related to the diversity and abundance of soil microorganisms (Sherriff, 2005; Mummey et al., 2002; Mendez et al., 2008). In addition, the study of microbial community structure in contaminated soils may be helpful in isolating strains of bacteria that are tolerant to heavy metals and aid in the development of heavy metal resistant genes (Gremion et al., 2003; Pawlowska et al., 2000). The Earth Microbiological Project has demonstrated that the V4 region can be widely supported as a standard 16S rRNA region for general community assessment across a range of very different environments (Gilbert et al., 2014). This helps us to explore the interaction between heavy metals and soil microbial communities to elucidate the mechanism by which heavy metal pollution changes microbial community structures.

For this purpose, an experiment was conducted in contaminated fields located in Zhen'an County of Shangluo City, Shaanxi Province, China. Soils at these sites were mostly polluted from lead–zinc and gold mining wastes, which caused concentrations of soil heavy metals such as Cd, As, Hg, Zn and Pb to exceed the national standard. Thus, the selected experimental site was an ideal environment for studying

* Corresponding author at: College of Natural Resources and Environment, Northwest A & F University, Yangling, Shaanxi 712100, China.

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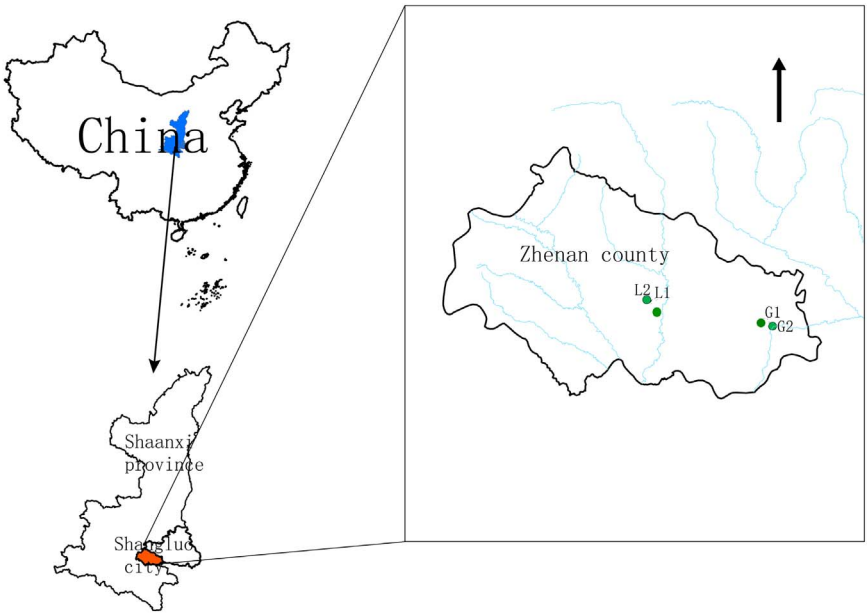

Fig. 1. Locations of all sampling sites contaminated with heavy metals.

microbial communities under in-situ conditions. The main objective of our study was to characterize the behavior of microbial communities in heavy metal contaminated soil and determine the effect of environmental factors on bacteria.

2. Materials and methods

2.1. Field description and soil collection

Twenty-four soil samples were collected from four farmlands contaminated with heavy metals, located in Zhen'an County of Shangluo City, Shaanxi Province, China (Fig. 1). The fields (G1, G2) and (L1, L2) were contaminated from gold mining and lead–zinc mining, respectively. Soils samples were collected from top 0 to 15 cm soil layer in December 2015 (L1-W, L2-W, G1-W, G2-W) and June 2016 (L1-S, L2-S, G1-S, G2-S). Five soil cores were collected for each sample and thoroughly mixed into a single composite sample. All composite samples were packed into plastic bags and taken to the lab for further processing. Soil samples were immediately stored in a freezer at −80 °C prior to DNA extraction and at 4 °C prior to soil chemical analysis. The total metal concentration of the sampling sites is shown in Table 1.

2.2. Soil chemical analysis

Soil pH was measured with a calibrated pH meter (soil: water ratio of 1:2.5). Soil moisture content was determined by the oven drying method at 105 °C for 8 h. Cation exchange capacity (CEC) was determined using NaOAc flame photometry. Soil organic matter (SOM) was determined by the $K_2Cr_2O_7$ colorimetric method. Total nitrogen (TN) was analyzed with a CN analyzer. Available phosphorus (AP) was extracted with 0.5 mol/L $NaHCO_3$ and measured with a spectrophotometer. Available potassium (AK) was extracted with 1 mol/L NH_4OAc and measured using flame photometry. Total heavy metals (Cd, Pb, Zn, Hg and As) and extractable heavy metals (Cd, Pb, and Zn) were measured using standard soil testing procedures (Bao, 2000). Extractable Hg extracted with 0.03% TGA-1/15 mol/L Na_2HPO_4 (Wang et al., 1983) and extractable As extracted with 0.05 mol/L NaH_2PO_4 (Wang, 2012a), were measured with an atomic fluorescence spectrophotometer.

2.3. High-throughput sequencing

Community DNA from soil samples was extracted in accordance with the manufacturer's protocol using the MoBio Power soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). The concentration and purity of DNA were tested with 1% agarose gel, and DNA was diluted with sterile water to 1 ng/μl.

The V4 region of the bacterial 16S rRNA genes was amplified using the specific primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) which yields accurate taxonomic information and has few biases among various bacterial taxa (Bates et al., 2010). A previously described protocol for DNA amplification was followed (Caporaso et al., 2011). Purified PCR amplicons with a bright main band of 300 bp were sequenced with the Illumina MiSeq platform at Novogene (Beijing, China). After sequencing, initial DNA fragments were assembled by fast length adjustment of short reads (Flash) (Magoč and Salzberg, 2011). Chimeras were removed and 97% similarity OTUs was picked up with UPARSE software package (Edgar, 2010). Representative sequence analysis was performed using the QIIME pipeline (Caporaso et al., 2010) and distributed ribosomal database project (RDP) (Wang et al., 2007b) with the latest Greengenes database (Mcdonald et al., 2012). For each representative sequence, the Greengenes database was used based on RDP classifier algorithm to assign putative taxonomic position. The raw reads generated in the study has been submitted to the NCBI's SRA. Accession: PRJNA376845 ID: 376845.

Table 1
Concentration of total heavy metals in all fields.

Location	Total Cd (mg kg^{-1})	Total Pb (mg kg^{-1})	Total Zn (mg kg^{-1})	Total Hg (mg kg^{-1})	Total As (mg kg^{-1})
L1	1.97 ± 0.17	59.86 ± 13.35	50.68 ± 2.26	2.09 ± 1.12	26.00 ± 0.24
L2	1.12 ± 0.35	63.59 ± 12.44	45.98 ± 2.15	0.87 ± 1.42	34.59 ± 0.35
G1	0.37 ± 0.02	30.91 ± 1.71	28.63 ± 1.54	0.12 ± 0.73	19.83 ± 0.02
G2	0.36 ± 0.04	34.41 ± 1.58	32.42 ± 1.68	0.24 ± 1.52	79.81 ± 0.08