Response of soil microbial communities to elevated antimony and arsenic contamination indicates the relationship between the innate microbiota and contaminant fractions

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ABSTRACT: We selected two sites in Southwest China with different levels of Sb and As contamination to study interactions among various Sb and As fractions and the soil microbiota, with a focus on the microbial response to metalloid contamination. Comprehensive geochemical analyses and 16S rRNA gene amplicon sequencing demonstrated distinct soil taxonomic inventories depending on Sb and As contamination levels. In addition, metagenomics revealed the potential metabolic pathways of the soil microbial ecosystems.

1 INTRODUCTION

The extensive mining and smelting activities at the Banpo Sb mine, Southwest China, have made this area one of the most severely Sb-contaminated areas in the world. Elevated concentrations of Sb and contamination of both Sb and As have been observed in surface waters, aquatic sediments, and tailing dumps with effects on their bacterial communities (Sun *et al.*, 2016a, 2016b). Therefore, these sites provided excellent natural laboratories to study the microbial response to different concentrations of Sb and As (Xiao *et al.*, 2016a, 2016b). However, the innate terrestrial soil bacterial microbiota of these sites has not yet been examined. In this study, we obtained soil samples from two contaminated sites with various levels of contamination. The aims were to: i) examine the impact of Sb and As contamination on the soil microbial community composition and diversity, ii) elucidate the correlation between soil microbial assemblages with environmental factors, especially those directly linked to Sb and As contamination, and iii) explore the potential metabolic pathways of the soil microbial ecosystems.

2 METHODS/EXPERIMENTAL

The study sites are located in eastern Dushan County, Guizhou Province, southwest China, with different levels of Sb and As contamination. Comprehensive geochemical analyses and 16S rRNA gene amplicon sequencing demonstrated distinct soil taxonomic inventories depending on Sb and As contamination levels. We used Random Forest (RF) and Stochastic Gradient Boosting (SGB) analyses to estimate the contributions of environmental factors and quantify the strengths of connections. Shotgun metagenomic

libraries from five soil samples were constructed on an Illumina Hiseq 4000 platform to explore the potential metabolic pathways of the soil microbial ecosystems.

3 RESULTS AND DISCUSSION

3.1 Correlation between environmental factors and microbial communities

SGB and RF models were used to interpret the relative importance of environmental variables to the community diversity as expressed by Shannon index. As(V)-C, nitrate, C/N, total N, and Sb(V)-C were

Figure 1. Co-occurrence network showing the correlation between bacterial taxa (OTUs) and contaminant fractions as nodes colored by modularity class.

Figure 2. Heatmap of Spearman's rank correlations coefficients between the contaminant fractions and (A) the annotated As-related genes as indicated in the bracket; (B) abundant genera (red genera were identified as “core” genera).

Figure 3. Percentage of reads annotated to Arsenic-related genes for five shotgun metagenomics sequencing libraries.

among the top five factors influencing the Shannon index by SGB. The co-occurrence network further demonstrated correlations between individual phylotypes and contaminant fractions (Fig. 1). Among these identified genera, many contain members relating to Sb and As redox reactions such as *Arthrobacter*, *Janthinobacterium*, *Thiobacillus*, and *Pseudomonas*.

3.2 Microbial response to contamination at the genus level

All of the five selected core genera have members with reported capabilities in As redox transformations and Sb resistance. In addition, four core genera were classified in module II of the co-occurrence network, and three core genera were significantly enriched at the more contaminated site. The correlation of these “core” genera with multiple contaminant fractions suggests their selection by Sb/As contamination (Fig. 2).

3.3 Metabolic functions revealed by metagenomics

Genes related to *aoxA*, *aoxB* and As(V) reductase (*ARSC1* and *ARSC2*) were detected in all libraries, suggesting possible biogeochemical As redox reactions mediated by the microbial communities in these soils (Fig. 3). Because As and Sb share structural similarities, microorganisms may use similar metabolic pathways to transform both As and Sb. In addition to the presence of As-related genes across all samples,

we observed significant positive correlations among As-related genes and various contaminant fractions – not only of As fractions, but those of Sb as well.

4 CONCLUSIONS

Stochastic gradient boosting indicated that citric acid extractable Sb(V) and As(V) contributed 5% and 15%, respectively, to influencing the community diversity. Random forest predicted that low concentrations of Sb(V) and As(V) could enhance the community diversity but generally, the Sb and As contamination impairs microbial diversity. Co-occurrence network analysis indicated a strong correlation between the indigenous microbial communities and various Sb and As fractions. A number of taxa were identified as core genera due to their elevated abundances and positive correlation with contaminant fractions (total Sb and As concentrations, bioavailable Sb and As extractable fractions, and Sb and As redox species). Shotgun metagenomics indicated that Sb and As biogeochemical redox reactions may exist in contaminated soils. All these observations suggest the potential for bioremediation of Sb- and As-contaminated soils.

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