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Depth-resolved microbial community analyses in two contrasting soil cores contaminated by antimony and arsenic

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ABSTRACT

Investigation of microbial communities of soils contaminated by antimony (Sb) and arsenic (As) is necessary to obtain knowledge for their bioremediation. However, little is known about the depth profiles of microbial community composition and structure in Sb and As contaminated soils. Our previous studies have suggested that historical factors (i.e., soil and sediment) play important roles in governing microbial community structure and composition. Here, we selected two different types of soil (flooded paddy soil versus dry corn field soil) with co-contamination of Sb and As to study interactions between these metalloids, geochemical parameters and the soil microbiota as well as microbial metabolism in response to Sb and As contamination. Comprehensive geochemical analyses and 16S rRNA amplicon sequencing were used to shed light on the interactions of the microbial communities with their environments. A wide diversity of taxonomical groups was present in both soil cores, and many were significantly correlated with geochemical parameters. Canonical correspondence analysis (CCA) and co-occurrence networks further elucidated the impact of geochemical parameters (including Sb and As contamination fractions and sulfate, TOC, Eh, and pH) on vertical distribution of soil microbial communities. Metagenomes predicted from the 16S data using PICRUSt included arsenic metabolism genes such as arsenate reductase (*ArsC*), arsenite oxidase small subunit (*AoxA* and *AoxB*), and arsenite transporter (*ArsA* and ACR3). In addition, predicted abundances of arsenate reductase (*ArsC*) and arsenite oxidase (*AoxA* and *AoxB*) genes were significantly correlated with Sb contamination fractions, These results suggest potential As biogeochemical cycling in both soil cores and potentially dynamic Sb biogeochemical cycling as well.

1. Introduction

Antimony (Sb) and arsenic (As) are naturally occurring metalloids and often co-occurred in sulfide ores (Mitsunobu et al., 2006). Sb and As are considered as suspected carcinogens and listed as priority pollutants of the US Environmental Protection Agency and the European Union (Filella et al., 2009; Fu et al., 2016). The mining and metallurgic processes of Sb and As minerals or combustion of fossil fuels may lead to significant releases of Sb and As to the environment, a large fraction of which impacts soils via atmospheric deposition (Tian et al., 2011; Yang et al., 2015). The enrichment of Sb and As could potentially cause health risk to exposed population via food chain by consuming crops grown in contaminated soils (He and Yang, 1999; Ngo et al., 2016). For

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example, elevated Sb concentrations in agriculture soils potentially caused dermatitis and pneumoconiosis for the local residents living around the Xikuangshan Sb mine in Hunan Province, China (He and Yang, 1999; Ngo et al., 2016).

Sb and As belong to group 15 in elementary periodic tables and share similar geochemical behavior in the environment (Filella et al., 2009; Fu et al., 2016). In general, the geochemical behavior of Sb and As in soils are governed by a suite of physico-chemical factors, including pH, oxidation-reduction potential (Eh), organic matter (OM), clay minerals, cation exchange capacity (CEC), and FeMn oxides (Mitsunobu et al., 2006, 2010; Shsharma et al., 2010; Wilson et al., 2010; Filella, 2011). Such geochemical factors may vary along the vertical profile in the soil core. Therefore, the migration and speciation of Sb and As could vary by soil type or depth (Duester et al., 2005; Ettler et al., 2010; Yang et al., 2015). In addition to geochemical parameters, soil microbiota may also influence the behavior of Sb and As, by altering soil geochemistry or Sb/As themselves. For example, microorganisms can produce secondary minerals with high surface area, which could adsorb and precipitate metal(loid)s (Mitsunobu et al., 2010; Shsharma et al., 2010; Learman et al., 2011; Nitzsche et al., 2015; Li et al., 2016). Furthermore, it has been reported that microorganisms may mediate the biogeochemical cycling of Sb and As by detoxification, precipitation, and mobilization (Majzlan et al., 2010; Hamamura et al., 2013; Kulp et al., 2014). Therefore, the fate and transport of Sb and As represent a complex interplay among the microbiota, geochemical conditions, and the metal(loid)s themselves in soils. However, most previous studies have been focused on chemical behaviors of Sb and As, few attentions have been paid for biogeochemical cycling of Sb and As mediated by bacteria in contaminated soils.

In the current study, we selected two soil cores (one from a flooded rice paddy field and the other from a dry corn field) near the Xiaohe antimony smelting facility, Guizhou Province, Southwest China. In our previous studies, we characterized the co-contamination of Sb and As in various environmental compartments including surface water, sediments, and tailing dumps (Sun et al., 2016a,b; Xiao et al., 2016a,b). Relatively sharp contaminant gradients and geochemical parameters may occur in vertical soil profiles, providing opportunities to untangle how the microbial communities interact with their geochemical environments, and especially the Sb and As contamination gradients. The purpose of this work is to investigate the physiochemical parameters and microbial community compositions along the vertical profiles of both soil cores. More specifically, our aims were to i) examine the response of innate soil microbiota in soil core to Sb and As contamination in vertical profiles; ii) predict the metabolic potentials of the indigenous soil microbiota, especially for potential Sb and As metabolism, and iii) explore the interaction between the soil microbiota and environmental conditions including the contaminant fractions as a function of depth in the soil cores.

2. Materials and methods

2.1. Site locations and sample collection

Two soil cores were collected near an Sb smelting plant in Dushan County, Guizhou Province, Southwest China (Fig. S1). Smelting at this facility has been ongoing since 1992, resulting in large amounts of Sb and As deposition to nearby. One soil core was taken from the flooded rice paddy soils (SA), located about 1000 m north of the smelting plant. The other was collected in a corn field (SB), located about 500 m east of the smelting plant. Both profiles were sampled by a sterile soil core sampler from the surface to 1 m below grade. Nineteen soil samples were collected from SA, and 20 samples from SB. Each soil sample was collected at interval of 5 cm from surface to bottom along the soil profile for both geochemical and molecular analyses. Each soil sample was homogenized with 3 parallel samples at site, and the microbial subsample was immediately stored into an icy cooler in the field and then shipped to laboratory within 3–4 °C to store at a −80 °C freezer. Then the vertical profiles of both soil cores were then divided into three layers according to their depths. These layers were indicated as SA-1 (0–30 cm), SA-2 (30–60 cm), SA-3 (60–95 cm) and SB-1(0–30 cm), SB-2 (30–65 cm), SB-3 (65–100 cm) from the surface to the bottom in the soil cores SA and SB, respectively.

2.2. Geochemical analysis

All the 39 Soil samples were freeze-dried (vacuum freeze dryer, Scientz, Ningbo, China) for 48 h. Leaves, plant roots, and gravel in the soils were removed through a 2-mm sieve. Soil samples were then further thoroughly ground by a mortar and pestle before passing a 200-mesh sieve. Ten grams of dry soils were placed in a 100-ml Erlenmeyer flask with 25 ml distilled water. The mixture was shaken for 5 min, and then stand for 20 min to equilibrate. A calibrated HACH HQ30d pH meter (HACH, Loveland, USA) was used to measure pH and oxidation reduction potential (Eh) in the soil samples. For nitrate and sulfate measurements, ten grams of dry samples were placed in a 100-ml Erlenmeyer flask with 50 ml distilled water. The mixture was then shaken for 5 min, followed by 4 h of equilibration. Later, the mixture was centrifuged at 3500 rpm for 10 min. The supernatant was collected and filtered through a 0.45-μm membrane to measure the concentrations of sulfate and nitrate, which were determined by ion chromatography (DIONEX ICS-40, Sunnyvale, CA, USA). Total sulfur, soluble sulfur, total organic carbon, total carbon, total hydrogen, and total nitrogen in the soils were measured by an elemental analyzer (Vario MACRO cube, Elementar, Hanau, Germany). Total Fe and Fe(II) were measured as described previously (Liu et al., 2015; Yu et al., 2016). Briefly, one gram ground soil samples were mixed with 10 ml 1 M HCl for 30 min with constant shake, followed by 4 h of equilibration. The supernatants were collected after centrifuging at 3500 rpm for 10 min, and then filtered through a 0.45-μm membrane. Fe(II) and total Fe concentrations were determined spectrophotometrically by UV-9000s (METASH, Shanghai, China) with 1,10-phenanthroline at 510 nm (Tamura et al., 1974). Trace elements were determined by ICP-MS (Agilent, 7700x, California, USA) after fully digesting the soils with HNO_3 and HF (5:1,v/v) (Edgell, 1989). For accuracy test, the certified reference materials (SLRS-5) and internal standards (Rh, 500 μg/L) were used. For analytical quality control, standard reference material GBW07310 (Chinese National Standard) was applied (Sun et al., 2016a). The analytical precision for trace metals was better than ±10%.

2.3. Sb and As contaminant fraction analysis

The citric acid extractable Sb and As redox species (Sb(III)C and As(III)C, Sb(V)C and As(V)C) measurement was described previously (Fuentes et al., 2003; Ge and Wei, 2013). Briefly, 0.2 g of soil were placed in a 15 ml Erlenmeyer flask, mixed with 10 ml of 100 mM citric acid (pH: 2.08) and shaken for 1 h, followed by 4 h of equilibration at room temperature. After centrifuging at 3500 rpm for 30 min, the supernatant was filtered through a 0.45-μm membrane. The Sb(III) and As(III) concentrations in the soil extracts were determined by measuring the supernatant via HG-AFS (AFS-920, Jitian, Beijing) directly. Total Sb and As were determined by mixing 0.2–1.0 ml of the supernatant (depending on the total concentration) with 2.5 ml of 5% thiourea, 2.5 ml of 5% ascorbic acid, and 3 ml of concentrated HCl. Deionized water was added to complete the

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