Coupling metabolisms of arsenic and iron with humic substances through microorganisms in paddy soil

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Abstract:

Humic acid (HA) and fulvic acid (FA) are dominating humic substances (HS) in soil. In this study, the effects of HA and FA addition (0.2%-1.5%) on arsenic (As) mobility and microbial community composition in paddy soil were investigated. FA significantly increased the concentrations of As (12-fold), iron (Fe; 20-fold), manganese (Mn; 3-fold) and acetic acid (3-fold) in soil porewater, and also caused significant enrichment of *Desulfitobacterium* (41-fold). Furthermore, the FA addition significantly increased the relative abundance of *Bathyarchaeota* (4-fold), a microorganism that is suggested to be important for FA degradation. In contrast, HA slightly increased As (1.2-fold) in porewater, had little effect on Fe, Mn and acetic acid, and 1.5% HA addition significantly decreased As in porewater at day 14 (45%). Both HA and FA addition promoted As methylation. HA increased dimethylarsenate concentration and FA increased monomethylarsenate concentration in porewater. These results highlight the contrasting effects of different (HA vs. FA) organic substances on As fate in paddy soil and advance our understanding of the associations among As, Fe and organic substances through microorganisms in paddy soil.

Keywords: Arsenic; humic acid; fulvic acid; *Bathyarchaeota*

1. Introduction

Arsenic (As) is of great concern in the environment due to its toxicity and carcinogenicity [1]. Arsenic contamination in soil could be caused by both natural and anthropogenic activities, such as mining, irrigation with waste water and solid waste deposit [2,3]. Arsenic contamination of paddy soil has been reported to pose a serious threat to human health through rice consumption, which has been shown to be the main route for human exposure to As. In China, rice consumption contributes about 60% of daily human As intake [4,5]. It is known that rice is efficient in accumulating As in its grains because the high As availability in flooded condition and efficient transport system in plant [6-9].

Soil organic substances play an important role in global carbon cycle, which contain more carbon than the atmosphere and the global vegetation combined [10]. Organic substances, such as rice straw, are recommended to be added to the soil for carbon sequestration and soil fertility [11-13]. However, when the soil is contaminated with As, the effects of organic substance application on the mobility of As should not be ignored. It has been demonstrated that rice straw addition to As contaminated soil could remarkably increase As mobility in soil and enhance As methylation [14,15]. These studies suggested that the content of soil organic substances play a key role in regulating As mobility and transformation in paddy soil.

Organic substances can affect As cycling via various ways. Several studies have shown that organic substances can form ternary complexes with As via iron (Fe) bridging, which could influence the mobility of As [16-18]. In addition, organic substances can be degraded to humic substances (HS) by microorganisms in the soil, and the further decomposition of HS is coupled to dissimilatory iron reduction (DIR) [19]. It is known that HS are redox-active owing to HS-quinone moieties and can serve as electron shuttle for DIR [20,21]. These coupled processes should be involved in the releasing of As. When the reductive dissolution of the ferric oxides is accelerated by HS addition, more mineral-bounded As is released out [22-24]. In addition, HS can enhance As release directly through promoting As reduction by arsenate-respiring microorganisms which can use arsenate (As(V)) as electron acceptor for DAsR [25,26]. It is demonstrated that arsenate-respiring bacterium in the deep sediments in Jianghan Plain played key roles in the mobilization and release of insoluble As into the groundwater [27]. Moreover, acetic acid is the product of anaerobic fermentation of various organic matter, which can work as electron donor for DIR and dissimilatory As reduction (DAsR) [25,26,28], resulting As release from soil minarals.

Humic substances are the major fraction of soil organic carbon, and humic acid (HA) and fulvic acid (FA) are dominating components of HS in paddy soil. However, so far it is still unknown how HS is coupled with As release in paddy soil, and what are the mechanisms governing HS and As interactions, as well as what microorganisms are involved in these processes. To answer these questions, microcosms were set up by incubating humic acid (HA) and fulvic acid (FA) with As-contaminated paddy soil. During the different incubating stages, As concentration and speciation in soil porewater was monitored, and after the incubation, soil microbial community, including bacteria and archaea, was investigated through high throughput sequencing. The results of this study would expand our understanding of the mechanisms about how HS regulate As behavior in paddy soil system.

2. Materials and Methods

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2.1. Properties of Soil, HA, FA and facility for trapping volatile As

Soil was collected from a paddy field in Qiyang, Hunan province, China. The soil is contaminated with As by geologically elevated levels of As [29]. The concentration of As in the soil was 69.8 mg kg^{-1}. The surface soil (0-20cm) of paddy field was sampled, air dried, passed through 2 mm nylon sieve and stored in dark. HA and FA used in the experiment were purchased from Aldrich (Switzerland) and Cramar (China), respectively. The functional groups of HA and FA were characterized by a Fourier transform infrared spectrometer (FTIR) (Nicolet 8700, Thermo Fisher Scientific, USA). Cyclic voltammetry analysis was carried out on electrochemical workstation (CHI1040B, Chenhua, China). The detailed methods are described in the supplementary material (SI). To collect volatile As during incubation, trapping tubes were set up. Silica gel particles (Sigma-Aldrich, Germany) were soaked in 2% AgNO$_3$ solution, then dried in the oven, and stored at room temperature. During the whole experiment, silica gel particles were filled in 1 mL pipette tips and covered with tin foil to protect them from light. Absorbent cotton was used to fix silica gel particles. The suction flask system was conducted as described by Huang et al. (2012) with some modification [14]. In order to sample porewater and the volatile As at the same time, the special pump bottles were customized (Fig. S1). All the solution required in the experiment was made with ultrapure water.

2.2. Incubation experiments

Incubation experiments were conducted in a greenhouse at 25 ˚C. A total of 200 g soil was added into each flask. Different doses of HA and FA were added into the soils. There were totally nine treatments: (1) untreated paddy soil; (2) paddy soil amended with 0.2%, 0.5%, 1.0%, 1.5% (*w/w*) HA; (3) paddy soil amended with 0.2%, 0.5%, 1.0%, 1.5% (*w/w*) FA. Each treatment had four replicates. When the soil and HS were well mixed, 240 mL deionized water was added and mixed, forming a 2 cm layer of standing water. To collect volatile As, the trapping tubes were connected to the bottles through glass tubes and rubber hose. The air was pumped into the bottle at the speed of 0.5 L min^{-1} to refresh the air in the headspace (involve volatile As). The volatile As was blown out from the bottles and preserved in trapping tubes by AgNO$_3$ on the silica gel particles [30]. During the incubation, the pump was on working for 24 h every day. Trapping tubes with new silica gel particles were replaced once a week. A rhizosphere soil solution sampler (Rhizosphere Research Products bv, Netherlands) was used to sample porewater on day 0, 7, 14, 21 and 28 during the incubation. The flasks were weighed every week after collecting samples, and deionized water was added to maintain the weight. At the end of the incubation, standing water was removed, soil in each flask was mixed, and 10 g soil was sampled and stored at -20 °C for microbial DNA extraction.

2.3. Analysis of As, Fe, Manganese and acetic acid concentrations in porewater

The volatile As adsorbed on silica gel particles in the trapping tubes was washed down by 1% HNO$_3$, and digested in a microwave digest oven (MARS 5, CEM Corporation, Matthews, North Carolina). Porewater was filtered with 0.45 μm filters, and then digested with 2% HNO$_3$ in microwave. The detail methods of digestion are described in SI. After digestion, the samples were passed through 0.45 μm filter membrane and stored at 4 °C until analysis of As, Fe, Mn. Total As concentration was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500, Agilent Technology, USA). Arsenic species were analyzed using high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS, Agilent Technology, USA). Fe and Mn concentrations were

determined using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, PerkinElmer, USA). Acetic acid in porewater was determined by high performance liquid chromatography (HPLC, Agilent Technology, USA). Standards and blanks were run with each batch. Scanning electron microscope-Energy Dispersive Spectroscopy (SEM-EDX, Hitachi, Japan) were used to analyze the kinds and contents of elements in HS.

2.4. DNA extraction and high-throughput sequencing of 16S rRNA gene

After incubation, the soil samples were freeze-dried and stored at -20 °C before DNA extraction. Soil DNA was extracted by FastDNA Spin Kit for Soil (MP bio, CA, USA). The DNA was dissolved in 50 μL sterilized deionized water and stored at -20 °C. DNA concentration and OD260/OD280 ratio were analyzed by NanoDrop (ND-1000, USA). After amplified by Polymerase Chain reaction (PCR), samples were sequenced targeting the V4 hypervariable regions of 16S rRNA gene (ArBa515F_Arch806R) at Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China [31]. In order to analyze the communities of bacteria and archaea separately, 338F_806R and 524F10extF_Arch958RmodR were used as primer to do high throughput sequencing again [32,33]. The details of PCR system and primers are shown in SI (Table S1).

2.5. Statistical analysis

The figures in the article were drawn with Origin 8.0. The significance of difference among treatments was detected via one-way analysis of variance (ANOVA) by LSD tests performing in SPSS 22.0. The Spearman and Pearson correlation between As and Fe, Mn in the porewater were conducted by SPSS 22.0 and the correlation coefficients were calculated using Microsoft Excel 2010. Statistical analysis of taxonomic and functional profiles (STAMP) was used to analyze the difference of OTUs between control and treatments. The sequences of the corresponding OTUs were determined by doing a blast from National Center for Biotechnology Information (NCBI) at 97% similarity level. The software of Mega 5.0 was used to set up the phylogenetic tree.

3. Results

3.1. Physicochemical, Electrochemical and Morphological properties of HS in the porewater

Different HS has different physicochemical, morphological and electrochemical characteristics. FTIR was used to identify the functional groups of HA and FA. The FTIR results showed the presence of C-O streching (900 cm^{-1}-1200cm^{-1}), C=C, C=O stretching (1600 cm^{-1}) in quinones and hydroquinone (Fig. 1a). The overall FTIR results suggested that HA and FA had carboxyl, ketone and phenol functional groups which have been reported to link with metal adsorption site and DIR via electron shuttling [20,21,35].

As for electrochemical properties, reversible charge-discharge current peaks showed that HA and FA could act as electron donators and electron acceptors. The maximum current of FA was obviously higher than that of HA, which indicated that the charge and discharge capacity of FA was higher than that of HA (Fig. 1b). The results of SEM-EDX showed that HA were circular particles and FA were flaky. Moreover, the distribution of As and Fe showed similar pattern at the surface of HA and FA (Fig. 2). The consistent distribution of Fe and As suggested that As might complex with Fe at the surface of HS.

Acetic acid concentration in porewater of all treatments increased from 0 to the 7th day of incubation, and then decreased after the 7th day of incubation (Fig. 1c and

1d). HA addition had no significant effect on acetic acid concentration (Fig. 1c) while FA addition significantly increased acetic acid concentration in porewater. The maximum concentration of acetic acid in porewater reached 1782 mg L^{-1} on the 7th day under 1.5% FA treatment, which was 3 times higher than that of the control (Fig. 1d). Higher concentration of acetic acid in porewater of FA treatments indicated that FA might be more active through acetic acid which can serve as electron donor for DIR or DAsR processes.

3.2. Effects of HA and FA addition on As transformation

The HA and FA addition significantly affected As volatilization (Fig. 3a). For HA treatments, high doses of HA addition (1.0% and 1.5%) significantly enhanced As volatilization. The total As volatilization reached the maximum value of 229.7 ng at 1.5% HA addition, which increased by about 74% comparing with the control (Fig. 3a). However, only 0.001% of total As in the soil was volatilized under 1.5% HA addition. In FA treatments, only low doses of FA addition significantly increased As volatilization. The total As volatilization reached 166.3 ng under 0.5% FA addition, which increased by about 25% compared with the control (Fig. 3a).

Arsenic concentration and species in porewater were also monitored during incubation. Under HA treatments, As variation exhibited similar pattern with that of control, i.e. gradually increased until 14 days of incubation, then declined by about 80% at 21 days of incubation, and maintained stable at this level during late incubation stages (Fig. 3b). After 14 days incubation, low doses of HA addition (<1.0%) increased total As content in porewater by about 10-20%, while 1.5% HA addition decreased total As concentration in porewater by about 30% compared with the control. Regarding to As species, high doses of addition increased dimethylarsenate (DMA) concentration after 21 days' incubation, resulting to higher total As concentration in porewater than control (Fig. 3b).

In FA treatments, As concentrations in porewater maintained at high level until the end of incubation. Total As content in porewater increased with the increasing doses of FA addition (Fig. 3b). After 28 days incubation, the average As concentration in porewater of 1.5% FA treatment was about 12-fold and 8-fold higher than that of control and 1.5% HA treatment, respectively (Fig. 3b). Regarding to As species, FA addition significantly increased monomethylarsenate (MMA) concentration after 21 days of incubation. However, the amount of organic DMA and MMA were at lower level comparing with inorganic As in both HA and FA treatments which counted for 25% and 9% of the total As in porewater in HA and FA treatments, respectively.

3.3. Effects of HA and FA addition on Fe and Mn release in porewater

Iron concentration in porewater exhibited similar dynamic pattern to that of As (Fig. 4a). HA addition did not significantly affect Fe concentration in porewater, except 1.5% HA addition which significantly decreased Fe concentration after 14 days' incubation. FA addition significantly increased Fe concentration in porewater. After 28 days' incubation, Fe concentration in porewater of 1.5% FA treatment was about 20-fold higher than that of control (Fig 4a). In both HA and FA treatments, As concentrations in porewater were significantly positively correlated with Fe concentrations in porewater (P<0.01, Fig. 4c and 4d).

Manganese concentration in porewater showed different dynamic pattern with Fe and As, which maximized after 7 days' incubation, then declined in both HA and FA treatments (Fig. 4b). HA addition had little effect on Mn concentration. In the whole incubation, FA addition significantly increased Mn concentration in porewater.

3.4. Effects of HA and FA addition on soil microbial community

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By clustering at 97% similarity level, 4982 OTUs (operational taxonomic units) were classified，which were derived from totally 37857 16S rRNA gene sequences. Principal co-ordinates analysis (PCoA) at genus level showed that the low dose of HA addition (0.2%) had no significant effect on the microbial community, and high doses of HA (0.5-1.5%) significantly changed the microbial community in the soil (Fig. 5a). FA addition (all treatments) significantly changed the microbial community (Fig. 5b). Wayne diagram showed that HA increased the microbial community richness at the genus level, and FA reduced the microbial community richness at the genus level (Fig. S2).

Regarding to the bacterial communities at phylum level, the dominant phylum in control were *Firmicutes, Proteobacteria,* accounting for 38%, 23%, respectively (Fig. S3). HA and FA addition had no significant effect on bacterial communities structure at phylum level, except 1.5% FA addition, which significantly increased the average relative abundance of *Firmicutes* (from 41.1% to 54.6%) (Fig. S3). At genus level of bacterial communities, HA addition had no significant effect on bacterial community structure, either. FA addition significantly increased the average relative abundance of *Desulfitobacterium* (from 0.1% to 4.7%), *Azospirillum* (0.4%-5.1%), *Christensenellaceae_R_7_group*(1.0%-7.7%) and *Hydrogenispora* (2.7%-9.0%) (Fig. 5c).

As for the archaeal communities at phylum level, the dominant phylum in control were *Euryarchaeota, Bathyarchaaeota* (Fig. S4). High doses of HA addition (1.0% and 1.5%) and all doses of FA addition (from 0.2% to 1.5%) significantly increased the relative abundance of *Bathyarchaeota* (from 8.9% to 45.6%). At genus level of archaeal communities, high doses of HA addition (1.0% and 1.5%) significantly increased the relative abundance of *Methanobacterium* (from 13.7% to 23.7%) (Fig. 5d). FA addition significantly decreased the relative abundance of *Methanobacterium* (from 13.7% to 8.9%) and *Methanosarcina* (from 26.9% to 21.7%) (Fig. 5d).

To further characterize the microbial community involved in As behavior, OTUs were selected and analyzed by STAMP. At OTU level, 1.5% HA treatment increased the relative abundance of OTU4880 by 2-fold (Fig. 6a), which had a high similarity with *Clostridium* (Fig. 6c). Compared with control, 1.5% FA addition increased the relative abundance of OTU4002, OTU2845, OTU4027, OTU3111 and OTU4004 by 20-fold, 2-fold, 3-fold, 2-fold, 45-fold, respectively (Fig. 6b). OTU4027 and OTU4004 were similar to *Clostridia* bacteria and iron-reducing bacteria, respectively. OTU2845 and OTU3111 were similar to *Methanosarcina sp.* and *Methanobacterium bryantii.*, respectively (Fig. 6b). These OTU might belong to the genus of *Methanobacterium* and *Methanosarcina*, repectively. OTU4002 was similar to *Methanosarcinaceae*.

4. Discussions

We investigated the impacts of two key components of HS on As speciation, Fe, Mn, acetic acid and microbial communities in paddy soils. Our results showed that FA addition elevated As levels in soil porewater (Fig. 3b). FA could mobilize As in paddy soil in a variety of ways. In term of biochemistry, the quinone structure in FA can mediate the process of DIR, which would promote the release of arsenic from iron mineral [23]. The results of FTIR suggested the presence of quinones in FA (Fig. 1a), which have been reported to associate with DIR via electron shuttling [25,34,35]. In addition, FA treatments significantly increased the concentrations of acetic acid in

porewater (Fig. 1d). It is known that acetic acid can serve as electron donor for DIR process reducing Fe(III) oxides or DAsR process reducing As(V) to As(III) [25,26,28]. Both DIR and DAsR processes result in arsenic release from soil minerals, and mobilize As [22]. The results of higher charge-discharge current of FA, i.e. higher charge and discharge capacity, further suggested that FA could increase As mobility in soil through serving as electron shuttle for DIR or DAsR processes. Therefore, in our study, FA addition significantly increased As, Fe and Mn concentrations in porewater, and there were significant positive correlation between As and Fe concentration (Fig. 4d). In term of physico-chemistry, our results suggested that FA can promote arsenic release via forming complex with As via Fe bridge. Our SEM-EDX results suggested the co-presence of As and Fe on FA (Fig. 2). The complex of As-Fe-FA is stable and soluble in soil porewater. Therefore, under FA treatments, As concentration in porewater maintained at high level until the end of incubation (Fig. 3b).

Compared with FA treatments, HA addition had minor influence on total As concentration in porewater (Fig. 3b).The FTIR analysis also suggested the presence of quinones in HA structure (Fig. 1a), which demonstrates that HA can serve as electron shuttle for DIR [19]. However, the released As and Fe could be complexed to form As-Fe-HA, as showed in Fig. 2 through SEM-EDX analysis, and this complex was insoluble and precipitated. Therefore, in our study, the highest dose of HA addition decreased As and Fe concentration in soil porewater on day 14 (Fig. 3b).

Our previous studies have shown that application of different organic substances, such as rice straw, to paddy soil could significantly increase DMA concentration in soil porewater, and enhance As volatilization [14,15,36]. In this study, we also found that both HA and FA addition promoted arsenic methylation. However, under high FA treatments, As volatilization was not increased as that under low FA treatments (Fig. 3a). We speculate that under FA treatments, more As was complexed with additional FA via Fe bridge, thus less As was available as substrate for arsenic methylating enzyme, such as *arsM*. Therefore, the process of As methylation could be inhibited under high FA treatments. And it is interesting that at the late incubation (after 21 days incubation) of FA treatments, MMA concentration was significantly increased with the increasing dose of FA addition (Fig. 3b). We speculate that MMA could also be complexed by FA, and the soluble MMA -FA or MMA-Fe-FA cannot be further methylated to DMA. Anyway the detailed MMA forms in FA treatments remain to be investigated in the future.

Microorganisms play essential roles on arsenic mobility and transformation in paddy soil [37,38]. In this study, we found that both HA and FA addition significantly changed the microbial community in the soil (Fig. 5a and 5b). Especially under FA treatments, even at the lowest dose of addition (0.2%), microbial community in soil was significantly modified (Fig. 5b). The addition of FA significantly increased the relative abundance of *Desulfitobacterium* (from 0.2% to 4.7%) (Fig. 5c). It is generally accepted that *Desulfitobacterium* can use various electron acceptors, such as As(V), Fe (III) and a variety of oxidized sulfur species [26,39,40]. It is demonstrated that *Desulfitobacterium* can promote As release by reducing As(V) on the solid phase into soluble As(III) via DAsR [26]. In our study, there was significant correlation between As(III) concentration in porewater and the relative abundance of *Desulfitobacterium* (Fig. S5). These results indicated that *Desulfitobacterium* might be involved in arsenic release via DAsR process in FA treated soils. The relative abundance of *Azospirillum* also increased under FA addition. *Azospirillum* have been proved to be able to degrade desferrioxamines [41], indicating that *Azospirillum* may

be related to the release of arsenic and iron in porewater. Previous studies have shown that *Azospirillum* had the potential to oxidize As(Ⅲ) to As(Ⅴ) [42,43]. In this study, FA addition also increased the relative abundance of fermentation-related microorganisms. *Christensenellaceae_R_7_group* is the member of Christensenellaceae which can utilize a variety of sugars and produce acetic acid [44]. *Hydrogenispora* is a kind of hydrogen-producing bacterium which can ferment various sugars other than aromatic compounds and amino acids [45]. Therefore, *Christensenellaceae_R_7_group* and *Hydrogenispora* might be involved in FA degradation. In addition, under 1.5% FA treatment, the relative abundance of OTU4004 increased by 45-fold when compared with control. OTU 4004 had a high similarity with iron-reducing bacteria (Fig. 6b). These results indicated that iron-reducing bacteria might play important role in As release via DIR process in FA treated soils. The significant increase of Fe concentration in porewater under FA treatments, and the positive correlation between Fe and As concentration in porewater further suggested the important role of iron-reducing bacteria on As release under FA treatments.

Compared with FA, HA addition showed much smaller effect on microbial community. Under 1.5% HA treatment, the relative abundance of OTU4880 increased by 2-fold when compared with control. The OTU4880 showed a high similarity with *Clostridium,* which has been reported to be involved in iron reduction (Fig. 6a) [46,47]. These results indicated that DIR process could be also involved in As release under some stages of HA treatment. However, the effects of HA on iron-reducing bacteria (the relative abundance increased by 2-fold) was much smaller than that of FA (the relative abundance increased by 45-fold). This result suggested that the contribution of DIR process to As release under HA treatments was much lower than that under FA treatments, which explained why Fe and As concentrations in porewater of HA treatments were much lower than those of FA treatments (Fig. 3b and Fig. 4a).

In addition to bacteria, HA and FA addition also significantly modified archaeal communities (Fig. 5d). HA addition increased the relative abundance of *Methanobacterium* by about 2-fold, and high dose of FA addition also significantly increased the relative abundance of OTU3111 and OUT 2845, which might belong to the genus of *Methanobacterium* and *Methanosarcina*, repectively (Fig. 6d). It has been recently reported that methanogenic microorganisms are involved in As methylation via *arsM* contained in methanogens or other enzymes mediating methyl transfer during methanogenesis [48-50]. Therefore, in our study, Both HA and FA addition promoted As methylation. The amount of volatile As was significantly increased at the late stages of incubation (21 and 28 days), as well as the concentrations of metylated As species in soil porewater significantly increased by HS addition (Fig. 3).

Interestingly, FA addition remarkably elevated the relative abundance of *Bathyarchaeota.* Under 1.5% FA treatment, the relative abundance of *Bathyarchaeota* was 45.6% at phylum level (Fig. 5d). More impressive, when we use the special primer for archaea 16s rDNA (524F10extF_Arch958RmodR), the relative abundance of *Bathyarchaeota* was more than 80% (Fig. S6). These results suggested that the absolutely dominated microorganism, *Bathyarchaeota,* might play an important role in the culture system. It has been reported that *Bathyarchaeota* was not only abundant in the marine sediments, but also widely distributed in hot spring, freshwater, terrestrial and other environments [51-53]. However, so far *Bathyarchaeota* has not been successfully isolated from the environment [54-56]. A recent study showed that

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lignin can enhance the abundance of *Bathyarchaeota,* and which may play an important role in the degradation of lignin under anaerobic environment [55]. By using lignin, the abundance of *Bathyarchaeota* doubled in 2-3 months, in our study, after 42 days' incubation with 1.5% FA, the relative abundance of *Bathyarchaeota* increased about 4 times when using ArBa515F_Arch806R (Fig. 5d), and 8 times when using 524F10extF/Arch958RmodR (Fig. S6). The dramatic increase of the relative abundance of *Bathyarchaeota* suggested that FA could be more suitable carbon source for the growth of *Bathyarchaeota*. In the future, the specific function of *Bathyarchaeota* on FA degradation and As biotransformation is worth to be investigated.

5. Conclusions

This study demonstrated that different HS exhibited varied effects on As mobilization. FA application could significantly mobilize As by acting as electron shuttle or donor, and the released As can be stable in soil environment which might cause non-negligible environmental risks. While HA application did not significantly affected As mobility in paddy soil. Both FA and HA had minor effects on As methylation. These results suggest that FA should not be used in As contaminated paddy soil in terms of food safety, proposing new concerns of soil carbon sequestration under heavy metal contaminated conditions. Furthermore, we found that FA could be suitable carbon source for the growth of *Bathyarchaeota*. Above all, our results extend our understanding of the mechanisms governing the interactions of As with Fe and HS in soil mediated by microorganisms.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

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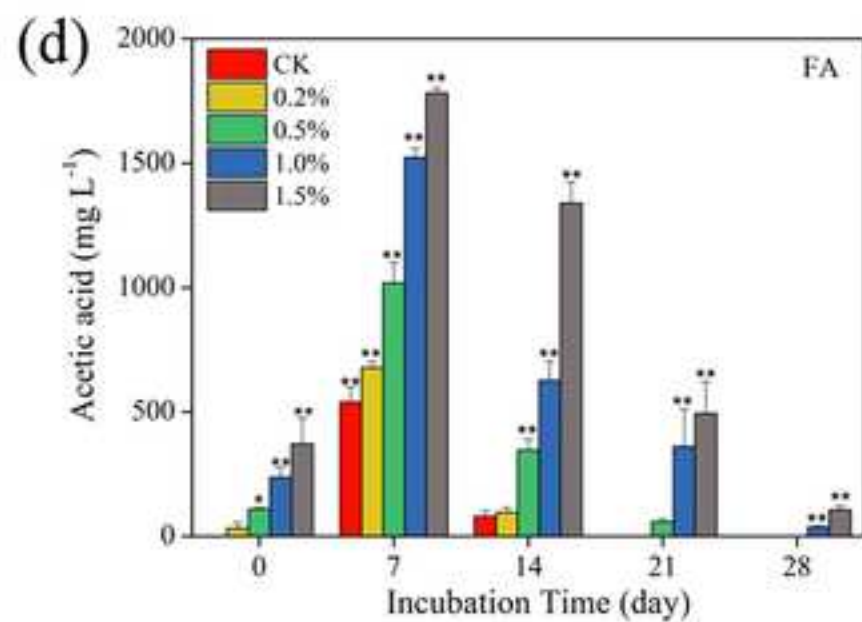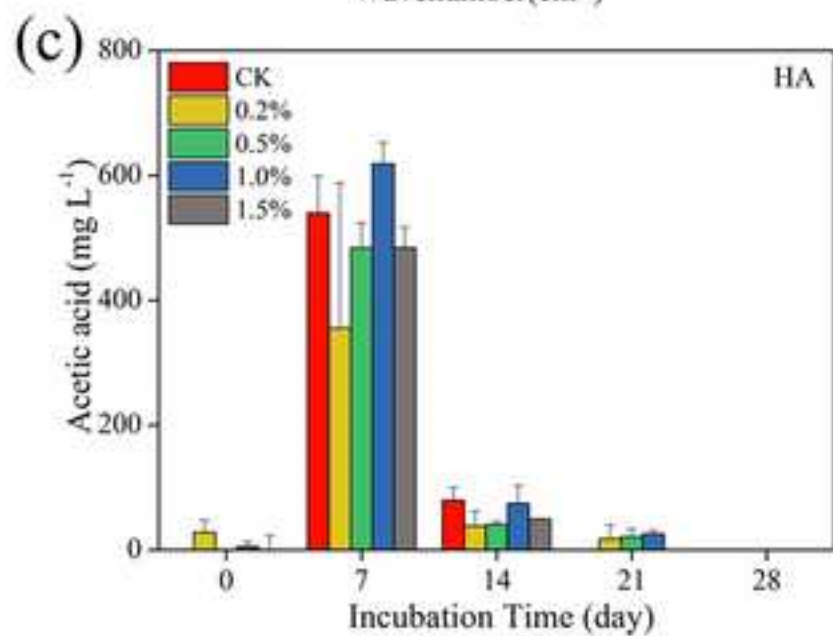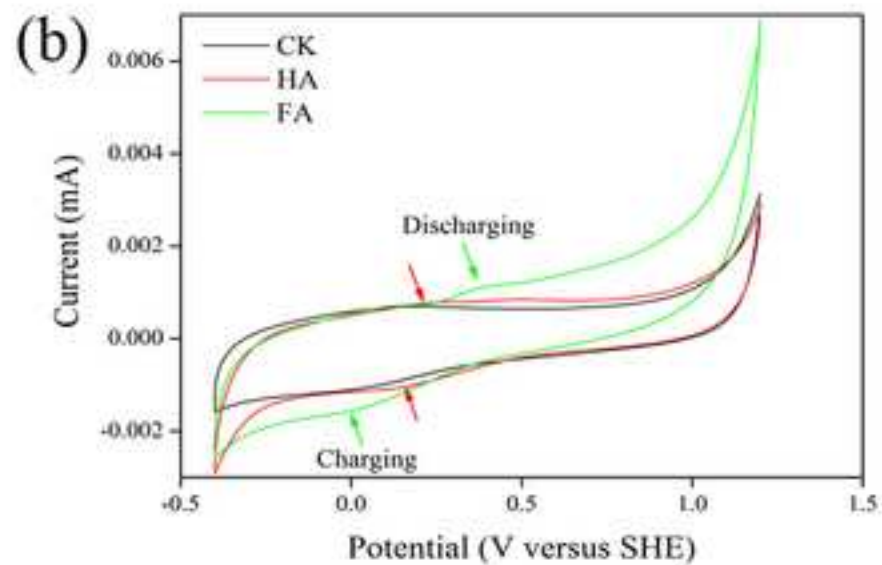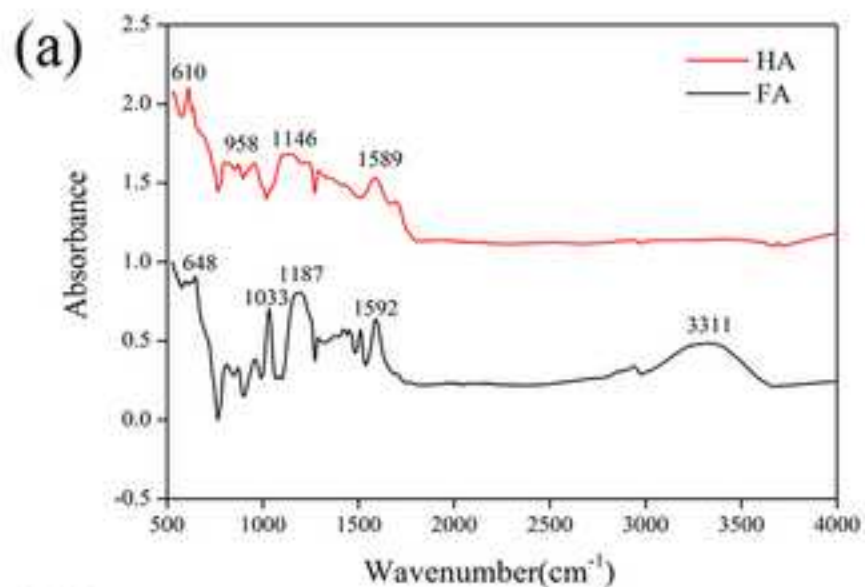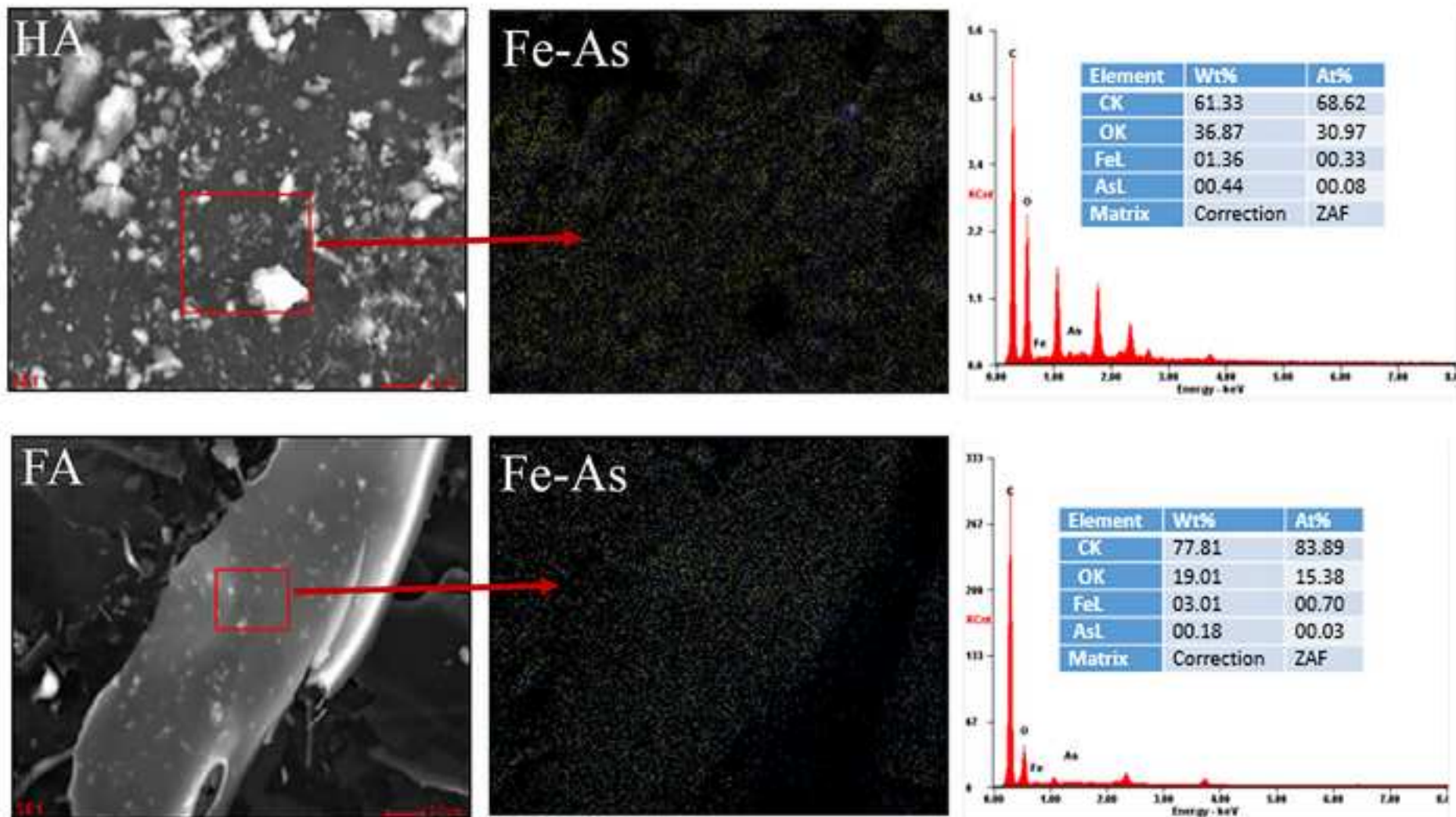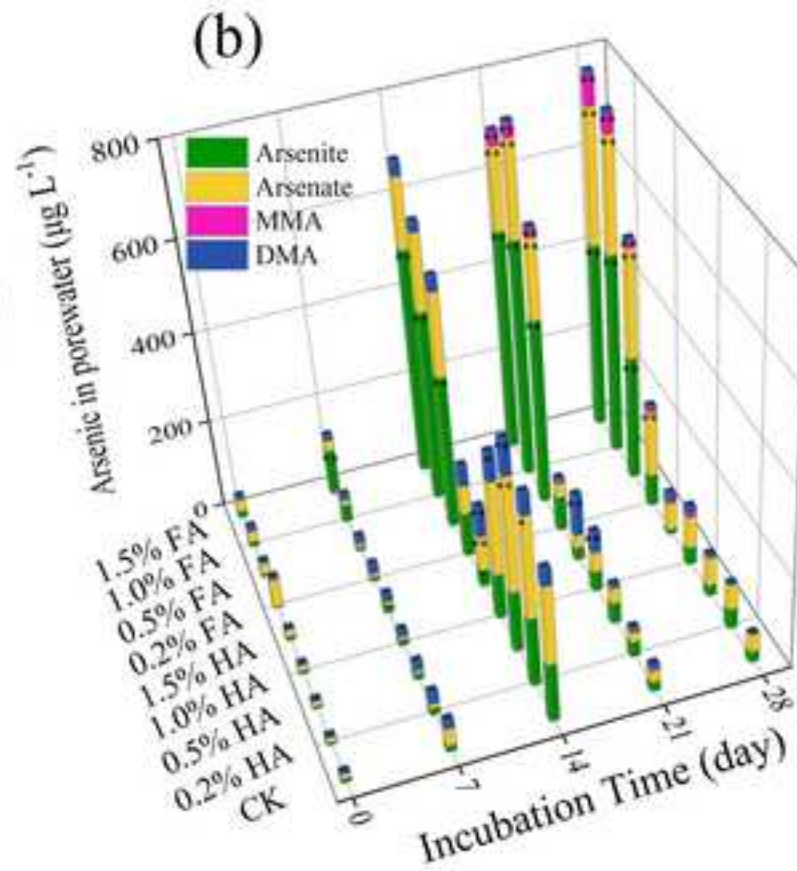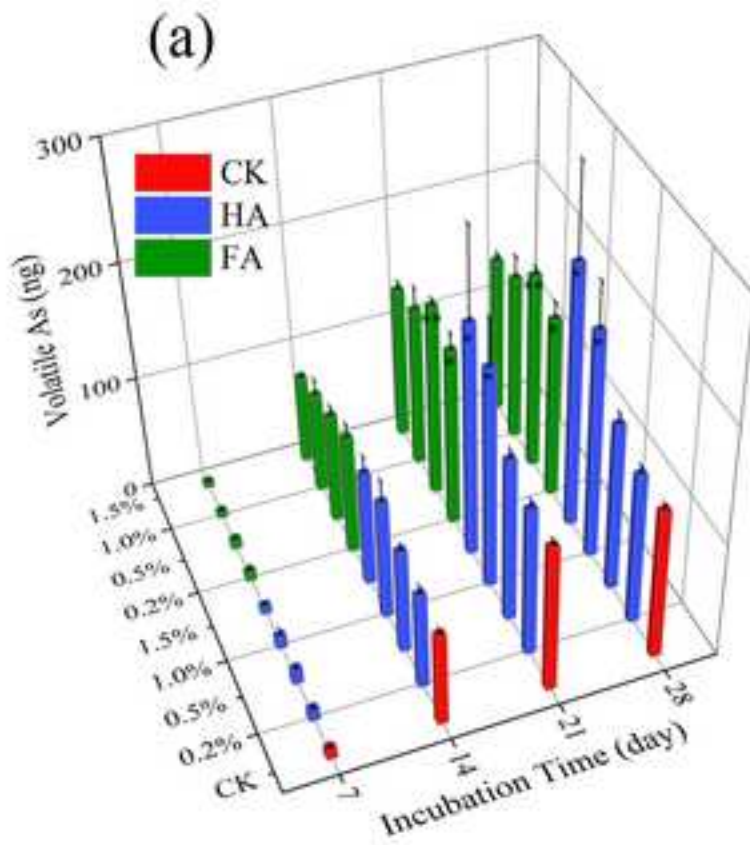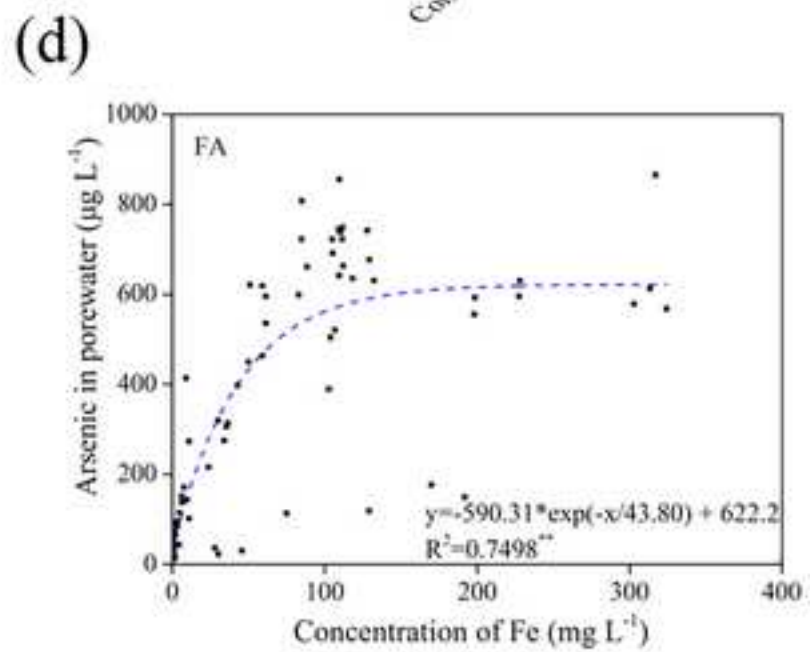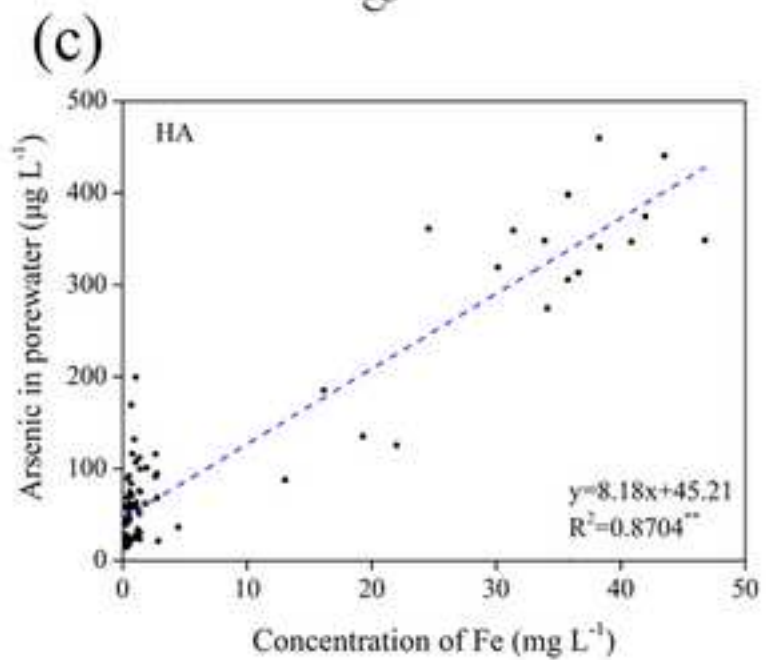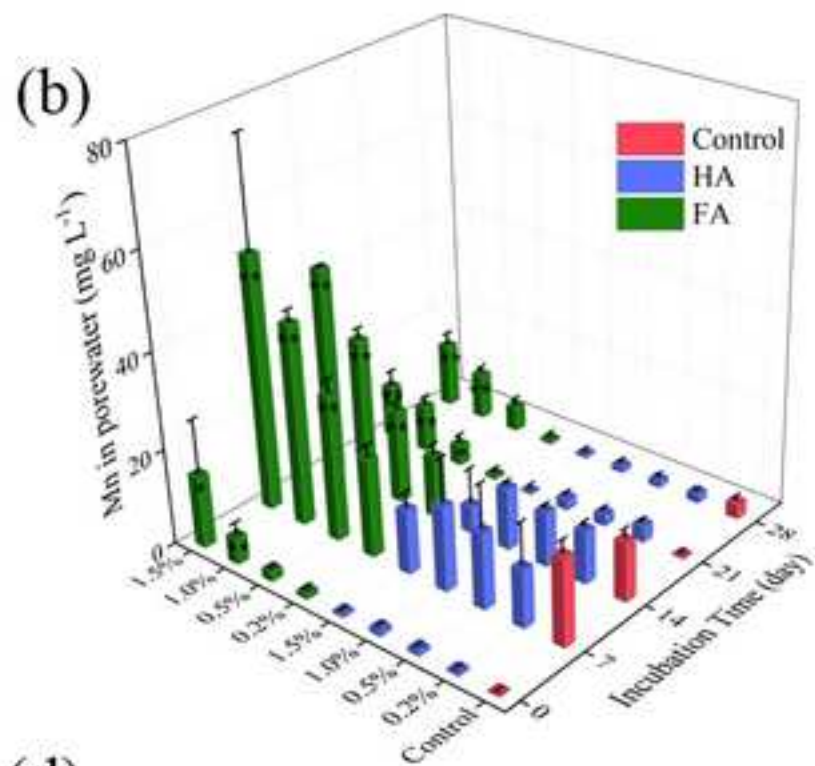
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Figure 2
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Figure 3
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Figure 5

(a) PCoA on Genus level — HA

PC2(13.14%) vs PC1(59.09%)

(b) PCoA on Genus level — FA

PC2(24.39%) vs PC1(49.8%)

(c) Bacteria

Relative abundance(%)

(d) Archaea

Relative abundance(%)

CK
0.2%
0.5%
1.0%
1.5%

Bacteria

others
unclassified_c__Gammaproteobacteria
Clostridium_sensu_stricto_8
unclassified_k__norank_d__Bacteria
norank_f__Christensenellaceae
Oxobacter
Desulfitobacterium
Christensenellaceae_R-7_group
Hydrogenispora
Azospirillum
unclassified_o__Clostridiales
norank_c__Bacteroidetes_vadinHA17
norank_f__RSV40
Fonticella
Clostridium_sensu_stricto_10

Archaea

others
Candidatus_Methanoperedens
norank_p__Woesearchaeota__DHVEG-6_
Methanomassiliicoccus
norank_c__Soil_Crenarchaeotic_Group_SCG_
Methanosaeta
norank_f__Rice_Cluster_II
Methanobacterium
Rice_Cluster_I
Methanocella
Methanosarcina
g__norank_p__Bathyarchaeota

Figure 6
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Supplementary Material