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RESEARCH ARTICLE

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Raphaël Lami[1,2,3], L. Camille Jones[4], Matthew T. Cottrell[1], Brandon J. Lafferty[4], M. Ginder-Vogel[4], Donald L. Sparks[4] & David L. Kirchman[1]

[1]School of Marine Science and Policy, University of Delaware, Lewes, DE, USA; [2]UPMC Univ Paris 06, UMR 7621, LOBB, Observatoire Océanologique, Banyuls-sur-Mer, France; [3]CNRS, UMR 7621, LOBB, Observatoire Océanologique, Banyuls-sur-Mer, France; and [4]Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA

**Correspondence:** David L. Kirchman, School of Marine Science and Policy, University of Delaware, Lewes, DE 19958, USA. Tel.: +302-645-4375; fax: +302-645-4007; e-mail: kirchman@udel.edu

**Present addresses:** L. Camille Jones, Department of Environmental Earth System Science, Stanford University, Stanford, CA, USA
Brandon J. Lafferty, Calera Corporation, Los Gatos, CA, USA

M. Ginder-Vogel, Department of Civil and Environmental Engineering, University of Wisconsin, Madison, WI, USA

Received 4 December 2011; revised 2 December 2012; accepted 8 December 2012. Final version published online 8 January 2013.

DOI: 10.1111/1574-6941.12061

Editor: Cindy Nakatsu

**Keywords**
arsenite oxidation; soil bacterial communities; molecular approaches; 16S rRNA pyrosequencing.

## Abstract

The influence of arsenite [As(III)] on natural microbial communities and the capacity of exposed communities to oxidize As(III) has not been well explored. In this study, we conducted soil column experiments with a natural microbial community exposed to different carbon conditions and a continuous flow of As(III). We measured the oxidation rates of As(III) to As(V), and the composition of the bacterial community was monitored by 454 pyrosequencing of 16S rRNA genes. The diversity of As(III)-oxidizing bacteria was examined with the *aox* gene, which encodes the enzyme involved in As(III) oxidation. Arsenite oxidation was high in the live soil regardless of the carbon source and below detection in sterilized soil. In columns amended with 200 μmol $kg^{-1}$ of As (III), As(V) concentrations reached 158 μmol $kg^{-1}$ in the column effluent, while As(III) decreased to unmeasurable levels. Although the number of bacterial taxa decreased by as much as twofold in treatments amended with As(III), some As(III)-oxidizing bacterial groups increased up to 20-fold. Collectively, the data show the large effect of As(III) on bacterial diversity, and the capacity of natural communities from a soil with low initial As contamination to oxidize large inputs of As(III).

## Introduction

Arsenic (As) contamination of soils and waters is a major public health issue in many parts of Southeast Asia and around the world (Nickson *et al.*, 1998; Nordstrom, 2002; Smedley, 2003; Duker *et al.*, 2005; Bhattacharya *et al.*, 2007; Fendorf *et al.*, 2010). This contamination is due to natural and anthropogenic release of various chemical forms of As. The environmental mobility and toxicity of inorganic As in soils is dependent on oxidation state, which is typically either arsenite [As(III)] or arsenate [As (V)] (Cullen & Reimer, 1989; Masscheleyn *et al.*, 1991; Borch *et al.*, 2010). At circumneutral pH, As(V), an oxyanion, can sorb to clay and mineral component of soil such as iron (Fe) oxides. Because arsenite is more mobile and more toxic than As(V) due to its neutral charge in soil, As(III) oxidation is a key reaction in As detoxification and immobilization (Oremland & Stolz, 2003). Because arsenite oxidation by oxygen ($O_2$) is kinetically slow, soil minerals and bacteria are the relevant As(III) oxidants (Eary & Schramke, 1990). As a result of this complexity, the influence of As(III) on microbial communities and the role of microbes in As(III) oxidation in soil remain poorly characterized.







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A few studies have examined the effects of As(III) contamination on bacterial composition and community structure (Jackson *et al.*, 2001; Macur *et al.*, 2004; Bruneel *et al.*, 2006; Lorenz *et al.*, 2006; Bouskill *et al.*, 2010; Halter *et al.*, 2011). However, because various other metals, such as cadmium or copper, also contaminated these environments examined by previous studies, the effects of specifically As(III) on microbial communities remain unclear. Additionally, most studies on As(III)-oxidizing bacteria have focused on cultivated strains, which are a small (< 1%) percentage of the total bacterial community of natural environments (Liao *et al.*, 2011). A few studies examined uncultivated potential As(III) oxidizers as detected by 16S rRNA genes (Jackson *et al.*, 2001). However, these genes are poor predictors of potential As(III) oxidation because this process is potentially carried out by many different bacteria (reviewed in Oremland & Stolz, 2003). Furthermore, few published studies have linked As(III) oxidation rates with As(III)-oxidizing gene diversity and abundance.

Arsenite oxidation is mediated by arsenite oxidase, a heterodimeric periplasmic enzyme containing molydopterin (Ellis *et al.*, 2001) encoded by *aox* genes (Silver & Phung, 2005). The *aoxB* genes have been found in cultivated bacteria from diverse prokaryotic groups, including *Alphaproteobacteria, Gammaproteobacteria,* and *Archaea* (Turner, 1954; Osborne & Enrlich, 1976; Oremland & Stolz, 2003; Quéméneur *et al.*, 2008). These genes have been used successfully to monitor As(III) oxidizers in natural environments (Oremland & Stolz, 2003; Inskeep *et al.*, 2007; Quéméneur *et al.*, 2010; Heinrich-Salmeron *et al.*, 2011). Culture-independent studies of *aox* genes have suggested that a few *Alphaproteobacteria* (mainly *Bosea* sp.), *Betaproteobacteria* (mainly *Acidovorax*), and *Gammaproteobacteria* (mainly *Pseudomonas*) are the major As(III) oxidizers in soil and freshwater environments (Oremland & Stolz, 2003). These reports suggest that the capacity for As(III) oxidation is widespread and could be important in the microbial world.

The goal of this study was to quantitatively evaluate the impact of As(III) on microbial communities in flow-through column experiments with and without an additional carbon source. We measured the time dependency of bacterial vs. abiotic driven As(III) oxidation by comparing live and sterile soil columns. We then examined the diversity of 16S rRNA genes by 454 pyrosequencing to monitor bacterial community structure before and after amendment with As(III) and organic material. The diversity and abundance of As(III)-oxidizing genes were examined with *aox* gene targeted clone libraries, and the abundance of these oxidizers was estimated using quantitative PCR (qPCR) analysis of *aox* genes. Our results show the importance of biological processes in As oxidation even though As(III) selected against many bacteria and reduced overall diversity of soil microbial communities.

## Materials and methods

### Design of soil columns experiments

Soil samples of the Matapeake series were collected from a depth of 5–10 cm at the University of Delaware Farm, Newark, DE (39° 39′57″ N; 75° 45′05″ W). Aliquots of soil samples were air-dried for soil analysis and sterilization. The Matapeake soil is a silt loam with 13.5% sand, 64.5% silt, and 22% clay. The cation-exchange capacity (CEC) was 16.34 cmol $kg^{-1}$, pH was 6.2, bulk density of the column soil was 1.32 g $cm^{-3}$, and organic matter content, estimated by loss on ignition, was 2.6%. Air-dried soil was acid-digested (nitric and hydrochloric acid) with microwave heating following the protocol for EPA 3051 soil analysis and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (US EPA, 1994). Total Fe was 15 476 mg $kg^{-1}$, and total Mn was 400 mg $kg^{-1}$, total Pb was 76.4 mg $kg^{-1}$, and total As was 15.5 ± 4.9 mg $kg^{-1}$. Air-dried samples of the Matapeake soil were sterilized by autoclaving (1 h, 121 °C) and γ-irradiation, which is considered to have minimal geochemical effect on soil (McNamara *et al.*, 2003). Air-dried soil was irradiated with a dosage of 25 000 Gy using a Sheperd Mark 1 γ-irradiator.

Soil columns were packed immediately after sampling to preserve the activity of native bacteria and were prepared in triplicate. All columns were assembled in a sterile aerobic environment at ambient room temperature. Fresh soil was gently crushed at field moist conditions, large (> 200 μm) particles were removed, and remaining soil was mixed with sterile coarse quartz sand in a 30 : 70 soil to sand ratio by mass. Column soils were not dried to minimize changes that may affect microbial community. Quartz sand was added to increase the porosity and prevent development of preferential flow paths. The soil–sand mixture was packed into sterile 20-mL plastic syringes (8.5 cm long) and capped with sterile glass wool on bottom and rubber stoppers on top. Influent entered at the bottom of the columns to prevent preferential flow paths and to reduce the effect of gravity on flow velocity.

The soil columns were amended with As(III)-spiked media with and without additional organic material. The concentration of As(III) used in all columns was 200 ± 50 μM added as $NaAsO_2$. One measurement of As concentration was carried out per column. Organic carbon-amended media (OM media) included 5 mg $L^{-1}$ yeast extract, 10 mg $L^{-1}$ tryptic soy broth, and 500 mg $L^{-1}$ NaCl. Organic carbon-free media contained

only 500 mg $L^{-1}$ NaCl. All media had trace metals added at the following concentrations: 0.100 mg $L^{-1}$ $MnCl_2$-$4H_2O$, 0.120 mg $L^{-1}$ $CoCl_2$-$6H_2O$, 0.070 mg $L^{-1}$ $ZnCl_2$, 0.060 mg $L^{-1}$ $H_3BO_3$, 0.025 mg $L^{-1}$ $NiCl_2$-$6H_2O$, 0.015 mg $L^{-1}$ $CuCl_2$-$2H_2O$, 0.025 mg $L^{-1}$ $Na_2MoO_4$-$2H_2O$, and 0.001 mg $L^{-1}$ 12 M HCl. The pH of all media was 6.5–7. Media solutions were sterilized by autoclaving.

A peristaltic pump was used to flow media through the columns at a rate of 10 mL $h^{-1}$, resulting in a pore water velocity of 4.25 cm $h^{-1}$. Aerobic conditions in the columns were verified by monitoring Fe(II)/Fe(III) in the effluent solution using the ferrozine spectrophotometric method (Stookey, 1970). Samples of the effluent were taken daily, filtered with a 0.22-μm nylon filter, and refrigerated prior to As speciation analysis. Arsenic speciation [As(III) and As(V)] was determined by high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP/MS) (HPLC: Agilent 1200; ICP/MS: Agilent 7500cx) following the protocol described in Parikh *et al.* (2010). Arsenite concentration over time was measured in triplicate columns, and all As chemical data errors are the standard deviations of these replicates.

The experiment consisted of four treatments: (1) sterilized soil and As(III) in the flow-through media; (2) live soil and As(III) and without the OM media; (3) live soil with the OM media; and (4) live soil with As(III) and organic material in the flow-through media. The experiment was run for 22 days after which the columns were sacrificed for chemical and molecular analyses of bacterial communities. All samples for microbial analyses were preserved at −80 °C until analyses. The columns were run in triplicate. The molecular biology analyses were conducted on one column per treatment, but the qPCR assays were run in triplicate. The As(III) and As(V) kinetics were very similar in the triplicate columns, suggesting that the biological variation among the three columns was minimal. Also, our conclusion about the effect of As on bacterial diversity was based on two As-treated columns vs. two samples without added As (the initial soil and the column receiving only organic material; see Results).

## Nucleic acids extraction and identification of *aoxB* genes

DNA was extracted using an UltraClean Soil DNA Isolation Kit (MoBIO) and quantified in triplicate via a standard picogreen assay (Invitrogen) on a POLARstar Optima fluorometer (BMG Labtech). The *aoxB* genes were recovered using universal *aoxB* genes primers (Table 1) described previously (Quéméneur *et al.*, 2008). A detailed review and list of phyla targeted by these primers are discussed by Quéméneur *et al.* (2008). Ten nanograms of DNA was used in PCR amplifications, with the following conditions: one cycle of 94 °C, 2 min, followed by 30 cycles of 94 °C, 30 s; 63 °C, 30 s; and 72 °C, 30 s. Final concentrations of primers and $MgCl_2$ were 1.87 mM and 2 mM, respectively. The PCR products were subjected to electrophoresis (1.5% agarose), and the products of the expected size were excised from the gel and eluted with a Gene Clean gel extraction kit (Q-Biogene). The purified fragments were cloned with a TOPO TA cloning kit for sequencing (Invitrogen) according to the manufacturer's instructions. Colonies were screened via PCR to determine the insert size, and 120 clones of the expected size were sequenced by standard Sanger sequencing. Sequences were trimmed of the vector sequence and aligned using the CLUSTAL program in MEGA version 3.1 (Tamura *et al.*, 2007). Sequences were added to an alignment containing most of the known *aox*B sequences. Within-group mean distances were calculated in MEGA 3.1. Phylogenetic trees of the translated sequences were constructed in MEGA 3.1 with the default parameters, based on both neighbor-joining and minimum evolutionary algorithms with 500 bootstrap replicates. Rarefaction analysis, coverage estimations, and Shannon diversity index calculations were performed with DOTUR (Schloss & Handelsman, 2005). The libraries were compared using libshuff (Singleton *et al.*, 2001).

## Quantification of *aoxB* and 16S rRNA gene abundance

One group of *aoxB* genes (Group 1), related to *Alphaproteobacteria,* was targeted by qPCR. This group was

**Table 1.** List of PCR and qPCR primers used in this study

| Name | Target | Sequence (5′ -> 3′) | Annealing (T °C) | Reference |
|---|---|---|---|---|
| w49 dir | All bacterial 16S rRNA genes | CGG TCC AGA CTC CTA CGG | 61° | Delbès *et al.* (2001) |
| w34 rev | | TTA CCG CGG CTG CTG GCA C | | Lee *et al.* (1996) |
| aoxBM1-2F | All *aox* genes carrying bacteria | CCA CTT CTG CAT CGT GGG NTG YGG NTA | 52° | Quéméneur *et al.* (2008) |
| aoxBM″-2R | | TGT CGT TGC CCC AGA TGA DNC CYT TYT C | | Quéméneur *et al.* (2008) |
| G1_27F | *Aox* genes from bacterial group 1 | GTC GTC CAG GAA CAA GGT | 69° | This study |
| G1_209R | | TCG CCT ACT CCC ATA TCA | | This study |

selected because of its relative abundance in *aoxB* clone libraries (see Results). Primers for this *aoxB* groups were designed using ARB (Ludwig *et al.*, 2004) with an alignment containing a large set of environmental and strain-derived *aoxB* sequences. One *aoxB*-containing plasmid was used as a positive control and for standard curves in the qPCR assays. The *aoxB*-containing plasmid related to the targeted *Alphaproteobacteria* Group 1 was isolated from environmental clones (Qiaprep kit, Qiagen) and linearized with PstI (Invitrogen). Products were quantified in triplicate using the picogreen assay. Standard reactions with linearized plasmid contained approximately $10^1$–$10^6$ copies per reaction. All standard curves were linear within the ranges tested. All qPCR reactions were performed in triplicate or quadruplicate with 1 μL of diluted DNA (0.1 ng $mL^{-1}$) in a final volume of 12.5 μL using the Stratagene SYBR green mix with an ABI 7500 (Applied Biosystems). The qPCR conditions were an initial denaturation step at 95 °C (10 min), followed by amplification at 95 °C for 15 s, the indicated annealing temperature for 45 s (Table 1), and 72 °C for 45s, with a final dissociation step. Final primer concentrations were 0.2 mM. Only single peaks were observed in the dissociation curves for both the standards and samples (data not shown), indicating specific amplification with each set of primers. Amplification efficiencies were between 95% and 105%, and no inhibition was detected when a known quantity of standard was added to each sample (data not shown). The detection limit of qPCR assays was about 10 copies. To verify the specificity of the *aoxB* primers, the qPCR primer pair was used to amplify *aoxB* genes from the column experiments, and the resulting products were cloned. The PCR conditions were as outlined above and in Table 1. PCR products were cloned and sequenced as described above. Of 10 clones that were analyzed, all contained the expected *aox*B sequences related to the targeted group. We evaluated the coverage of our new primer set targeting Group 1. These tests indicated that our primers match 60% of sequences relative to this group in GenBank allowing for one mismatch for our probes. 16SrRNA genes were quantified following a similar protocol, using primers indicated in Table 1.

### Sequencing of 16S rRNA genes

All 16S rRNA genes were pyrosequenced on a Roche 454 FLX instrument (Research and Testing Laboratory, Lubbock, TX) using a mixture of Hot Start and HotStar high-fidelity taq polymerases, which generated amplicons of 250–550 bp starting from position 27 (*Escherichia coli* 16S rRNA number). Using QIIME (Caporaso *et al.*, 2010), the 16S rRNA sequences were demultiplexed and quality filtered, including homopolymer removal. Sequences were denoised using PyroNoise (Quince *et al.*, 2009), and chimeras were removed using ChimeraSlayer (http://sourceforge.net/projects/microbiomeutil). The data were randomly resampled to check whether the observed patterns were similar to those observed when each sample had the same number of sequences. Diversity indices were also calculated using QIIME (Quince *et al.*, 2009). All 16S rRNA genes sequences were deposited in GenBank (JN388250–JN388300).

## Results

### As(III) oxidation

We examined the rates of biotic and abiotic As(III) oxidation and the effect of As(III) on microbial communities over 22 days in soil columns. Arsenite oxidation was not detected in the sterilized soil, as As(III) was near the added concentration in the effluent over the entire experiment, while As(V) remained below 18 μmol $kg^{-1}$ (Fig. 1a). In contrast, oxidation was apparent in live columns where As(III) was added (Fig. 1b and c). After the first day, the concentration of As(III) in the effluent from the live soil column amended with only As(III) was 140 μmol $kg^{-1}$ or about 60% of the As(III) concentration in the influent (Fig. 1b). Arsenite oxidation remained steady in the live soil column for the first 10 days of incubation. After approximately 12 days, As(III) oxidation increased substantially, and after 16 days As(III) oxidation was high enough to reduce the concentration of As(III) to only 1% of the added concentration in the influent (Fig. 1b). Microbial As(III) oxidation resulted in a concentration of As(V) equal to 180 μmol $kg^{-1}$ in the effluent, which accounted for 80% of the decrease of As (III) within the column. High rates of As(III) oxidation continued for the remaining 8 days of incubation (Fig. 1b).

Based on concentrations of As(III) in the effluent, As (III) oxidation appeared to be similar in soils with and without the organic material (OM) addition, at least for the first 8 days of the incubation (Fig. 1B and C). By the end of the experiment, the concentration of As(III) in the effluent from the soils with and without OM averaged 158 ± 36 μmol $kg^{-1}$ and 128 ± 10 μmol $kg^{-1}$, respectively. The OM addition resulted in more As(V) being eluted after 10 days compared with the column without OM added (Fig. 1B and C). After 10 days, the concentration of As(III) in the effluent from columns with and without the OM addition averaged 10 ± 18 μmol $kg^{-1}$ and 44 ± 40 μmol $kg^{-1}$, respectively (Fig. 1B and C), and the majority of As recovered was As(V).

Total As content was measured in the initial soil, and after the column experiments were completed to examine

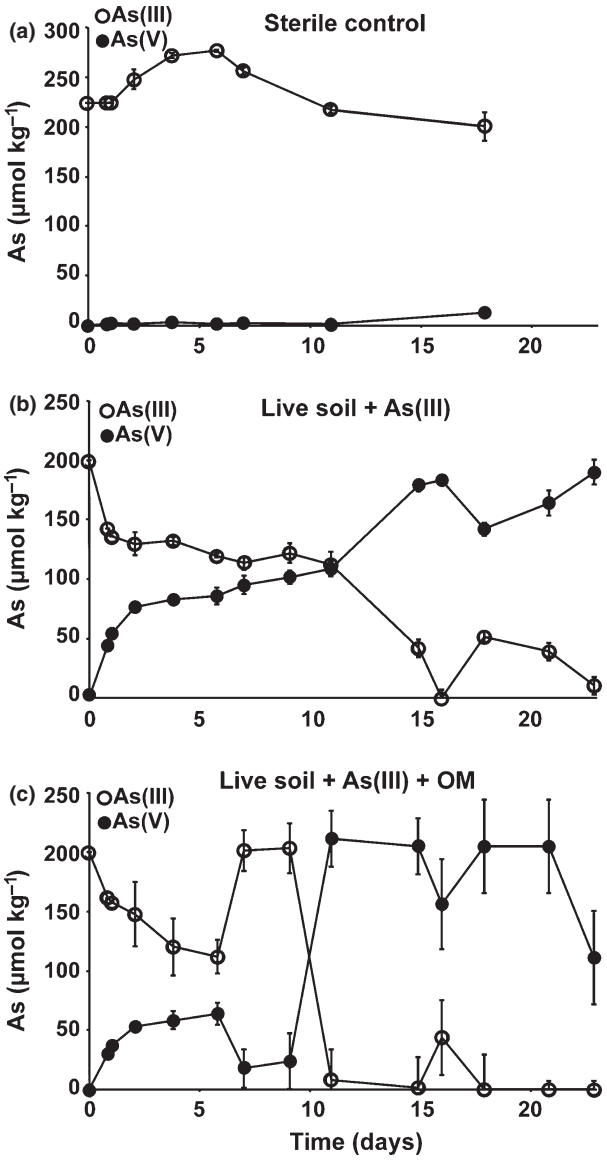


**Fig. 1.** As(V) and As(III) in effluent from column experiments. (a) Sterile soil, (b) arsenite-amended soil, and (c) arsenite and OM media-amended soil. Error bars represent the standard deviation of As concentrations over time in triplicate columns.

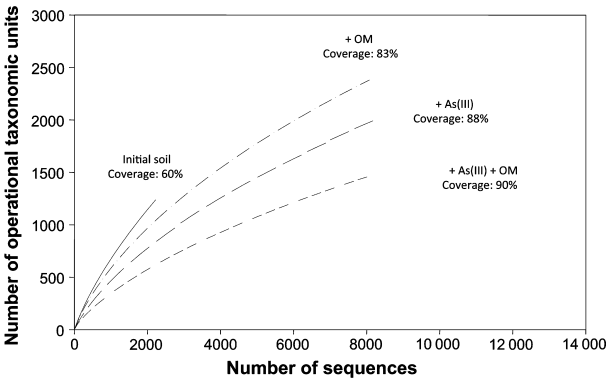


**Fig. 2.** Rarefaction analysis of 16S rRNA genes in the various treatments and in the initial soil. Operational taxonomic units (OTU) were defined as those sequences that were $\geq$ 97% similar. Coverage is given from a Chao1 analysis.

As retention. The initial soil contained approximately $0.21 \pm 0.0013$ mmol $kg^{-1}$ of As, which originated from historical pesticide use at the soil collection site. The column treated with only As(III) retained the most As ($2.05 \pm 0.26$ mmol $kg^{-1}$) followed by the column treated with As(III) and OM ($1.78 \pm 0.20$ mmol $kg^{-1}$). The sterile soil had about half the amount of residual As(III) ($0.98 \pm 0.07$ mmol $kg^{-1}$) compared with the live soils. Retention of As indicates that As(III) oxidation was accompanied by sorption of As(V) to mineral or organic phases in the soil. Sorption of As was enhanced in columns with live soil and significant but lower in sterile soils.

## Bacterial community structure

A total of 1300 operational taxonomic units (OTUs, grouped at $\geq$ 97% similarity) were identified in the soil used for the column experiments. The coverage index indicated that only 60% of the total 16S rRNA gene diversity was sampled (Fig. 2). The rarefaction curves and diversity indices indicated that diversity was lowest in the columns with added As(III) (Fig. 2). The Shannon index was highest in the initial soil ($7.1 \pm 0.32$) and lower in all treatments (< 7). When As(III) was added, the Shannon index was $5.4 \pm 0.09$, lower than the value ($6.1 \pm 0.21$) found in the columns with As(III) and OM. In the OM treatment without added As(III), this index was $6.9 \pm 0.23$. Similar trends were found using the Simpson index, and after sample size was equalized among all samples by random resampling (data not shown). This decrease of bacterial diversity was also evident in Chao1 values, which were as much as 50% lower in soils with added As(III) than in the initial soil (data not shown).

We analyzed the degree of similarity among the different communities by UPGMA clustering of the 16S rRNA gene sequences (Fig. 3). The communities were similar in the top, middle, and bottom section of the columns (similarity index > 70%), and any differences were small compared with the variations between the different columns (similarity index $\leq$ 55%). The sterilized soil differed the most from the initial soil. The communities in OM amended and As(III)+OM amended columns were the most similar (55%), while the columns with only added As(III) differed the most (Fig. 3). An analysis based on

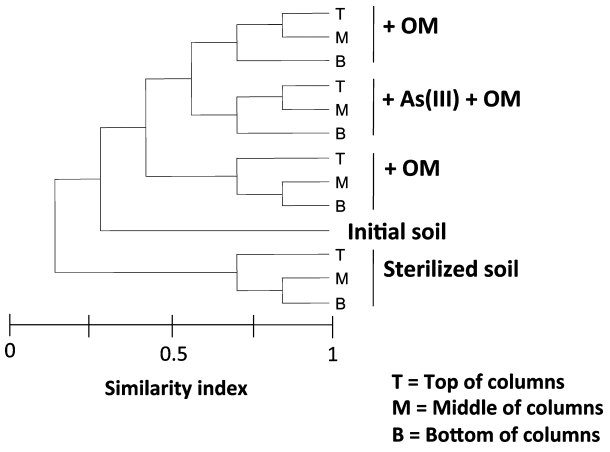


**Fig. 3.** UPGMA dendrogram of 16S rRNA genes in the various treatments and in the initial soil, based on OTUs sharing 97% similarity (Bray–Curtis distance).

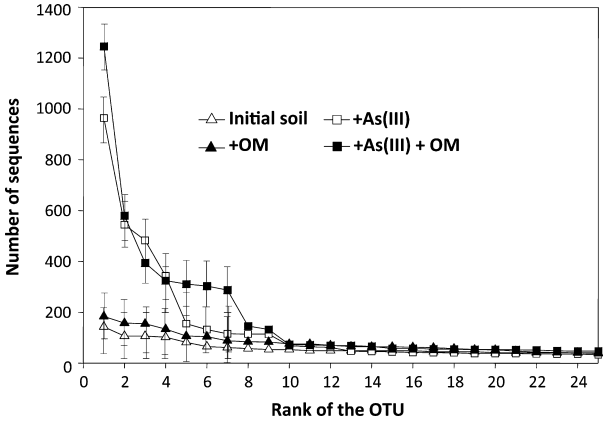


**Fig. 4.** Abundance-rank curves for the different treatments. For clarity, the figure gives only the 25 most abundant OTUs, which accounted for 40% of all sequences. The OTUs are defined at 97% similarity. The curves from the two As(III)-amended treatments were significantly higher than the OM-only amended treatment and the initial soil (Student's *t*-test, $P < 0.005$).

OTU presence/absence rather than abundance was similar (data not shown).

We used rank-abundance curves to examine the evenness of the soil microbial communities exposed to As (III). In the As(III)-amended columns, three OTUs dominated the community at the end of the experiment, while the abundance of all other OTUs was low (Fig. 4). By contrast, the microbial communities not exposed to As(III) were more even, and the rank-abundance curves were below those for the As(III)-exposed communities. The rank-abundance curves of microbial communities not exposed to As(III) were very similar to the rank-abundance curves for the initial soil community (Fig. 4). In the treatments where only As(III) was added, an OTU affiliated with the genus *Acidovorax* dominated the bacterial community, accounting for 9.6% of total 16S rRNA gene sequences. Similarly, in the treatment where As(III) and OM were added, *Chitinophaga* dominated the community, with 12% of all 16S rRNA gene sequences. By contrast, no single OTU dominated the community in the other treatments or in the initial soil. In those soils, the relative abundances of six OTUs were 1–2% of all 16S rRNA gene sequences, while all other sequences were < 1% of the total community (data not shown).

## Diversity of arsenite-oxidizing genes

Most of the *aoxB* gene sequences found in this study were difficult to assign to known As oxidizers because they were very different from *aox*B genes in cultivated strains (Fig. 5). In all treatments, the As(III)-oxidizing genes were most similar to those from *Alphaproteobacteria* (Group 1 and 8, for example) and *Betaproteobacteria*-related taxa (Group 8 and 9). No *aoxB* genes related to *Gammaproteobacteria* or *Chloroflexi* were detected (Fig. 5). Overall, the diversity and composition of *aoxB* genes did not differ significantly among the different treatments and from the initial soil ($P > 0.05$; libshuff analysis).

## Abundance of Group I *aoxB* genes and 16S rRNA genes

The *aoxB* sequence data were used to design primers to target alphaproteobacterial Group 1 (defined in Fig. 5) and to estimate its abundances using qPCR. Bacterial abundance differed significantly among the different treatments. We detected $2.4 \times 10^6 \pm 1.7 \times 10^6$ copies of Group1 *aoxB* related genes per g of soil in the initial sampled soil (Fig. 6a). This abundance sharply increased in the As(III)-only column (up to $1.7 \times 10^7 \pm 1.3 \times 10^6$ copies per g of soil) and in the As(III)+OM column (up to $8.07 \pm 1.3 \times 10^6$ copies per g of soil). In contrast, the abundance of Group 1 *aoxB* genes did not differ significantly between initial soil and the OM-only treatment (Fig. 6a).

Bacterial abundance in the different treatments was assessed by qPCR assays of 16S rRNA genes. The initial soil had $5.2 \times 10^7 \pm 8.9 \times 10^6$ copies of 16S rRNA genes per g of soil (Fig. 6b). Abundance of 16S rRNA genes increased in all treatments and in all sections of the columns, reaching about $10^8$ copies of 16S rRNA genes per g of soil. Abundances were highest for the middle section of the AsIII + OM treatment, with $1.1 \times 10^9 \pm 1.1 \times 10^8$ copies of 16S rRNA genes per g of soil.

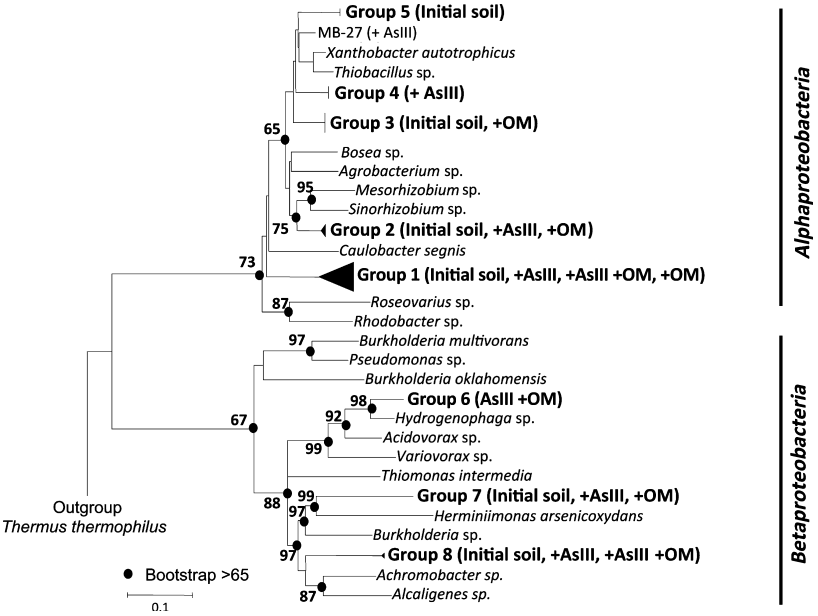


**Fig. 5.** Phylogenetic tree of *aox*B amino acid sequences based on a neighbor-joining analysis of translated nucleotide sequences. Numbers on branches represent bootstrap proportions from 500 replicates. The phylogenetic identification was based on cultured representatives containing *aoxB* genes.

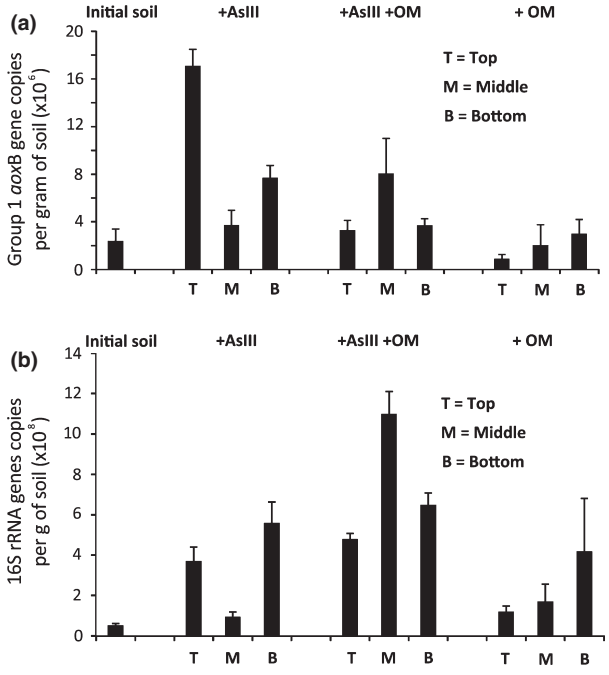


**Fig. 6.** (a) Abundance of *aoxB* genes related to Group 1, as defined in Figure 5. Top, middle, and bottom refer to the different sections of the columns. (b) Abundance of total 16SrRNA genes. Top, middle, and bottom refer to the different sections of the columns. Errors bars indicate the standard deviations of triplicate measurements.

## Discussion

In this study, our objective was to quantitatively evaluate the impact of As(III) on natural soil microbial communities by measuring microbiologically driven As(III) oxidation, the effect of As(III) toxicity on microbial diversity, and the growth of potential As(III) oxidizers over time in soil columns. We found that As(III) oxidation was primarily driven by biological or coupled biogeochemical processes, as oxidation was not measurable in sterile controls. Because of microbially driven oxidation, As(III) decreased to unmeasurable levels, while As (V) increased to nearly the maximum possible concentrations. With added As(III), bacterial diversity decreased by nearly one-half, and the dominant bacterial species shifted, resulting in a bacterial community enriched in As (III)-oxidizing genes. The abundance of some types of arsenite-oxidizing genes increased nearly 20-fold over initial levels.

Arsenite oxidation in the two treatments with live soil was greater than in the two controls, suggesting that purely chemically driven oxidation of As(III) in these experiments was limited. Because soil minerals such as manganese (Mn) oxides can oxidize As(III) rapidly (Lafferty *et al.*, 2010), we expected that the abiotic component would have a greater role in our column experiments. However, the soil used in these experiments had relatively low Mn content (Jones, 2011), and oxidization by Mn oxides may have been low due to age, crystallinity, and surface passivation of the oxides (Post, 1999; Lafferty *et al.*, 2010). As(III) oxidation by $O_2$ was likely low because it is a very slow process (Cherry *et al.*, 1979; Langner *et al.*, 2001; Eary & Schramke, 1990). The rapid biological As(III) oxidation observed in this study is similar to rates observed previously with As(III)-oxidizing bacteria (Langner *et al.*, 2001; Kulp *et al.*, 2004; Macur *et al.*, 2004; Jones *et al.*, 2012). Our observation that adding

OM enhanced oxidation of As(III) is consistent with a previous study using isolated strains (Santini *et al.*, 2002). Also, the OM addition probably led to the appearance of copiotrophic bacteria, including Gammaproteobacteria, as previous shown in other studies (Cleveland *et al.*, 2007).

Soil minerals still probably have a role in As redox reactions, and they were likely involved in the retention of As in our soils (Macur *et al.*, 2004). Soil minerals including Mn and Fe oxides and hydroxides are known to sorb As, and those minerals were likely responsible for some of the sorbed As found after digesting the soil at the end of our experiment (Fendorf *et al.*, 1997; Tournassat *et al.*, 2002). The live soils retained about twofold more As than the sterile soil, suggesting that removal of As from solution in the columns was a coupled biogeochemical process.

We found that As had a large impact on bacterial communities in these soil columns. Among the few relevant previous studies, one also found major variations in bacterial community structure due to As(III) mixed with other metals in riverine waters (Bouskill *et al.*, 2010). The toxicity of As(III) probably explains the large decrease in bacterial diversity in our soil columns. However, some of the bacteria in our experiments seem to have had enough time, given a generation time of days (Demoling *et al.*, 2008), to adapt to the added As(III). Longer experiments are needed to determine whether diversity recovers to levels seen in the absence of added As(III).

Addition of As(III) led to a microbial community dominated by a few types of bacteria, based on analyses of 16S rRNA genes. By contrast, no phylotype dominated the community in the treatments without As(III). Interestingly, the dominant bacteria in the As(III) and As(III)+OM treatments were affiliated with the genera *Acidovorax* and *Chitinophaga,* respectively, which have previously been reported in As(III)-contaminated environments (Santini *et al.*, 2002; Shivaji *et al.*, 2011). These results suggest that some phylotypes are able to grow in the presence of As(III), at least at the concentration of 200 μM provided in the study. Our qPCR measurements confirmed that some bacterial groups were able to grow in the treatments, as 16S rRNA gene abundance increased substantially during the experiment, up to 20-fold. The phylotypes that dominated the bacterial community in As(III) treatments were probably resistant to this metalloid (Paez-Espino *et al.*, 2009). Our analysis revealed that in all treatments, some *Alphaproteobacteria* and *Betaproteobacteria* phylotypes were *aox* gene carriers. Some of these phylotypes may be chemolithotrophs able to oxidize As(III). Interestingly, the diversity of *aoxB* genes did not vary substantially among the different treatments and in comparison with the initial soil. Previous studies demonstrated that As(III) oxidizers are able to grow over a large range of environmental conditions, including a variety of carbon and energy sources (Santini *et al.*, 2002).

Our data on the abundance of *aoxB* genes show that As(III)-oxidizing population increased when As(III) was added. This result is not surprising because As(III) oxidizers in As(III) contaminated environments have been easily cultivated (Santini *et al.*, 2000; Liao *et al.*, 2011). Our observation is also consistent with Quéméneur *et al.* (2008), which found that the total abundance of *aoxB* genes as measured by qPCR correlated with As(III) concentrations in French rivers. These results demonstrate that high fluxes of As(III) lead to bacterial communities enriched in As(III)-oxidizing genes.

In this study, we show that the oxidation of As(III) in soil were driven by microbially mediated processes. Also, we found that As(III) shifted the dominant bacterial species and was probably toxic to some species because bacterial diversity decreased substantially. These effects led to the selection of adapted As(III) oxidizers, which increased in the whole community and maintained rapid As(III) oxidation, especially in the presence of an additional source of organic carbon. Our study suggests that microbially driven oxidation and detoxification of As(III) in the natural environment can be driven at high rates by natural microbial populations.

## Acknowledgements

We thank Liying Yu for help in soil DNA extraction and Dr. Cameron Koch of the University of Pennsylvania Perelman School of Medicine for performing soil gamma irradiation. This work was supported by grants from the National Science Foundation (EPSCoR EPS-0814251 and EAR 0544246), the State of Delaware, and the Partners for Universities Fund and the Environmental Protection Agency (EPA) Science to Achieve Results (STAR) graduate fellowship.

## References

Bhattacharya P, Welch AH, Stollenwerk KG, McLaughlin MJ, Bundschuh J & Panaullah G (2007) Arsenic in the environment: biology and chemistry. *Sci Total Environ* **379**: 109–120.

Borch T, Kretzschmar R, Kappler A, Cappellen PV, Ginder-Vogel M, Voegelin A & Campbell K (2010) Biogeochemical redox processes and their impact on contaminant dynamics. *Environ Sci Technol* **44**: 15–23.

Bouskill N, Barker-Finkel J, Galloway T, Handy R & Ford T (2010) Temporal bacterial diversity associated with metal-contaminated river sediments. *Ecotoxicology* **19**: 317–328.

Bruneel O, Duran R, Casiot C, Elbaz-Poulichet F & Personne JC (2006) Diversity of microorganisms in Fe-As-rich acid mine drainage waters of Carnoules, France. *Appl Environ Microbiol* **72**: 551–556.

Caporaso JG, Kuczynski J, Stombaugh J, *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.

Cherry JA, Shaikh AU, Tallman DE & Nicholson RV (1979) Arsenic species as an indicator of redox conditions in groundwater. *J Hydrol* **43**: 373–392.

Cleveland CC, Nemergut DR, Schmidt SK & Townsend AR (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* **82**: 229–240.

Cullen WR & Reimer KJ (1989) Arsenic speciation in the environment. *Chem Rev* **89**: 713–764.

Delbès C, Moletta R & Godon JJ (2001) Bacterial and archaeal 16S rDNA and 16S rRNA dynamics during an acetate crisis in an anaerobic digestor ecosystem. *FEMS Microbiol Ecol* **35**: 19–26.

Demoling F, Nilsson LO & Bååth E (2008) Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biol Biochem* **40**: 370–379.

Duker AA, Carranza EJM & Hale M (2005) Arsenic geochemistry and health. *Environ Int* **31**: 631–641.

Eary LE & Schramke JA (1990) Rates of inorganic oxidation reactions involving dissolved oxygen. *Chemical Modeling of Aqueous Systems II. ACS Symposium Series*, Vol. **416**, pp. 379–396.

Ellis PJ, Conrads T, Hille R & Kuhn P (2001) Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 Å and 2.03 Å. *Structure* **9**: 125–132.

Fendorf S, Eich MJ, Grossl P & Sparks DL (1997) Arsenate and chromate retention mechanisms on goethite. 1. Surface structure. *Environ Sci Technol* **31**: 315–320.

Fendorf S, Michael HA & van Geen A (2010) Spatial and temporal variations of groundwater arsenic in South and Southeast Asia. *Science* **328**: 1123–1127.

Halter D, Cordi A, Gribaldo S *et al.* (2011) Taxonomic and functional prokaryote diversity in mildly arsenic-contaminated sediments. *Res Microbiol* **162**: 877–887.

Heinrich-Salmeron A, Cordi A, Brochier-Armanet C *et al.* (2011) Unsuspected diversity of arsenite-oxidizing bacteria revealed by a widespread distribution of the *aoxB* gene in prokaryotes. *Appl Environ Microbiol* **77**: 4685–4892.

Inskeep WP, Macur RE, Hamamura N, Warelow TP, Ward SA & Santini JM (2007) Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environ Microbiol* **9**: 934–943.

Jackson CR, Langner HW, Donahoe-Christiansen J, Inskeep WP & McDermott TR (2001) Molecular analysis of microbial community structure in an arsenite-oxidizing acidic thermal spring. *Environ Microbiol* **3**: 532–542.

Jones LC (2011) Coupling of biotic and abiotic arsenite oxidation in soils. MS Thesis, University of Delaware, Newark, DE.

Jones LC, Lafferty BJ & Sparks DL (2012) Additive and competitive effects of bacteria and Mn oxides on arsenite oxidation kinetics. *Environ Sci Technol* **46**: 6548–6555.

Kulp TR, Hoeft SE & Oremland RS (2004) Redox transformations of arsenic oxyanions in periphyton communities. *Appl Environ Microbiol* **70**: 6428–6434.

Lafferty BJ, Ginder-Vogel M & Sparks DL (2010) Arsenite oxidation by a poorly crystalline manganese-oxide 1. Stirred-flow experiments. *Environ Sci Technol* **44**: 8460–8466.

Langner HW, Jackson CR, McDermott TR & Inskeep WP (2001) Rapid oxidation of arsenite in a hot spring ecosystem, Yellowstone National Park. *Environ Sci Technol* **35**: 3302–3309.

Lee DH, Zo YG & Kim SJ (1996) Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single strand conformation polymorphism. *Appl Environ Microbiol* **62**: 3112–3120.

Liao VHC, Chu YJ, Su YC, Hsiao SY, Wei CC, Liu CW, Liao CM, Shen WC & Chang FJ (2011) Arsenite-oxidizing and arsenate-reducing bacteria associated with arsenic-rich groundwater in Taiwan. *J Contam Hydrol* **123**: 20–29.

Lorenz , Hintemann T, Kramarewa T, Katayama A, Yasuta T, Marschner P & Kandeler E (2006) Response of microbial activity and microbial community composition in soils to long-term arsenic and cadmium exposure. *Soil Biol Biochem* **38**: 1430–1437.

Ludwig W, Strunk O, Westram R, Richter L *et al.* (2004) ARB: a software environment for sequence data. *Nucl Acids Res* **32**: 1363–1371.

Macur RE, Jackson CR, Botero LM, McDermott TR & Inskeep WP (2004) Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environ Sci Technol* **38**: 104–111.

Masscheleyn P, Delaune RD & Patrick WH (1991) Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environ Sci Technol* **25**: 1414–1419.

McNamara NP, Black HIJ, Beresford NA & Parekh NR (2003) Effects of acute gamma irradiation on chemical, physical, and biological properties of soils. *Appl Soil Ecol* **24**: 117–132.

Nickson R, McArthur J, Burgess W, Ahmed KM, Ravenscroft P & Rahmann M (1998) Arsenic poisoning of Bangladesh groundwater. *Nature* **395**: 338.

Nordstrom DK (2002) Worldwide occurrences of arsenic in ground water. *Science* **296**: 2143–2145.

Oremland RS & Stolz FJ (2003) The ecology of arsenic. *Science* **300**: 939–944.

Osborne FH & Enrlich HL (1976) Oxidation of arsenite by a soil isolate of *Alcaligenes*. *J Appl Bacteriol* **41**: 295–305.

Paez-Espino D, Tamames J, de Lorenzo V & Canovas D (2009) Microbial responses to environmental arsenic. *Biometals* **22**: 117–130.

Parikh SJ, Lafferty BJ, Meade TG & Sparks DL (2010) Evaluating environmental influences on As(III) oxidation kinetics by a poorly crystalline Mn-oxide. *Environ Sci Technol* **44**: 3772–3778.

Post JE (1999) Manganese oxide minerals: crystal structures and economic and environmental significance. *PNAS* **96**: 3447–3454.

Quéméneur M, Heinrich-Salmeron A, Muller D, Lievremont D, Jauzein M, Bertin PN, Garrido F & Joulian C (2008) Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidising bacteria. *Appl Environ Microbiol* **74**: 4567–4573.

Quéméneur M, Cébron A, Billard P, Battaglia-Brunet F, Garrido F, Leyval C & Joulian C (2010) Population structure and abundance of arsenite-oxidizing bacteria along an arsenic pollution gradient in waters of the upper Isle River basin, France. *Appl Environ Microbiol* **76**: 4566–4570.

Quince C, Lanzen A, Curtis TP, Davenport RJ, Hall N, Head IM, Read LF & Sloan WT (2009) Accurate determination of microbial diversity from 454 pyrosequencing data. *Nat Methods* **6**: 639–641.

Santini JM, Sly LI, Schnagl RD & Macy JM (2000) A new chemolithoautotrophic arsenite-oxidising bacterium isolated from a gold mine: phylogenetic, physiological, and preliminary biochemical studies. *Appl Environ Microbiol* **66**: 92–97.

Santini JM, Sly LI, Wen A, Comrie D, De Wulf-Durand P & Macy JM (2002) New arsenite-oxidizing bacteria isolated from Australian gold mining environments–phylogenetic relationships. *Geomicrobiol J* **19**: 67–76.

Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71**: 1501–1506.

Shivaji S, Pratibha M, Sailaja B, Hara Kishore K, Singh A, Begum Z, Anarasi U, Prabagaran S, Reddy G & Srinivas T (2011) Bacterial diversity of soil in the vicinity of Pindari glacier, Himalayan mountain ranges, India, using culturable bacteria and soil 16S rRNA gene clones. *Extremophiles* **15**: 1–22.

Silver S & Phung LT (2005) Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl Environ Microbiol* **71**: 599–608.

Singleton DR, Furlong MA, Rathbun SL & Whitman WB (2001) Quantitative comparisons of 16S rDNA sequence libraries from environmental samples. *Appl Environ Microbiol* **67**: 4373–4376.

Smedley P (2003) Arsenic in groundwater – South and East Asia. *Arsenic in Ground Water*, (Welch A & Stollenwerk K, eds), pp. 179–209. Kluwer Academic Publishers, New York.

Stookey LL (1970) Ferrozine: a new spectrophotometric reagent for iron. *Anal Chem* **42**: 779–781.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol Biol Evol* **24**: 1596–1599.

Tournassat C, Charlet L, Bosbach D & Manceau A (2002) Arsenic(III) oxidation by birnessite and precipitation of manganese(II) arsenate. *Environ Sci Technol* **36**: 493–500.

Turner AW (1954) Bacterial oxidation of arsenite. I. Description of bacteria isolated from arsenical cattle-dipping fluids. *Aust J Biol Sci* **7**: 452–478.

US Environmental Protection Agency (1994). Microwave assisted acid digestion of sediments, sludges, soils and oils. EPA Methods 3051, National Technical Information Service, Springfield, VA.