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Indigenous arsenic(V)-reducing microbial communities in redox-fluctuating near-surface sediments of the Mekong Delta

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ABSTRACT

Arsenic (As) cycling within soils and sediments of the Mekong Delta of Cambodia is affected by drastic redox fluctuations caused by seasonal monsoons. Extensive flooding during monsoon seasons creates anoxic soil conditions that favor anaerobic microbial processes, including arsenate [As(V)] respiration—a process contributing to the mobilization of As. Repeated oxidation and reduction in near-surface sediments, which contain 10–40 mg kg^{-1} As, lead to the eventual downward movement of As to the underlying aquifer. Amplification of a highly conserved functional gene encoding dissimilatory As(V) reductase, *arrA*, can be used as a molecular marker to detect the genetic potential for As(V) respiration in environmental samples. However, few studies have successfully amplified *arrA* from sediments without prior enrichment, which can drastically shift community structure. In the present study, we examine the distribution and diversity of *arrA* genes amplified from multiple sites within the Cambodian Mekong Delta as a function of near-surface depth (10, 50, 100, 200, and 400 cm), where sediments undergo seasonal redox fluctuations. We report successful amplification of 302 *arrA* gene sequences (72 OTUs) from near-surface Cambodian soils (without prior enrichment or stimulation with carbon amendments), where a large majority (>70%) formed a well-supported clade that is phylogenetically distinct from previously reported sequences from Cambodia and other South and Southeast Asian sediments, with highest sequence similarity to known *Geobacter* species capable of As(V) respiration, further supporting the potentially important role of *Geobacter* sp. in arsenic mobilization in these regions.

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INTRODUCTION

Millions of people in South and Southeast Asia are currently exposed to arsenic (As) concentrations as high as three orders of magnitude greater than the World Health Organization (WHO) suggested limit of 10 μg L^{-1}. Arsenic-bearing minerals derived from the Himalayas are transported down river channels and deposited into deltas below, including the Ganges–Brahmaputra–Meghna (Nickson *et al.*, 2000; Polizzotto *et al.*, 2008), Red River (Berg *et al.*, 2001), and Mekong River deltas (Buschmann *et al.*, 2007; Kocar *et al.*, 2008; Polizzotto *et al.*, 2008). Aquifer As concentrations in one of the most densely populated areas of the Mekong Delta (between the Mekong and Basaac Rivers) range from 100 to >1000 μg L^{-1}, with an average of ~500 μg L^{-1} (Kocar *et al.*, 2008; Polizzotto *et al.*, 2008).

Redox processes within near-surface sediments are responsible for the supply and release of As into groundwater (Kocar *et al.*, 2008; Polizzotto *et al.*, 2008). Oxidation of Himalayan-derived As-bearing sulfur minerals deposited in the surface sediments releases As, which is temporarily immobilized through adsorption onto Fe(III) oxides, hydroxides, and oxyhydroxides (collectively referred to as oxides) in the surrounding sediment matrix. Subsequent reductive dissolution of Fe(III) oxides and As(V) reduction under reducing conditions during the wet season leads to desorption and partitioning of As into the aqueous phase. Arsenic(V) is the predominant oxidation state under oxic conditions and is generally considered the less mobile species. In contrast, As(III) dominates under reducing conditions and is more labile and thus more mobile under flow conditions (Tufano & Fendorf, 2008; Tufano *et al.*,

2008). Therefore, characterization of factors and processes responsible for the reduction in As(V) is crucial toward understanding As transport.

Under anaerobic conditions, a major pathway contributing to the transformation of As(V) is microbial respiration of As(V), which has been shown to provide greater energetic yield than respiration on the common iron oxides goethite and hematite under environmental conditions in Cambodian sediments (Kocar & Fendorf, 2009). Dissimilatory As(V)-reducing bacteria (DARB) have been isolated from a wide range of environments and are phylogenetically and physiologically diverse (Oremland & Stolz, 2003; Oremland *et al.*, 2005; Hollibaugh *et al.*, 2006; Stolz *et al.*, 2006; Kulp *et al.*, 2008). A number of studies have identified and characterized enzymes that catalyze As(V) respiration (Krafft & Macy, 1998; Afkar *et al.*, 2003; Saltikov & Newman, 2003; Saltikov *et al.*, 2003). The dissimilatory As(V) reductase is a periplasmic heterodimer composed of the molybdenum-containing terminal reductase, ArrA (87–110 kDa), and a Fe-S cluster subunit, ArrB (25.7–34 kDa), which provides electrons to ArrA from c-type cytochromes (Krafft & Macy, 1998).

While model organisms are invaluable for deciphering biochemical mechanisms responsible for As transformation under constrained laboratory conditions, these organisms and their functional genes may not be representative of those found in the environment. To this end, the diversity of *arrA* has been explored in a variety of environments, including estuarine sediments of Chesapeake bay (Song *et al.*, 2009), aquifer sediments from West Bengal (Héry *et al.*, 2008), As-rich soda lakes (Kulp *et al.*, 2008; Hoeft *et al.*, 2010), and various groundwater sources (Barringer *et al.*, 2010; Giloteaux *et al.*, 2013). However, the extent of diversity of native *arrA* sequences surveyed in many of these studies has been limited due to difficulty in amplifying sequences from untreated soil samples, and hence a large majority of *arrA* sequences documented to date are from incubations studies. Previously, Lear *et al.* (2007) examined *arrA* genes in an acetate-amended Cambodian sediment core collected from 9 m depth after 16 and 30 days of incubations, but were unable to amplify any products from unamended samples. Héry *et al.* (2014) discovered 12 new *arrA* phylotypes in an unamended Holocene soil core taken at 11 m depth from Cambodia. However, further investigation into the overall As(V)-reducing bacteria communities in the Mekong Delta is needed, particularly at shallower depths (≤4 m below surface) where reduction–oxidation processes are responsible for As release to the aquifer (Kocar *et al.*, 2008; Polizzotto *et al.*, 2008). Here, we report the discovery of highly diverse communities of As(V)-respiring bacteria in unamended near-surface sediments from the Mekong Delta of Cambodia, at four sites and multiple depths (10, 50, 100, 200, and 400 cm). We report successful amplification and analysis of 302 *arrA* sequences from naturally occurring, unamended surface sediments, increasing the existing sequence database of *arrA* phylotypes in unaltered sediments by more than twofold. Statistical analysis shows that communities are clustered by sample site rather than by depth, likely indicating arsenic concentrations do not dictate *arrA* phylotype distribution.

RESULTS AND DISCUSSION

Phylogenetic analysis of *arrA* genes in Cambodian sediments

The diversity of As(V)-reducing bacteria was assessed in near-surface (≤4 m) Cambodian sediments by amplifying the *arrA* functional gene from sediment samples without prior enrichment (e.g., carbon amendment, incubations). The concentration of aqueous constituents in porewater extracted from sediments can be found in Table 1. A total of 302 non-chimeric sequences were included in a neighbor-joining tree of *arrA* phylogeny (Fig. 1). OTUs were assigned based on 90%, 95%, and 99% sequence similarity yielding a total of 72, 106, and 174 *arrA* OTUs, respectively, where more dissimilar sequences are defined as the same OTU under a lower percentage cutoff value, hence leading to greater number of OTUs as the percentage of sequence similarity required increases. Only OTUs identified using the 90% sequence similarity cutoff were used for phylogenetic analysis, where asymptotic behavior of rarefaction curves demonstrates that sequencing depth was sufficient (Fig. S1). Use of 99% sequence similarity cutoff would have required additional sequencing efforts to accurately capture the diversity, which supports our finding that near-surface Cambodian sediments harbor previously undocumented *arrA* diversity. In general, *arrA* sequences from Cambodian near-surface sediments formed a separate monocladistic group (Cluster A) that is most similar to the *arrA* sequences of *Geobacter uranireducens* (sequence similarities ranges from 48.9 to 74.8%), *Geobacter* sp. OR-1 (48.5 to 76.2%), and *Geobacter lovleyii* (49.5 to 75%). A great majority of these sequences (71% of sequences; 80% of OTUs) formed a cluster distinct from previously reported Southeast Asian *arrA* sequences (Fig. 1). Generation of a maximum-likelihood amino acid tree supports the topology of near-surface Cambodian sequences relative to DARB isolates (Fig. S3).

The distant relationship of our Cambodian near-surface sequences to other Southeast Asian sequences is likely due to enrichment of strains capable of metabolizing specific carbon sources (i.e., acetate or lactate) or selection for As-tolerant strains in previous studies (Lear *et al.*, 2007; Héry *et al.*, 2014), with the exception of OTU 31, which falls in close proximity to clones from unamended Cambodian sediments retrieved from 11 m depth (Héry *et al.*, 2014). Interestingly, Héry *et al.* (2014) showed that *Desulfosporosinus* sp. was the closest cultivated DARB relative to sequences

Table 1 Concentration of aqueous constituents in porewater samples

Site	Season	Depth cm	As μg L^{-1}	Fe	Mn	K	Mg	Ca	Na	P	S
				mg L^{-1}							
A	Dry	10	8.3	3.5	5.3	0.6	0	165	44.3	0.002	9.5
A	Wet	10	9.7	2.1	4.7	0.6	0	163	49.6	0.003	12.5
A	Dry	50	11.4	2.6	4.3	0.5	38.7	176	36.0	0.04	4.9
A	Wet	50	13.7	1.6	2.0	10.2	38.1	84.6	30.0	1.2	14.1
A	Wet	100	7.5	0.71	0.3	24.7	34.6	7.8	20.2	0.2	3.7
A	Dry	200	333	16.1	1.0	2.4	23.5	86.7	22.4	0.4	0.5
A	Wet	200	196	15.5	1.1	1.2	30.7	107	20.6	0.3	0.6
A	Dry	400	367	16.9	1.1	0.6	32.9	119	28.4	0.4	0.5
B	Wet	10	5.8	1.0	0.3	10.1	14.8	5.7	16.3	0.1	19.7
B	Wet	100	4.7	0.45	0.07	133	47.0	11.9	45.0	0.1	62.4
B	Wet	200	26.9	1.9	0.03	5.4	27.0	8.5	44.1	0.2	102
C	Dry	10	6.1	0.07	0.1	1.1	6.08	20.4	6.2	0	0.7
C	Dry	400	162	8.9	1.0	1.5	40.0	73.6	86.9	1.4	12.0
T	Wet	50	34.2	0.3	3.8	3.4	48.6	149	113	0.07	21.1
T	Wet	100	24.7	0.6	4.2	19.8	52.0	154	68.9	0.03	4.3

amplified from 11-m-deep unamended samples, followed by a transition toward a community dominated by *Geobacter*-like *arrA* sequences after either acetate or lactate amendments. By contrast, our samples from shallower, more carbon-rich depths show highest *arrA* resemblance to *Geobacter* sp. without amendment. Interspersed within the near-surface Cambodian cluster (Fig. 2) are sequences from biogeochemically diverse environments including inner coastal plain groundwater (New Jersey) (Barringer *et al.*, 2010), Chesapeake Bay sediments (Song *et al.*, 2009), Cache Valley Aquifer sediment (Mirza *et al.*, 2014), and Japanese paddy soils, demonstrating the complexity of factors that likely dictate *arrA* distribution.

The majority of remaining sequences (13% of sequences, 15% of OTUs) fell within Cambodian near-surface Cluster B, a strongly supported cluster containing no cultured representatives. This group is more distantly related to existing DARB isolates than Cluster A sequences and is almost entirely composed of Cambodian sequences. Héry et al. (2014) stated that 10 *arrA* sequences acquired from Cambodian sediments following enrichment/stimulation with As(V) and acetate amendments cluster separately from West Bengal sequences; however, sequences from that study are not publicly available and thus we are unable to make comparisons to those obtained during our current study. Nevertheless, our current dataset supports the conclusion that clones from Cambodia are indeed distinct from those amplified from West Bengal.

Community diversity analysis

Beta diversity of As(V)-reducing communities was examined using NMDS to visualize community similarity through ordination, and analysis of similarity (ANOSIM) was used to quantitatively test whether communities were significantly different between sites and depths (diversity indices for each sample is provided in Table 2). NMDS results show that As(V)-reducing communities can generally be clustered based upon site more so than by depth, particularly for sites A, T, and B (Fig. 3). Although executing the ordination with only two axes resulted in relatively high stress (>1.3, results not shown), resultant clusters in two dimensions are consistent with use of three dimensions. To quantitatively test the significant difference between As(V)-reducing communities based upon site and depth variables, ANOSIM was employed using Bray–Curtis dissimilarity distances (Fig. S2). ANOSIM results show that sequences are more similar within sites than between sites ($P < 0.07$), but with no significance when grouped by depth ($P > 0.2$), where pore water arsenic concentrations are more similar, demonstrating that similar geochemical conditions are not necessarily indicative of the presence of a specific *arrA* phylotype. These results may complement recent findings from *Giloteaux et al.* (2013) demonstrating that factors other than As availability regulate the transcription of *arrA*. Cluster analysis based upon season also did not produce significant results (results not shown).

Dry season conditions in Cambodia lead to the formation of large cracks, due to the high shrink-swell capacity of clays, promoting aeration and presence of As(V) species, with subsequent adsorption of As on Fe(III) oxides at shallow depths (<1 m) (Kocar *et al.*, 2008). Arsenic(V)-respiring micro-organisms are likely most active in these near-surface sediments where fresh carbon sources are deposited annually (e.g., detritus from plants and animals) and As(V) is available. The surface sediments are reduced upon wetting during the monsoon season, giving rise to biogeochemically diverse conditions, which is reflected in the

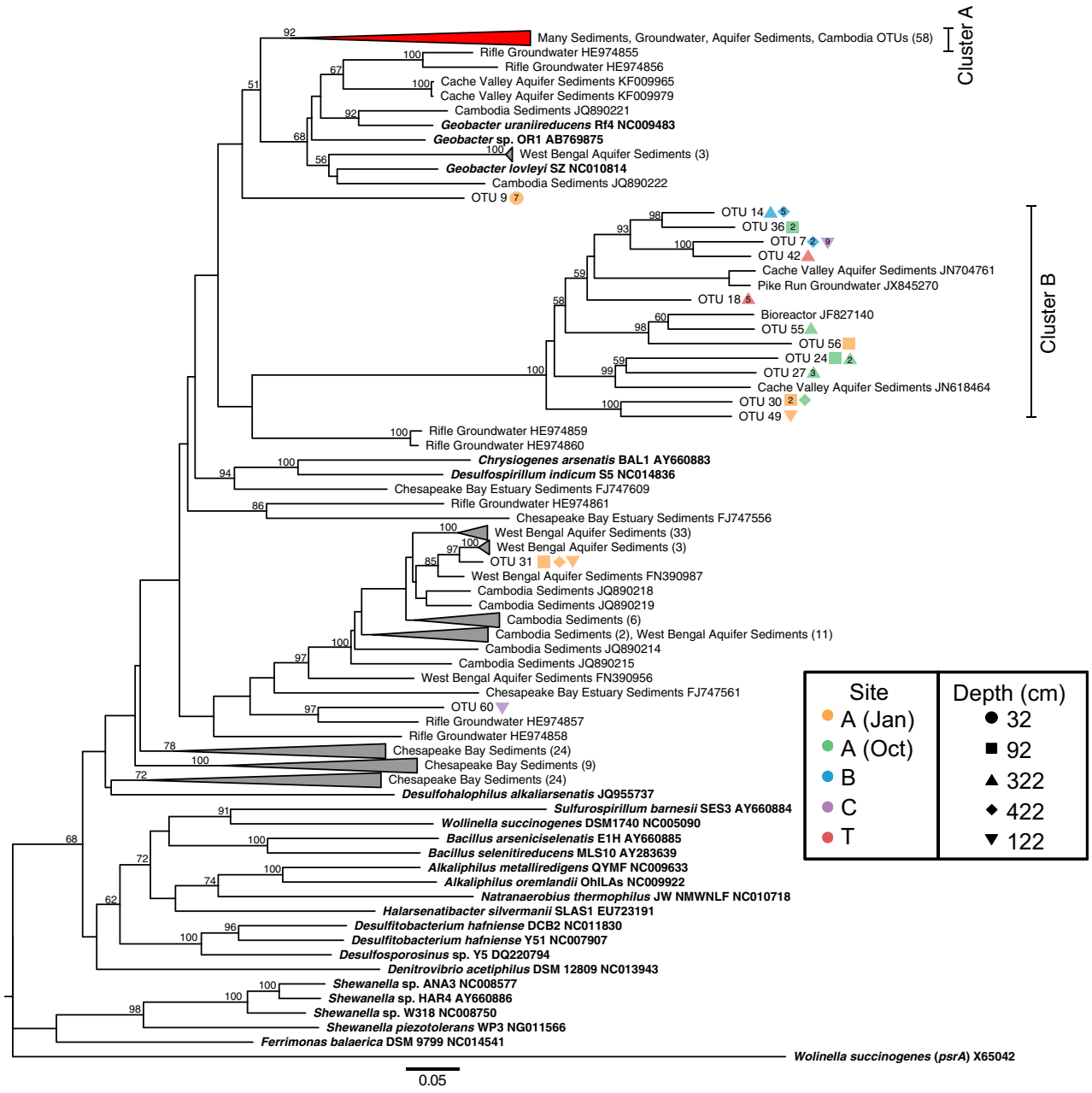

Fig. 1 Neighbor-joining phylogenetic comparison of 302 *arrA* DNA sequences from unamended Cambodian near-surface sediments at four sites and five depths to *arrA* amplified from other environments and cultivated As(V)-respiring bacteria (in bold; clone names are prefixed OTU). The total number of sequences within reference wedges is indicated in parentheses; the number of sequences from the present study are shown within colored symbols. Parentheses indicate number of OTUs within the distinct Mekong Delta near-surface cluster (shown in red, expanded Fig. 2) or the number of reference sequences in reference wedges. Percentage of trees with repeatable taxa clusters (as determined by 1000 replicates of bootstrap test) is noted at each cluster node. Scale bar represents 0.05 substitutions per nucleotide position.

diversity of *arrA* phylotypes. However, deeper depths (>4 m) remain reduced throughout both the dry and wet seasons, where As concentrations are no longer strongly correlated with Fe and alkalinity (Kocar *et al.*, 2008; Stuckey *et al.*, 2015). Further, because arsenic is present mostly in reduced forms [as As(III)] at these depths, As (V)-respiration plays a minor role in As cycling. Our findings provide an updated catalog of the *arrA* phylotypes that may have more prevalent roles in As(V) reduction in near-surface, redox-fluctuating sediments in Cambodia. Future studies utilizing transcriptomic approaches will be helpful in elucidating more directly whether there is a clear

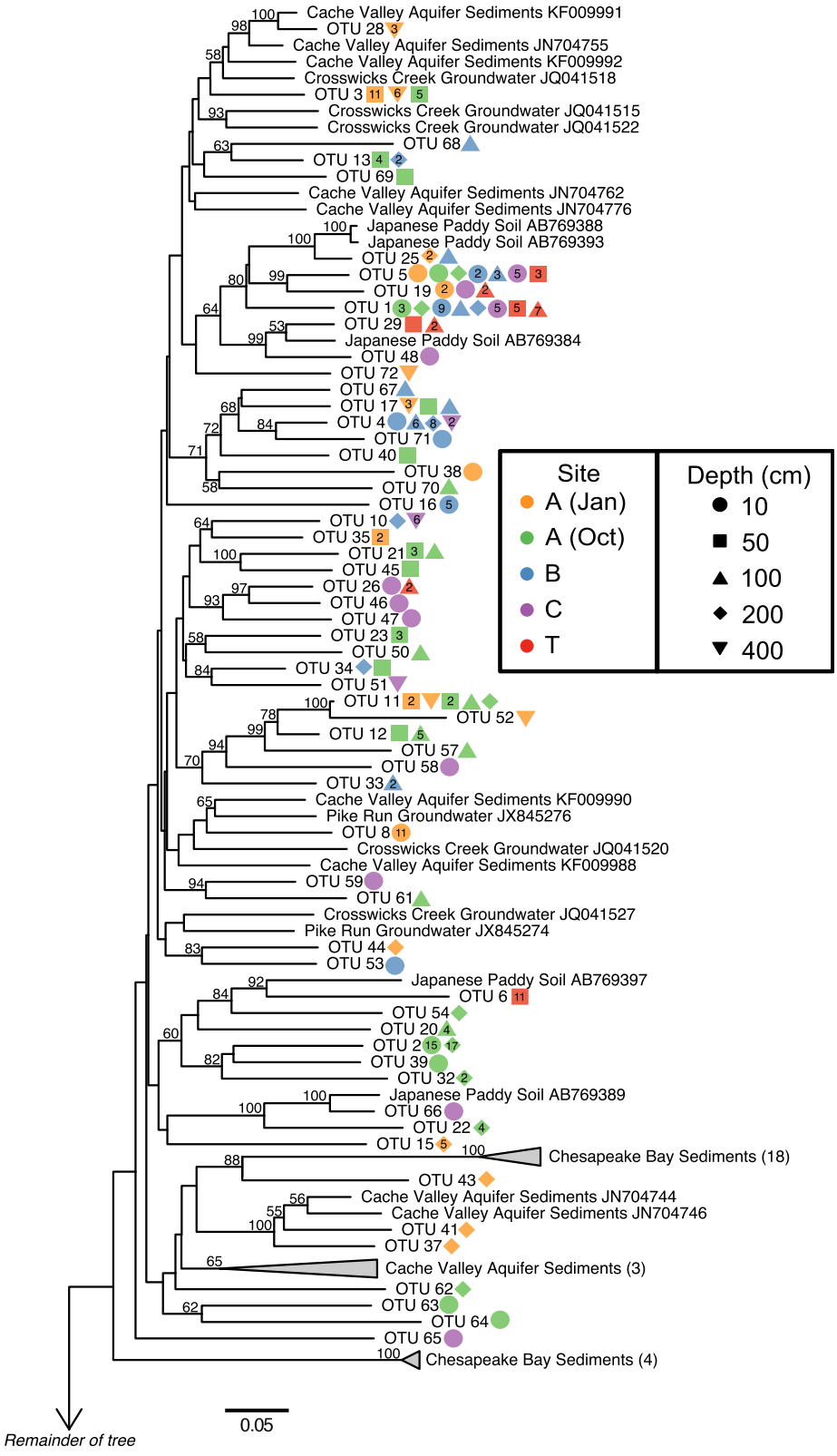

Fig. 2 Expanded view of Cambodian near-surface *arrA* cluster A calculated using neighbor-joining method. The total number of sequences within references wedges is indicated in parentheses; a number of sequences from the present study are shown within colored symbols. Percentage of trees with repeatable taxa clusters (as determined by 1000 replicates of bootstrap test) is noted at each cluster node. Scale bar represents 0.05 substitutions per nucleotide position.

Table 2 Diversity indices of Cambodia samples in current study

Site	Season	Depth (cm)	No. Clones	No. OTUs*	Chao1†	Shannon	Unique OTUs	Singletons
All sites			302	72	128.6		51	37
A	Dry	10	22	4	4 (100%)	1.1	4	2
A	Wet	10	22	5	8 (63%)	1	3	3
A	Dry	50	19	5	5.3 (90%)	1	1	1
A	Wet	50	26	13	20 (65%)	2.4	5	3
A	Wet	100	21	9	14 (64%)	1.9	7	5
A	Dry	200	12	6	7 (90%)	1.6	5	4
A	Wet	200	29	8	10 (80%)	1.4	4	2
A	Dry	400	17	7	8.5 (80%)	1.7	4	3
B	Wet	10	19	5	5 (100%)	1.3	3	2
B	Wet	100	17	8	13 (60%)	1.8	3	2
B	Wet	200	20	6	6.3 (95%)	1.5	0	0
C	Dry	10	19	9	16.5 (55%)	1.9	7	7
C	Dry	400	19	4	4 (100%)	1.1	2	2
T	Wet	50	20	3	3 (100%)	0.8	1	0
T	Wet	100	20	4	4 (100%)	1	2	1

*OTUs are defined as >90% similarity.

†Values in parentheses are percentage of estimated OTUs observed (No. OTUs/Chao1).

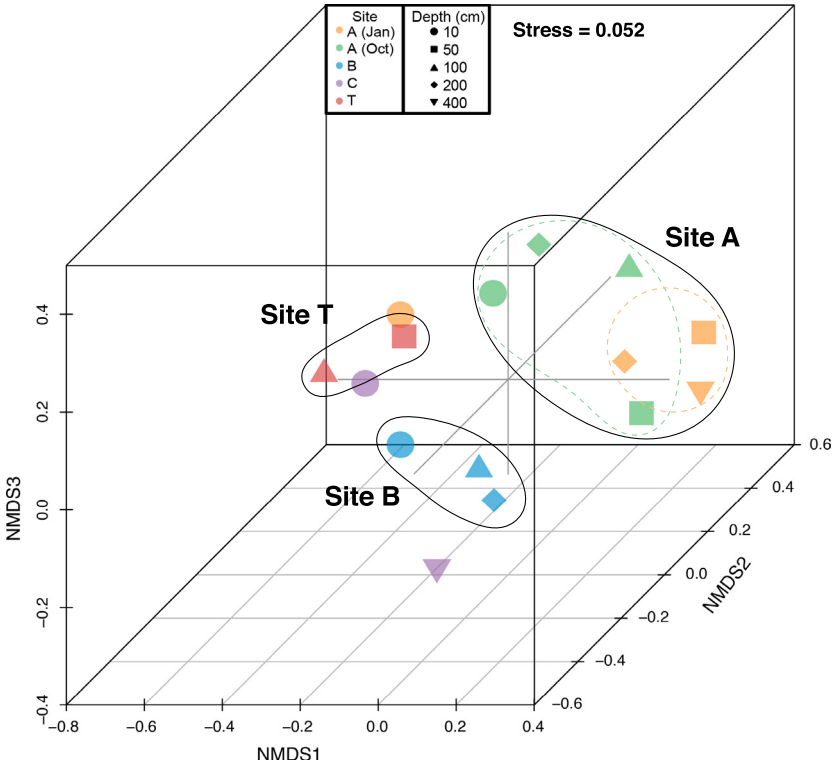

Fig. 3 Non-metric multidimensional scaling (NMDS) ordination diagram of site and depth variations in *arrA* community structure. The ordination is based on a Bay–Curtis similarity matrix. The stress value for the tridimensional NMDS ordination is shown in bold.

distinction between those *arrA* phylotypes active in redox-fluctuating soils compared to permanently reduced soils and sediments.

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REFERENCES

Afkar E, Lisak J, Saltikov C, Basu P, Oremland RS, Stolz JF (2003) The respiratory arsenate reductase from Bacillus selenitireducens strain MLS10. *FEMS Microbiology Letters* **226**, 107–112.

Barringer JL, Mumford A, Young LY, Reilly PA, Bonin JL, Rosman R (2010) Pathways for arsenic from sediments to groundwater to streams: biogeochemical processes in the Inner Coastal Plain, New Jersey, USA. *Water Research* **44**, 5532–5544.

Berg M, Tran HC, Nguyen TC, Pham HV, Schertenleib R, Giger W (2001) Arsenic contamination of groundwater and drinking water in Vietnam: a human health threat. *Environmental Science & Technology* **35**, 2621–2626.

Buschmann J, Berg M, Stengel C, Sampson ML (2007) Arsenic and manganese contamination of drinking water resources in Cambodia: coincidence of risk areas with low relief topography. *Environmental Science & Technology* **41**, 2146–2152.

Giloteaux L, Holmes DE, Williams KH, Wrighton KC, Wilkins MJ, Montgomery AP, Smith JA, Orellana R, Thompson CA, Roper TJ, Long PE, Lovley DR (2013) Characterization and transcription of arsenic respiration and resistance genes during in situ uranium bioremediation. *The ISME Journal* **7**, 370–383.

Héry M, Gault AG, Rowland HAL, Lear G, Polya DA, Lloyd JR (2008) Molecular and cultivation-dependent analysis of metal-reducing bacteria implicated in arsenic mobilisation in south-east Asian aquifers. *Applied Geochemistry* **23**, 3215–3223.

Héry M, Rizoulis A, Sanguin H, Cooke DA, Pancost RD, Polya DA, Lloyd JR (2014) Microbial ecology of arsenic-mobilizing Cambodian sediments: lithological controls uncovered by stable-isotope probing. *Environmental Microbiology* **17**, 1857–1869.

Hoeft SE, Kulp TR, Han S, Lanoil B, Oremland RS (2010) Coupled arsenotrophy in a hot spring photosynthetic biofilm at Mono Lake, California. *Applied and Environmental Microbiology* **76**, 4633–4639.

Hollibaugh JT, Budinoff C, Hollibaugh RA, Ransom B, Bano N (2006) Sulfide oxidation coupled to arsenate reduction by a diverse microbial community in a Soda Lake. *Applied and Environmental Microbiology* **72**, 2043–2049.

Kocar BD, Fendorf S (2009) Thermodynamic constraints on reductive reactions influencing the biogeochemistry of arsenic in soils and sediments. *Environmental Science & Technology* **43**, 4871–4877.

Kocar BD, Polizzotto ML, Benner SG, Ying SC, Ung M, Ouch K, Samreth S, Suy B, Phan K, Sampson M, Fendorf S (2008) Integrated biogeochemical and hydrologic processes driving arsenic release from shallow sediments to groundwaters of the Mekong delta. *Applied Geochemistry* **23**, 3059–3071.

Krafft T, Macy JM (1998) Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. *European Journal of Biochemistry* **255**, 647–653.

Kulp TR, Hoeft SE, Asao M, Madigan MT, Hollibaugh JT, Fisher JC, Stolz JF, Culbertson CW, Miller LG, Oremland RS (2008) Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. *Science* **321**, 967–970.

Lear G, Song B, Gault AG, Polya DA, Lloyd JR (2007) Molecular analysis of arsenate-reducing bacteria within Cambodian sediments following amendment with acetate. *Applied and Environmental Microbiology* **73**, 1041–1048.

Mirza BS, Muruganandam S, Meng X, Sorensen DL, Dupont RR, McLean JE (2014) Arsenic(V) reduction in relation to Iron(III) transformation and molecular characterization of the structural and functional microbial community in sediments of a basin-fill aquifer in Northern Utah. *Applied and Environmental Microbiology* **80**, 3198–3208.

Nickson RT, McArthur JM, Ravenscroft P, Burgess WG, Ahmed KM (2000) Mechanism of arsenic release to groundwater, Bangladesh and West Bengal. *Applied Geochemistry* **15**, 403–413.

Oremland RS, Stolz JF (2003) The ecology of arsenic. *Science* **300**, 939–944.

Oremland RS, Kulp TR, Blum JS, Hoeft SE, Baesman S, Miller LG, Stolz JF (2005) A microbial arsenic cycle in a salt-saturated, extreme environment. *Science* **308**, 1305–1308.

Polizzotto ML, Kocar BD, Benner SG, Sampson M, Fendorf S (2008) Near-surface wetland sediments as a source of arsenic release to ground water in Asia. *Nature* **454**, 505–508.

Saltikov CW, Newman DK (2003) Genetic identification of a respiratory arsenate reductase. *Proceedings of the National Academy of Sciences* **100**, 10983–10988.

Saltikov CW, Cifuentes A, Venkateswaran K, Newman DK (2003) The ars detoxification system is advantageous but not required for As(V) respiration by the genetically tractable Shewanella species strain ANA-3. *Applied and Environmental Microbiology* **69**, 2800–2809.

Song B, Chyun E, Jaffé PR, Ward BB (2009) Molecular methods to detect and monitor dissimilatory arsenate-respiring bacteria (DARB) in sediments. *FEMS Microbiology Ecology* **68**, 108–117.

Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. *Annual Review of Microbiology* **60**, 107–130.

Stuckey JW, Schaefer MV, Kocar BD, Dittmar J, Pacheco JL, Benner SG, Fendorf S (2015) Peat formation concentrates arsenic within sediment deposits of the Mekong Delta. *Geochimica et Cosmochimica Acta* **149**, 190–205.

Tufano KJ, Fendorf S (2008) Confounding impacts of iron reduction on arsenic retention. *Environmental Science & Technology* **42**, 4777–4783.

Tufano KJ, Reyes C, Saltikov CW, Fendorf S (2008) Reductive processes controlling arsenic retention: revealing the relative importance of iron and arsenic reduction. *Environmental Science & Technology* **42**, 8283–8289.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Rarefaction on curves computed using 90% (A) 95% (B) and 99% (C) sequence similarity cutoff values.

Fig. S2 Shepard plot for 3---D Bray Cur)s ordina)on shown in Fig. 3 of the main text.

Fig. S3 Maximum likelihood phylogene)c comparison of known DARB isolates (black) with translated *arrA* sequences from the present study (red).

Appendix S1 Materials and methods.