Land scale biogeography of arsenic biotransformation genes in estuarine wetland

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Summary

As an analogue of phosphorus, arsenic (As) has a biogeochemical cycle coupled closely with other key elements on the Earth, such as iron, sulfate and phosphate. It has been documented that microbial genes associated with As biotransformation are widely present in As-rich environments. Nonetheless, their presence in natural environment with low As levels remains unclear. To address this issue, we investigated the abundance levels and diversities of

***aioA*, *arrA*, *arsC* and *arsM* genes in estuarine sediments at low As levels across Southeastern China to uncover biogeographic patterns at a large spatial scale. Unexpectedly, genes involved in As biotransformation were characterized by high abundance and diversity. The functional microbial communities showed a significant decrease in similarity along the geographic distance, with higher turnover rates than taxonomic microbial communities based on the similarities of 16S rRNA genes. Further investigation with niche-based models showed that deterministic processes played primary roles in shaping both functional and taxonomic microbial communities. Temperature, pH, total nitrogen concentration, carbon/nitrogen ratio and ferric iron concentration rather than As content in these sediments were significantly linked to functional microbial communities, while sediment temperature and pH were linked to taxonomic microbial communities. We proposed several possible mechanisms to explain these results.**

Introduction

Arsenic (As) is a toxic and carcinogenic element that is widely distributed in the environment. Microbe-mediated As biotransformation is an ancient mechanism that emerged at least 2.72 billion years ago (Lebrun *et al.*, 2003; Duval *et al.*, 2008; Sforza *et al.*, 2014) and significantly modulate the fate of As in the environment via As respiratory oxidation and reduction, arsenate (As(V)) cytoplasmic reduction and arsenite (As(III)) methylation (Zhu *et al.*, 2014). As(III) oxidase (Aio) catalyses the respiratory oxidation of As(III) by either detoxifying it to less toxic As(V), or coupling it to ATP production (Slyemi and Bonnefoy, 2012). Aio's large subunit (AioA) has been well characterized in both bacteria and archaea (Lebrun *et al.*, 2003). As(V) respiratory reduction in bacteria couples As(V) reduction to anaerobic heterotrophic growth (Ahmann, 1994; Bhattacharjee and Rosen, 2007), for which *arrA* gene encoding the large catalytic subunit (ArrA) serves as a reliable marker (Malasarn *et al.*, 2004). Cytoplasmic As(V) reduction is a detoxification mechanism followed by As(III) efflux, and it is catalysed by As(V) reductase (ArsC) (Mukhopadhyay *et al.*, 2002; Silver and

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Phung, 2005). As(III) methylation catalysed by As(III) S-adenosylmethionine methyltransferase (ArsM) is an alternative As detoxification mechanism that methylates inorganic As(III) to less toxic organic As and volatile As such as mono-, di- and trimethylarsines (Qin *et al.*, 2006; Yin *et al.*, 2011b; Wang *et al.*, 2014; Zhu *et al.*, 2014).

Microbial genes involved in arsenate (As(V)) respiratory reduction (*arrA*), As(V) detoxification reduction (*arsC*), arsenite (As(III)) oxidation (*aioA*) and As(III) methylation (*arsM*) are detected with high abundance and diversity in As-rich environments such as paddy soils (Zhang *et al.*, 2015), mine fields (Luo *et al.*, 2014) and spring or brine sediments (Escudero *et al.*, 2013), demonstrating the presence of microbes capable of As biotransformation in these environments. The underlying mechanisms of these microbial biogeographic patterns can be classified into two types: deterministic (also termed niche-based) processes, and stochastic or neutral processes (Martiny *et al.*, 2006). The deterministic processes imposed by environmental filters select certain species according to their environmental preferences (Aguilar *et al.*, 2014; Hambright *et al.*, 2015), while stochastic processes are driven by ecological drift and dispersal limitation (Martiny *et al.*, 2011; Zhou *et al.*, 2014). Notably, most of the current biogeographic microbial studies are focussed on 16S rRNA genes, despite a resurging interest in understanding the microbial biogeographic patterns of functional genes (Green *et al.*, 2008), which serve as the basis of the biotransformation of various elements on the earth and contribute to the elemental cycle.

Arsenic biotransformation has been detected in natural environments with low As levels (Zhang *et al.*, 2017). In estuarine and coastal wetlands, at interface between terrestrial and coastal ecosystems (Zedler and Kercher, 2005), the activity of As(V)-reducing microbes is thought to be the key driver for As release from sediments to aquifers (Islam *et al.*, 2004), while As(III)-oxidizing microbes are able to decrease As mobility and bioavailability (Bhattacharya *et al.*, 2007). Together, these two microbial groups determine the dynamic release of inorganic As species from estuarine sediments to coastal aquifers. As(III) methylation has been suggested as a link between surface and subsurface As cycling (Maguffin *et al.*, 2015), and the volatile As species generated during this process can further facilitate As emissions to the atmosphere (Chilvers and Peterson, 1987; Mestrot *et al.*, 2011). Therefore, it is of great interest to investigate the presence and geographic distribution patterns of microbes involved in As biotransformation in estuarine sediments.

Here, we examined the microbial biogeography associated with As biotransformation in 70 samples collected from 14 estuarine sediments in Southeastern China. To our knowledge, this is the first study of microbial

biogeography associated with As biotransformation in natural environments, which typically with low As levels that we encounter today (Kulp, 2014). Quantitative PCR (qPCR) methods and terminal restriction fragment length polymorphism (T-RFLP) analysis were used to investigate the abundance levels and geographic distribution patterns of *aioA*, *arrA*, *arsC* and *arsM* genes respectively. High-throughput MiSeq sequencing was performed to profile the taxonomic microbial community via the 16S rRNA genes and the functional microbial community via the *arsM* genes, owing to its important role in linking surface and subsurface arsenic cycling (Maguffin *et al.*, 2015), and the limited knowledge of the *arsM* genes in the environment since it was amplified only from the paddy soils three years ago (Jia *et al.*, 2013). We aim to address the following questions: (i) Are microbial genes involved in As biotransformation widely present in the estuarine wetlands with low As levels? (ii) Do As biotransformation genes have biogeographic patterns? (iii) Which process drive the biogeographic pattern of As biotransformation genes?

Results

Physiochemical characteristics of sediment samples

A total of 70 samples were collected from 14 estuarine sediments across 4 provinces in Southeastern China, ranging from 21.6° to 30.4° N and 108.3° to 121.7° E (Supporting Information Fig. S1 and Table S2). Tm varied from 20.4°C to 30.2°C, and pH varied from 6.5 to 8.1. The C/N ratio varied from 9.2 to 26.8. Total As concentrations in these samples were below the Class I Sediment Quality Criteria (20 mg kg⁻¹, National Standard of P.R. China, 2002), and half of the samples were below the low interim sediment quality value for As concentrations in wetlands (8.2 mg kg⁻¹, Hongkong ISQVs). Ferric iron (Fe(III)) was dominant in total Fe, which ranged from 15.1 to 27.2 g kg⁻¹ and accounted for 94%–99% of the total Fe. Significant positive correlations for Tm and pH values with geographic indicators (latitude and longitude) were detected by Spearman's rank-order correlation analysis ($P < 0.05$). Total C concentrations significantly correlated with total N concentrations ($P < 0.05$) (Supporting Information Table S3).

Abundance and biodiversity of genes involved in as biotransformation

Wide range of gene abundance levels and diversities of *aioA*, *arrA*, *arsC* and *arsM* genes were detected in all 70 samples (Fig. 1 and Supporting Information Table S4). The relative abundance levels of *arsC* genes were significantly ($P < 0.01$) higher than the other three genes (Supporting Information Fig. S2), ranging from 2.3×10^{-3} to 2.9×10^{-1} . Unexpectedly, *arsC* genes were even more

Fig. 1. Detected levels of *aioA*, *arrA*, *arsC* and *arsM* genes normalized to copy numbers of 16S rRNA genes present in 14 estuarine sediments collected across Southeastern China. The absolute gene copy numbers of *aioA*, *arrA*, *arsC* and *arsM* were normalized to that of ambient 16S rRNA genes, in order to minimize variances caused by different background bacterial abundances, extraction and analytical efficiencies. Error bars indicate the standard deviation of five replicates.

abundant than in As-contaminated paddy soils (Jia *et al.*, 2014; Zhang *et al.*, 2015). The relative gene abundance levels of the *aioA*, *arrA* and *arsM* genes varied from 2.1×10^{-4} to 4.2×10^{-3} , 4.4×10^{-5} to 5.0×10^{-3} and 3.3×10^{-4} to 3.4×10^{-3} , respectively, one magnitude lower than (*aioA* gene) or comparable to (*arrA* and *arsM* genes) those detected in paddy soil.

Functional microbial communities involved in As biotransformation were examined by terminal restriction fragment length polymorphism (T-RFLP) analysis (Supporting Information Fig. S3). The *aioA* genes were dominated by terminal restriction fragments (T-RFs) 80, 88 and 130 bp, while the *arsC* genes were dominated by T-RFs 53 and 146 bp, and the *arsM* genes were dominated by T-RFs 59 and 83 bp. For *arrA*, T-RF 34 bp was the dominant T-RF in almost all samples except sample E (FJ-HTX), which was dominated by T-RF 58 bp. Shannon diversity indices based on the T-RFLP profiles indicated that the alpha-diversities of the *aioA* genes were significantly higher than the others ($P < 0.001$) (Supporting Information Fig. S4), but all As biotransformation genes showed comparable alpha-diversities to those detected in As contaminant paddy soils (Jia *et al.*, 2014; Zhang *et al.*, 2015).

Geographic distribution patterns of genes involved in As biotransformation

Principle coordinates analysis (PCoA) of functional microbial communities revealed four clusters for *aioA*, *arrA* and *arsC* genes based on the T-RFLP profiles (Fig. 2). However, for the *arsM* genes, no clear clustering of the functional microbial communities was observed based on the T-RFLP profiles (Supporting Information Fig. S5), possibly due to the low resolution of the T-RFLP technique for *arsM* genes. In addition, the biases associated with the widely used digestion enzyme (*TaqI*) in T-RFLP analysis for *arsM* genes should also be considered since the choice of different digestion enzymes could generate highly variable species richness and diversity indices (Zhang *et al.*, 2008). Therefore, MiSeq sequencing analysis of *arsM* genes was performed to generate a high-resolution profile. The rarefaction curves of Shannon index approached the plateau phase, indicating adequate sequencing depth (Wang *et al.*, 2012) for *arsM* in these samples (Supporting Information Fig. S6), and microbial community richness and diversity across sampling sites were revealed (Supporting Information Table S5). Functional microbial communities based on *arsM* MiSeq sequencing data were

Fig. 2. Principal coordinate analysis (PCoA) of Bray-Curtis distances generated from T-RFLP profiles for *aioA*, *arrA* and *arsC* genes, and MiSeq sequencing data for *arsM* genes. Proportion of variance explained by each principal coordinate axis is denoted in the corresponding axis label. Data sets coloured by different colours indicate samples from different locations (red: Fujian province, green: Guangdong province, blue: Guangxi province, purple: Zhejiang province). [Color figure can be viewed at wileyonlinelibrary.com]

clustered into four groups (Fig. 2), corresponding to the geographic patterns for microbes involved in As redox.

The Adonis dissimilarity test corroborated that the geographic distribution patterns imposed changes in microbial community compositions involved in As biotransformation. Samples located at 28.0° to 30.4° N and 120.6° to 121.7° E (Zhejiang province), 24.4° to 26.6° N and 117.9° to 119.6° E (Fujian province), 22.8° to 23.4° N and 113.6° to 116.2° E (Guangdong province) and 21.6° to 21.9° N and 108.3° to 109.1° E (Guangxi province) showed significant ($P < 0.01$ or 0.05) dissimilarities in the functional microbial community compositions for *aioA*, *arrA* and *arsC* genes based on T-RFLP profiles and for *arsM* genes based on MiSeq sequencing data (Supporting Information Table S6). Microbes involved in As(III) methylation displayed a significant, negative distance-decay curve (Pearson correlation value = 0.512, $P < 0.001$) (Fig. 3), corresponding to the significant decay of the microbial community similarity over

geographic distance. The distance-decay relationship was also significant for the 16S rRNA genes (Pearson correlation value = 0.371, $P < 0.001$), but it was weaker than that of *arsM* (Supporting Information Fig. S7).

Taxonomic profiling of microbes involved in As(III) methylation and their co-occurrence patterns

Microbes involved in As(III) methylation were mostly assigned to *Proteobacteria* (*Alpha*-, *Beta*-, *Delta*- and *Gamma*-), *Methanomicrobia*, *Actinobacteria*, *Clostridia*, *Planctomycetia*, *Halobacteria*, *Thermomicrobia* and *Opitutae* (Fig. 4). However, 6% to 33% (average 17%) of the total microbes were unclassified, and 28% to 58% (average 42%) of the total microbes were identified as uncultured bacteria from the environmental samples. The top 100 abundant As(III)-methylating microbes at the family level are classified in a heatmap (Supporting

Fig. 3. Distance-decay curve for microbial community similarity based on *arsM* genes. Bray-Curtis distances for microbial community and geographic distances among samples were calculated and plotted to generate Pearson correlation values (*cor*). The shadow indicates the 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

Information Fig. S8), and their abundance levels in total microbes involved in As(III) methylation were calculated (Supporting Information Table S7). *Bradyrhizobiaceae* and *Methanosaetaceae* were the most abundant microbes involved in As(III) methylation, followed by *Clostridiales XVIII*, *Planctomycetaceae*, *Halobacteriaceae*, *Nocardiodaceae*, *Sphaerobacteraceae*, *Hyphomicrobiaceae*, *Hydrogenophilaceae* and *Polyangiaceae*. Eukaryota including *Euteleostomi* and *Chlorellaceae* were also

detected, but with relatively low abundance (0.3% and 0.04% respectively).

The co-occurrence patterns of microbes involved in As(III) methylation are shown by network inference (Fig. 5). The resulting network consisted of 49 nodes (OTUs) and 129 edges with a node connectivity of 2.63. The clustering coefficient (Newman, 2003) was 0.22, and the modularity index was 0.50 (values > 0.4), suggesting that the network has a modular structure (Newman, 2006). At the family level, microbes represented by the 49 OTUs in the network included *Bradyrhizobiaceae*, *Clostridiales XVIII*, *Opitutaceae*, *Sphaerobacteraceae*, *Hyphomicrobiaceae*, *Planctomycetaceae*, *Polyangiaceae*, *Nocardiodaceae*, *Halobacteriaceae*, *Desulfobacteraceae*, *Methanosaetaceae*, and some uncultured and unclassified microbes. Further analysis of the relative abundance levels of these microbes in random occurrence in all samples and in the co-occurrence network (Supporting Information Fig. S9) showed that *Clostridiales XVIII*, *Opitutaceae*, *Sphaerobacteraceae*, *Hyphomicrobiaceae*, *Polyangiaceae* and *Desulfobacteraceae* were more abundant in co-occurrence network (14.9%, 11.9%, 9.9%, 6.9%, 5.0% and 2.0%, respectively) than random occurrence in the total microbes involved in As(III) methylation (10.6%, 1.8%, 4.8%, 3.8%, 3.0% and 1.3% respectively). This finding suggests that these microbes tend to co-occur more than what would be expected by chance. *Bradyrhizobiaceae* was the most abundant microbe both in the total (36.7%) microbes and in the co-occurrence network (31.7%). The second most abundant microbe, *Methanosaetaceae*, in total microbes (18.9%) was the least abundant in the co-occurrence network (2.0%).

Fig. 4. Class-level diversity chart for microbial communities involved in As(III) methylation in different sediment samples arranged by hierarchical cluster analysis of Bray-Curtis distances. [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 5. Network of co-occurring 97% cut-off OTUs based on correlation analysis. A connection stands for a strong (correlation coefficient > 0.6) and significant (P -value < 0.01) correlation. The 52 OTUs in the network were forward selected with abundance $> 0.3\%$ to total OTUs based on *arsM* genes. The size of each node is proportional to the number of connections (that is, degree). [Color figure can be viewed at wileyonlinelibrary.com]

Environmental factors affecting gene abundance levels and microbial biogeographic patterns involved in *as* biotransformation

Redundancy analysis (RDA) of the environmental factors affecting *As* biotransformation gene abundances and microbial biogeographic patterns involved in *As* redox (*aioA*, *arrA* and *arsC* genes based on T-RFLP profiles) revealed that significant models had a confidence level of $P < 0.01$ (Supporting Information Fig. S10). Latitude, Tm and total N and C/N ratio significantly correlated with the variation in the relative *As* gene abundance levels, and the first axis accounted for the majority of the total variance (96.4%). For *As*(III)-oxidizing microbes (*aioA*), latitude, pH and Tm were the significant environmental factors affecting the microbial community compositions, and the first and second axis explained 26.1% and 11.9% of the total variances respectively. For microbes involved in *As*(V) respiratory reduction (*arrA*), latitude, pH, Fe(III) and Tm were significantly linked to the variances in the microbial community compositions with 34.8% and 14.3% explained by the first and second axis. The variances in microbial

community compositions involved in *As*(V) detoxification reduction (*arsC*) were significantly shaped by longitude, latitude, pH, C/N ratio and Tm. The first and second axis explained 31.3% and 10.2% of total variances.

The soil properties were further separated to soil attributes, including pH, C/N ratio, total C, N, Fe, As and Fe(III) concentrations, and geographic factors including latitude, longitude and Tm. Tm was included in geographic factors because Tm and latitude are not independent and will nearly always co-vary in coastal environments, which was also demonstrated by the significant Spearman rank-order correlation ($\rho = -0.705$) of Tm and latitude across all sites (Supporting Information Table S3). Canonical correspondence analysis (CCA) based on *arsM* MiSeq sequencing data revealed that latitude, longitude, C/N ratio, pH, Tm, total N and Fe(III) concentrations significantly explained the variation in the microbial community compositions involved in *As*(III) methylation ($P = 0.003$), and the first and second axis accounted for 14.0% and 12.6% of the total variance respectively (Fig. 6A). Variance partition analysis revealed that a total of 62.9% of the

Fig. 6. A. Canonical correspondence analyses (CCA) of *arsM* microbial communities based on Miseq sequencing profiles with environmental factors (arrows). Environmental explanatory variables were forward selected based on the significance test performed by Monte Carlo permutation test ($P < 0.05$). Symbols in different colours indicate samples from different provinces (green: Zhejiang province, red: Fujian province, blue: Guangdong province, yellow: Guangxi province). The values of CCA1 and 2 are percentages that the corresponding axis can explain.

B. Variation partitioning analysis that partitions relative influence of environmental factors on microbial community structure. Environmental factors are divided into groups of geographic factors (Latitude, Longitude, Tm) and soil attributes (C/N, pH, N, Fe(III)). The overlap represents the joint effect of the two groups.

C. The correlations between microbial community and soil attributes. Bray-Curtis distances for microbial community and Euclid distances for soil attributes among samples were calculated and plotted to generate Pearson correlation values (cor). The shadow indicates the 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

community variations were explained by these selected variables (Fig. 6B). Among them, soil attributes (C/N, pH, total N, Fe(III)) independently accounted for 33.0% of the total variances, and showed strong (Pearson correlation value = 0.424) and significant correlations ($P < 0.001$) with microbial compositions based on the *arsM* genes in the similarity test (Fig. 6C). Geographic factors (latitude, longitude and Tm) independently explained 22.9% of the total variances. Soil attributes and geographic factors interactively explained 7.1% of the total variances, which possibly resulted from the significant correlation ($P < 0.01$ or 0.05)

of pH values with the geographic factors (Supporting Information Table S3). A total of 37.1% of the total variances remained unexplained by these environmental factors.

The environmental factors revealed in CCA and RDA were verified by Mantel tests on the basis of their significant correlation with microbial gene abundance levels and community compositions involved in As biotransformation (Table 1 and Supporting Information Table S8). The heterogeneity of local environmental factors was demonstrated using a significant distance-decay relationship (Pearson correlation value = 0.263, $P < 0.01$) of soil attributes (pH,

Table 1. Correlation between microbial community involved in As(III) methylation (*arsM*) based on MiSeq sequencing profiles and environmental factors as shown by Mantel tests.

Environmental factor (unit)	<i>r</i> value	Environmental factor (unit)	<i>r</i> value
Latitude	0.670**c	N (g kg^{-1})	0.338*
Longitude	0.373**	C/N ratio	0.346*
Tm ($^{\circ}\text{C}$)	0.502**	Fe (g kg^{-1})	0.220*
pH	0.582***	Fe(III) (g kg^{-1})	0.231*
C (g kg^{-1})	0.079	As (mg kg^{-1})	0.056
Geographic factors ^a	0.627***	Soil attributes ^b	0.565***

a. Geographic factors: Latitude, Longitude, Tm (Temperature).

b. Soil attributes: pH, N, C/N ratio, Fe(III).

c. Significant differences ($P < 0.05$) are indicated in bold. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. AIC values for 6 rank abundance distribution models based on MiSeq sequencing profiles of *arsM* genes. Lowest AIC value for each sample represents the best fit model.

Sample ID	Niche					Neutral
	Broken-stick	Pre-Emption	Log-Normal	Zipf	Zipf-Mandelbrot	ZSM
A	37 559	17 183	1652*	3181	1835	8689
B	24 814	15 259	2438*	3325	2596	5975
C	21 814	11 248	1613*	2449	1672	4633
D	47 922	29 270	5035	5293	4045*	6887
E	16 338	12 379	2889*	4665	4132	7356
F	38 792	27 486	4053	3660	3173*	8031
G	29 261	26 289	6275	4988*	4990	11 013
H	27 753	24 502	5299	3521*	3523	9506
I	15 133	11 617	3311*	4049	3399	9947
J	26 316	18 941	4957	5822	3536*	10 410
K	17 848	9541	3344	5447	2376*	7898
L	13 483	10 440	3833	5543	3725*	11 063
M	34 303	27 466	4857	3912	3751*	11 242
N	22 960	18 636	3569	3146*	3148	8774

*The best fit model with lowest AIC value.

total N, C/N ratio and Fe(III) (Supporting Information Fig. S11).

A niche-based theory explained the assembly of microbial communities

The comparison of different rank abundance distribution models based on AIC values showed that the data based on *arsM* genes in different sediments best fit either with the Log-normal, Zipf or Zipf-Mandelbrot models (Table 2), indicating that the niche-based mechanism best explained the composition and structure of the microbial communities involved in As(III) methylation. Similarly, the data based on the 16S rRNA genes in different sediments were also fit with the Zipf-Mandelbrot model, suggesting that the niche-based mechanism primarily shaped the taxonomic microbial community (Supporting Information Table S10).

Discussion

Prevalence of microbes involved in as biotransformation distributed in estuarine sediments

The ubiquitous presence of microbes capable of As(III) respiratory oxidation, As(V) respiratory reduction, As(V) detoxification reduction and As(III) methylation in all estuarine sediments was demonstrated in this study (Fig. 1). Among them, *arsC* had the highest abundance among the four As biotransformation genes (Supporting Information Fig. S2); strikingly, it was even more abundant than those detected in As contaminant paddy soils (Jia *et al.*, 2014; Zhang *et al.*, 2015). The Great Oxidation of the Earth resulted in more As(V) in the environment. To detoxify As(V), microbial *ArsC* enzymes evolved to reduce As(V) to As(III) and subsequently pump it out (Mukhopadhyay

et al., 2002). The high abundance levels of As(V) reduction-related genes (*ars*) have also been observed in low As environments (Cai *et al.*, 2013; Xiao *et al.*, 2016), corroborating the prevalence of microbes capable of As(V) detoxification via reduction in these estuarine sediments. Additionally, estuaries are adjacent to the marine environment, in which As(V) accumulation by microbes is elevated via the phosphate uptake system due to the low phosphate levels in the marine environment (Sanders, 1980; Takahashi *et al.*, 1990; Zhang *et al.*, 2013a). Although it is not experimentally validated in this study, it is likely that microbes in these environments may acquire more abundant *arsC* genes to detoxify the highly accumulated As in their cytoplasm.

The abundance of As(III) respiratory oxidizing microbes (*aioA*) and respiratory As(V) reducing microbes (*arrA*) were lower in sediments (Fig. 1) but still comparable to those detected in As-contaminated paddy soils (Jia *et al.*, 2014; Zhang *et al.*, 2015). The low As levels in these estuarine sediments could explain the lower relative abundance, suggesting that As respiration may not be an important process to either detoxify As or couple it to ATP production (Ahmann, 1994; Slyemi and Bonnefoy, 2012) due to the lack of available As. Nevertheless, the highest Shannon diversity detected in *aioA* genes (Supporting Information Fig. S4) indicated that As(III)-oxidizing microbes are still very diverse. In addition, the previously identified dominant T-RFs for *aioA* genes (Jia *et al.*, 2014; Zhang *et al.*, 2015) were also detected in this study (Supporting Information Fig. S3), demonstrating the presence of dominant As(III)-oxidizing microbes in these estuarine sediments.

The surface of ancient earth might have had more abundant As than it has today (Oremland *et al.*, 2009), and As

metabolic pathways subsequently evolved as important processes for early cellular life to either couple the element to energy production or mitigate its toxicity (Lebrun *et al.*, 2003; Oremland and Stolz, 2003; Silver and Phung, 2005; Zhu *et al.*, 2014). Therefore, it is likely that the prevalence of As biotransformation genes in the environment resulted from this history.

In-depth investigation of microbes involved in As(III) methylation distributed in estuarine sediments

The *arsM* genes responsible for As(III) methylation are considered to be involved in an important As detoxification mechanism (Zhu *et al.*, 2014). The comparable abundant *arsM* genes detected in this study showed great potential for As(III) methylation in the estuarine environment. However, the present study of *arsM* genes is still limited since *arsM* gene references in the NCBI database are insufficient, thus resulting in the high percentage of unclassified *arsM* gene sequences (17%) or those identified as uncultured bacteria from environmental samples (42%) (Supporting Information Table S7). Among the classified microbes involved in As(III) methylation were microbes belonging to *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia* and *Eukaryotes* (Fig. 4), along with those belonging to *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Euryarchaeota*, which were previously identified in paddy soils (Jia *et al.*, 2013; Zhao *et al.*, 2013; Zhang *et al.*, 2015). To the best of our knowledge, *arsM* genes assigned to *Eukaryotes* have never been observed in environmental samples, possibly due to the limited number of sequences based on clone library analysis (Jia *et al.*, 2013; Zhang *et al.*, 2015), the limited *arsM* sequences on the GeoChip (Zhao *et al.*, 2013), or the reduced sensitivity of metagenomics for identifying functional genes (Cai *et al.*, 2013; Luo *et al.*, 2014; Xiao *et al.*, 2016). However, *Eukaryotes* are prevalent in estuarine environments, and their importance in As(III) methylation warrants further investigation, especially considering the confirmed As(III) methylation capacity of *Eukaryotes*, such as *Tetrahymena thermophila* (Yin *et al.*, 2011a) and *Ostreococcus tauri* (Zhang *et al.*, 2013b).

Close associations of *Alphaproteobacteria* with *Opitutae* and *Thermomicrobia*, and *Actinobacteria* with *Deltaproteobacteria* and *Halobacteria* were observed for microbes involved in As(III) methylation (Fig. 5), possibly contributing to the relative higher abundance of these microbes detected in sediments from Zhejiang province (Sample A, B, C and D, Fig. 4). While previous studies showed that co-occurrence patterns mostly occurred in closely related phylogenetic taxa (Barberan *et al.*, 2012), our study demonstrated the relatively high co-occurrence patterns of microbes from different taxa, indicating a different niche for functional microbial communities. Nevertheless, the

relatively high fraction of unclassified OTUs in the network might be an important reason why we hardly detected co-occurrence patterns derived from closely related phylogenetic taxa. The co-occurrence patterns could be potential indicators of competitive interactions, non-overlapping niches or historical effects (Horner-Devine *et al.*, 2007). *Clostridiales* XVIII, *Opitutaceae*, *Sphaerobacteraceae*, *Hyphomicrobiaceae*, *Polyangiaceae* and *Desulfobacteraceae* tend to co-occur more than expected by chance (Supporting Information Fig. S9), suggesting niche adaptation of these microbes involved in As(III) methylation. However, *Methanosaetaceae* tended to co-occur less than expected by chance, suggesting their independence and restricted habitat preference in sediments from the Fujian province (Sample E, F, G and H, Supporting Information Table S7).

Processes and factors driving microbial biogeography associated with as biotransformation

Our results showed that the mechanisms for shaping both taxonomic microbial communities (based on the 16S rRNA gene amplicons) and the functional microbial communities involved in As(III) methylation (based on the *arsM* gene amplicons) were consistent with deterministic processes (Table 2 and Supporting Information Table S10). Intriguingly, the variations in functional microbial community compositions involved in As biotransformation were unrelated to the As levels (Fig. 6A and Supporting Information Fig. S10). Our results collectively suggest that at large spatial scales, other environmental factors, rather than As levels, are crucial in shaping the functional microbial communities involved in As(III) methylation. This conclusion is reasonable because As levels in all samples were relatively low and similar; such correlation would be expected in As-rich environment and with various *in situ* bioavailable As.

The significant correlations between soil attributes (with high spatial heterogeneity, Supporting Information Fig. S11), and the microbial community based on *arsM* genes (Fig. 6C) corroborated the concept that the assembly based on niche-based mechanisms is mainly determined by the niche requirements and local habitat conditions (Chase and Myers, 2011). The reason for latitude as the main factor influencing both abundance and diversity of As biotransformation genes is possibly due to that factors showing strong linkages with As biotransformation microbes such as Tm, pH and N concentration, which exhibit significant correlation with latitude (Supporting Information Table S3). The significant effect of pH on microbial community compositions involved in As biotransformation could be due to the fact that soil pH was important for determining the ionized and associated forms of As in As biotransformation (Vahter, 2002), and As

biotransformation processes in paddy soils were sensitive to pH variation (Stolz and Oremland, 1999). In addition, we found that Fe(III) in estuarine sediments was linked to microbial biogeographic patterns associated with As biotransformation, especially for microbes involved in As(V) respiratory reduction and As(III) methylation (Fig. 6 and Supporting Information Fig. S10). It is well known that iron oxyhydroxides could sequester As(V) (Liu *et al.*, 2006), reducing As bioavailability to microbes, and possibly contribute to the less abundance of *arrA* genes detected in samples from Fujian province (E, F, G and H) (Fig. 1) in which Fe(III) concentrations were higher compared with the other samples (Supporting Information Table S2). Since microbe-mediated redox processes are strongly related to the microbial accessibility of C and N (Koegel-Knabner *et al.*, 2010), it is reasonable to expect that C/N ratio would also be significant environmental factor affecting the abundance of As biotransformation microbes. Moreover, the effect of N concentration on microbial community compositions involved in As(III) methylation has also been demonstrated previously (Zhang *et al.*, 2015).

Microbial biogeography: Taxonomic and functional communities

The functional similarity (Pearson correlation value = 0.512 for *arsM* genes) showed a higher distance-decay rate than the phylogenetic similarity (Pearson correlation value = 0.371 for 16S rRNA genes), suggesting a quicker turnover of the functional genes than that found in microbial species. The higher fraction of variances (33.0%) in the microbial community involved in As(III) methylation was explained independently by soil attributes, including pH, total N, C/N ratio and Fe(III), rather than by geographic factors (Fig. 6B). In contrast, among the soil attributes, only pH was significantly correlated with the taxonomic microbial community (Supporting Information Table S9). Considering the substitutability of microorganisms, microbial taxa distributions have been suggested to be of little value in predicting environmental changes (Green *et al.*, 2008). Rather, a function-based approach might be essential for understanding the importance of the specific habitats of microbial functional traits in the environment (Burke *et al.*, 2011). It has been shown that metabolic flexibility can be a major predictor for the spatial distribution within microbial communities (Carbonero *et al.*, 2014), representing a distinct microbial ecology principle that differs from macro-ecology. We reinforce this viewpoint in our study by showing that the functional composition of microbes is correlated to soil geochemical parameters. Therefore, in-depth metagenomics and metatranscriptomics profiling will provide a large amount of data that is amenable for exploration to recognize bio-indicators of environmental conditions.

Implications of functional biogeography to as biotransformation genes evolution and as biogeochemical cycle

This study is the first to demonstrate the wide presence of As biotransformation genes (*aioA*, *arrA*, *arsC* and *arsM*) in an environment with a low As level, which is possibly a legacy of active As metabolism in early life (Sforza *et al.*, 2014). While previous studies have demonstrated the diversity of As biotransformation microbes (Oremland and Stolz, 2003), we further disclosed the biogeographic microbial patterns associated with As biotransformation at a large spatial scale. Since the low As level in these sediments was not strong enough to impose selection on As biotransformation genes, heterogeneity of local soil attributes may lead to variable selection for taxonomic microbial communities (Dini-Andreote *et al.*, 2015), which may become more important in determining functional microbial biogeographic patterns. Compared with microbial taxonomy, environmental selection for functional genes seems to be less complicated, rendering functional microbial communities as an ideal model to study the important role of deterministic processes in shaping microbial biogeographic patterns. Moreover, our study revealed the ubiquity of the abundant and diverse microbes involved in As redox reactions and methylation in estuarine sediments at a large spatial scale. While As redox reactions determine the dynamic of inorganic As species in estuarine wetlands (Islam *et al.*, 2004; Bhattacharya *et al.*, 2007), As(III) methylation can facilitate the emission of As from sediments to the atmosphere because the end products of this process are volatile As species (Chilvers and Peterson, 1987; Mes-trot *et al.*, 2011). Therefore, it is not surprising that high methylating capacity was found in sediments where As concentrations were low. Considering the prevalence of microbes capable of As biotransformation, the high capacity of As biotransformation in environments and the large, global area of estuarine wetlands, these microbes are critical to the global As biogeochemical cycle.

Experimental procedures

Sites description and soil chemistry

A total of 70 sediment samples from depths of 0–15 cm were collected in 2013 from 14 estuarine wetlands (five replicates for each sample) distributed in 4 provinces in Southeastern China (Supporting Information Fig. S1). These sites included, the regions of Zhejiang-Qiantangjiang (A: ZJ-QTJ), Zhejiang-Yongjiang (B: ZJ-YJ), Zhejiang-Jiaojiang (C: ZJ-JJ), Zhejiang-Ouijiang (D: ZJ-OJ), Fujian-Huotongxi (E: FJ-HTX), Fujian-Minjiang (F: FJ-MJ), Fujian-Jinjiang (G: FJ-JJ), Fujian-Jiulongjiang (H: FJ-JLJ), Guangdong-Hanjiang (I: GD-HJ), Guangdong-Longjiang (J: GD-LJ), Guangdong-Zhujiang (K: GD-ZJ), Guangxi-Nanlijiang (L: GX-NLJ), Guangxi-Qinjiang (M: GX-QJ) and Guangxi-Fangchengjiang (N: GX-FCJ). Sediments samples were placed in sterile plastic bags and

transported to the laboratory on ice for DNA extraction and soil chemistry analysis. Soil properties, including pH, the concentration of total carbon (C), nitrogen (N), As, iron (Fe) and ferric iron (Fe(III)), and the C/N ratio were analysed. Details of the measurement methods are shown in the Supporting information.

DNA extraction and PCR-based analysis of aioA, arrA, arsC and arsM

DNA was extracted from 0.5 g of the samples using the FastDNA® SPIN kit for soil (MP Biomedicals) according to the manufacturer's instructions and was checked for quality by agarose gel electrophoresis and ultraviolet-absorbance (ND1000, Nanodrop, Thermo Fisher Scientific, Inc.). DNA yields of the 70 sediment samples were between 4 to 116 ng μL^{-1} with most of them arranged from 20 to 60 ng μL^{-1} . The ratio of absorbance at 260 nm and 280 nm was around 1.8. Quantitative real-time polymerase chain reaction (qPCR) performed on an iQTM5 Thermocycler (Bio-Rad, USA) was conducted to investigate the *aioA*, *arrA*, *arsC*, *arsM* and 16S rRNA gene abundance levels. The *aioA*, *arrA*, *arsC* and *arsM* genes were normalized by dividing the absolute gene copy numbers by that of 16S rRNA. Terminal restriction fragment length polymorphism (T-RFLP) analysis was conducted to investigate *aioA*, *arrA*, *arsC* and *arsM* gene diversities. For each sampling site, the relative abundance levels of terminal restriction fragments (T-RFs) were calculated by normalizing the peak areas of each individual T-RF to the peak areas of the total T-RFs. T-RFs that occurred in at least five sampling replicates and with percentages > 2% were listed. Details are shown in the Supporting information.

High-throughput MiSeq sequencing of the arsM and 16S rRNA genes

Total genomic DNA was PCR amplified using the primers *arsMF1* and *arsMR2* (Jia *et al.*, 2013) with barcodes, targeting a conserved ~347 bp region of the *arsM* genes. PCR conditions were: 95°C for 5 min, 35 amplification cycles of 95°C for 30 s, 62°C for 35 s, 72°C for 1 min, and a final single extension cycle of 72°C for 1 min. Three replicates PCRs were performed for each DNA sample, and amplicons generated from each set of the three reactions were subsequently pooled and quantified using PicoGreen. Negative controls and no template controls were included for DNA extraction and PCR amplification. Finally, the five barcoded amplicons for each sample site were pooled in equal concentrations for sequencing. The amplicon pool was purified using the MoBio UltraClean PCR Clean-up kit and was sequenced on the Illumina MiSeq sequencing platform (MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18) at Majorbio Bio-pharm Technology Company in Shanghai, China. The 14 pools of amplicons were sequenced using the 300PE protocol, which generated a total of 393 980 raw read pairs. The sequence read pre-processing was performed in FLASH (Magoč and Salzberg, 2011), yielding a total of 316 975 trimmed sequences that varied from 17 371 to 28 468 per sample. The final average sequence length after the barcode, heterogeneity spacer and primer removal was 324 bp.

Processing of the *arsM* gene's sequence reads was performed using QIIME (version 1.6.0) (Caporaso *et al.*, 2010), including additional quality trimming and demultiplexing. Operational taxonomic units (OTUs) were calculated at the 97% sequence identity level and resulted in 3211 OTUs. The most abundant sequence from each OTU was selected as a representative sequence for that OTU. Taxonomy identification was performed using the Basic Local Alignment Search Tool (BLAST) for each representative sequence against the *arsM* gene sequences from the NCBI database. Shannon and Simpson diversity, abundance-based coverage estimators (ACE) and Chao richness for each sample of the sites were calculated in R 3.1.2 with the fossil package (Vavrek, 2012). Details of the 16S rRNA gene sequencing process were shown in the Supporting information. Gene sequences data of *arsM* and 16S rRNA genes were submitted to NCBI under the accession number: KP678028 - KP681238 for *arsM* genes and SRP076214 for 16S rRNA genes.

Data analyses

Principle coordinates analysis (PCoA) and the Adonis dissimilarity test based on T-RFLP or MiSeq sequencing data were conducted to examine the distribution patterns of microbial community compositions involved in As biotransformation. Monte Carlo permutation was used to test the statistical significance. The distance-decay analysis was applied for the *arsM* genes to investigate the declining microbial community similarity with geographic distance, and tested by Pearson correlation coefficient. The distance-decay analysis based on the 16S rRNA genes was also performed to compare the distance-decay rates for functional and taxonomic microbial communities, and details are shown in the Supporting information. The similarity and Mantel tests were performed to evaluate the linkages between functional or taxonomic microbial groups and environmental factors, followed by calculation of the Pearson correlation coefficient. In these analyses, the Bray-Curtis distance for microbial communities and the Euclid distance for environmental factors were used to calculate the distance between samples. Redundancy analysis (RDA) or Canonical correspondence analysis (CCA) were chosen according to the result of the detrended correspondence analysis (DCA). Environmental explanatory variables were forward selected based on the significance test performed by the Monte Carlo permutation test ($P < 0.05$). The variation partitioning analysis resulted in three geographic factors (latitude, longitude and sediment temperature (Tm)) and four soil attributes (C/N, pH, N, Fe(III)). Spearman's rank-order correlation was used to test the correlations between the environmental factors. This non-parametric method was used because these data did not follow a normal distribution. All of the analyses were performed in R 3.1.2 with vegan (Oksanen *et al.*, 2013) and ggplot2 (Wickham, 2009) packages.

To explore the co-occurrence patterns in soil microbial communities involved in As(III) methylation, network analysis was conducted by calculating the inferred correlations between the forward selected 52 OTUs (with abundance > 0.3% to total OTUs) using Sparse Correlations for Compositional data (SparCC) (Friedman and Alm, 2012; Weiss *et al.*, 2016). If the correlation coefficient was > 0.6 and statistically significant (P -value < 0.01), a valid co-occurrence event was identified as a

robust correlation. The nodes in the reconstructed network represent the OTUs at 97% identity, and the edges correspond to a strong and significant correlation between nodes. The network was explored and visualized with the interactive platform Gephi (Bastian *et al.*, 2009).

The rank abundance distribution was examined to test whether niche-based or neutral models best explain the assembly of the microbial community involved in As(III) methylation. The niche-based theory predicts that the rank abundance distribution would fit the broken stick, pre-emption, log-normal, Zipf and Zipf-Mandelbrot models (Motomura, 1932; MacArthur, 1957; McGill *et al.*, 2007). The neutral theory assumes that the rank abundance distribution would be consistent with ZSM (Hubbell, 2001). A measure of the relative quality of a statistical model, i.e., the Akaike Information Criterion (AIC) value, was used to compare which model provided the best fit to the empirical data (Dumbrell *et al.*, 2010; Mendes *et al.*, 2014). The best fit model was indicated by the lowest AIC value. The analyses of the broken stick, pre-emption, log-normal, Zipf and Zipf-Mandelbrot models were performed in R 3.1.2 with the vegan package (Oksanen *et al.*, 2013), and the ZSM model was performed using Tetame (Jabot *et al.*, 2008).

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Supporting information

Additional supporting information can be found in the online version of this article at the publisher's web-site:

Fig. S1. Location of 14 estuarine sediments collected across Southeastern China. The base map used is from the National Fundamental Geographic Information System of China.

Fig. S2. Boxplot of the relative abundance of *aioA*, *arrA*, *arsC* and *arsM* genes. The red triangles indicate average relative abundance of each gene. One-way analysis of variance (ANOVA) was conducted to test the difference of relative gene abundance for each gene (** $P < 0.01$).

Fig. S3. Community structure of *aioA*, *arrA*, *arsC* and *arsM* genes reflected by the terminal restriction fragment length polymorphism (T-RFLP) analysis in 14 estuarine sediments collected across Southeastern China. Terminal restriction fragments (T-RFs) were digested by *TaqI* enzyme of PCR products of *aioA*, *arrA*, *arsC* and *arsM* genes from sediment samples.

Fig. S4. Boxplot of shannon diversity of *aioA*, *arrA*, *arsC* and *arsM* genes based on T-RFLP profiles. The red triangles indicate average shannon diversity of each gene. One-

way analysis of variance (ANOVA) was conducted to test the difference of average shannon diversity for each gene (** $P < 0.001$, * $P < 0.05$).

Fig. S5. Principal coordinate analysis (PCoA) of Bray-Curtis distances generated from *arsM* genes T-RFLP profiles. Proportion of variance explained by each principal coordinate axis is denoted in the corresponding axis label. Data sets colored by different colors indicate samples from different locations (red: Fujian province, green: Guangdong province, blue: Guangxi province, purple: Zhejiang province).

Fig. S6. Rarefaction curves for shannon index based on *arsM* MiSeq sequencing data in samples collected from 14 estuarine sediments across Southeastern China.

Fig. S7. Distance-decay curve for microbial communities based on 16S rRNA genes. Bray-Curtis distances were used for microbial community. Pearson correlation values were calculated and plotted. The shadow indicates the 95% confidence intervals.

Fig. S8. Family-level diversity and abundance of microbe involved in As(III) methylation in 14 estuarine sediments. Complete linkage clustering of samples were calculated based on the composition and relative abundance of *arsM* genes. Scales of completely blue, green, yellow, orange, and red indicated the abundance of 0–0.01%, 0.01–0.14%, 0.14–2.79%, and 2.79–57.66%, respectively.

Fig. S9. Relative abundance of different microbial taxonomic groups (at the family level) involved in As(III) methylation. (a) number of sequences assigned with different microbial taxonomic groups in all sediment samples. (b) number of significant co-occurrent OTUs assigned with different microbial taxonomic groups (node from Figure 5).

Fig. S10. RDA correlation biplot of relative gene abundances of *aioA*, *arrA*, *arsC* and *arsM* and *aioA*, *arrA* and *arsC* microbial communities based on T-RFLP profiles with environmental factors (arrows). Environmental explanatory variables were forward selected based on the significance test performed by Monte Carlo permutation test ($P < 0.05$). Symbols in different colors indicate samples from different provinces (green: Zhejiang province, red: Fujian province, blue: Guangdong province, yellow: Guangxi province). The values of RDA1 and 2 are percentages that the corresponding axis can explain.

Fig. S11. Distance-decay curve for soil attributes (C/N, pH, N, Fe(III)) similarity. Euclid distances for soil attributes and geographic distances among samples were calculated and plotted to generate Pearson correlation values. The shadow indicates the 95% confidence intervals.

Table S1. Details of primer pairs and qPCR thermal cycling parameters for *aioA*, *arrA*, *arsC*, *arsM* and 16S rRNA genes.

Table S2. The location and soil properties of the 14 estuarine sediments collected across Southeastern China.

Table S3. Spearman rank-order correlation (ρ) of environmental parameters.

Table S4. Shannon and Simpson diversity for *aioA*, *arrA*, *arsC* and *arsM* genes detected in each sample site calculated based on T-RFLP profiles.

Table S5. Shannon and Simpson diversity, ACE and Chao richness, and identified OTUs for each sample site based on *arsM* MiSeq sequencing data.

Table S6. The effect of distribution pattern on microbial community compositions involved in As biotransformation as examined by the dissimilarity test of Adonis.

Table S7. Microbes involved in As(III) methylation detected in 14 estuarine sediments at family-level, and the percentage of their members to total microbes in all samples.

Table S8. Correlation between T-RFLP data (*aioA*, *arrA* and *arsC* genes) and relative gene abundances (*aioA*, *arrA*, *arsC* and *arsM* genes) with environmental factors as shown by Mantel tests.

Table S9. Correlation between microbial community based on 16S rRNA gene MiSeq sequencing profiles and environmental factors as shown by Mantel tests.

Table S10. AIC values for 6 rank abundance distribution models based on MiSeq sequencing profiles of 16S rRNA genes. Lowest AIC value for each sample represents the best fit model.