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Analysis of the functional gene structure and metabolic potential of microbial community in high arsenic groundwater

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Highlights

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Samples were divided into low and high arsenic (As) groups based on geochemical parameters and microbial functional structures.

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Genes *arsC*, *arrA*, *dsrA/B*, *ureC*, *amoA*, *hzo*, *mcrA*, *hdrB* were correlated with As, SO₄²⁻, NH₄⁺ or CH₄, respectively.

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As, TOC, SO₄²⁻, NH₄⁺, ORP and pH were important factors shaping the functional microbial community structure.

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Alkaline and reducing conditions could be associated with As enrichment in groundwater.

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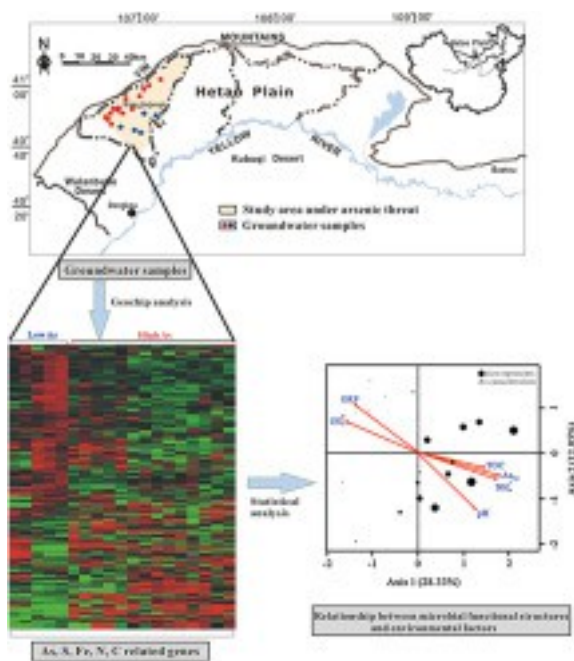
An overall picture of functional microbial communities in high As aquifers is provided.

Abstract

Microbial functional potential in high [arsenic](#) (As) groundwater ecosystems remains largely unknown. In this study, the [microbial community](#) functional composition of nineteen groundwater samples was investigated using a functional gene array (GeoChip 5.0). Samples were divided into low and high As groups based on the clustering analysis of geochemical parameters and microbial functional structures. The results showed that As related genes (*arsC*, *arrA*), sulfate related genes (*dsrA* and *dsrB*), nitrogen cycling related genes (*ureC*, *amoA*, and *hzo*) and [methanogen](#) genes (*mcrA*, *hdrB*) in groundwater samples were correlated with As, SO₄²⁻, NH₄⁺ or CH₄ concentrations, respectively. Canonical [correspondence](#)

[analysis](#) (CCA) results indicated that some geochemical parameters including As, total organic content, SO_4^{2-} , NH_4^+ , [oxidation-reduction](#) potential (ORP) and pH were important factors shaping the functional microbial community structures. Alkaline and reducing conditions with relatively low SO_4^{2-} , ORP, and high NH_4^+ , as well as SO_4^{2-} and Fe [reduction](#) and [ammonification](#) involved in microbially-mediated geochemical processes could be associated with As enrichment in groundwater. This study provides an overall picture of functional microbial communities in high As groundwater [aquifers](#), and also provides insights into the critical role of microorganisms in As biogeochemical cycling.

Graphical abstract



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Keywords

Arsenic
Groundwater
Functional genes
GeoChip5.0
Inner Mongolia

1. Introduction

[Arsenic](#) (As) in groundwater is a serious environmental issue due to its widespread distribution and high toxicity, which threatens the health of millions of people in many countries such as Bangladesh, West Bengal, China, Cambodia, Japan, Argentina, Chile and USA ([Pi et al., 2017](#), [Schaefer et al., 2016](#), [Michael, 2013](#), [Rodríguez-Lado et al., 2013](#), [Fendorf et al., 2010](#), [Chakraborti et al., 2010](#), [Nordstrom, 2002](#)). Long term ingestion of As groundwater can result in arseniasis, which causes many chronic diseases including cardiovascular, [respiratory diseases](#), and skin, lung, liver and kidney cancers ([Chen, 2014](#), [Chung et al., 2013](#)). Over the last few decades, numerous studies focusing on [hydrology](#), [mineralogy](#), and [geochemistry](#) have been undertaken to detect As mobilization and determine transformation mechanisms in high As groundwater [aquifers](#). The explanation of As generation in groundwater aquifers is not trivial due to the complex sets of hydrogeological conditions and biogeochemical processes within the aquifers. The absorbed and sequestered As on Fe oxides/hydroxides is one of the most common As reservoirs in some [sedimentary basins](#). Reductive dissolution of the Fe [oxide minerals](#) in sediments, as well as [reduction](#) of As(V) to highly mobile As(III) leads to the release of bound As into the groundwater ([Guo et al., 2011](#), [Kocar et al., 2010](#), [Deng et al., 2009](#)). Generation of HS⁻ by sulfate reduction was proposed to promote As mobilization through HS⁻ abiotic reductive dissolution of the Fe oxide minerals and formation of As-sulfur compounds in high HS⁻ concentration water ([Wang et al., 2014b](#); [Stauder et al., 2005](#)). In addition, anthropogenic activities such as agricultural irrigation which releases chemicals including organic matter, nitrogen, and other chemicals into groundwater systems, were found with important roles on As release in groundwater ([Weng et al., 2017](#), [Gao et al., 2014](#), [Neidhardt et al., 2012](#), [Itai et al., 2008](#), [Bose and Sharma, 2002](#)). Previous studies showed that As mobilization and transformation could be ascribed to the complex interactions between microbes and geochemical processes and that microbes were likely to play key roles in driving the [biogeochemical cycle](#) in high As groundwater aquifers ([Chen et al., 2017](#), [Zhang et al., 2015](#), [Ghosh and Sar, 2013](#), [Gorra et al., 2012](#), [Mumford et al., 2012](#), [Kocar et al., 2010](#), [Barringer et al., 2010](#), [Sutton et al., 2009](#)).

Understanding [microbial community](#) structures and their associations with geochemical processes is one of the central topics in [microbial ecology](#). Previous studies investigated microbially-mediated As transform and release mechanisms using pure microbial cultures under laboratorial conditions ([Flynn et al., 2014](#), [Sutton et al.,](#)

2009, [Saalfield and Bostick, 2009](#)). Recently, with the methods of [sequencing](#) technologies and qPCR, some studies explored the diversity and structure of *in situ* microbial communities and functional genes of As reducing and oxidizing bacteria, [sulfate reducing bacteria](#) and [methanogens](#) in high As groundwater aquifers including Bangladesh, Cache Valley Basin Utah, Jiangnan and Hetao Plain of China ([Ye et al., 2017](#), [Chen et al., 2017](#), [Mirza et al., 2017](#), [Wang et al., 2015](#), [Wang et al., 2016](#), [Hassan et al., 2015](#), [Li et al., 2014](#), [Li et al., 2015](#), [Sultana et al., 2011](#)). These previous studies provided useful but limited information on the mechanisms of microbially-mediated As transformation and mobilization in groundwater aquifers. It is still not fully understood which functional microbial populations mediate As geochemical processes *in situ*. However, microbial communities in groundwater aquifers contaminated with high levels of As are complex and largely unknown. Therefore, there is an urgent need to more fully investigate the functional potential of microbial communities in these As contaminated aquifers. In this study, we used GeoChip 5.0, a powerful and high-throughput technology which targets more than 1400 functional gene families, and involves in geochemical cycling (N, C, S, and P), metal [homeostasis](#), and [organic contaminant](#) degradation genes ([Van Nostrand et al., 2016](#)) to investigate the functional potential microbial communities in high As groundwater aquifers. Our study area is located in Hetao Plain, an arid region with annual precipitation 140–180 mm and evaporation from 2000 to 2500 mm. About half of the soils are saline, due to strong [evapotranspiration](#) ([Deng et al., 2009](#)). It has been one of the largest irrigation districts using delivered Yellow [River water](#). The groundwater for drinking is taken from the late Pleistocene and [Holocene](#) layer in a complex organic-rich reductive environment which was detected with many gases such as H₂S and CH₄ ([Li et al., 2014](#), [Tang et al., 2017](#)). The water chemical types were mostly Na(–Mg)–Cl(–HCO₃) and Na–Mg–HCO₃. Most high-As groundwater in the region occurs at the depth 20–30 m. Groundwater from this area contains As concentrations up to 1.74 mg/L ([Deng et al., 2009](#)), which greatly exceeds the upper limit (10 µg/L) recommended by world health organization guidelines ([WHO, 2011](#)).

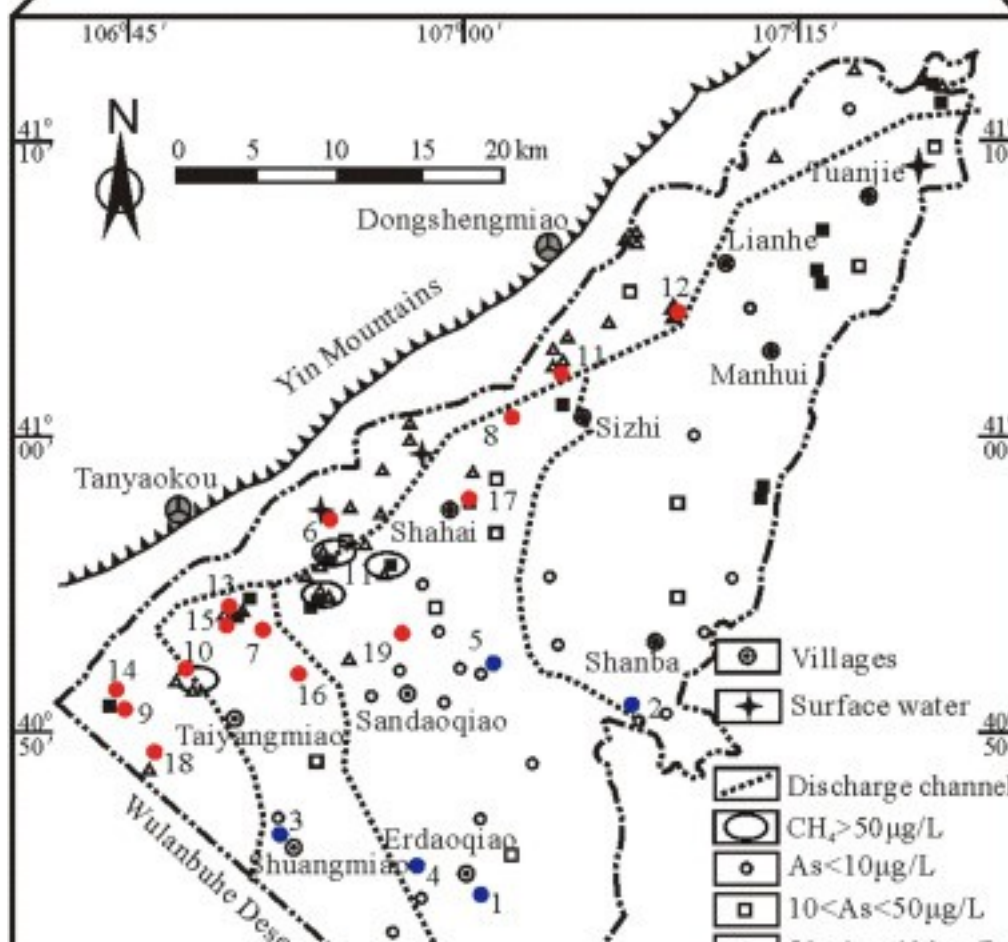
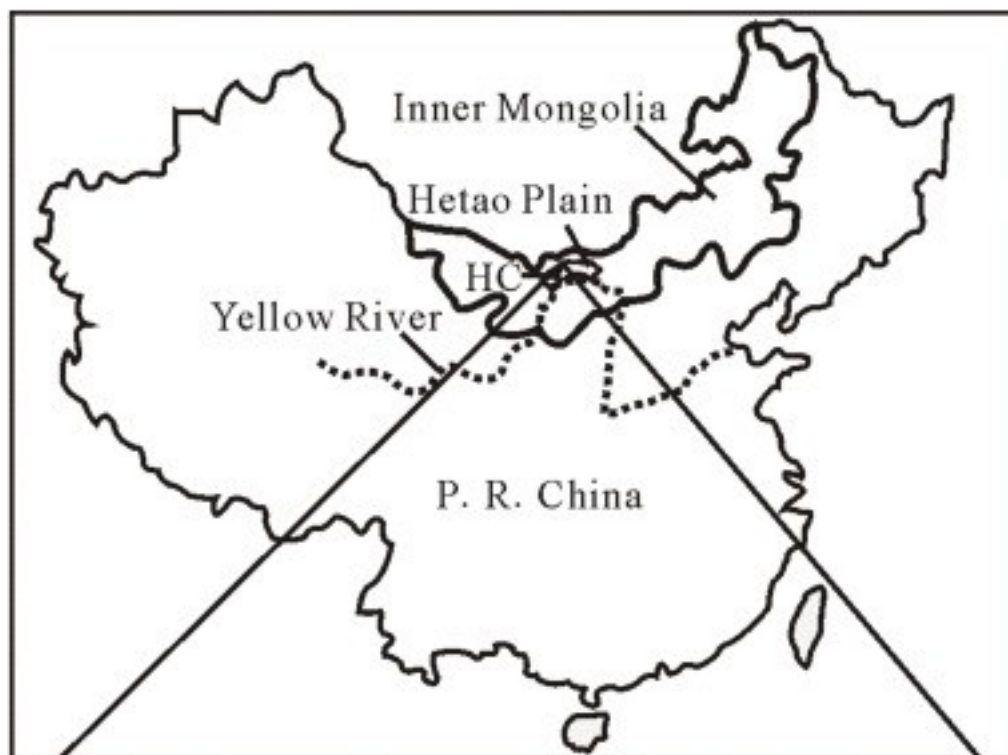
The primary objectives of this study were to: (1) investigate the [groundwater chemistry](#) with respect to As and other components, characterize the high As groundwater geochemistry comparing to the low arsenic samples, (2) examine functional diversity and structure of the *in situ* microbial communities, (3) assess the metabolic potential of microbial communities, and (4) determine the relationships between the functional microbial community and [environmental factors](#) in As-rich aquifers. To achieve these objectives, a coordinated geochemical and molecular survey

was conducted for 19 groundwater samples in Hangjinhouqi County, Inner Mongolia, China. The results of this study fill a much needed knowledge gap regarding the relationship between the functional microbial community and geochemical processes in natural As aquifers and expand the current understanding of microbial ecology in these aquifers.

2. Materials and methods

2.1. Site description

The study site is located in the western part of the Hetao Basin (the Great Bend of Yellow River) Inner Mongolia, China ([Fig. 1](#)) in an area that has been seriously affected by As poisoning ([Wei et al., 2016](#), [Wade et al., 2009](#)). The Basin was formed at the end of the [Jurassic Period](#) and contains fine [clastic sediments](#). Frequent channel changes deposited sediments and generated [oxbow lakes](#) replete with accumulated [humus](#) and organic mud. The targets of this study are shallow [aquifers](#) which are composed of late Pleistocene and [Holocene](#) alluvial and lacustrine deposits. Groundwater is recharged by lateral flowing groundwater from [bedrocks](#), vertical infiltrating [meteoric water](#), and/or by irrigation return flow and leakage from the Yellow River from the south. Discharge occurs mainly via [evapotranspiration](#) and pumping. These aquifers have been widely used for drinking water by local residents. Our case study was performed in Hangjinhouqi County (HC) in the western part of the Hetao Plain. Local residents have been drinking the As contaminated groundwater for over 30 years. Some of the residents have been affected with serious skin cancer which was caused by arseniasis ([Wei et al., 2016](#), [Wade et al., 2009](#)).



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Fig. 1. Geographic map of Hangjinhouqi County (HC), Inner Mongolia of China. Map showing location of study area (HC) and the sampling sites. Numbers 1–19 refer to groundwater samples S1-19.

2.2. Sample collection and field measurements

Nineteen groundwater samples were collected from tube wells with a depth range of 20–30 m in June 2013 from nine towns in Hangjinhouqi County ([Fig. 1](#)) and named S1-19 based on As concentration. The groundwater samples consisted of one from Manhui (sample S1), three from Shahai (sample S2, S5 and S19), one from Sizhi (sample S3), five from Sandaoqiao (sample S4, S6, S15, S17 and S18), one from Shanba (sample S7), four from Taiyangmiao (sample S8, S9, S10 and S12), one from Shuangmiao (sample S11), two from Erdaoqiao (sample S13 and S14), and one from Menghai (sample S16) ([Fig. 1](#)). Water samples were pumped out and then filtered or acidified. The tubing was flushed with copious amounts of the sampling groundwater before each use. Water pH, electronic conductivity (EC), [oxidation-reduction](#) potential (ORP), total dissolved solids (TDS) and [dissolved oxygen](#) (DO) were measured in the field at the site of [water collection](#) using a hand-held meter (Horiba W-23X D, Japan). Concentrations of [ammonium](#), [sulfide](#), ferrous iron (FeII) and total iron were also determined in the field with a Hach [spectrophotometer](#) (DR850, Hach Corp., USA) according to the manufacturer's instructions. Samples used for laboratory measurements, e.g., [cations](#), [anions](#) and [dissolved organic carbon](#) (DOC) were filtered through 0.22 μm mixed [cellulose ester](#) membranes and then the filtrates were collected into 50 mL acid-washed [polypropylene](#) bottles or brown glass bottles. Water samples for cations and DOC were acidified with 1% v/v HNO_3 . AsIII (arsenite) and AsV (arsenate) species were separated with an [anion exchange](#) cartridges (Supelco, USA) ([Li et al., 2013](#)). Microbial samples were collected by on-line filtering of 5–10 L water through 0.22- μm filters (Millipore). Filtered biomass-containing membranes were placed in 50 mL sterile tubes and immediately stored in dry ice. All microbial samples (biomass-containing membranes) were immediately frozen and stored on dry ice in the field and during transportation, and then kept at $-80\text{ }^\circ\text{C}$ in laboratory until further analyses.

2.3. Geochemistry measurements

Anion and As measurements were performed according to [Li et al. \(2013\)](#). Briefly, pre-separated AsIII and AsV were determined by ion chromatography-hydride generation

atomic fluorescence [spectrometry](#) (IC-HG-AFS-9700, Haiguang, China). Anions, including SO_4^{2-} , NO_3^- , NO_2^- and Cl^- were determined by [ion chromatography](#) (DX-120, Dionex, USA). Dissolved organic carbon (DOC), total carbon (TC) and total nitrogen (TN) of the samples were determined using a TOC analyzer (Vario MICRO cube, Elemental, Germany), respectively. Samples for CH_4 determination were collected in 100 mL brown serum bottles without filtration, which were fully filled with no headspace and sealed with gas impermeable butyl rubber [septum](#) stoppers, secured with a 20-mm aluminum seal. Two drops of saturated mercury chloride were added to the samples to retard [microbial activity](#). Samples were kept on blue ice during transportation and then kept in 4 °C in laboratory before analysis. The head space was created by purging with pure [helium](#) (99.999%) ([Johnson et al., 1990](#)). Soluble CH_4 concentrations were analyzed from the head space by [gas chromatography](#) (Thermo Trace Ultra) at the Third Institute of Oceanography, State Oceanic Administration. All samples were run in triplicate and then averaged.

2.4. DNA extraction and quantitation

In the laboratory, [DNA](#) extractions for all groundwater were conducted using FastDNA spin kits for soil (MP Bio, USA) according to the manufacturer's manual. DNA yield was quantified based on spectral [absorbance](#) at 260 nm, ratios of 260/280 nm and 260/230 nm, respectively (Thermo Scientific NanoDrop, 2000), and then stored at -80 °C until required for PCR amplification. The final DNA concentrations were quantified by PicoGreen, using a FLUO star Optima instrument (BMG Labtech, Jena, Germany).

2.5. GeoChip analysis

The new generation of functional gene array (GeoChip 5.0) was used to analyze the functional potential of microbial samples. The purified DNA (500 ng) was labeled with Cy 3 as described previously ([Sun et al., 2014](#)). Briefly, the labeled DNA was re-suspended in [hybridization](#) solution, and then hybridized in an Agilent hybridization oven at 67 °C for 24 h. After hybridization, the slides were washed with buffers to remove unbound DNA. The arrays were scanned with a NimbleGen MS200 Microarray Scanner (Roche NimbleGen, Inc., Madison, WI, USA). The images were extracted by the Agilent [Feature Extraction](#) program. Poor quality spots with a [signal-to-noise ratio](#) of less than 2.0 were removed before statistical analysis. The positive signals were normalized within each sample and across all samples.

2.6. Statistical analysis

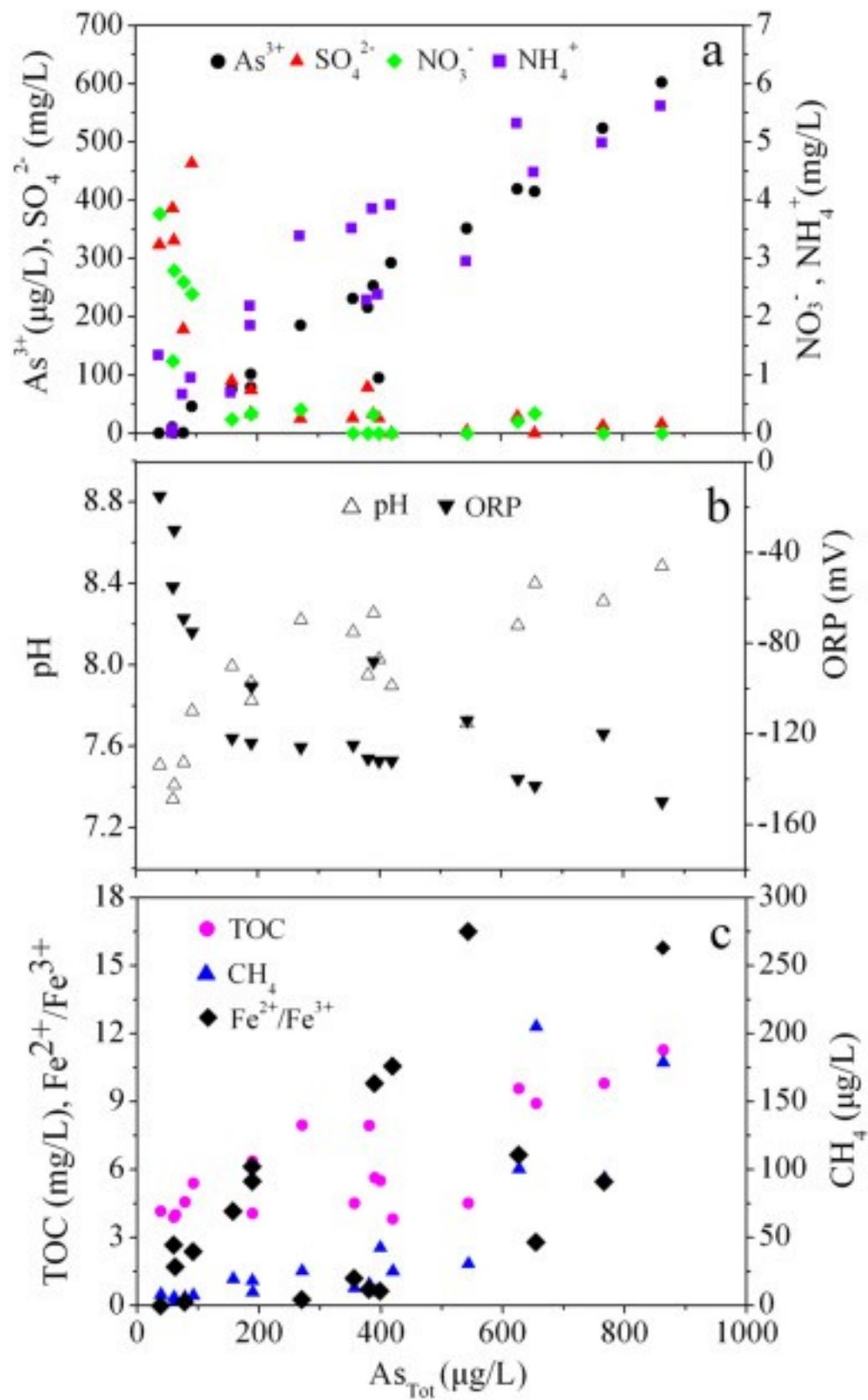
Pre-processed GeoChip data were further analyzed by the Vegan package in R 3.1.1 (<http://www.r-project.org/>) unless otherwise stated. Hierarchical clustering was performed with CLUSTER 3.0 using uncentered correlations and the complete average linkage for both genes and environmental variables, and trees were visualized in TREEVIEW ([de Hoon et al., 2004](#)). Functional gene diversity was calculated using Simpson's 1/D, Shannon-Weiner's H' and evenness. The effects of As on functional [microbial communities](#) were analyzed by response ratio (RR) using the formula described by [Zhang et al. \(2013\)](#). Based on the standard error, the 90% confident interval for each response variable was obtained and the statistical difference between the high As and low As groups was estimated. The total abundance of each gene category or family was simply the sum of the normalized intensity for the gene category or family. Three non-parametric multivariate analyses (analysis of similarities (ANOSIM), non-parametric [multivariate analysis](#) of variance (ADONIS) using distance matrices, and multi-response permutation procedure (MRPP)) were used to examine whether As has significant effects on microbial communities. Canonical [correspondence analysis](#) (CCA) and partial CCA for co-variation analysis (variation partitioning analysis, VPA) were performed to link microbial communities to environmental variables ([Low et al., 2016](#), [Zhou et al., 2008](#)). Selection for CCA modeling was conducted by an iterative procedure of eliminating redundant environmental variable based on variance inflation factor (VIF).

3. Results

3.1. Sample characteristics and chemical composition

The groundwater geochemical parameters were measured. Total [arsenic](#) (As_{Tot}) concentrations ranged from 38.93 to 863.42 $\mu\text{g/L}$ ([Table S1](#)), and were positively correlated with NH_4^+ , TOC, CH_4 and the ratio of Fe(II/III) ($r = 0.917$, $p < 0.001$; $r = 0.785$, $p < 0.001$; $r = 0.807$, $p < 0.001$; $r = 0.582$, $p < 0.01$, respectively) ([Fig. 2](#), [Table S2](#)). Negative correlations were observed between As_{Tot} and sulfate and nitrate concentrations, and ORP ($r = -0.699$, $p < 0.001$; $r = -0.669$, $p < 0.01$; $r = -0.734$, $p < 0.01$, respectively) ([Fig. 2](#), [Table S2](#)). Hierarchical clustering analyses separated the [geochemistry](#) of the 19 samples into two groups ([Fig. 3](#)). The first group contained five samples (S1-5), which were characterized by relatively low As (38.93–92.17 $\mu\text{g/L}$), relatively high oxidation [reduction](#) potential (ORP), and low TOC, FeII, and NH_4^+ . The remaining 14 samples were grouped together, and were characterized by relatively high As concentrations (157.71–863.42 $\mu\text{g/L}$). Most samples in this group were either neutral or slightly alkaline in pH. Some samples in the group had a strong [hydrogen](#)

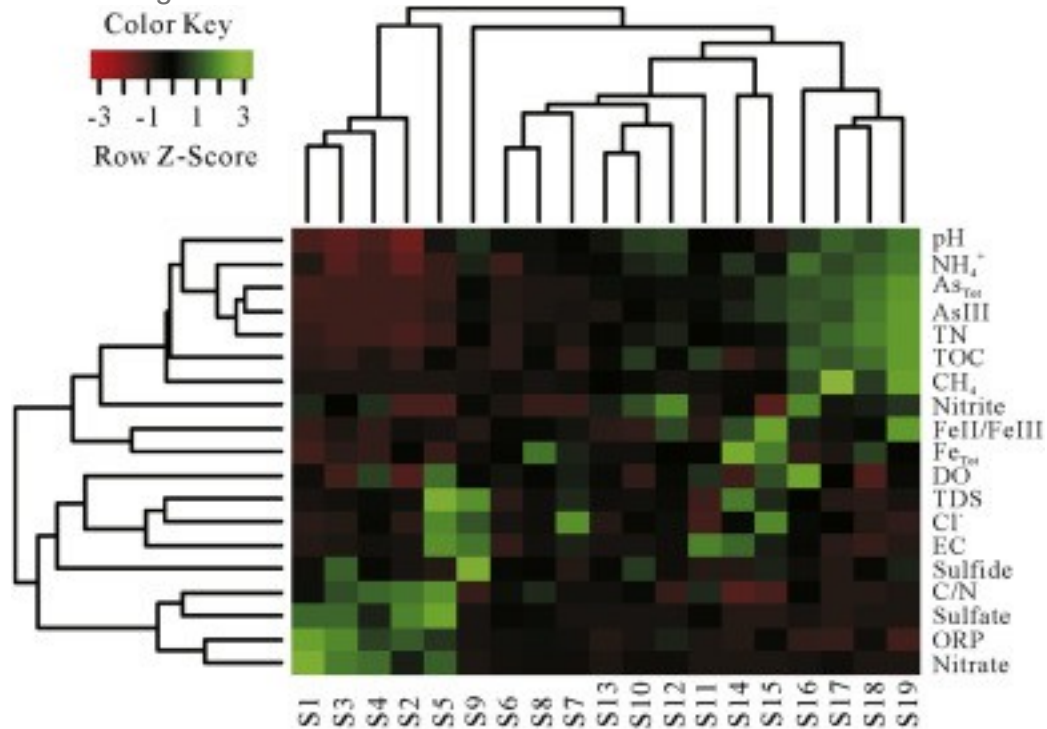
[sulfide](#) smell, consistent with their high [sulfide](#) concentrations and low ORP ([Table 1](#)). Samples with high As concentrations generally had low concentrations of sulfate, negative ORP, and relatively high concentrations of AsIII, FeII, CH₄, NH₄⁺ and TOC contents. These geochemical characteristics indicated that strong reducing conditions prevailed in the high As groundwater. The results of pairwise comparisons using ANOSIM (bray-cutis) also suggested that there was a significant difference in geochemistry between these two groups of samples ($r = 0.6526$, $p < 0.001$) ([Table 1](#)).



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Fig. 2. Variation of [arsenic](#) concentrations with (a) AsIII, SO₄²⁻, NO₃⁻ and NH₄⁺ (b) pH and ORP, (c) TOC, CH₄ and ratios of FeII to FeIII in the groundwater sample S1-19 from Inner Mongolia.



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Fig. 3. Hierarchical [cluster analysis](#) of geochemical parameters of sample S1-19 from Inner Mongolia. Results were generated in CLUSTER and visualized using TREEVIEW. Red indicates signal intensities below background, whereas blue indicates signal intensities above background. Brighter blue color indicates higher signal intensities. Sample S1-5 with lower [arsenic](#) (As) concentrations clustered together and were well separated from other samples S6-19 with higher As concentrations. EC: [electrical conductivity](#); DO: [dissolved oxygen](#), ORP: [oxidation reduction](#) potentials; TDS: total dissolved solids, As_{Tot}: total arsenic; AsIII: [arsenite](#); Fe_{Tot}: total dissolved iron, FeII: ferrous iron; TOC: [total organic carbon](#), C/N: ratio of total carbon to total nitrogen. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1. Significance test of the overall [microbial community](#) structures and geochemical patterns between different sample groups using three statistical analyses. Samples S1-5 with relatively low concentration of [arsenic](#) were grouped together and sample S6-19 with higher arsenic were included into another group.

Method	Geochemical parameters ^a		Microbial community	
	Statistic	P-value	Statistic	P-value
MRPP ^b	0.4708	0.002	0.0469	0.018
ANOSIM ^c	0.6526	0.001	0.4776	0.007
Adonis ^d	0.3852	0.001	0.1264	0.060

Abbreviations: ANOSIM, analysis of similarity; MRPP, multi response permutation procedure. All three tests are non-parametric multivariate analyses based on dissimilarities among samples.

a

Geochemical parameters included DO (dissolved oxygen), ORP (oxidation reduction potentials), As_{tot} (total arsenic), AsIII (arsenite), Fe_{tot} (total dissolved iron), FeII (ferrous iron), TOC (total organic carbon) and etc (see [Table S1](#)).

b

Multiple response permutation procedure.

c

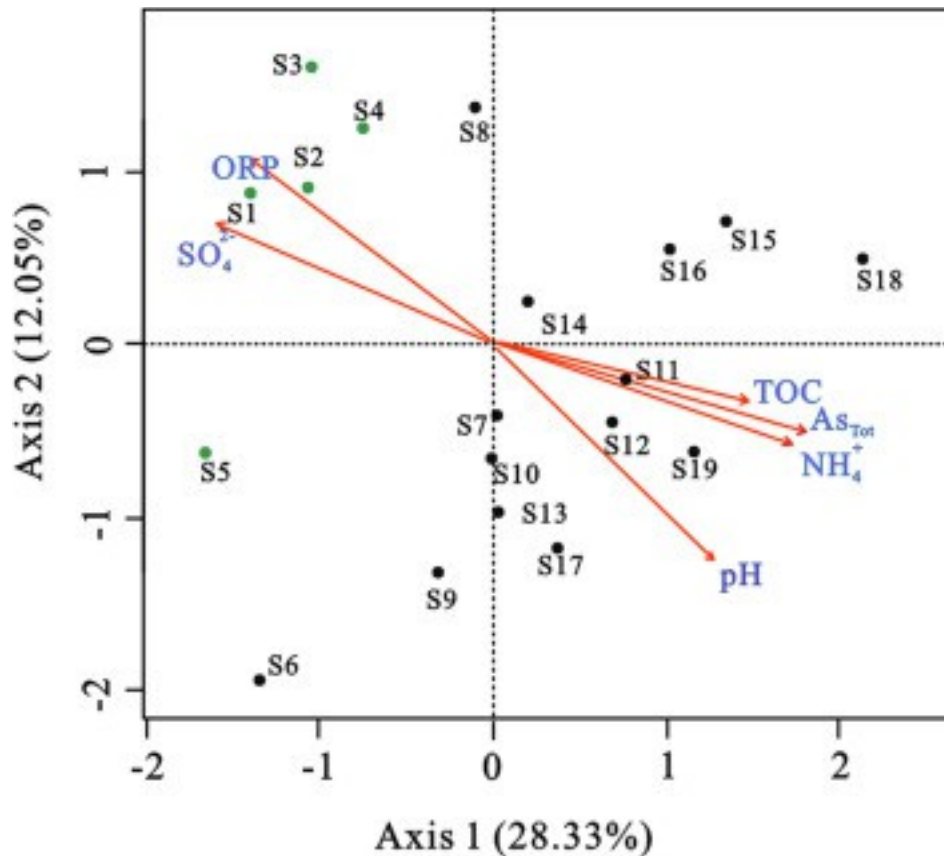
Analysis of similarities.

d

Non-parametric multivariate analysis of variance (MANOVA) with the adonis function.

3.2. Functional microbial community

The functional potential of the [microbial communities](#) was measured using a high-throughput functional gene array, GeoChip5.0. Significant differences were observed in functional microbial structure between low and high As groups ($r = 0.4776$, $p = 0.007$) ([Table 1](#)). Consistent with geochemical ordination patterns, CCA showed a differentiation in functional microbial structure between samples with different levels of As ([Fig. 4](#)). Samples were mainly divided into two groups which were characterized by relatively low and high As concentrations, respectively. The microbial functional structure of samples with low As clustered more tightly than those of the higher As samples ([Fig. 4](#)). Similarly, hierarchical clustering analysis showed similar clustering of microbial community functional structure ([Figure S1](#)).



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Fig. 4. Canonical [correspondence analysis](#) (CCA) analysis of GeoChip data (symbols) and environmental variables (arrows). Environmental variables were chosen based on significance calculated from individual CCA results and variance inflation factors (VIFs) calculated during CCA. The percentage of variation explained by each [axis](#) is shown, and the relationship is significant ($P = 0.021$).

The numbers of functional gene probes detected in these 19 groundwater samples were all in the magnitude of 10^5 ([Table S3](#)). The functional gene distribution among the samples was variable ([Figure S1](#)). For example, some genes involved in nitrogen cycling, sulfate reduction and [methanogenesis](#) from groups 9, 11 and 12 had a distinctly higher abundance in high As samples than samples with low As. Arsenic related genes were mainly distributed in group 8, 11 and 12. Three complimentary non-parametric multivariate statistical tests including MRPP (multi response permutation procedure), ANOSIM (analysis of similarity), and adonis further confirmed the significant differences of geochemical patterns and microbial communities between different sample groups (high and low As groups) ([Table 1](#)).

3.3. Overall functional microbial community structure in relation to geochemistry

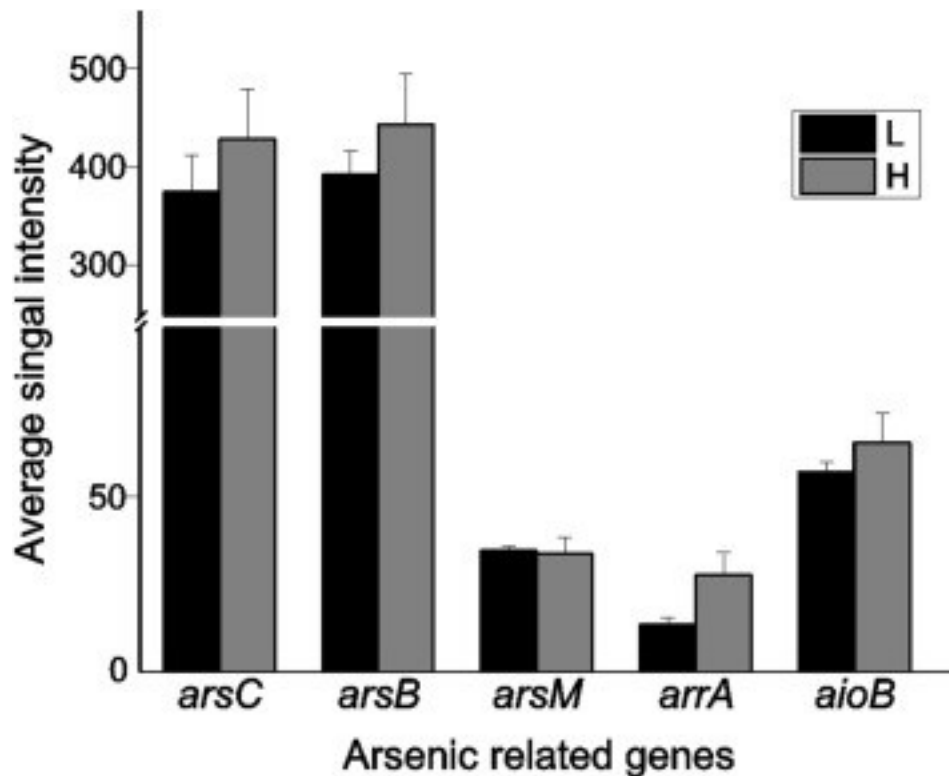
CCA ordination results showed that six variables, As_{Tot} , SO_4^{2-} , NH_4^+ , pH, ORP, and TOC were the most significant environmental variables shaping the microbial community structure ($p = 0.021$, [Fig. 4](#)). The length and direction of vectors represent the influence of a given geochemical vector to samples. For microbial community samples with high As, As_{Tot} , TOC and NH_4^+ were the most significantly correlated [environmental factors](#), while ORP and SO_4^{2-} were correlated with low As samples. To better understand the influence of the relative contributions from environmental variables to the microbial community structure, a CCA-based variation partitioning analysis was performed ([Figure S2](#)) ([Zhou et al., 2008](#)). Six environmental variables were divided into three group, including group 1 (SO_4^{2-} , ORP), group 2 (As_{Tot} , TOC and NH_4^+) and group three (pH). Both groups 1 and 2 showed significant correlation with the functional gene structure of the communities. Group 1 (SO_4^{2-} , ORP) explained 22.63% ($p = 0.010$), and group 2 (As_{Tot} , TOC and NH_4^+) explained 24.98% ($p = 0.010$), the pH environmental variable (group 3) independently explained 17.64% ($p = 0.005$) of the observed variation ([Figure S2](#)). About 58.80% of the community functional variation remained unexplained by the above selected variables.

3.4. Functional genes for arsenic cycling

Arsenic related genes including As-resistance (*arsC/arsB*), dissimilatory arsenate-reducing (*arrA*), As-oxidation (*aoxB*) and As [methylation](#) genes (*arsM*) were detected in these samples. Compared with the other As related genes, As-resistance *arsC/arsB* genes were distinctly more diverse. A total of 855 As-resistance *arsC/arsB* gene sequences derived from 418 bacterial genera were detected. Of these, 438 genes were shared among all the samples. *arsC* genes with percentages greater than 2% of the total intensity of *arsC* genes were from *Rhodanobacter*, *Rhodococcus*, *Stenotrophomonas*, *Aurantimonas*, [Nitrobacter](#), *Xanthobacter*, *Agrobacterium*, *Rhodococcus*, *Acinetobacter*, *Thioalkalivibrio*, and *Mycobacterium* ([Table S4](#)). *arsB* genes with percentages higher than 2% of the total intensity of *arsB* genes were from *Methylobacterium*, *Micromonospora*, *Ethanoligenens*, *Acidovorax*, *Methylobacterium*, and *Oceanimonas*. Thirty-nine dissimilatory arsenate-reducing *arrA* genes were detected mainly from *Azoarcus*, *Aromatoleum*, *Chlorobium*, *Thauera*, *Geobacter*, *Alkaliphilus*, *Desulfonatospira*, *Desulfitobacterium* and some uncultured bacteria ([Table S4](#)). Of these, 15 genes were shared among all the samples. Thirty-nine As methylation genes *arsM* were detected and 15 genes were shared among all the samples. A majority (81%) of the *arsM* probes were derived

from *Conexibacter*, *Desulfohalobium*, *Thiocapsa*, *Nitrosomonas*, *Cupriavidus*, *Leptonema*, *Halomonas* and *Pelotomaculum*. The 96 As-oxidization genes, *aoxB*, detected were mainly from *Vibrio*, *Acidiphilium*, *Halomonas*, *Variovorax* and *Burkholderia*. Of these, 50 were shared among all the samples. Multiple As-related genes from the same dissimilatory arsenate-reducing microorganisms were detected. For example, *arrA* and *arsCBM* derived from the genus *Geobacter* were detected in the same sample. Similarly, multiple genes derived from *Aromatoleum* (*arrA* and *arsC*), *Azoarcus* (*arrA* and *arsCB*), *Chlorobium* and *Thauera* (*arrA* and *arsB*), *Alkaliphilus* (*arrA* and *arsM*), and *Desulfitobacterium* (*arrA* and *arsB*) were also detected. The predominated As metabolic related populations were significantly different with those in high As groundwater of Cache Valley Basin Utah and Bangladesh ([Mirza et al., 2017](#), [Hassan et al., 2015](#)).

Most of the As related genes including the As [detoxification](#) genes *arsC* and *arsB*, and the respiratory [arsenate](#) (AsV) reductase gene *arrA* had higher [relative abundances](#) in the high As group than low As group ([Fig. 5](#)). Positive correlations were observed between As concentration and the number of detected As related genes *arsC* ($r = 0.462$, $p < 0.01$) and *arrA* ($r = 0.486$, $p < 0.01$). There was no correlation between *arsB*, AsIII S-adenosylmethionine methyltransferase gene *arsM*, [arsenite](#) (AsIII) [oxidase](#) gene *aoxB* and As concentrations. More *arsC/arsB* genes were detected than the other genes ([Fig. 5](#)), such as *arrA* (around 5–8 folds), indicating that the most common detoxification mechanism was reduction of AsV to AsIII rather than respiratory arsenate reduction in the high As groundwater [aquifers](#), which was consistent with results based on phylogenetic information detected with 454 ([Li et al., 2015](#)). Similar results were observed for [paddy soils](#) in a previous study ([Zhao et al., 2013](#)).



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Fig. 5. [Relative abundance](#) of the genes involved in [arsenic](#) metabolisms in the groundwater samples. The signal intensity for each gene was the average of the total signal intensity in each group. L: low arsenic group samples; H: high arsenic group samples.

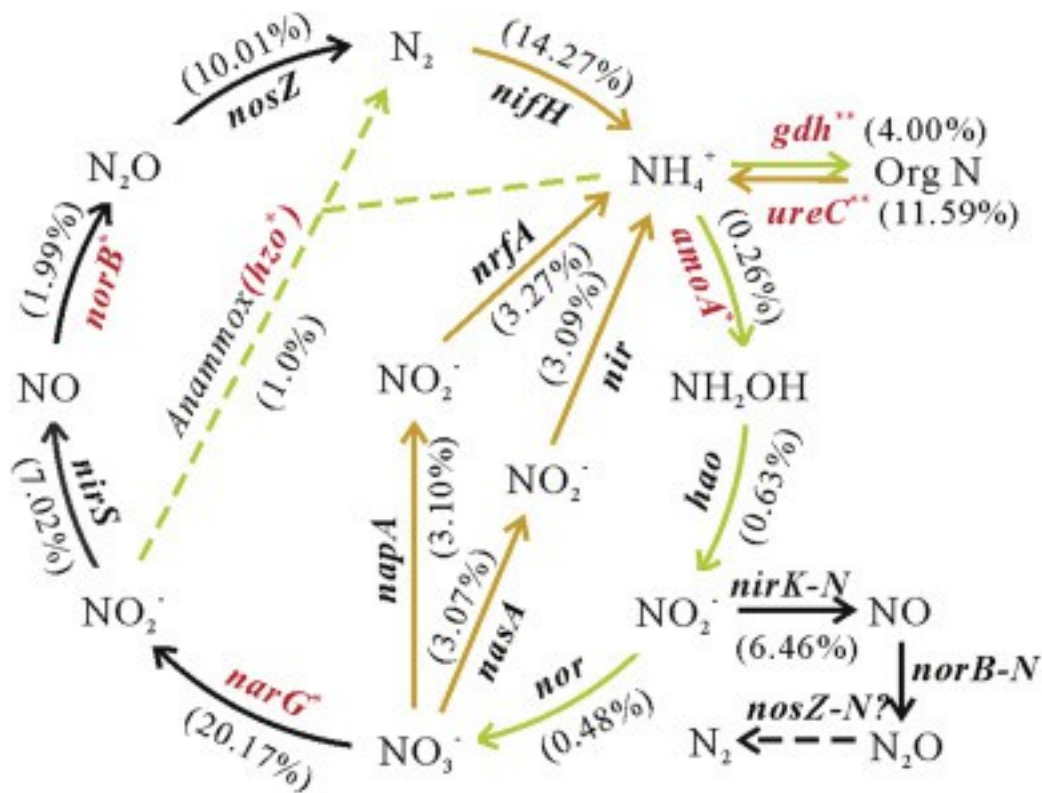
3.5. Functional genes for sulfur cycling

Of the 1211 [sulfur](#) related genes detected in this study, most were for [sulfur reduction](#) (the average gene intensity for the *dsrAB* probes was 53.48%, and was 25.33% for *soxY*). Microbial populations including *Desulfovibrio*, *Desulfobulbus*, *Desulfohalobium*, *Desulfotomaculum*, *Chlorobium*, *Clostridium*, *Geobacter*, *Magnetospirillum*, *Thiobacillus*, *Thioalkalivibrio*, *Syntrophobacter*, *Pyrobaculum*, and *Acetobacterium* were frequently detected in samples, while most of the detected *dsrAB* were from some uncultured [sulfate-reducing bacteria](#). Both As related genes and sulfate reductions genes were detected from *Desulfohalobium*, *Geobacter*, *Thioalkalivibrio*, and *Chlorobium*. The diversity of the sulfur reduction population in this study was much higher than that of high As groundwater in Bangladesh ([Gorra et al., 2012](#)). There were positive correlations between As and sulfur reduction genes (*dsrA* and As: $r = 0.455$, $p < 0.01$; *dsrB* and As:

$r = 0.327$, $p < 0.05$). These results were consistent with our previous studies using qPCR ([Li et al., 2014](#)).

3.6. Functional genes for the nitrogen cycling

Nitrogen (N) cycling, an important process in high As groundwater, is generally of concern for its roles of [microbial activity](#) ([Kurosawa et al., 2013](#), [Natarajan et al., 2009](#)). Previous studies found that microbial nitrate-dependent oxidation of FeII enhances the immobilization of As in the anoxic environments, ammonium-N could enhance microbial activity, and then improve As release in a reducing condition ([Weng et al., 2017](#), [Kurosawa et al., 2008](#)). There are many genes involved in N cycling, including those for [denitrification](#) (*narG*, *nirS*, *nirK*, *norB*, and *nosZ*), [nitrification](#) (*amoA* and *hao*), dissimilatory N reduction (*napA* and *nrfA*, *nasA* and *nir*), [ammonification](#) (*ureC*, *gdh*), anammox (*hzo*) and N fixation (*nifH*). The dissimilatory N reduction gene (*nasA*) was positively correlated with As ($r = 0.357$, $p < 0.01$). The *ureC* gene that transforms organic nitrogen to NH_4^+ was positively correlated with As and NH_4^+ ($r = 0.571$, $p < 0.01$; $r = 0.323$, $p < 0.01$, respectively). The NH_4^+ oxidization gene, *amoA*, was marginal negatively correlated with As ($r = -0.311$, $p < 0.05$), while the anammox gene, *hzo*, was positively correlated with As concentration ($r = 0.452$, $p < 0.05$), which implies that anaerobic bacterial oxidation might be a survival strategy oxidizing [ammonia](#) in high As groundwaters. The signal intensities were significantly different ($p < 0.05$) between the high and low As groups for the genes *ureC*, *gdh*, *amoA*, *hzo*, *narG* and *norB* ([Fig. 6](#)). The signal intensities of the other nitrogen related genes exhibited no significant correlation between As and nitrate or NH_4^+ and the intensities in high and low As groups were not significantly different ([Fig. 6](#)).



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Fig. 6. The relative change of detected nitrogen cycling genes from the high [arsenic](#) group samples. The signal intensity for each gene was normalized by the mean signal of all detected gene sequences in the high arsenic samples. Percentages indicate the normalized total intensity of the functional genes. Red color indicates the difference in gene abundance between high and low arsenic groups was significant (** $p < 0.01$, * $p < 0.05$). Orange arrows indicate [ammonium](#) source processes, green arrows indicate ammonium utilization processes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.7. Methanogenesis genes

The methanogenesis genes detected (248) were mainly from *Methanosaeta*, *Methanoculleus*, *Methanocella*, *Desulfatibacillum*, and *Moorella*. Among these genes, 59–65% were *mcrA*, encoding the α subunit of methyl [coenzyme](#) M reductase, and *hdrB*, encoding cytoplasmic heterodisulfide reductase. Most of the methanogenesis genes were more abundant in the high As group samples than in the low As group. The average abundance of methanogenesis genes was 1.37% in the low As group and 3.06% in the high arsenic group. Positive correlations were observed between methanogenesis genes and arsenic (As_{tot})

and [methane](#) concentrations (*mcrA*: $r = 0.549$, $p < 0.05$; $r = 0.314$, $p < 0.05$, and *hdrB*: $r = 0.387$, $p < 0.05$; $r = 0.389$, $p < 0.05$, respectively). These results indicated that methanogenesis might be an important metabolic process and accelerate As release and accumulation in high As aquifers, consistent with our previous study ([Wang et al., 2015](#)). The composition of [methanogens](#) in this study is totally different with high As groundwater in Bangladesh ([Gorra et al., 2012](#)). Fifty two methane oxidation genes were detected, mainly from *Methylosinus*, *Methylocapsa* and some uncultured bacteria (*pmoA*), as well as *Methylomonas*, *Citricella*, *Mesorhizobium* and *Methylocella* (*mmox*). There was no correlation between methane oxidation genes abundance and As concentrations.

3.8. Genes related to phosphorus, carbon degradation, organic remediation and metal resistance

There was no correlation between As concentration and the quantity of carbon degradation genes, although As was positive correlated with TOC contents ([Fig. 2](#)). A potential explanation is that dissolved organic matter, such as fulvic or [humic acids](#) could form stable complexes with [mineral surfaces](#), effectively blocking As from [adsorption](#) onto minerals, which would improve As keeping in the aqueous phase ([Natarajan et al., 2009](#)). Similarly, no obvious changes in relative abundance across the nineteen samples were detected between As and [phosphorus, organic](#) remediation genes, or metal resistance genes (data not shown), indicating that organic remediation and metal resistance mechanisms contributed little to the change observed in the functional microbial communities.

4. Discussion

In this study, a large number of genes with a tremendous diversity of sequences were detected in our samples. Such functional gene information is useful for assessing the impacts of microbially-mediated As [geochemistry](#). Our study highlights that the geochemistry is a strong driver of a [microbial community's](#) functional structure, with As being one of the key [environmental factors](#) contributing to the differences in the geochemistry and microbial community structure in groundwater. Groundwater samples could be divided into well-defined high and low As groups based on both geochemistry and the functional composition of the microbial communities. Similar results were found in our previous [phylogeny](#) based studies on microbial communities in this sites using high throughput and traditional [sequencing](#) technologies ([Wang et al., 2014a](#), [Wang et al., 2016](#), [Li et al., 2015](#), [Li et al., 2013](#)). This could be explained by As release and

mobilization. Our study provides evidence for possible links between functional potential of *in situ* microbial communities and As, S, N and C chemicals [reduction](#) which promoted As release in groundwater. The results are in agreement with previous studies that reported As enrichment was related to nitrogen, carbon, [sulfur](#) and iron geochemistry, all of which are usually considerable drivers of specific metabolic pathways in microbes, and the composition of associated microbial communities ([ThomasArrigo et al., 2016](#), [Wang et al., 2016](#), [Li et al., 2015](#), [Ghosh et al., 2015](#), [Wang et al., 2014a](#), [Wang et al., 2014b](#), [Li et al., 2013](#), [Nordstrom, 2002](#), [Kocar et al., 2010](#), [Xiong et al., 2010](#)). The current investigation supported the finding that alkaline and reducing conditions with relatively low ORP could be linked to natural As enrichment ([Rodríguez-Lado et al., 2013](#)). Our study indicated that alkaline pH was one of the most important environmental variables shaping the functional microbial community structure in high As samples ([Fig. 4](#) and [Figure S2](#)). The elevated pH might be associated with the rise of NH_4^+ and TOC in high As groundwater. The pH value of most of the high As samples in this study were slightly alkaline (7.77–8.48). Water chemical types were mostly $\text{Na}(-\text{Mg})-\text{Cl}(-\text{HCO}_3)$ and $\text{Na}-\text{Mg}-\text{HCO}_3$. Most groundwater samples in our study area are unsaturated with [gypsum](#) and close to saturation with respect to [calcite](#) and [dolomite](#) ([Deng et al., 2009](#)). The rise of NH_4^+ increased [microbial activities](#), which might in turn promote the biotic dissolution of calcite and [dolomite minerals](#), and then increases pH value in groundwater. Reducing conditions associated with As, sulfate and iron reduction, have long been considered the prevailing geochemical processes in As enrichment mechanisms in many high As groundwater [aquifers](#) and some other anoxic natural systems ([Wang et al., 2014a](#), [Wang et al., 2014b](#), [Ehlert et al., 2014](#), [Kocar et al., 2010](#), [Razzak et al., 2009](#), [Deng et al., 2009](#), [Stauder et al., 2005](#)). Reductive dissolution of Fe [oxide minerals](#) and the oxidation of [pyrite](#) and mineral [phase transition](#) in sediments were demonstrated to promote the release of bound As into the groundwater in our study area ([Zhang et al., 2017](#), [Guo et al., 2011](#), [Guo et al., 2016](#)). Additionally, As(III) reducing, sulfate reducing and [methanogenesis](#) were proposed to promote As mobilization in the study sites ([Dai et al., 2016](#), [Wang et al., 2015](#), [Li et al., 2014](#)). Our current study connected the reducing geochemical conditions with functional gene profiles including As, S, C and N related functional genes in the high As groundwater aquifers.

In this study our data implied that As, sulfate and iron reduction might occur in high As samples at the same sites ([Fig. 2](#)). In our previous study demonstrated that indigenous As/iron reducing bacteria from the high As aquifer could release As from sediments to

aqueous phase ([Dai et al., 2016](#)). Moreover, the positive correlation between *dsrAB* gene abundance and As concentration was found in our study. Some As and sulfate reduction genes from the same organism were also found, which was in agreement with previous findings that some microorganisms were capable of reducing both As and sulfate ([Macy et al., 2000](#), [Newman et al., 1997](#)). In high As groundwater aquifers, [arsenic](#), sulfur and iron often coexist and can be important for As transformation and mobilization ([Kocar et al., 2010](#), [Saalfield and Bostick, 2009](#)). The [homeostasis](#) of dissolved As may be controlled by biotic reduction of Fe-(hydr)oxides and FeIII minerals and the solubility of [sulfide](#) phases ([Wang et al., 2014a](#), [Wang et al., 2014b](#), [Xiong et al., 2010](#)). Biotic sulfate reduction could drive reductive dissolution of Fe-(hydr)oxides as well as As reduction, resulting in increased As release and mobilization when As is not incorporated into [iron sulfides](#) ([Li et al., 2014](#)).

[Ammonification](#) is an important biological process providing N in high As groundwater by transforming organic nitrogen to [inorganic nitrogen](#). In high As groundwater aquifers, $\text{NH}_4^+\text{-N}$ was found in high concentrations. The gene *ureC* was in relatively high abundance in high As groundwater samples in this study ([Fig. 6](#)). Although there is no direct evidence of $\text{NH}_4^+\text{-N}$ in groundwater from agriculture in our study sites, previous studies indicated that the $\text{NH}_4^+\text{-N}$ (mainly urea) in some other high As groundwater aquifers is from fertilizer in agriculture practices ([Weng et al., 2017](#), [Uddin and Kurosawa, 2014](#), [Mayorga et al., 2013](#), [Itai et al., 2008](#)). Our current evidence of NH_4^+ accumulation in high As groundwater might be due to the higher abundance of genes such as *ureC* that transform organic nitrogen to NH_4^+ and the lower abundance of NH_4^+ utilization genes (*hzo* and *gdh*) ([Fig. 6](#)). The rise in ammonium-N concentration, as well as elevated TOC, might enhance microbial activities, which in turn would lower ORP, and then enhance As reduction, microbial reduction of Fe(III) oxides/hydroxides, and sulfate reduction which improved As mobilization through dissolution of Fe(III) oxide minerals or formation of As-S compounds in high HS^- concentration water ([Kurosawa et al., 2008](#), [Deng et al., 2009](#), [Wang et al., 2014a](#), [Wang et al., 2014b](#)). This might explain why NH_4^+ was significantly correlated with As concentrations in groundwater aquifers. In addition, organic matter from agricultural irrigation was reported to release into groundwater in this study area ([Gao et al., 2014](#)). Previous studies proposed high concentrations of TOC might be in favor of microbial mediated As enrichment ([Uddin and Kurosawa, 2014](#), [Mayorga et al., 2013](#), [Barringer et al., 2010](#), [Reza et al., 2010](#)). These results suggested again that human agriculture activities might be one of the

most important factors of improving microbial activities, causing As contamination in groundwater ([Gao et al., 2014](#), [Neidhardt et al., 2012](#)).

It is worth noting that genes specific for anammox were detected, indicating that [ammonium](#) and [nitrite](#) can be converted directly into nitrogen gas via anammox pathways in high As groundwater. Anammox can happen without requiring substantial amounts of organic-C, reduced iron, or mineral phase electron donors.

Groundwater [hydrology](#) could create expanded zones suitable for anammox communities and widespread [spatial distribution](#) of anammox activity ([Smith et al., 2015](#)). Arsenic concentrations were positively correlated with anammox gene numbers, suggesting anammox was a survival strategy oxidizing [ammonia](#) in high As groundwaters. However, the importance of anammox in groundwater aquifers had not been considered previously. Further [tracer studies](#) of the anammox pathway in the groundwater might help elucidate nitrogen cycling in this ecosystem.

5. Conclusion

Our results reveal that environmental variables account for the majority of variation in microbial functional potential. This study provides evidence for possible links between As, S, N and C related microbial functional gene abundances and As [geochemistry](#). Alkaline and reducing conditions as well as SO_4^{2-} and Fe [reduction](#) and [ammonification](#) associated with microbially-mediated geochemical processes could be linked to As enrichment in groundwater in this study area. These results suggest that the indigenous [microbial communities](#) could have a significant role in As release and transformation in high As groundwater [aquifers](#) of the Hetao Basin, Inner Mongolia. This study expands our current understanding of [microbial ecology](#) in high As aquifers.

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Appendix A. Supplementary data

The following is the supplementary data related to this article:

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