Geographic distance and amorphous iron affect the abundance and distribution of *Geobacteraceae* in paddy soils in China

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Abstract

Purpose *Geobacteraceae* are important dissimilatory Fe (III)-reducing microorganisms, influencing the cycling of metals, nutrients as well as the degradation of organic contaminants. However, little is known about their distribution, diversity, and abundance of *Geobacteraceae* and the effects of environment factors and geographic distance on the distribution and diversity of *Geobacteraceae* in paddy soils remain unclear. Therefore, the objectives of this study were to investigate the distribution, diversity, and abundance of *Geobacteraceae* in paddy soils and to determine key factors in shaping the *Geobacteraceae* distribution, environmental factors,

geographic distance, or both and to quantify their contribution to *Geobacteraceae* variation.

Materials and methods Illumina sequencing and quantitative real-time PCR using a primer set targeting 16S rRNA genes of bacteria affiliated with the family *Geobacteraceae* were employed to measure the community composition, diversity, and abundance patterns of 16S rRNA genes of *Geobacteraceae* in 16 samples collected from north to south of China. MRT, Mantel test, and VPA were used to analyze the relationship between communities of *Geobacteraceae* and environmental factors and geographic distance.

Results and discussion Quantitative PCR showed that the abundance of 16S rRNA genes of *Geobacteraceae* ranged from $(1.20 \pm 0.18) \times 10^8$ to $1.13 \times 10^9 \pm 2.25 \times 10^8$ copies per gram of soil (dry weight) across different types of soils. Illumina sequencing results showed *Geobacter* was the dominant genus within the family of *Geobacteraceae*. Multivariate regression tree (MRT) analysis showed that soil amorphous iron contributed more (22.46 %) to the variation of dominant species of *Geobacteraceae* than other examined soil chemical factors such as pH (14.52 %), ammonium (5.12 %), and dissolved organic carbon (4.74 %). Additionally, more geographically distant sites harbored less similar communities. Variance partitioning analysis (VPA) showed that geographic distance contributed more to the variation of *Geobacteraceae* than any other factor, although the environmental factors explained more variation when combined. So, we detected the uneven distribution of *Geobacteraceae* in paddy soils of China and demonstrated that *Geobacteraceae* community composition was strongly associated with geographic distance and soil chemical factors including aFe, pH, Fe, DOC, C:N, and NO_3^- -N. These results greatly expand the knowledge of the distribution of *Geobacteraceae* in environments, particularly in terrestrial ecosystems.

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Conclusions Our results showed that geographic distance and amorphous iron played important roles in shaping *Geobacteraceae* community composition and revealed that both geographic distance and soil properties governed *Geobacteraceae* biogeography in paddy soils. Our findings will be critical in facilitating the prediction of element cycling by incorporating information on functional microbial communities into current biogeochemical models.

Keywords Amorphous iron (aFe) · *Geobacteraceae* · Illumina HiSeq sequencing · Iron reduction · Paddy soil

1 Introduction

Biogeochemical redox processes of iron (Fe) are important in controlling the fate and behavior of contaminants and nutrients in the environment (Borch et al. 2009). Fe is the most abundant redox-active element on the Earth, and the vast majority is distributed as iron minerals in natural environments (Davison 1993; Stumm and Sulzberger 1992). Ferric iron (Fe (III)) and ferrous iron (Fe (II)) are the main redox states in the environments, and the transition between Fe (III) and Fe (II) is of particular interest in dynamic environments (Mejia et al. 2016). The microbially mediated redox cycling of Fe plays an important role in environmental biogeochemistry (Lalonde et al. 2012; Melton et al. 2014).

Paddy soil represents an intermediate between terrestrial and aquatic ecosystems and is usually employed as a model to study wetland biogeochemistry (Wang et al. 2015). Paddy soils experience regular and specific management practices such as submergence and drainage. The alternation between oxic and anoxic conditions results in periodically occurring redox reactions. Due to the unique characteristics of paddy soils and the great abundance of iron, dissimilatory Fe (III) reduction is prevalent and thought to be central to many other biogeochemical processes in paddy soils (Hori et al. 2015). It has been established that dissimilatory Fe (III) reduction strongly influences carbon (C), nitrogen (N), and sulfur (S) cycling and indirectly affects nutrient availability, greenhouse gas emissions, and contaminant transformation (Burgin et al. 2011; Coates et al. 1996; Hori et al. 2010; Yi et al. 2013).

Geobacteraceae represents a rare example of a family of microorganisms that play an important role in Fe redox processes in a diverse environment (Holmes et al. 2002). Most of the known dissimilatory Fe (III)-reducing microbes are in the family *Geobacteraceae*, which permits *Geobacteraceae* species to fill key niches in various anaerobic environments (Holmes et al. 2002; Snoeyenbos-West et al. 2000). However, previous studies on *Geobacteraceae* in paddy soils are mainly conducted in batch culture, in which microbes grow rapidly with high substrate concentrations (Yi et al. 2013). These conditions cannot reflect the natural conditions

well, where microbes usually grow slowly due to nutrient limitation (Pierra et al. 2015). Meanwhile, those studies were conducted within an individual site, and it remains unclear how the distribution of *Geobacteraceae* is affected by geographic distance under field conditions. So far, our knowledge about the distribution and diversity of *Geobacteraceae* in paddy soils across a wide range of scales is limited, and the effects of geographic distance and environment variables on the distribution and diversity of *Geobacteraceae* in paddy soils remain unclear.

High-throughput sequencing technologies have opened new frontiers in microbial community analysis by providing a cost-effective means for identifying microbial phylotypes which are present in samples. Illumina HiSeq sequencing produces higher quality tags, greater throughput, and more user-friendly process compared with 454 pyrosequencing (Caporaso et al. 2010, 2012). Therefore, in our study, the distribution and diversity of *Geobacteraceae* in paddy soils under field conditions within a rather broad range spanning over 3500 km from north to south of China were examined by 16S rRNA gene-based Illumina HiSeq sequencing, and also the abundances of targeted microbes were analyzed by real-time PCR. The primary objectives of the present study were (i) to investigate the distribution, diversity, and abundance of *Geobacteraceae* in these soils and (ii) to determine key environment factors in shaping the *Geobacteraceae* distribution and quantify their contribution to *Geobacteraceae* variation.

2 Materials and methods

2.1 Soil samples

Soil samples were collected from various paddy soils at 16 different locations in China (20° 92' N to 47° 34' N and 105° 27' E to 132° 45' E) in October 2014 (Table S1, Electronic Supplementary Material). The study area and sampling sites are presented in Fig. S1 (Electronic Supplementary Material). In each location, three soil cores (5-cm diameter and 10-cm depth) were collected and mixed to form one composite sample. Soil samples were placed in sterile plastic bags, sealed, and transported to the laboratory on ice. Upon arrival at the laboratory, each soil sample was divided into two subsamples after homogenization. One subsample was stored at 4 °C in sealed containers for about 1 to 2 weeks before the soil physicochemical analysis, and the other was frozen at −80 °C for the molecular analysis.

2.2 Chemical analysis of soil samples

Nitrate and ammonium were extracted with 2 M KCl and determined by a continuous flow analyzer (SAN++, Skalar, Holland). Soil pH was measured in a 1:2.5 soil:water (w/v)

suspension with a digital pH meter (PHS-3C, Shanghai Lida Instrument Company, China). The C:N ratio was calculated based on the total C and total N contents that were measured on an element analyzer (Vario EL III, Elementar, Germany). The dissolved organic carbon (DOC) concentration was determined using total organic carbon (TOC) analyzer (Liquic TOC, Elementar, Germany). Total Fe and Mn contents were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES, Optimal 2000 DV, Perkin-Elmer, USA). Amorphous iron (aFe) was extracted with oxalic acid described by Chao et al. (Chao and Zhou 1983). Soil electrical conductivity (EC) was determined by a portable conductivity meter (MP521, China).

2.3 Genomic DNA extraction, PCR amplification, and community analysis

Total DNA in the soil samples was extracted using the FastDNA SPIN kit for soils (MP Biomedicals, USA). The extracted DNA was visualized on a 1 % agarose gel using ethidium bromide staining. DNA quality and quantity was assessed using a NanoDrop ND-2000 spectrophotometer (NanoDrop Co., USA).

PCR was conducted in triplicate to detect the *Geobacteraceae* 16S rRNA genes, using the primers Geo494F (5'-barcode AGG AAG CAC CGG CTA ACT CC-3') and Geo825R (5'-TAC CCG CRA CAC CTA GT-3') with sample-identifying barcodes (Holmes et al. 2002). PCR amplifications were performed in 50 μ l reaction mixtures containing ~40 ng of template DNA, 25 μ l Dream Taq Green PCR master mix (2 $\times$), 20.5 μ l H₂O, 0.5 μ l 1 % bovine serum albumin (BSA), and 0.2 μ M each primer. The thermal conditions of PCR were as follows: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, and extension at 72 °C for 30 s and the final extension step at 72 °C for 7 min. The amplicons were pooled from the triplicate reactions, purified using the universal DNA purification kit (Tiangen, China), and normalized in equimolar amounts. Finally, the PCR amplicons were submitted to Novogene Bioinformatics Institute (Beijing, China) for sequencing on the Illumina HiSeq 2500 platform.

Raw reads with contaminated adapters, or an undetermined nucleotide (N), or one base occurring more than 10 times successively were removed. Low-quality reads (containing >20 low-quality bases) were further filtered from the data, and then primer regions were removed. The above processes were conducted by Novogene Bioinformatics Institute in a pre-bioinformatics analysis. The resulted sequences were subsequently analyzed using Quantitative Insights Into Microbial Ecology (QIIME) (version 1.6) according to a previous study (Caporaso et al. 2010). Operational taxonomic units (OTUs) were picked at 97 % sequences similarity level on the basis of

an open reference by searching reads against the Greengenes database. Representative sequence for each OTU was then chosen for subsequent alignment and taxonomic assignment with the RDP classifier. Chimeric sequences, chloroplast and mitochondrial OTUs (around 1 %), and singleton OTUs were discarded from the final OTU table. OTUs with the same taxonomy were combined at different taxonomic levels. Both α -diversity (including Shannon, Simpson, and Phylogenetic diversity (PD)) and β -diversity calculations were performed based on the OTU table with a random sampling depth of the minimal sequencing number (11919) among samples. For β -diversity analysis, dissimilarity of *Geobacteraceae* was calculated using principal-coordinate analysis (PCoA) on pairwise, weighted UniFrac distances among all samples. Additionally, PCoA based on pairwise Bray-Curtis dissimilarity was performed using QIIME.

2.4 Quantitative real-time PCR for 16S rRNA gene sequences of *Geobacteraceae* and bacteria

The abundance of *Geobacteraceae* bacteria in the paddy soils was evaluated by quantitative PCR (qPCR) assay using a primer set targeting 16S rRNA genes of bacteria affiliated with the family *Geobacteraceae*: Geo494F and Geo825R (Holmes et al. 2002). qPCR was performed on an iQTM5 Thermocycler (Bio-Rad, USA) in 25 μ l-reaction mixtures containing 12.5 μ l SYBR Premix Ex TaqTM (Takara Bio Inc., Japan), 1 μ l DNA template, and 1 μ l of each 10 μ M primers. As thermal conditions, after an initial denaturation at 94 °C for 5 min, targeted DNA was amplified in 35 cycles; each cycle consisted of denaturation for 30 s at 94 °C, annealing for 30 s at 51 °C, and extension for 40 s at 72 °C. Fluorescence was collected at the end of the extension step. Following PCR, amplicons were assessed by a melting-curve analysis to check for successful amplification.

The standard template DNA was prepared using the primer pair Geo494F/Geo825R to amplify 16S rRNA genes of *Geobacteraceae*. The PCR amplicons were ligated to pGEM-T Easy vector (Promega, USA) and transformed into *Escherichia coli* JM109 cells. The positive clones with the targeted gene insert were sequenced, and the most abundant one was used for plasmid DNA extraction. After concentration measurement with Nanodrop ND-2000 UV-vis spectrophotometer (NanoDrop Co., USA), the purified plasmid DNA was serially tenfold diluted and subjected to qPCR in triplicate to generate an external standard curve. The abundance of 16S rRNA genes was estimated using a TaqMan assay with the primer set 1369 F/1492R and a probe 1389 F as described previously (Zhang et al. 2015). Target gene copy numbers were calculated from the standard curves and presented per gram of dry weight of soil. Moreover, the relative abundance of *Geobacteraceae* DNA to the total extracted DNA was also determined.

2.5 Statistical analysis

All statistical analyses were conducted using QIIME (<http://qiime.sourceforge.net/>), R (<http://www.r-project.org/>) and SPSS (SPSS statistics 17.0). Nonmetric multidimensional scaling (NMDS) was performed to cluster the different sites by their soil physicochemical similarities. Spearman correlation coefficient was used to test the relationships between soil physicochemical parameters and the abundance and diversity indices of *Geobacteraceae*. Multivariate regression tree (MRT) was used to illustrate the community dissimilarities among different paddy soil samples. MRT is a constrained classification approach which is well suited for ecological data sets and has been successfully used for exploring key environmental variables that influence microbial communities (Ge et al. 2012). Heatmap was generated using the software of HemI (HemI 1.0, Heatmap Illustrator). The OTUs with relative abundances >1 % (the percentage of each OTU to total OTUs) were normalized and used to generate heatmap. The pairwise geographic distances between different paddy soil sites were calculated based on geographic coordinates. The relationships between the similarity of community (taxonomic similarity and phylogenetic similarity) and geographical distance were measured by linear models. The taxonomic community similarity was based on the Bray-Curtis distance and the phylogenetic community similarity was based on weighted UniFrac distance (Rodrigues et al. 2013). Partial Mantel test was performed to analyze the relationships between communities of *Geobacteraceae* and soil variables or spatial patterns, using Euclidean distance. BioEnv and canonical correspondence analysis (CCA) were also used to identify the abiotic factors most important to the composition of *Geobacteraceae*, and they were used to construct the soil property matrix for variance partitioning analysis (VPA).

3 Results

3.1 Physicochemical properties of the soil samples

Physicochemical properties of paddy soils are listed in Table 1. Soil characteristics varied considerably with sampling sites. Concentration of aFe ranged from 2.16 to 19.86 g kg⁻¹. Concentrations of total Fe and Mn ranged from 17.04 to 41.43 g kg⁻¹ and 0.16 to 0.97 g kg⁻¹, respectively. Concentrations of dissolved organic carbon (DOC), ammonium-N (NH₄⁺-N), and nitrate-N (NO₃⁻-N) were highly variable across the samples, averaging at 232.62±93.90, 12.01±3.51, 17.37±6.64 mg kg⁻¹, respectively. Soil pH (5.58±1.13) and C:N ratio (11.10±1.85) were also subjected to considerable fluctuations. Concentrations of EC ranged from 360 to 2527 µs cm⁻¹. Additionally, NMDS analysis also

confirmed the high heterogeneity among the paddy soils (Fig. S2, Electronic Supplementary Material).

3.2 Abundance of *Geobacteraceae*

The abundance of *Geobacteraceae* was estimated on the basis of quantification of 16S rRNA genes of the *Geobacteraceae* using qPCR. The copy numbers of *Geobacteraceae* in soil samples ranged from $1.20 \times 10^8 \pm 1.85 \times 10^7$ to $1.13 \times 10^9 \pm 2.25 \times 10^8$ copies per gram of dry weight (Fig. 1 and Fig. S3, Electronic Supplementary Material). The relative abundance of *Geobacteraceae* in paddy soil samples ranged from 0.27 to 9.90 % (Fig. 1 and Fig. S4, Electronic Supplementary Material). Spearman correlation analysis showed that the abundance of *Geobacteraceae* was significantly correlated with Fe and aFe (Table S2, Electronic Supplementary Material). Moreover, spearman correlation analysis indicated that the relative abundance of *Geobacteraceae* was significantly correlated with the DOC, NH₄⁺-N, latitude, longitude, Fe, and aFe (Table S3, Electronic Supplementary Material).

3.3 Community composition and diversity of *Geobacteraceae*

A total of 572,112 high-quality sequences of *Geobacteraceae* were obtained, and all of them were assigned to 2908 operational taxonomic units (OTUs) at a 97 % similarity level. The community structure of *Geobacteraceae* was dominated by the genus *Geobacter* (more than 99 % of the total sequences). Additionally, 0.19 % sequences belonged to the genus *Geopsychrobacter* and 0.60 % to the genus *Geothermobacter*. The diversity of the *Geobacteraceae* was highly variable across the 16 samples, with higher diversity in JSJ, SY, MY, JS, JX, JL, and AX (Simpson index ranged from 100.86 to 113.81) and the lowest diversity in DH (Table S4, Electronic Supplementary Material). Spearman correlation analysis showed that the Shannon and Simpson estimators did not significantly correlate with any examined environmental factors, while PD was significantly related with DOC, NH₄⁺-N, and NO₃⁻-N (Table S5, Electronic Supplementary Material).

3.4 Relationships of community composition of *Geobacteraceae* to soil chemical properties and geographical distance

We used MRT to identify the relative importance of soil variables contributing to the distribution of main species of *Geobacteraceae* in different paddy samples. The main species were composed of OTUs detected in every sample, making up 89 to 92 % of the total reads in these soil samples (Table S6, Electronic Supplementary Material). MRT analysis showed a

Table 1 Physicochemical properties of the 16 paddy soils

Sample	pH	mg kg ⁻¹ dry paddy soil			g kg ⁻¹ dry paddy soil				μs cm ⁻¹
		DOC	NH ₄ ⁺ -N	NO ₃ ⁻ -N	Fe	aFe	Mn	C:N	
HL	6.58±0.58	210.56±6.20	20.42±3.38	8.81±0.38	30.68±2.49	9.65±0.28	0.93±0.37	12.48±0.29	594±82
JSJ	5.89±0.24	181.27±6.76	26.11±1.34	14.11±0.66	23.07±1.21	16.71±1.69	0.97±0.22	10.78±0.12	452±99
BC	8.06±0.15	113.49±5.39	17.26±4.54	12.05±0.78	21.69±1.88	2.17±0.27	0.36±0.04	10.67±0.79	1406±39
WC	6.29±0.64	140.52±4.09	21.77±2.34	9.94±0.68	29.40±2.50	14.69±0.11	0.66±0.05	11.93±0.12	494±75
DH	7.85±0.34	130.64±6.02	16.01±5.06	12.62±0.97	32.74±1.88	12.46±0.47	0.79±0.21	11.69±0.06	2391±412
SY	6.67±0.62	104.85±8.11	9.84±1.91	8.81±0.34	28.82±2.91	5.88±0.32	0.46±0.10	11.14±0.03	821±252
MY	7.92±0.04	354.35±11.52	10.05±0.85	12.55±0.05	20.63±0.98	4.48±0.13	0.45±0.02	14.89±0.09	2527±49
JS	5.72±0.30	224.13±6.02	6.73±0.42	20.58±0.46	32.35±0.12	7.62±0.16	0.40±0.01	10.52±0.06	623±31
JX	6.27±0.22	211.20±5.18	26.22±5.82	9.39±0.54	35.71±0.42	13.6±0.16	0.66±0.04	8.57±0.07	629±148
AH	5.22±0.03	251.34±7.54	16.70±4.83	9.08±0.69	41.43±1.30	16.89±0.85	0.56±0.01	9.48±0.07	360±63
JL	7.93±0.17	248.15±6.20	12.74±4.54	17.88±3.90	29.47±2.08	7.63±0.03	0.56±0.05	15.32±0.03	1725±559
AX	7.93±0.02	250.60±11.84	10.94±2.15	14.59±0.25	40.02±0.48	12.03±0.8	0.86±0.00	11.19±0.01	2036±186
TY	5.02±0.05	278.16±4.98	19.30±4.12	9.93±0.57	20.33±0.62	2.61±0.09	0.21±0.02	9.01±0.10	615±86
YT	5.22±0.07	484.85±22.13	22.84±4.94	9.76±0.52	17.04±0.45	2.64±0.02	0.16±0.01	10.21±0.95	553±46
BJ	7.40±0.85	308.53±6.88	15.83±1.97	13.29±2.01	36.79±9.32	19.86±1.38	0.70±0.32	10.16±0.03	1723±552
LZ	5.32±0.04	229.30±5.30	25.11±2.99	8.79±0.14	23.70±0.63	16.22±0.7	0.18±0.00	9.48±0.04	419±26

nested structure, with communities splitting first by aFe, then by pH, aFe (on the left split), NH₄⁺-N, and DOC (Fig. 2). aFe explained 22.46 % of the variation in *Geobacteraceae* community composition. pH explained 14.52 % and aFe (on the left split) explained 5.58 %. Then, NH₄⁺-N and DOC explained 5.12 and 4.74 %, respectively. Heatmap showed the dominant species of *Geobacteraceae* in these paddy soils (Table S6, Electronic Supplementary Material). Patterns of

each OTU in six splits were different, which illustrated the effect of aFe, pH, NH₄⁺-N, and DOC on the distribution of the main species of *Geobacteraceae*. Together, these results suggested that aFe was an important indicator for the distribution of the dominant species of *Geobacteraceae* in paddy soils of China.

The partial Mantel test revealed a significant correlation between the *Geobacteraceae* communities and the

Fig. 1 Abundance and relative abundance of *Geobacteraceae* in paddy soils in China. The base map used is from the National Fundamental Geographic Information System of China. The red and green columns represent estimated abundance and relative abundance of *Geobacteraceae*, respectively

Fig. 2 Multivariate regression tree (MRT) to reveal the hierarchical environmental determinants on communities of *Geobacteraceae* with amorphous iron (aFe), pH, amorphous iron (aFe), $\text{NH}_4\text{-N}$, and DOC. In an MRT, each split is represented graphically as a branch that is labeled with the levels of the classification variable; *bar plots* show the multivariate means of OTUs at each branch; the *numbers* of samples included in the splits are shown under the bar plot. The heatmap shows the normalized abundances of OTUs (the OTUs higher than 1 % percentage) that occurred in 48 samples

environmental factors including Fe, aFe, Mn, latitude, and longitude (Table 2). Due to the significant correlation between *Geobacteraceae* communities and latitude or longitude, we linked the communities of *Geobacteraceae* with geographic distance. The relationships were calculated by determining the slope between geographic distance and community similarity of *Geobacteraceae*. It showed that both taxonomy similarity (Bray-Curtis) and phylogenetic similarity (weighted UniFrac) were significantly correlated with geographic distance. Plots of taxonomic similarity ($R^2 = 0.0301$, $P = 0.032$) or phylogenetic similarity ($R^2 = 0.052$, $P = 0.007$) versus geographic distance for each pairwise set of samples revealed that the *Geobacteraceae* displayed a significant, negative distance-decay curve across the scales from 92 km up to approximately 3515 km (Fig. 3).

VPA was performed to quantify the relative contribution of geographic distance and soil properties to the taxonomic structure of *Geobacteraceae*. A subset of environmental

Table 2 Mantel analysis of the relationship between the communities of *Geobacteraceae* and soil properties

Family	Variable	<i>r</i>	<i>P</i>
<i>Geobacteraceae</i>	pH	0.039	0.128
	DOC	−0.009	0.442
	$\text{NH}_4^+\text{-N}$	−0.059	0.868
	$\text{NO}_3^-\text{-N}$	−0.026	0.535
	C:N	−0.079	0.867
	EC	0.040	0.249
	Mn	0.132	0.050
	Fe	0.129	0.045
	aFe	0.184	0.009
	Latitude	0.092	0.050
	Longitude	0.251	0.001

Significant ($P \leq 0.05$) values are presented in bold

Fig. 3 The relationship between the beta-diversity with geographic distance. **a** Decay of taxonomic similarity (Bray-Curtis) with geographic distance. **b** Decay of phylogenetic similarity (weighted UniFrac) with geographic distance

parameters (aFe, pH, Fe, DOC, C:N, and NO_3^- -N) was selected by the BioEnv procedure, which provides the highest Pearson correlation with *Geobacteraceae*. These variables explained 23.3 % of the observed variation, leaving 76.6 % of the variation unexplained. The soil properties explained 18.2 % and geographic distance explained 5.1 % variations, and no interaction effect was detected. Although the soil properties explained more of the variation, geographic distance by itself explained 5.1 % of the variation observed, more than any of the other 6 of the individual soil properties (Fig. 4). Thus, both soil properties and geographic distance are important factors in shaping the structure of *Geobacteraceae*.

4 Discussion

In the present study, the copy numbers of *Geobacteraceae* and the relative abundances of *Geobacteraceae* in paddy soil samples varied greatly among soil samples. We detected the uneven distribution of *Geobacteraceae* in paddy soils of China and demonstrated that *Geobacteraceae* community composition was

Fig. 4 Variance partition analysis of the effects of geographic distance and soil properties on the phylogenetic structure of *Geobacteraceae*

strongly associated with soil chemical factors including aFe, pH, Fe, DOC, C:N, and NO_3^- -N and geographic distance. These results greatly expand the knowledge of the distribution of *Geobacteraceae* in environments, particularly in terrestrial ecosystems.

Environmental heterogeneity and geographic distance are considered to be major factors driving current differences in microbial diversity (Liu et al. 2015). Our results showed that the 16S rRNA genes of *Geobacteraceae* exhibited a biogeographic pattern that agreed with previous work on the biogeography of soil bacterial communities in soils (Ge et al. 2008; Griffiths et al. 2011). We can consider our results within the framework of factors that drive the biogeography of microbial communities, including selection, drift, dispersal, and mutation (Hanson et al. 2012). Selection and dispersal both have the effect on increasing distinctiveness of a community and push distance-decay slopes more negative. Meanwhile, endemism is a widely recognized phenomenon in microbial ecology and could be facilitated by the broad dissimilarities of soil physicochemical properties and climate conditions that serve as clear barriers to dispersal in many locations, which indicated that geographic distance and environmental selection and dispersal may act on the 16S rRNA genes of *Geobacteraceae* (Jiang et al. 2016).

It is interesting to note that aFe explained more than the other examined soil physicochemical parameters in the distribution of *Geobacteraceae* especially the dominant species of *Geobacteraceae* in paddy soils. aFe can be used as an electron acceptor to facilitate the respiration of *Geobacteraceae* (Lovley et al. 2004). Previous studies have showed that higher level of aFe favored the growth of iron-reducing bacteria, especially the growth of *Geobacteraceae* species (Ding et al. 2015; Kato et al. 2010).

Fe (III) reduction couples the Fe, C, S, P, and N cycles and occurs in paddy soils (Melton et al. 2014). For example, *Geobacteraceae* could inhibit methane production (Jäckel et al. 2005). The suppression of methanogenesis is mostly from out-competition of methanogens by iron-reducing microorganisms for common electron donors (Hori et al.

2010). The availability of iron for microbial reduction is an important factor controlling the extent of organic matter decomposition, with Fe (III) serving as the terminal electron acceptor (Lovley 1987). *Geobacteraceae* can metabolize organic matter that otherwise would be metabolized by methanogenic organisms in paddy soils (Deng et al. 2015). Due to the importance of dissimilatory Fe (III) reduction to many other biogeochemical processes, our findings have far-reaching implications for understanding of iron cycling in paddy soils and its impacts on the environment.

In addition to aFe, our results also showed that the community structure of *Geobacteraceae* was significantly correlated with soil pH. Previous studies have demonstrated that soil pH is a major factor in determining the soil bacterial biodiversity and composition. For instance, soil pH has been shown to influence bacterial communities in British soils (Griffiths et al. 2011), Arctic soils (Chu et al. 2010), soils across North and South American (Lauber et al. 2009), and soils of Northeast China (Liu et al. 2014). pH seems to be a key variable in the soil environment (Feng et al. 2014). Fe (III) primarily exists as insoluble, solid-phase minerals in circumneutral pH environment. The evidence suggested that Fe (III) oxides provide an electron sink and are reduced biologically in neoteric environmental systems in the anoxic zone at pH >4.0 (Weber et al. 2006). Therefore, soil pH is a reasonable predictor of Fe (III)-reducing microorganisms at the continental scale. In the current study, pH was also a predictor of the community structure of *Geobacteraceae*.

This is the first study to investigate the distribution of *Geobacteraceae* by Illumina HiSeq sequencing. It has been suggested that genomics data can be integrated into biogeochemical model to predict environmental changes modulated by diverse functional microbes (Reed et al. 2014). The importance of microbe-mediated iron redox changes in controlling the biogeochemistry of elements in the environment, particularly in sediments and wetland, is well established (Melton et al. 2014). Paddy soils are subjected to regular dry-wet cycles, and long-term paddy cultivation will lead to the accumulation of amorphous iron, which is a common terminal electron acceptor (Ding et al. 2015; Li et al. 2011). Therefore, understanding the diversity and distribution of *Geobacteraceae*, a major group of microbes involved in dissimilatory iron reducers in paddy soils, at large scale will be indispensable in predicting the biogeochemical cycling of contaminants and nutrients. Furthermore, our findings have implications for understanding the ecology of *Geobacteraceae* in terrestrial system and will provide information for predicting how the community of *Geobacteraceae* will respond to environmental perturbations, such as climate change.

5 Conclusions

In summary, this study gives insight into how geographic distance and environmental factors structure the community of *Geobacteraceae* within a rather broad range spanning over 3500 km from north to south of China. We reveal that the community composition of *Geobacteraceae* is modulated by geographic distance and soil properties. This is the first study to demonstrate that geographic distance can predict community structure of *Geobacteraceae* across a large scale. Moreover, aFe as a factor is also a contributor to community variation of *Geobacteraceae* especially the dominant species of *Geobacteraceae* in paddy soils. These results can potentially be integrated into current biogeochemical models in better predicting the fate and behavior of elements in terrestrial environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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