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ORIGINAL PAPER

Comparative evaluation of three archaeal primer pairs for exploring archaeal communities in deep-sea sediments and permafrost soils

Shiping Wei[1,2] · Hongpeng Cui[2] · Yuchen Zhang[2] · Xin Su[2] · Hailiang Dong[1] · Fang Chen[3] · Youhai Zhu[4]

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Abstract

16S rRNA gene profiling is a powerful method for characterizing microbial communities; however, no universal primer pair can target all bacteria and archaea, resulting in different primer pairs which may impact the diversity profile obtained. Here, we evaluated three pairs of high-throughput sequencing primers for characterizing archaeal communities from deep-sea sediments and permafrost soils. The results show that primer pair Arch519/Arch915 (V4–V5 regions) produced the highest alpha diversity estimates, followed by Arch349f/Arch806r (V3–V4 regions) and A751f/AU1204r (V5–V7 regions) in both sample types. The archaeal taxonomic compositions and the relative abundance estimates of archaeal communities are influenced by the primer pairs. Beta diversity of the archaeal community detected by the three primer pairs reveals that primer pairs Arch349f/Arch806r and Arch519f/Arch915r are biased toward detection of Halobacteriales, Methanobacteriales and MBG-E/Hydrothermarchaeota, whereas the primer pairs Arch519f/Arch915r and A751f/UA1204r are biased to detect MBG-B/Lokiarchaeota, and the primers pairs Arch349f/Arch806r and A751f/UA1204r are biased to detect Methanomicrobiales and Methanosarcinales. The data suggest that the alpha and beta diversities of archaeal communities as well as the community compositions are influenced by the primer pair choice. This finding provides researchers with valuable experimental insight for selection of appropriate archaeal primer pairs to characterize archaeal communities.

Keywords High-throughput sequencing · Archaeal primers · Archaeal communities · Deep-sea sediment · Permafrost soil

Introduction

The prokaryotic 16S rRNA gene is widely used for phylogenetic and microbial community analyses in environmental samples (Olsen et al. 1986; Head et al. 1998). It is approximately 1500 bp and contains nine variable regions (V1–V9) that demonstrate considerable sequence diversity to make phylogenetic classification (Wang and Qian 2009; Yang et al. 2016a). Moreover, the variable regions of 16S rRNA gene are flanked by several conserved regions, making it possible to design the broad targeting primers to amplify as many bacterial species as possible. The traditional approach is cloning and sequencing a full-length 16S rRNA gene using conserved broad-range PCR primers. With the advent of massive parallel sequencing technologies, the use of next-generation sequencing technology has enabled the large-scale investigation of microbial communities and stimulated broad interest in microbiota research. However, next-generation sequencing, also known as high-throughput sequencing, usually reads a shorter fragment of 250–600 bp, limiting it to classifying shorter reads from taxa (Ghyselinck

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✉ Shiping Wei
weishiping@cugb.edu.cn

[1] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Beijing 100083, China

[2] School of Marine Sciences, China University of Geosciences, Beijing 100083, China

[3] Guangzhou Marine Geological Survey, Guangdong 510760, China

[4] Oil and Gas Survey, China Geological Survey, Beijing 100029, China

et al. 2013; Schloss et al. 2016). In fact, the 16S rRNA gene sequences used in most studies are partial sequences, which only cover a part of the variable regions of the 16S rRNA gene. Therefore, selection of primers to amplify appropriate variable regions of the 16S rRNA gene is critical for studying bacterial community structure (Fredriksson et al. 2013; Ghyselinck et al. 2013; Klindworth et al. 2013).

Next-generation sequencing has revolutionized genomics research. It has proven to be an important discovery tool to investigate the microbial community by 16S rRNA gene sequencing in depth. Currently, the high-throughput sequencing approach covers a few variable regions of the full 16S rRNA gene, making it important to select appropriate variable regions for most accurate taxa identification (Liu et al. 2007). Previous studies show that a single or multiple variable regions of 16S rRNA gene can be targeted for bacterial community analysis, such as a single V3 (Bartram et al. 2011), V4 (Kuang et al. 2012), V5 (Lazarevic et al. 2009), V6 (Zhou et al. 2011), or multiple variable regions V1–V2 (Ravel et al. 2011), V3–V4 (Sinclair et al. 2015), V4–V5 (Fierer et al. 2012), V5–V6 (Liu et al. 2014a, b), and V6–V8 (Vavourakis et al. 2016). Different variable regions of the 16S rRNA gene vary in the accuracy of their taxonomic assignments (Liu et al. 2007; Chakravorty et al. 2007). Although a single-variable region of the 16S rRNA gene (e.g. V3, V4, V5 and V6) can be targeted for bacterial community analysis (Bartram et al. 2011; Kuang et al. 2012; Lazarevic et al. 2009; Zhou et al. 2011), however, significant loss of sensitivity and specificity in the taxonomic classification occurs when short 16S rRNA gene sequences are used (Martínez-Porchas et al. 2016). The combined variable regions are the most reliable regions for representing the full-length 16S rRNA sequences of bacteria (Yang et al. 2016a). The combined variable regions of V1–V2, V2–V3, V3–V4, V4–V5, V5–V6, and V7–V8 in silico evaluations show that the V3–V4 and V4–V5 regions would provide the highest classification accuracy (Claesson et al. 2010). Zheng et al. (2015) studied the oral bacterial community and the results show that V1–V3 provides with greater phylotype richness and evenness than V3–V4. Peiffer et al. (2013) tested four primer sets designed to amplify the V1–V2, V3–V4, V5–V8, and V6–V8 regions of the 16S rRNA gene from maize rhizosphere, and the results show that V3–V4 region yield the greatest diversity at the domain and bacterial phylum levels, suggesting the combined specific regions of the 16S rRNA gene that are used to characterize microbial diversity can also impact the diversity profile obtained.

In contrast to bacteria, the archaeal community are often not specifically addressed in the analyses of microbial communities. Determination of primers specifically amplifying archaeal 16S rRNA gene has been demonstrated in previous studies (Baker et al. 2003; De León et al. 2013; Gantner et al. 2011; Herfort et al. 2009; Hugoni et al. 2013; Klindworth et al. 2013; Takahashi et al. 2014; Takai and Horikoshi 2000). There are some reports about in silico evaluation of archaeal-specific primers designed for the next-generation sequencing platforms (Fischer et al. 2016; Gantner et al. 2011; Klindworth et al. 2013). However, in silico evaluation of archaeal primers covering different variable regions does not necessarily reflect the results obtained in experimental approaches (Fischer et al. 2016). It is imperative to evaluate them by experimental analyses based on the archaeal community structure obtained from different samples. In this study, we compare the performances of three archaeal primers, Arch340f/Arch806r (V3–V4 regions) (Galand et al. 2016; Hugoni et al 2013; Liu et al 2014a, b; Mesa et al 2017; Takai and Horikoshi 2000), Arch519f/Arch915r (V4–V5 regions) (Bai et al 2017; Coolen et al 2004; Ding et al 2017; Jiao et al 2019; Liu et al 2019; Yang et al 2016b) and A751f/UA1204r (V5–V7 regions) (Bell et al. 2016; Cui et al 2016, De León et al. 2013; Genderjahn et al 2018; McKay et al 2017), which are commonly used for exploring the archaeal communities in a wide range of environments, and get if whether they work well for detection of the archaeal communities under extreme environments, and then provide a promising primer set for surveying archaeal diversities in the deep-sea sediments and the permafrost environments.

Materials and methods

Sampling

Sediment gravity piston core DH-CL11 (767 cm in length) was taken below the water depth of 1607 m at an active methane seep site in the South China Sea (Cui et al. 2016). Thirteen subsamples, designated D1–D13, were sampled at 50-cm intervals for archaeal community analyses. The permafrost samples were obtained by digging to a depth of 1.6 m. Ten subsamples, designated P1–P10, were sampled at 15-cm intervals in the permafrost soil profile, and subject to archaeal community analyses.

DNA extraction and PCR amplification

The bulk community DNAs were extracted from 10 g sediments using an available soil DNA isolation kit according to the manufacturer's instruction (MP Biomedicals, USA). The concentration of the extracted DNA was determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, US). Three primer pairs were used to amplify the archaeal 16S rRNA partial gene sequence (Table 1). Each forward primer was barcoded with a unique eight-base sequence on its 5′ end for sample identification. The PCR amplification was performed in triplicate in a total volume of 20 μl mixture containing 4 μl of 5×FastPfu buffer, 2 μl

Table 1 Archaeal primers used in this study

Primer pair	Sequence	Variable regions	Amplicon size	References
Arch349f	CCCTACGGGGTGCASCAG	V3–V4	458	Takai and Horikoshi 2000; Hugoni et al. 2013
Arch806r	GGACTACVSGGGTATCTAAT			
Arch519f	CAGCCGCCGCGGTAA	V4–V5	397	Herfort et al. 2009
Arch915r	GTGCTCCCCCGCCAATTCCT			
A751f	CCGACGGTGAGRGRYGAA	V5–V7	454	Baker et al. 2003; De León et al. 2013
AU1204r	TTMGGGGCATRCNKACCT			

of 2.5 mM dNTPs, 0.8 µl of each primer (5 µM), 0.4 µl of FastPfu polymerase, and 10 ng of template DNA. The PCR condition consisted of an initial denaturation at 95 °C for 2 min, and 25 cycles at 95 °C for 30 s, a gradient of 50–55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min.

High-throughput 16S rRNA gene sequencing

PCR amplicons were checked on 2% agarose gels and purified with a DNA gel extraction kit according to the manufacturer's instructions (Axygen Biosciences, Union City, CA, USA). The purified PCR products from triplicated amplifications were quantified using QuantiFluor™-ST (Promega, USA) and then mixed accordingly to achieve equimolarity in the final mixture of each sample, which was used to construct PCR amplicon libraries. Sequencing was performed on an Illumina MiSeq platform (Majorbio, Shanghai, China).

Sequence data processing and analyses

Raw fastq files were de-multiplexed and the sequences were quality-filtered using QIIME (Quantitative Insights into Microbial Ecology, version 1.8.0) software package (Caporaso et al 2010). The 250-bp reads were truncated at any site receiving an average quality score <20 over a 10 bp sliding window. Sequences were discarded that were less than 50 bp in length, presented two nucleotide mismatches in the primer sequences, or had reads containing ambiguous bases. The effective reads were assembled according to their overlap sequence and assigned to each sample according to their unique barcode sequences. After removal of the chimeric sequences, the remaining sequences were clustered into Operational Taxonomic Units (OTUs) with 97% similarity cutoff using UPARSE (version 7.1 https://drive5.com/uparse/). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (https://rdp.cme.msu.edu/) against the SILVA (SSU115) 16S rRNA database using confidence threshold of 70%.

Statistical analyses

To calculate alpha diversity, three metrics were calculated: Chao1 metric (estimating the species richness), observed species metric (the count of unique OTUs found in the sample), and Shannon diversity index. Chao1 estimator, ACE (abundance-based coverage estimator), and Shannon diversity index were generated using Mothur (Schloss et al 2009). Principal component analysis was conducted to characterize dissimilar relationship of archaeal community versus the primer pairs using CANOCO 5.0, and a correlation coefficient matrix was generated by two-tailed *p* value statistics.

Results

Alpha diversity estimates of archaeal communities differ depending on primer pairs

The three primer pairs, Arch349f/Arch806r, Arch519f/Arch915r and A751f/UA1204r, gave no significant differences of sequence reads ($p > 0.05$) between the two sediment types (Fig. 1), with an average of 14,077, 17,468 and 14,190 in the deep-sea sediments (Fig. S1A) and 15,632, 13,390 and 17,927 in the permafrost soils (Fig. S1B), respectively. However, these primer pairs yield a significant change ($p < 0.01$) in the observed OTU numbers based on 97% sequence similarity level (Fig. 1 and Fig. S1). Specifically, a higher average of OTU numbers in the samples of deep-sea sediments was observed (434 ± 178, 884 ± 351, 134 ± 50) (Fig. S1C) than in the samples of permafrost soils (62 ± 48, 210 ± 108, 13 ± 5) (Fig. S1D). The primer pair Arch519f/Arch915r yields the highest OTU numbers in both of the two environmental samples, followed by the primer pair Arch349f/Arch806r, and then A751f/UA1204r. The alpha diversity indices, including Shannon and Simpson indices, ACE, and Chao1 richness estimators are calculated for archaeal communities, indicating different patterns in terms of alpha diversity depending on the environmental samples and the primer pair used (Fig. S2). The results reveal that the deep-sea sediments have higher alpha diversity estimates for

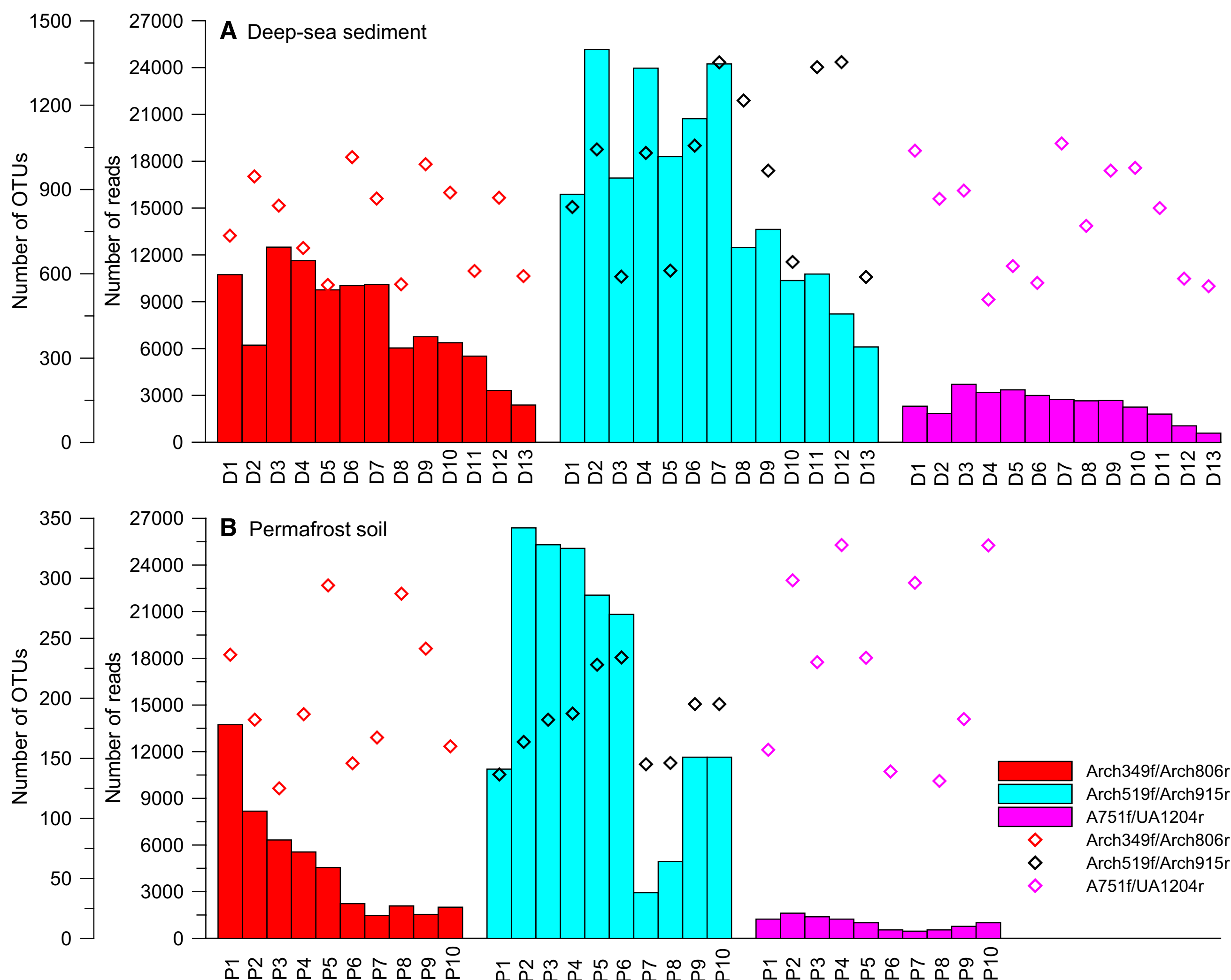


Fig. 1 Sequence reads (bars) and OTUs (diamonds) detected by the three primer pairs, Arch349f/Arch806r, Arch519f/Arch915r and A751f/UA1204r

archaeal community than those in the permafrost soils. Basically, the alpha diversity presents a decreasing trend with the sampling depth in both of the environmental samples. Besides the alpha diversity differences between the environmental samples, the data also reveal that alpha diversity varied with the primer pairs. Primer pair Arch519f/Arch915r yielded a higher alpha diversity estimates compared to the other two primer pairs. Primer pair A751f/UA1204r produces the lowest alpha diversity estimates in the both two environmental settings.

Primer pair influences the observed taxonomic composition of archaeal communities

All three primer pairs are compared to detect the archaeal community compositions among a total of 23 subsamples from deep-sea sediments and permafrost soils. The results show that archaeal community composition varies between the two environments, depending on the depth and the primer pair used (Fig. 2). The archaeal community composition is different between the deep-sea sediments and the permafrost soils. The dominant groups (>10%) in the deep-sea sediments are composed of Halobacteriales, MCG (now known as Bathyarchaeota) (Meng et al 2014), C3 (now known as Bathyarchaeota) (Spang et al 2015; Sollai et al 2019), MBG-B (now known as Lokiarchaeota) (Spang et al 2017) and SAGMEG (now known as Hadesarchaea) (Spang et al 2017) (Figs. 2 and S3A), whereas the dominant groups in the permafrost soils are composed of MCG/Bathyarchaeota, Methanomicrobiales, Methanosarcinales and Methanobacteriales (Figs. 2 and S3B). For the deep-sea sediments, the primer pair Arch349f/Arch806r detected the four most abundant groups Halobacteriales (26.9%), MCG/Bathyarchaeota (24.1%), C3/Bathyarchaeota (13.2%)

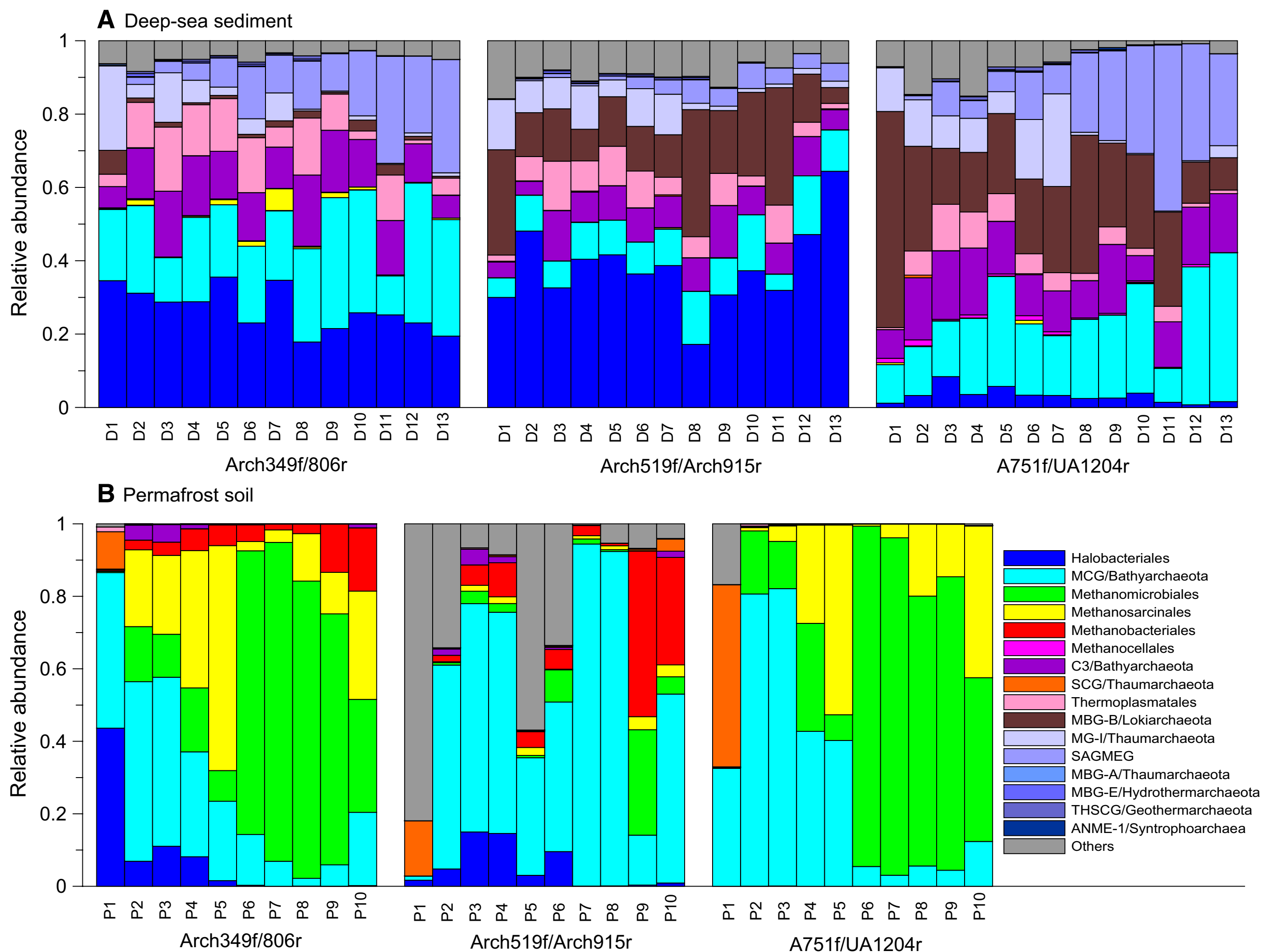


Fig. 2 Taxonomic profiles of archaeal community. Annotation was done using SILVA database. Unclassified sequences and other archaeal groups represented less than 1% of total archaeal populations were grouped as “Others”

and SAGMEG/Hadesarchaea (12.6%) (Fig. S3A). In contrast, the three most abundant groups detected by primer pair Arch519f/Arch915r are affiliated to Halobacteriales (38.2%), MCG/Bathyarchaeota (10.2%) and MBG-B/Lokiarchaeota (17.3%), whereas the four most abundant groups MCG/Bathyarchaeota (22.1%), C3/Bathyarchaeota (13.7%), MBG-B/Lokiarchaeota (24.3%) and SAGMEG/Hadesarchaea (16.9%) are detected by primer pair A751f/UA1204r (Fig. S3A). However, the most abundant groups in permafrost soils detected by each of those three primer pairs are significantly different from those the primer pairs detected in the deep-sea sediments. In the combined permafrost soils, the dominant groups detected by primer pair Arch349f/Arch806r are MCG (23.9%), Methanomicrobiales (40.2%) and Methanosarcinales (20.4%). The most abundant groups detected by primer pair Arch519f/Arch915r are MCG/Bathyarchaeota (50.8%) and Methanobacteriales (10.6%), whereas MCG/Bathyarchaeota (30.9%), Methanomicrobiales (45.5%) and Methanosarcinales (16.5%) are detected by primer pair A751f/UA1204r (Fig. S3B). In addition, the primer pair Arch519f/Arch915r amplifies a higher fraction of others sequences than the other primer pairs, both in the deep-sea sediments and in the permafrost soils (Figs. 2 and S3).

Beta diversity measurement

Beta diversity of the archaeal community was analyzed by principal component analysis based on the archaeal groups. The results demonstrate that significant clustering of the samples occurs according to those two categorical sample types. A clear separation of archaeal communities was observed between the deep-sea sediments and the permafrost soils ($p < 0.0001$) (Fig. 3a), indicating the most important source of variation in the archaeal beta diversity is the sample type. Figure 3b, c shows that beta diversity within the sample type is also different based on the primer pair used. In terms of primer pair effects, the differentiation patterns

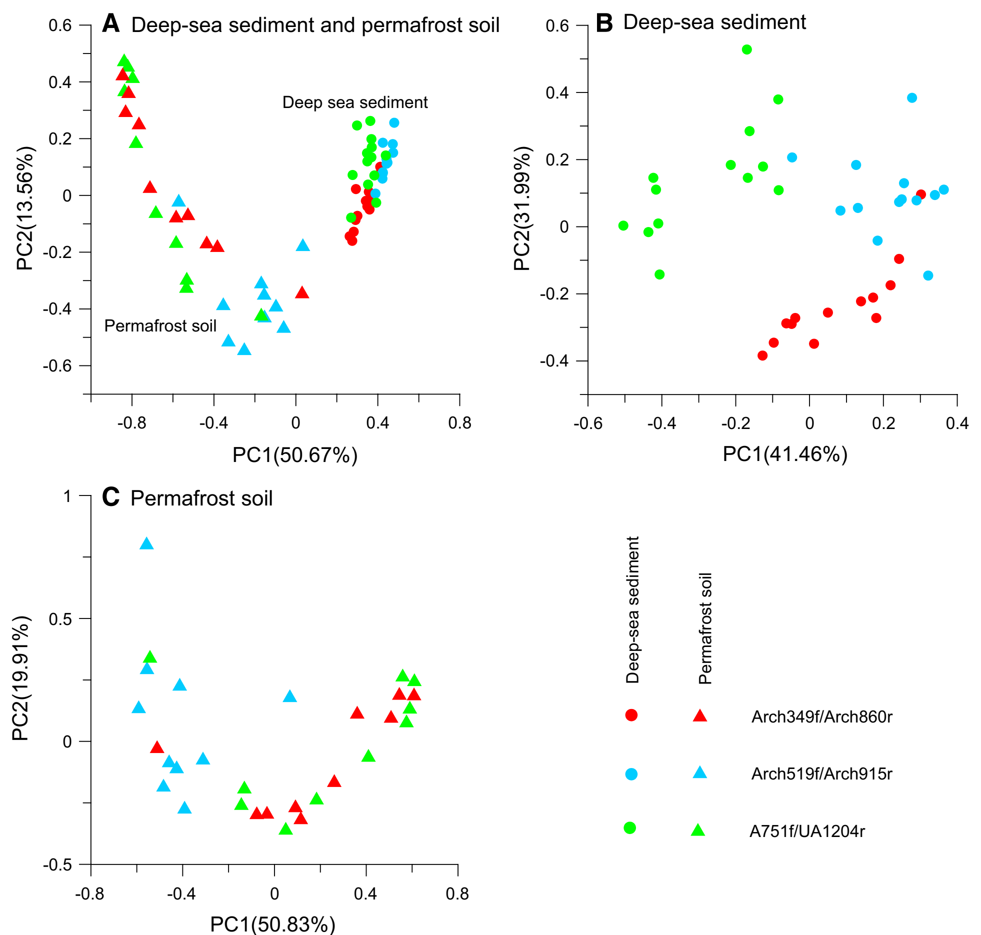


Fig. 3 Principal component analysis plots reflecting the dissimilar distance of archaeal community from deep-sea sediments and permafrost soils with the primer pairs Arch349f/Arch806r, Arch519f/Arch915r and A751f/UA1204r

among those three primer pairs in archaeal beta diversity from deep-sea sediment are significant at $p<0.001$ (Fig. 3b). However, different archaeal beta diversity patterns influenced by primer pairs are observed between the permafrost soils and in the deep-sea sediments. The results show that two clear clusterings, A751f/UA1204r, Arch349f/Arch806r and Arch519f/Arch915r, are observed in the permafrost soils ($p<0.001$), whereas not much difference is observed between Arch349f/Arch806r and Arch519f/Arch915r ($p<0.53$) (Fig. 3c), indicating the primer pairs effect on the variation in archaeal beta diversity depends on the sample types and the primer pair used.

The ternary plots of 12 archaeal groups were built to visualize the relative abundance of archaeal groups associated with the three primer pairs (Fig. 4). The results show that more archaeal groups were detected in the deep-sea sediments than in the permafrost soils using the three primer pairs. The groups of Halobacteriales, MCG/Bathyarchaeota, Methanomicrobiales, Methanosarcinales, Methanobacteriales, Methanocellales, C3/Bathyarchaeota, SCG (now known as Thaumarchaeota) (Lauer et al 2016) and Thermoplasmatales were detected both in the deep-sea sediments and in the permafrost soils (Fig. 4a–i), whereas the groups of MBG-A (now known as Thaumarchaeota), MBG-B/Lokiarchaeota, MG-I (now known as Thaumarchaeota) (Sollai et al 2019), MBG-E (now known as Hydrothermarchaeota) (Jungbluth et al 2017), SAGMEG/Hadesarchaea, THSCG (now known as Geothermarchaeota) (Spang et al 2017) and ANME-1 (now known as Syntrophoarchaea) (Spang et al 2017) tended to be found only in the deep-sea sediments (Fig. 4j–p). The relative abundance of each archaeal group is shown to be a sample-specific association with the environments and sampling depths, except for MCG/Bathyarchaeota, which is abundant both in the deep-sea sediments and in the permafrost soils (Fig. 4b). Most importantly, different primer pairs are significantly biased to detect different abundances of archaeal groups. The primer pairs Arch349f/Arch806r and Arch519f/Arch915r tend to detect Halobacteriales (Fig. 4a), Methanobacteriales (Fig. 4e), whereas the primer pairs Arch519f/Arch915r and A751f/UA1204r tend to detect MBG-B/Lokiarchaeota (Fig. 4k), and the primers pairs Arch349f/Arch806r and A751f/UA1204r tend to detect Methanomicrobiales, Methanosarcinales and SAGMEG/Hadesarchaea (Fig. 4c–d and n). However, the majority of archaeal groups is located at the middle of the ternary plots, such as MCG/Bathyarchaeota (Fig. 4b), C3/Bathyarchaeota

(Fig. 4g) and Thermoplasmatales (Fig. 4i), indicating that they are equally likely to be detected by the three primer pairs.

Discussion

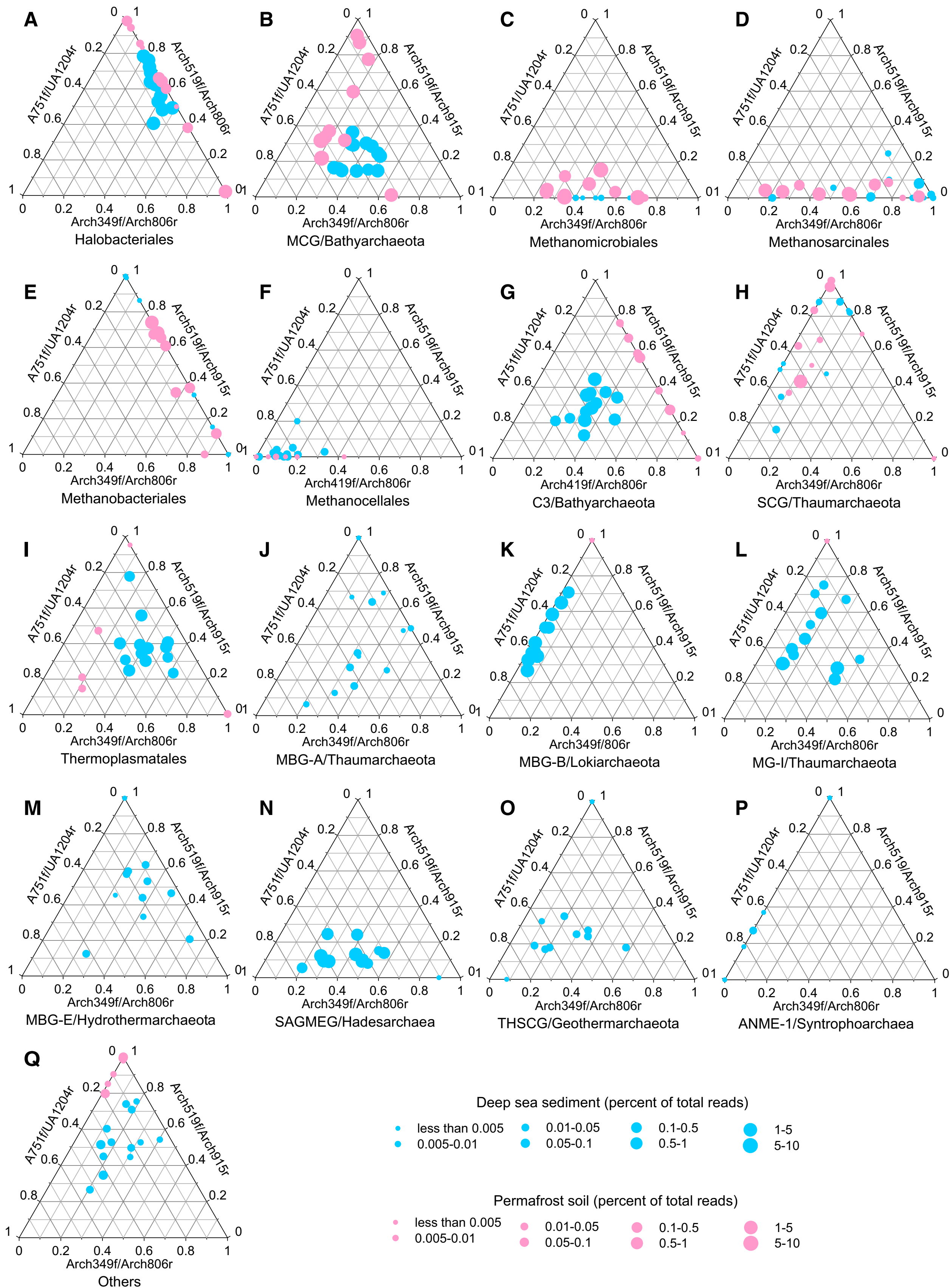
Effects of primer pairs on archaeal alpha and beta diversities

All three primer pairs, Arch349f/Arch806r, Arch519f/Arch915r and A751f/UA1204r, are found to be highly specific for the domain Archaea in both of the sample types, with exception of Arch349f/Arch806r, which detected a total average of 3.4% bacterial sequences in the permafrost soils. Not much difference of archaeal sequence reads were observed between the two sample types ($p > 0.5$, Fig. S1), indicating that the yield sequences of different primer pairs are not affected by the sample types (Thijs et al. 2017). However, alpha diversity analysis shows that the average observed OTUs in the deep-sea sediments are significantly higher ($p < 0.05$) than those in the permafrost soils using the three primer pairs. In the deep-sea sediments, comparison of the three primer pairs reveals the highest OTU richness for primer pair Arch519f/Arch915r, which obtained an average of 884 OTUs, in comparison with 434 OTUs which were retrieved with the primer pair Arch349f/Arch806r, and the primer pair A751f/UA1204r yielded the lowest OTU richness estimates at 133 OTUs (Fig. S1). A similar pattern of OTU richness estimates is observed in the permafrost soils with the three primer pairs (Fig. S1), indicating OTU richness varies significantly depending on the sample types. The Shannon and Simpson indices, ACE and Chao1 estimates also differed among the three primer pairs (Fig. S2). Higher alpha diversity is observed with the primer pair Arch519f/Arch915r targeting the variable regions V4–V5 for both the deep-sea sediments and in the permafrost soils, indicating the primer pair Arch519f/Arch915r yields a higher resolution of archaeal community in species level than that of Arch349f/Arch806r and A751f/UA1204r, targeting the V3–V4 and V5–V7 regions, respectively. A previous comparison of archaeal primer pairs shows that high diversities are observed for the primer pairs ArchV46 and PrkV4 covering the variable regions V4–V6 and V4, respectively. However, the primer ArchV34 targeting for V3–V4 regions yields the lowest alpha diversity (Fischer et al. 2016). Bahram et al. (2018) designed a couple of new archaeal primers targeting different variable regions of 16S rRNA genes, the results show that the primer pair SSU1ArF/SSU-1000ArR targeting V1–V5 regions is promising for high OTU and phylum richness for longer amplicons. For short amplicons, the primer pairs SSU1ArF/SSU520R (V1–V2) and 340F-806B (V4–V5) are recommended for Illumina sequencing platform (Bahram et al. 2018). Different variable regions of 16S rRNA genes yielding significant differences in bacterial alpha diversity estimates are often observed in various environments (Claessin et al. 2010; Peiffer et al. 2013; Zheng et al. 2015; Fischer et al. 2016; Thijs et al. 2017; Zhang et al. 2018), indicating the microbial diversity estimates measured from the same sample differed significantly depending on choice of variable regions (Claesson et al. 2010).

Comparison of variation in the observed archaeal beta diversity was subjected to PCA analysis based on abundance of taxonomic sequences. Both the sediment types and the primer pairs are influential on the beta diversity pattern of archaeal community (Fig. 3). The clearly different clustering of the deep-sea sediments and the permafrost soils separately indicates the sample types impose more influence on archaeal beta diversity pattern than the primer pairs (Fig. 3a). This finding is similar with the results reported by Peiffer et al. (2013), in which the majority of the variation in microbial beta diversity across the samples could be attributed to the sample types (Peiffer et al. 2013). In addition, the primer pairs could explain the second source of variation in beta diversity. PCA analyses show that the three primer pairs also separate the archaeal community into corresponding assemblages both in the deep-sea sediments and in the permafrost soils, resulting in differences in beta diversity based on the primer pair used when they are applied to the same samples (Fig. 3b, c). The deep-sea sediment samples cluster per primer pair in high concordance with the three primer pairs Arch349f/Arch806r, Arch519f/Arch915r and A751f/UA1204r (Fig. 3b). However, in the permafrost soils, the primer pair Arch519f/Arch915r primed samples form a clear cluster, whereas no significant difference is observed between the primer pairs Arch349f/Arch806r and A751f/UA1204r (Fig. 3c). This result is consistent with previous findings of microbial communities in various environments, the primer pair primed samples often cluster together (Peiffer et al. 2013; Fischer et al. 2016; Thijs et al. 2017; Zhang et al. 2018). It indicates that the primer pairs targeting for different variable regions of 16S rRNA gene strongly impact the microbial beta diversity patterns, suggesting to us that the primer choice is vital for beta diversity analysis. Primer pair choice depends on the research purpose and the sample types.

Effects of sample types on archaeal community composition

Two distinct environmental samples, deep-sea sediments and permafrost soils, which were previously shown to harbor a large quantity of archaea (Nunoura et al. 2012; Cui et al. 2016; Wei et al. 2018), were used to evaluate the performances of the three archaeal primer pairs, Arch349f/


A
Halobacteriales
B
MCG/Bathyarchaeota
C
Methanomicrobiales
D
Methanosarcinales
E
Methanobacteriales
F
Methanocellales
G
C3/Bathyarchaeota
H
SCG/Thaumarchaeota
I
Thermoplasmatales
J
MBG-A/Thaumarchaeota
K
MBG-B/Lokiarchaeota
L
MG-I/Thaumarchaeota
M
MBG-E/Hydrothermarchaeota
N
SAGMEG/Hadesarchaea
O
THSCG/Geothermarchaeota
P
ANME-1/Syntrophoarchaea
Q
Others
A751f/UA1204r
Arch519f/Arch806r
Arch519f/Arch915r
Arch349f/Arch806r
Arch419f/Arch806r
Arch349f/806r
Deep sea sediment (percent of total reads)
less than 0.005
0.005-0.01
0.01-0.05
0.05-0.1
0.1-0.5
0.5-1
1-5
5-10
Permafrost soil (percent of total reads)
less than 0.005
0.005-0.01
0.01-0.05
0.05-0.1
0.1-0.5
0.5-1
1-5
5-10

◂**Fig. 4** Ternary plots of relative abundances of archaeal communities related to the three primer pairs, Arch349f/Arch806r, Arch519f/Arch915r and A751f/UA1204r. Each point represents a different depth sampling site, and its position indicates the relevance of archaeal group to the three primer pairs. Point size indicates the relative abundance of each archaeal groups corresponding to their sampling sites

Arch806r, Arch519f/Arch915r and A751f/UA1204r. A significant difference in archaeal community composition between the two sediment types is observed among the primer pairs (Figs. 3, 4). The majority of the archaeal groups in the deep-sea sediments identified by all primers pairs are assigned to Halobacteriales, MCG/Bathyarchaeota, C3/Bathyarchaeota, SAGMEG/Hadesarchaea, MBG-B/Lokiarchaeota, MBG-E/Hydrothermarchaeota and MG-I/Thaumarchaeota, compared to Halobacteriales, MCG/Bathyarchaeota, Methanomicrobiales, Methanosarcinales and Methanobacteriales in the permafrost soils. Previous researchers demonstrated that the MCG/Bathyarchaeota group has a much wider habitats ranging from terrestrial environments to marine environments (Teske and Sørensen 2008; Kubo et al. 2012), whereas Halobacteriales are often observed in multiple saline environments (You et al. 2012). In addition, all the groups Thermoplasmatales, SAGMEG/Hadesarchaea, MBG-B/Lokiarchaeota, MBG-E/Hydrothermarchaeota and MG-I/Thaumarchaeota are frequently observed in marine environments including the deep-sea sediments (Vetriani et al. 1999; Takai and Horikoshi 1999; Teske and Sørensen 2008). In contrast, Methanomicrobiales, Methanosarcinales and Methanobacteriales are mainly detected in the permafrost soils, which are thought to be the most important groups for methane emissions (Mackelprang et al. 2011; Liebner et al. 2015; Wei et al. 2018).

Effects of primer pairs on archaeal community composition

Besides the sample types, the primer pairs could result in biased profiles of archaeal community composition and abundance when they are applied to the same samples. This phenomenon has been frequently observed in other studies (Klindworth et al. 2013; Peiffer et al. 2013; Zhang et al. 2018). In the deep-sea sediments, the relative abundances of major archaeal groups are also significantly different between primer pairs (Fig. 3a). Primer pairs Arch349f/Arch806r and Arch519f/Arch915r show bias to detect high abundance of Halobacteriales (Figs. 3a, 4a). A high relative abundance of MBG-B/Lokiarchaeota is detected by both of the primer pairs Arch519f/Arch915r and A751f/UA1204r (Figs. 3a, 4k), whereas MCG/Bathyarchaeota and SAGMEG/Hadesarchaea sequences are simultaneously more amplified by the primer pairs Arch349f/Arch806r and A751f/UA1204r (Figs. 3a, 4b, n). In an overall comparison of the three primer pairs coupled with their performances in archaeal species level, the primer pairs Arch519f/Arch915r and Arch349f/Arch806r are recommended for archaeal community analysis in the deep-sea sediments.

However, in the permafrost soils, the primer pair Arch519f/Arch915r poorly detected the groups of Methanomicrobiales and Methanosarcinales (Figs. 3b, 4c–d), which are thought to be the dominant methane-producing groups in the permafrost soils (Mackelprang et al. 2011; Liebner et al. 2015; Wei et al. 2018). Furthermore, this primer pair amplified a greater proportion of unclassified sequences than the other primer pairs, making it an obstacle for archaeal community analysis in the permafrost soils. The primer pair A751f/UA1204r failed to amplify Halobacteriales and amplified very few sequences of Methanobacteriales (Figs. 3b, 4a, e). However, it is specific for detection of the groups MCG/Bathyarchaeota, Methanomicrobiales and Methanosarcinales (Figs. 3b, 4c–d). In contrast, the primer pair Arch349f/Arch806r detected more taxonomic orders than the other two primer pairs (Fig. 3b). Although it shows only a moderate recovery of archaeal alpha diversity among the three primer pairs (Fig. 1b), this primer pair generates a more accurate profile of archaeal community structure (Fig. 4b), making it a reliable candidate for investigation of the archaeal community in the permafrost soils.

Conclusion

We evaluated three widely used archaeal primer pairs using experimental approaches to study the archaeal communities in deep-sea sediments and permafrost soils. The results show that the primer pairs strongly impact the archaeal community diversity and composition estimates. The primer pairs Arch349f/Arch806r and Arch519f/Arch915r, targeting the V3–V4 and V4–V5 regions in 16S rRNA gene, respectively, are suited for archaeal community analysis as they show high archaeal alpha diversity and taxonomic coverage in the deep-sea sediments. However, the primer pair Arch519f/Arch915r displays a high amplified proportion of other sequences and an inability to detect the most abundant groups Methanomicrobiales and Methanosarcinales in the permafrost soils. Even though primer pair Arch519f/Arch915r yields a high archaeal alpha diversity estimate, it is not recommended to characterize the archaeal community in the permafrost soils. Instead, the primer pair Arch349f/Arch806r exhibits a moderate alpha diversity detection, but it performs best in differentiating taxa of archaeal communities, making it a reliable primer pair to investigate the archaeal community in the permafrost soils. In addition, the ternary plot analyses show that each primer pair is biased for detection of specific archaeal groups. This finding provides researchers

with valuable experimental insight for selection of appropriate archaeal primer pairs for archaeal community analysis.

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