STRUCTURE, VARIATION, AND CO-OCCURRENCE OF ARCHAEAL COMMUNITIES ASSOCIATED WITH POLYMER- AND ASP-FLOODED PETROLEUM RESERVOIR BLOCKS

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Abstract. The response of archaeal communities to the enhancement of the oil recovery process in the extreme environments of oil reservoirs has rarely been investigated. In this study, archaeal communities in polymer- and alkaline-surfactant-polymer (ASP)-flooded production wells were evaluated via Illumina high-throughput sequencing targeting 16S rRNA genes. The composition of the archaeal community differed significantly among enhanced oil recovery production wells. *Methanothermobacter* was dominant in the polymer- and ASP-flooded production blocks. *Methanoculleus* accounted for numerous archaeal communities inhabiting the polymer-flooded blocks but were hardly detected in the ASP-flooded blocks; the trends for *Methanolinea* were the opposite. Redundancy analysis indicated that the archaeal communities in polymer- and ASP-flooded production blocks were closely related to physical and chemical factors, such as strong alkalinity and high salinity, which together accounted for 75.03% of the total variance. We applied network analysis to the co-occurrence pattern of archaeal communities in oil reservoirs. The archaeal communities in oil reservoirs were non-random, and that the community structure was more stable in the polymer- than in the ASP-flooded production wells. The polymer flooding process led to a more complex archaeal network than that of ASP flooding. Our results indicated that enhanced oil recovery mediates the coexistence of archaeal species in deep oil reservoirs. This study contributes to our understanding of the contemporary coexistence theory in microbial ecosystems in extreme environments. Revealing the influence of polymer flooding and ASP flooding on community composition and co-occurrence patterns of archaea could facilitate the implementation of MEOR technology in the future.

Keywords: *archaeal community, network analysis, polymer flooding, ASP flooding, enhanced oil recovery, deep subsurface environments*

Introduction

Subsurface petroleum reservoir ecosystems represent a deep subsurface environment with high salinity and pressure, low water activity, and high hydrophobicity, which is extreme for microbial life (Cai et al., 2015; Pannekens et al., 2019). Nevertheless, oil reservoirs consist of multiphase media (including water, organic materials, and crude oil), which allow microorganisms to thrive (Kobayashi et al., 2012; Abilio et al., 2021) and harbor a wide distribution of anaerobic microorganisms, including most hydrocarbon-oxidizing, sulfur-oxidizing, sulfate-reducing, fermentative, and methanogenic bacteria (Chia, 2014; Singh and Choudhary, 2021). In recent years, the microbial communities associated with deep oil reservoirs have received increasing attention from researchers (l'Haridon et al., 1995; Ollivier et al., 1997; Grabowski et al., 2005; Li et al., 2007; Zhao et al., 2021; Yun et al., 2022). Numerous anaerobic microorganisms, such as sulfidogens

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(Lenchi et al., 2021), sulfate reducers (Rajbongshi and Gogoi, 2021), methanogens (Nilsen and Torsvik, 1996; Dengler et al., 2022), and iron and manganese reducers (Dong et al., 2022) have been isolated and recognized from different oil reservoirs. Methanogenic archaea are very common in subsurface petroleum reservoir ecosystems, in which liquid petroleum hydrocarbons are the primary organic matter (Magot et al., 2000). In these oil reservoirs, hydrocarbons are degraded by various bacteria into methanogenic substrates, such as CO_2, acetate, and H_2. Methanogenesis is the last degradation procedure of organic matter by methanogenic archaea in the subsurface petroleum reservoir and, therefore, of great significance for understanding the ecological pattern of oil reservoir archaea from the perspective of scientific significance and industrial applications, such as microbial enhanced oil recovery (MEOR) (Aitken et al., 2004; Rathi et al., 2018; Tyne et al., 2021; Du et al., 2022).

Many large oil fields worldwide, including the DaQing, have recently entered the late stage of high water cut production. Oil recovery in such reservoirs faces differing degrees of annual decline. However, the consumption and demand for oil are growing, and oil will continue to be a major contributor to the world economy in the coming decades (Al Adasani and Bai, 2011; Chen et al., 2019; Baumeister et al., 2022). Approximately 70% of the total crude oil in a reservoir remains after the use of only conventional oil recovery methods (Yang et al., 2004; Kang et al., 2011). In recent years, chemical flooding has become a very important tertiary oil recovery technology. Chemical EOR flooding technologies, such as alkaline, surfactant, polymer, alkaline surfactant, alkaline polymer (AP), and alkaline-surfactant-polymer (ASP) flooding, are increasingly used in oil fields (Shutang and Qiang, 2010; Firozjaii and Saghafi, 2020). Polymer and ASP flooding technologies have been applied in oil reservoirs globally for 30 years (Chang et al., 2006). However, the diversity and composition of the archaea community in polymer- and ASP-flooded reservoirs have rarely been reported.

Due to the natural advantages of indigenous microbial communities, such as archaea in the process of microbial oil flooding, revealing the community composition and co-occurrence pattern of archaea in polymer flooding and ASP flooding reservoirs could facilitate the implementation of future oil recovery technologies. In this study, a 16S rRNA gene library was analyzed to study the archaeal community structure in different EOR processes at the DaQing Oilfield located in Heilongjiang province, China. We aimed to reveal additional archaeal diversity, which will increase our understanding of the complex community that inhabits the subterranean petroleum-rich system. The composition of archaeal communities present in two EOR production blocks (polymer- and ASP-flooded) was investigated to determine the archaeal co-occurrence pattern response to EOR treatment. Considering the contrasting processes and mechanisms of polymer and ASP flooding, we hypothesized that (I) the community composition of archaea was significantly different in polymer- and ASP-flooded production blocks, and (II) the community co-occurrence networks from these sample sets were non-random. EOR methods mediated the organization and complexity of co-occurrence networks and stability.

Materials and methods

Sample collection and total DNA extraction

The research was conducted in the DaQing Oilfield in Northeast China. The temperature of the oil-bearing layer of the reservoir was approximately 45 °C. Polymer and ASP flooding technologies were applied in 1995 and 2014 in the Daqing Oil Field, respectively. The pH of the production water samples obtained from polymer- and ASP-flooded blocks were 11.18 and 8.22, respectively (INESA Instrument, Shanghai, China). In August 2019, A total of 66 oil-water samples were collected from polymer-flooded (29 wells) and ASP-flooded (37 wells) production wells and were immediately stored in 10 L plastic containers. To avoid oxygen intrusion, the containers were filled to maintain anaerobic conditions. We transported the containers in a cooler filled with ice blocks within 48 h to the lab for DNA extraction. Before collecting the samples, we autoclaved all the containers and rinsed them with water samples for collection. The water samples (2 L) were centrifuged repeatedly at 12,000 g at 4 °C for 30 min to precipitate microbial cells (Beckman, United States). The filters were used for genomic extraction, placed in a buffer, and immediately frozen in a freezer at −80 °C until the DNA was isolated (Pham et al., 2009; Tully et al., 2012). The Axygene bacterial genomic DNA extraction kit (Takara) was used to extract genomic DNA from the precipitate. The quality of DNA was checked on an agarose gel and either used in the analysis described below or stored at −20 °C.

Amplification and analysis of 16S rRNA genes

Total genomic DNA was extracted from the production water and used to construct the archaeal library. Archaeal 16S rRNA sequences were amplified using archaea-specific primer set Arch524f (5’-TGYCAGCCGCCGCGGTAA-3’) and Arch958r (5’-YCC GGC GTT GAM TCC AAT T-3’) (DeLong, 1992). The polymerase chain reaction (PCR) mixture contained 2 μL of dNTP (2.5 mM), 0.2 μL of Ex Taq (5 U/μL), 2.5 μL of 10 × PCR buffer, primers 27f/1492r (20 μM) at 0.4 μl, 4 μL of template DNA (100 ng), and 15.5 μl of sterile water. The genomic DNA from the water sample was used as the template for amplification with final reaction concentrations of 1.5–3 ng/μL. The PCR amplification program contained a 5-min initial denaturation at 95 °C, followed by 5 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, 30 cycles at 92 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The 16S rRNA PCR clones were used to construct a library that was sequenced.

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar ratios and paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Illumina, SanDiego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Statistical analysis

The variation in the composition of the archaeal community inhabiting the ASP- and polymer-flooded production blocks was visualized using nonmetric multidimensional scaling (NMDS) with 1000 iterations, using the function metaMDS () of the VEGAN package in R (Van Geel et al., 2018). To explicitly test whether archaeal communities differed among different EOR application oil reservoirs, we used PerMANOVA (1000 permutations), applying the Adonis () function of VEGAN package in R on the archaeal OTU data matrix (Oksanen et al., 2017). The Wilcoxon rank-sum test was performed to determine the microbial populations with statistical differences. The relationships between environmental factors and archaeal communities were assessed via redundancy analysis (RDA), using the rda () function of the VEGAN package in R. The co-occurrence of operational taxonomic units

(OTUs) in archaeal communities across the two EOR production blocks was analyzed. To reduce the network complexity and facilitate the identification of the core soil community, we selected OTUs with more than 20 sequences for further analysis. The correlation between two OTUs was considered statistically robust if the Spearman correlation coefficient (ρ) was > 0.6 and the Benjamini Hochberg adjusted p-value was < 0.01 (Barberán et al., 2012). The visualization of the co-occurrence network was conducted using the Fruchtermann–Feingold layout of the interactive platform Gephi version 0.9.2. The within-module connectivity (Zi) and among-module connectivity (Pi) were used to divide the network nodes into four sub-categories: (1) nodes with Zi > 2.5 and Pi > 0.62 were defined as network hubs; (2) nodes with Zi < 2.5 and Pi < 0.62 were defined as peripherals; (3) nodes with Zi < 2.5 and Pi > 0.62 were defined as connectors; and (4) nodes with Zi > 2.5 and Pi < 0.62 were defined as module hubs (Olesen et al., 2007).

Results

Overall pyrosequencing information

After quality control, 1,936,270 sequences ranging from 202–549 bp (the length of most sequences was 445 bp) were obtained from Illumina MiSeq sequencing from 37 ASP-flooded production well samples, whereas 1,435,920 sequences ranging from 190–549 bp (the length of most sequences was 448 bp) were obtained from 29 polymer-flooded samples. Based on 97% similarity, 914 OTUs were detected (*Table A1*) that belonged to 8 phyla, 17 classes, 27 orders, 39 families, 57 genera, and 112 species. The species accumulation curves (*Fig. A1*) tended to reach saturation plateaus as the sample number increased, indicating that the number of archaeal sequences obtained represented the archaeal communities well.

Archaeal community composition in polymer-flooded and alkaline-surfactant-polymer (ASP)-flooded production wells

Six genera, including *Methanothermobacter* (18.11%), *Methanoculleus* (15.22%), *Methanolinea* (16.74%), *Methanofollis* (10.59%), *Methanobacterium* (8.50%), and *Candidatus Methanomethylicus* (5.60%) were predominant (relative abundance > 5%) in the polymer-flooded wells and accounted for 74.74% of the total sequences. Five genera, including *Methanothermobacter* (33.69%), *Methanosaeta* (16.70%), *Methanolinea* (13.14%), *Methanobacterium* (9.27%), and *Methanolobus* (7.58%) were predominant (relative abundance > 5%) in the ASP-flooded wells and accounted for 80.39% of the total sequences (*Fig. 1*).

The relative abundance of each archaeal taxonomic group varied between the two EOR technologies (*Fig. 2*). Remarkably, the relative abundance of archaeal genera associated with the polymer-flooded blocks differed significantly from that of the ASP-flooded production wells. Based on the Wilcoxon rank-sum test, the Methanosaeta phylotypes were less abundant in the polymer-flooded blocks than in the ASP production wells. Methanoculleus and Methanofollis were significantly higher in polymer- than in the ASP-flooded wells. The relative abundance of *Candidatus Methanomethylicus* and *Methanolinea*–the dominant archaeal populations in the water-flooded wells–decreased in the ASP-and polymer-flooded wells, whereas *Methanosaeta* became the dominant archaeal population in the ASP-flooded wells.

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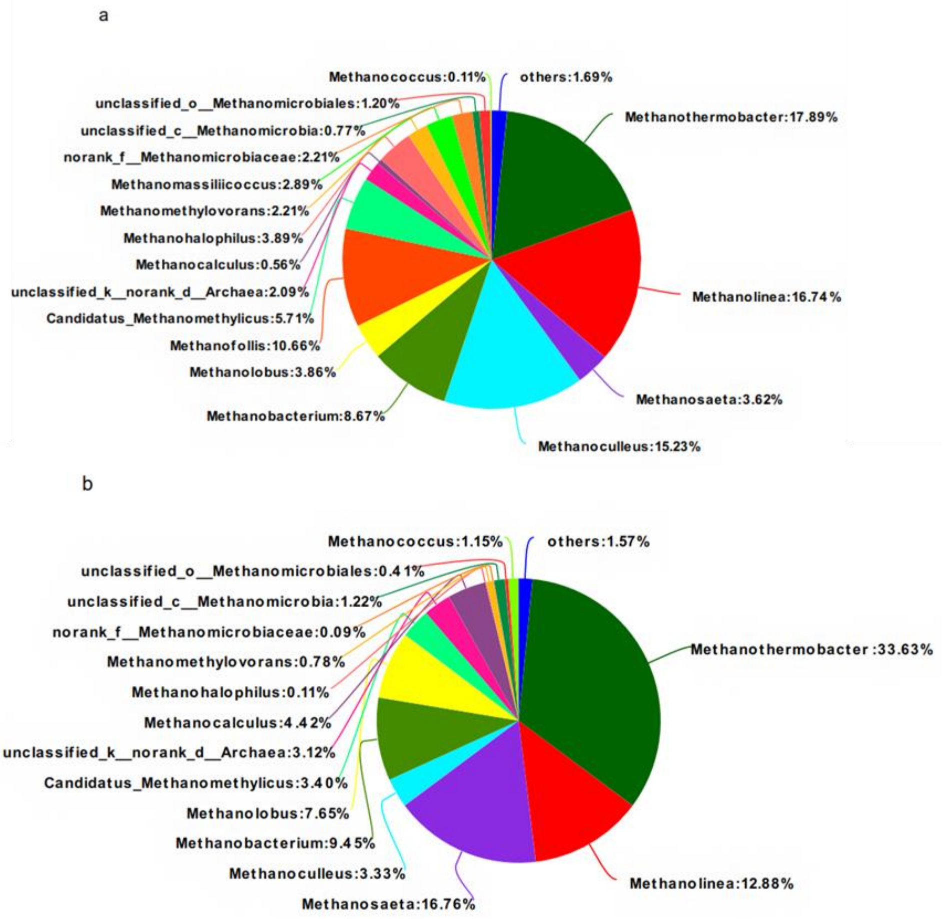

Figure 1. *Archaeal community compositions at the genus level in polymer flooded (a) and ASP flooded (b) production wells*

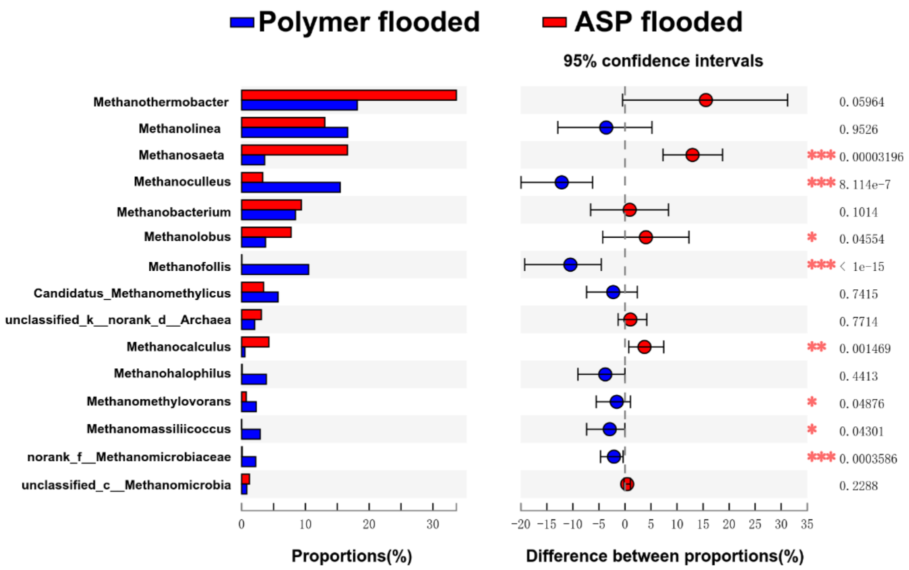

Figure 2. *Wilcoxon rank-sum test revealed the archaeal populations with significant differences in the relative abundance between water flooded, polymer flooded and ASP flooded production wells. *P < 0.05, **P < 0.01, ***P < 0.001*

NMDS ordination (*Fig. 3*) and PerMANOVA revealed a significant effect of EOR flooding technology on the taxonomic composition of archaeal communities ($R^2 = 0.0755$, $P = 0.001$). There was a clear separation between the archaeal communities in the polymer- and ASP-flooded wells from oil recovery.

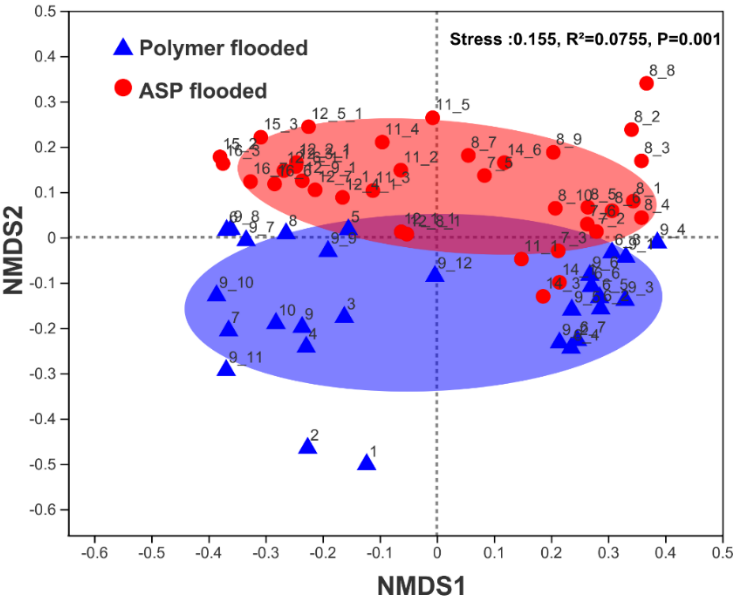

Figure 3. *Nonmetric multidimensional scaling (NMDS) ordination plot of archaeal communities from ASP flooded wells and polymer flooded wells. PERMANOVA analysis showed significant differences in archaeal communities between polymer flooded and ASP flooded production wells*

Archaeal co-occurrence network analysis

We applied correlation-based network analysis to explore the co-occurrence patterns of archaeal communities across two EOR practices by combining all archaea originating from each EOR technology production well (*Fig. 4*). The modularity indexes were 0.745 and 0.739 in the polymer- and ASP-flooded blocks, respectively (*Table A2*), revealing that the networks had a modular structure. The polymer-flooded network consisted of 539 nodes linked by 4665 edges. In contrast, the ASP-flooded network consisted of 689 nodes with 5031 edges. The genera in the network were primarily distributed into eight major genera, of which the most abundant were Methanosaeta (16.67%), Methanothermobacter (14.44%), Methanoculleus (14.25%), and Candidatus Methanomethylicus (8.21%). In the ASP network, Methanosaeta (19.71%), Methanothermobacter (17.10%), Candidatus Methanomethylicus (7.68%), and unclassified Archaea (11.15%) appeared most frequently, indicating environmental adaptation. Compared to that in the polymer-flooded network, the clustering coefficient of the ASP network decreased by 0.019, the network density of the polymer-flooded network increased by 0.011, and the average degree of the polymer-flooded network was higher than that of the ASP-flooded network (*Table A2*), indicating that the archaeal community structure was more complex in polymer-flooded than in ASP-flooded networks and that the application of ASP technology reduced the interspecific relationship of the archaeal community.

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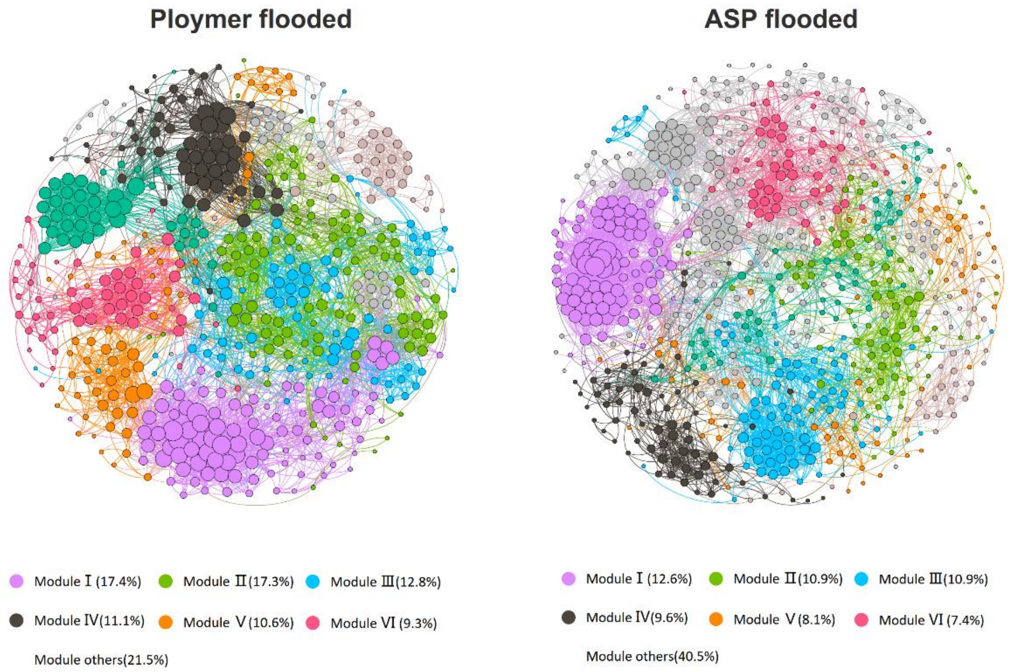

***Figure 4.** The co-occurrence patterns among OTUs revealed by network analysis. The nodes were colored according to different types of modularity classes, respectively. A connection stands for a strong (Spearman's r > 0.6 or r < −0.6) and significant (P-value < 0.01) correlation. The size of each node is proportional to the number of connections (i.e., degree)*

To evaluate the possible topological roles of taxa in the networks based on the within-module connectivity (Zi) and the among-module connectivity (Pi) values, we classified the network nodes into four categories: network hubs, module hubs, connectors, and peripherals (*Fig. A2*). Peripherals were the most abundant nodes in each network with most links inside their modules (*Fig. 4*). No network hubs or module hubs were identified in any of the two networks (*Fig. A2*). In contrast, multiple nodes were classified as connectors in polymer-flooded and ASP networks (*Table A3*). Seventy-five nodes, i.e., Methanosaeta (21 OTUs), Methanoculleus (14 OTUs), Candidatus Methanomethylicus (7 OTUs), Methanolinea (7 OTUs), Methanobacterium (5 OTUs), Methanothermobacter (3 OTUs), and others (18 OTUs), were defined as connectors in the polymer-flooded network. In contrast, the ASP network contained 66 connector nodes, primarily Methanosaeta (16 OTUs), Methanoculleus (7 OTUs), Candidatus Methanomethylicus (10 OTUs), Methanolinea (7 OTUs), Methanobacterium (5 OTUs), Methanothermobacter (3 OTUs), and others (18 OTUs).

Relationship between AMF and soil factors

The different EOR technologies had distinct effects on oil reservoir characteristics (*Table 1*). CO_3^{2-}, Na^+, and pH were significantly higher in ASP-flooded production wells than in polymer-flooded production wells ($p < 0.05$), whereas Ca^{2+} was significantly lower in polymer-flooded production wells. Cl^-, SO_4^{2-}, HCO_3^-, and Mg^{2+} between the polymer- and ASP-flooded processes were not significantly different. To further determine the environmental variables associated with changes in the archaeal community structure, RDA was applied to the contextual parameters in *Table A4*,

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revealing that the archaeal community structure was formed by primary environmental characteristics. The first two RDA axes explained 75.03% of the variance in the composition of archaeal communities among different EOR processes (*Fig. 5*). Forward selection in RDA revealed that pH (*P* = 0.011) was the strongest driver of archaeal community assembly in the oil reservoir.

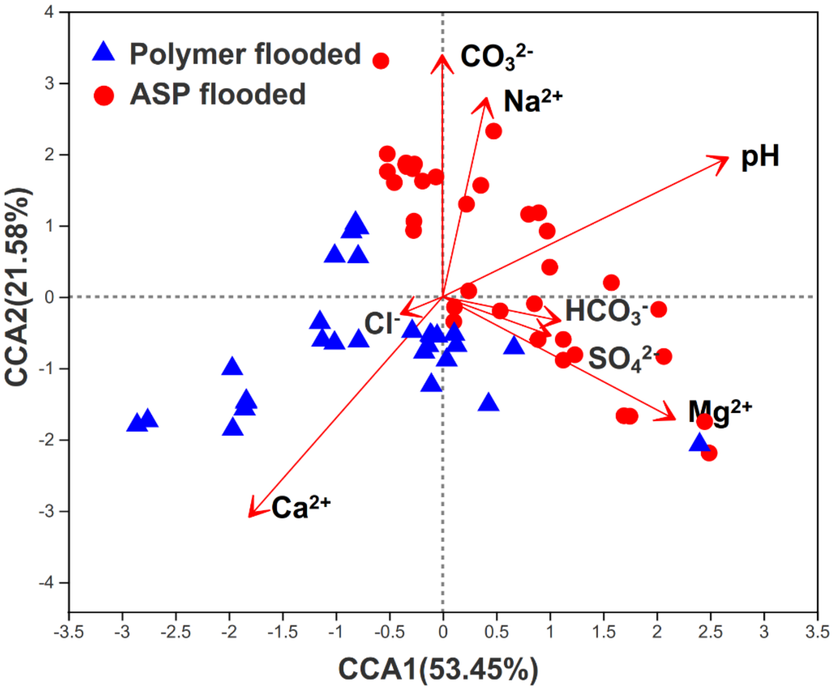

Figure 5. *Redundancy analysis (RDA) of the AMF community based on the OTU matrix from different treatments as affected by different EOR process. Different color points represent different treatments: Polymer flooded (blue) and ASP flooded (red). Arrows represent environmental factors*

Table 1. *Physical and chemical characteristics of samples collected from different EOR treatment*

	pH	**CO_3^{2-} (g/L)**	**HCO_3^- (g/L)**	**Cl^- (g/L)**	**SO_4^{2-} (g/L)**	**Mg^{2+} (mg/L)**	**Na^+ (g/L)**	**DOM (g/L)**	**Ca^{2+} (mg/L)**
Polymer	8.22 ± 0.46b	0.40 ± 0.06b	3.52 ± 0.43a	1.22 ± 0.35a	0.12 ± 0.08a	10.52 ± 1.12b	1.94 ± 0.20b	6.64 ± 0.56a	30.20 ± 2.54b
ASP	11.18 ± 1.09a	3.93 ± 1.4a	3.35 ± 0.19a	0.99 ± 0.24a	0.10 ± 0.01a	13.44 ± 0.96a	3.62 ± 0.21a	7.99 ± 1.12a	52.36 ± 1.37a

Data with different lowercase letters indicate significant levels at $P < 0.05$

Discussion

Archaeal communities in oil reservoirs have been investigated for many years (Zhao et al., 2012). This study investigated the influence of polymer and ASP flooding processes on the composition of archaeal communities inhabiting an oil reservoir exploited by decades of water flooding. The archaeal communities in the two EOR flooded oil reservoirs were dominated by *Methanothermobacteraceae* and *Methanosaetaceae*, in which *Methanothermobacter* and *Methanosaeta* were the principal groups (*Fig. 1*). Our results agreed with those of a previous investigation on

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archaeal communities in aqueous and oil phases from four production wells from the Chengdong petroleum reservoir in the Shengli Oilfield (Liang et al., 2018) and showed that *Methanothermobacter* was the most common archaea. In the polymer- and ASP-flooded production blocks, *Methanothermobacter*–a common type of *methanogen* in many high-temperature oil reservoirs–was the major division (Wang et al., 2012). *Methanothermobacter* is a type of hydrogenotrophic methanogen that produces methane from CO_2 (Wasserfallen et al., 2000). Some isolates use formate as an electron donor and sulfate as an electron acceptor (Wasserfallen et al., 2000). *Methanosaeta* and *Methanosarcina* are acetoclastic methanogens; *Methanosarcina* utilizes CO_2 and hydrogen to produce methane (Oren, 2014). The NMDS results suggested that the archaeal community structure was significantly different between polymer- and ASP-flooded production wells (*Fig. 3*) and corroborated previous investigations on microbial communities in diverse oil reservoirs, which showed that the microbial community was significantly influenced by reservoir environments. The bacterial and archaeal communities were remarkably affected by extreme reservoir environments, such as high temperature, steam soaking, high pressure, and hypersalinity (Gao et al., 2016, 2019). Crucially, the injection of alkali could result in an extremely alkaline environment in ASP-flooded oil reservoirs, with a pH of up to 11.18, which is much higher than the pH 8.22 of most polymers-flooded environments and exceeds the survival limits of most microbial populations. In the present study, archaeal communities were primarily affected by pH (*Fig. 5*). The strong correlation between pH and archaea distribution could be explained by the relatively narrow growth tolerance exhibited by most archaeal taxa. Indeed, each type of microorganism has an optimal pH value, and a slight change in pH might favor distinct archaeal taxa (Xue et al., 2017). The aforementioned factors made the environmental significance different in the polymer- and ASP-flooded production wells and affected the composition of the archaeal community.

The results of the co-occurring network analysis (*Fig. 4*) suggested the existence of non-random assembly patterns in archaeal communities in polymer- and ASP-flooded networks, as shown by the high modularity index of the network (*Fig. 4*). The non-random assembly pattern of the microbiota may be dominated by deterministic factors, such as nutritional and competitive interactions and niche differentiation (Barberán et al., 2012; Ju et al., 2014).

Compared to the ASP-flooded network, the clustering coefficient of the polymer-flooded network increased by 0.019 (*Table A3*). The average degree of polymer flooding increased by 2.71 compared to the ASP network (*Table A3*), indicating that archaeal associations were tighter in polymer-flooded than ASP-flooded production wells. This agrees with the results of our previous investigation on bacterial communities in diverse oil reservoirs: the bacterial community network was more complex in polymer-flooded than in ASP-flooded production wells (Ren G, 2020). The difference may be explained by changes in taxa that are sensitive to alkali, surfactants, or hydrolyzed polyacrylamide. In particular, the increase in the pH of formation water reduced the connections of the archaeal community in the network of the ASP-flooded blocks and may indicate that the pH of the formation water served as a major selective force in the EOR production blocks.

Multiple nodes were classified as connectors in the polymer- and ASP-flooded networks (*Table A4*). The significance of a taxon in a microbial network may be less influenced by its abundance and instead, depend on its connectivity (Peura et al., 2015). Euryarchaeota (70 OTUs nodes) and Nanoarchaeaeota (2 OTUs) were defined as connectors in the polymer-flooded network, whereas Euryarchaeota (58 OTUs) and Thaumarchaeota (2 OTUs) were defined in the ASP network. These “key” OTUs identified as hubs are rich OTUs, some of which are rare and may be important participants in ecosystem functions (Comte et al., 2016). In accordance with a previous study (2015), Keystone taxa, such as connectors, are defined by interaction with multiple members and are deemed to play important roles in the overall community (Berry and Widder, 2014).

Conclusions

This study revealed the characteristics of archaeal communities in polymer- and ASP-flooded production wells. Our results indicated that EOR technologies significantly altered the composition of the archaeal community and that different EOR methods significantly influenced the distribution of archaeal communities. Furthermore, the polymer flooding process could make the network of archaea communities more complex and tight than ASP flooding. Considering the critical roles of indigenous microbial communities, such as archaea, in microbial enhanced oil recovery (MEOR) process, revealing the influence of polymer flooding and ASP flooding on community composition and co-occurrence patterns of archaea could facilitate the implementation of MEOR technology in the future. Future work is needed to investigate how to use an upgraded method to stimulate the indigenous archaea, and increase the stability of archaeal co-occurrence patterns to enhance oil recovery.

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Conflict of interests. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interests.

Author contributions. WJL, QLN and RGL proposed and organized the overall project. WCJ, HM, BLH and QLN performed the majority of the experiments. WJL and RGL gave assistance in lab work and laboratory analyses. WJL and BLH wrote the main manuscript text. HM, WCJ and QLN contributed insightful discussions. All authors reviewed the manuscript.

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APPENDIX

***Table A1**. The rarefied sample OTU matrix data. See electronic appendix*

***Table A2.** Topological indices of each network in root AMF of the three grasslands*

	Polymer flooded	**ASP flooded**
Clustering coefficient	0.584	0.563
Modularity	0.745	0.739
Network density	0.032	0.021
Number of nodes	539	689
Number of edges	4665	5031
Average degree	17.31	14.60

Table A3. *Network nodes in polymer and ASP flooded production wells. See electronic appendix*

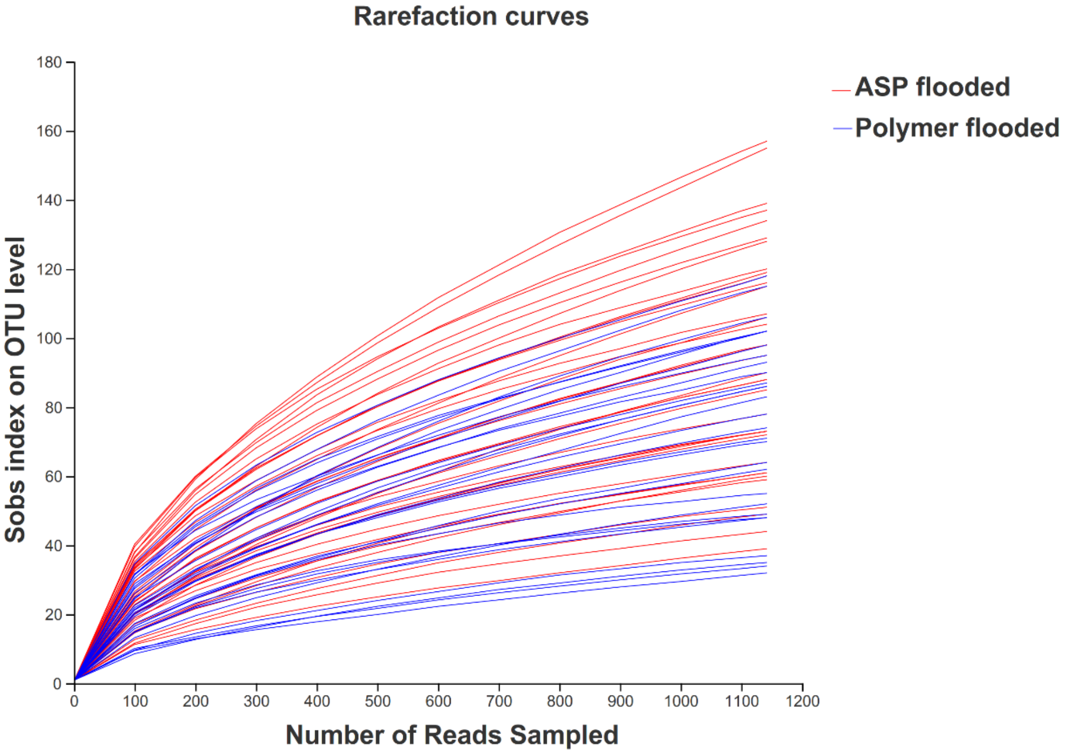

Figure A1. *Taxa accumulation curves of AMF in all samples*

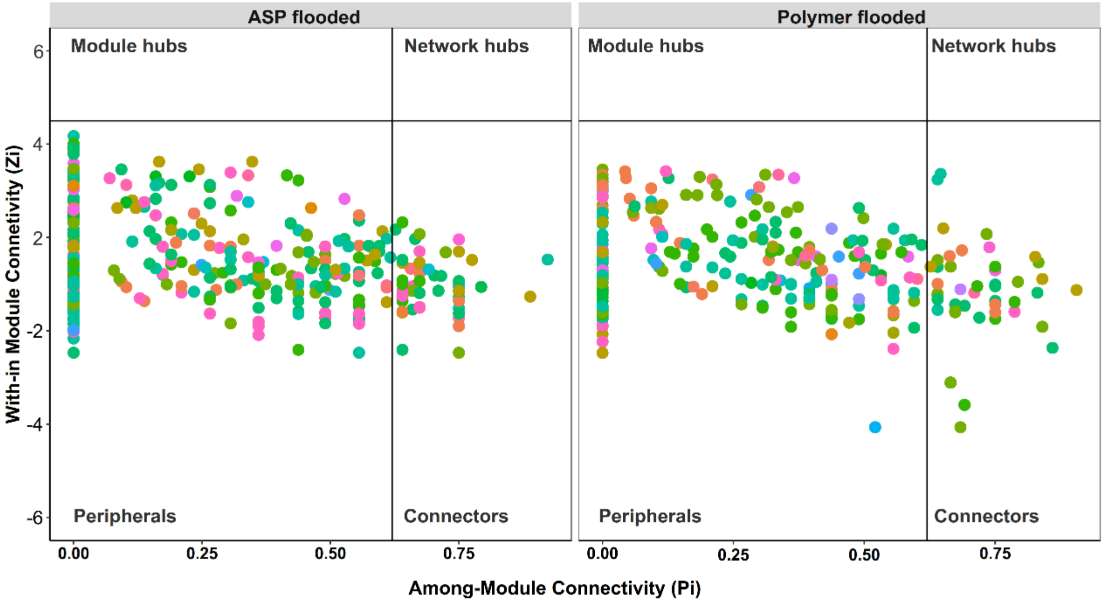

Figure A2. *Zi-Pi plot showing the distribution of OTUs based on their topological roles. Each symbol represents an OTU. The topological role of each OTU was determined according to the scatter plot of within-module connectivity (Zi) and among-module connectivity (Pi)*

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