Dear editor Jia

We thank you for the time and efforts to review our manuscript. We appreciate your comments, suggestions, and positive decision on our manuscript. We have addressed the issues raised according to your suggestions.

Sincerely yours,

Dr. Ting Ma

Authors' responses to editor Zhongjun Jia

Initial Decision: Publish subject to technical corrections (10 May 2015)

Comments to the Author:

The manuscript has been reviewed by two external experts and the Editor (myself). All reviewers find that your study is of interest for general readers, and the comments have been adequately addressed.

Before publication, there are few minor concerns for your attention as follows:

(1) Line 28. I guess it is not "injected" and "produced", but it is water in injection well and production. Please be careful about the usage of "produced" throughout the manuscript.

(2) Figure 6. please note that the circle should have been within box.

(3) Figure 6. please replace "I" with "bacteria", and replace "II" with "archaea"

Authors' response: Thank you for your comments and suggestions. We have made corrections in the revised manuscript. The changes were highlighted with color in the revised manuscript.

Differences in microbial community composition between injection and production water samples of water-flooding petroleum reservoirs

Author: P. K. Gao[1, 2], G. Q. Li[1, 2], H. M. Tian[1, 2], Y. S. Wang[1, 2], H. W. Sun[3], T. Ma[1, 2]

Affiliations:

[1] Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Tianjin 300071, P. R. China

[2] College of Life Sciences, Nankai University, Tianjin 300071, P. R. China

[3] College of Environmental Science and Engineering, Nankai University, Tianjin 300071, P. R. China

Corresponding author. T. Ma. Mailing address: College of Life Sciences, Nankai University, Tianjin 300071, P.R. China. Tel/Fax: 86-22-23498185.

E-mail: tingma@nankai.edu.cn

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Abstract. Microbial communities in injected water are expected to have significant influence on those of reservoir strata in long-term water-flooding petroleum reservoirs. To investigate the similarities and differences in microbial communities in injected water and reservoir strata, high-throughput sequencing of microbial partial 16S rRNA of the water samples collected from the wellhead and downhole of injection wells, and from production wells in a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir were performed. The results indicate that a small number of microbial populations are shared between the water samples from the injection and production wells in the sandstone reservoir, whereas a large number of microbial populations are shared in the conglomerate reservoir. The bacterial and archaeal communities in the reservoir strata have high concentrations, which are similar with those in the injected water. However, microbial population abundance exhibited large differences between the water samples from the injection and production wells. The number of shared populations reflects the influence of microbial communities in injected water on those in reservoir strata to some extent, and show strong association with the unique variation of reservoir environments.

Keywords 16S rRNA • Microbial community• Pyrosequencing • Miseq • MEOR

1 **Introduction**

Water-flooding is an efficient and least expensive oil recovery process that is employed worldwide. Water-flooding is believed to be a continued reinoculation of reservoir with surface microorganisms. After long-term water-flooding, microbial populations possessing exceptional survival abilities in injected water are expected to flow into oil-bearing strata, in where, exogenous and indigenous microbial populations form a new complex ecosystem (Zhang et al., 2012a). When injecting nutrients and air into reservoir strata, these microbial populations can be stimulated, and produce metabolites, such as polysaccharide, surfactants, acid, alcohol, and biogas. Because these metabolites can improve reservoir properties by blocking preferred water flow paths, lowering interfacial tension between brines and the oil phase, and decreasing oil viscosity (Youssef et al., 2009), microbial enhanced oil recovery (MEOR) have been applied to petroleum industry, and is currently studied extensively (Abdel-Waly, 1999;Zhang et al., 2012b;Bao et al., 2009;Gao et al., 2013;Li et al., 2014).

Microbial populations inhabiting petroleum reservoirs play critical roles in the microbial enhancing of the oil recovery process. As a result, an increasing number of studies, especially those based on culture-independent methods, have been performed, and broaden our knowledge of microbial diversity in oil reservoirs (Al-Bahry et al., 2013;Kumaraswamy et al., 2011;Lenchi et al., 2013;Okoro et al., 2014;Wang et al., 2012). However, to our best knowledge, detailed studies about the effects of the microbial communities in injected water on those in reservoir strata remains poorly understood. Based on the 16S rRNA gene clone library method, several studies to date have suggested that despite being flooded by the same injected water, there is a

significant difference in the communities between each production well (Tang et al., 2012;Ren et al., 2011). Zhang et al. compared microbial communities in samples of injection and production wells from reservoirs with different in situ temperatures, and pointed that the effects of microorganisms in the injected waters on microbial community compositions in produced waters are strong associated with reservoir temperature (Zhang et al., 2012a). However, because of the low throughput of the clone library method, many infrequent microbial taxa may not be detected, making it difficult to compare microbial communities in detail.

If microbial populations in injected water can flow into reservoir strata and reach production wells, is the microbial community in the injected water expected to have a similar community composition to those in the production wells? If there is a large difference in community composition, what is the difference and how many microbial populations are shared? To explore these issues, we investigated the microbial populations and their abundance in injection and production wells in a homogeneous sandstone petroleum reservoir with a permeability of 522×10^{-3} μm^2 and inter-well spacing of 300–425 m using 16S rRNA pyrosequencing and real-time fluorescent quantitative PCR (qPCR). At the same time, we analyzed microbial communities in water samples collected from the wellhead and downhole of injection wells, and from production wells in a heterogeneous conglomerate water-flooding petroleum reservoir with a permeability of 362×10^{-3} μm^2 and inter-well spacing of 100–150 m. High-throughput sequencing provides the opportunity to compare microbial populations with unprecedented levels of coverage and detail. The variation in permeability, inter-well spacing, and heterogeneity of the reservoirs is benefit for exploring the influence of reservoir physical properties on microbial distribution in

injected water and reservoir strata. The similarity among microbial communities was investigated using hierarchical clustering and Principal Coordinate Analysis. Microbial populations were also clustered according to injection and production wells to highlight the populations that showed the highest variability.

2 Materials and methods

2.1 Sampling locations

The Lu and Liu field block reservoirs are located in the Xinjiang Oil Field, in the Junggar Basin of Xinjiang Uygur Autonomous Region, Northwest China. The Lu field block is a homogeneous sandstone reservoir that has been water-flooded since 2001. The depth of the sampling horizon is approximately 1200 m with a temperature of 37^{o}C. The porosity of the reservoir is 29.9%, with an average permeability of 522×10^{-3} μm^2. The density of the crude oil is 0.846 g/cm^3, with an oil viscosity of 18 mPa•s. In the investigated well group (an injection well and four production wells), injection well Lu3084, located in the center of the production wells, has a direct influence on the neighboring producers, with inter-well distances of 300–425 m. The Liu field block is a heterogeneous conglomerate reservoir that has been water-flooded for approximately 30 years. The depth of the block horizon is approximately 1088 m, with a temperature of 22.6^{o}C. The porosity of the reservoir is 18.96 %, with an average permeability of 362×10^{-3} μm^2. The oil density is 0.912 g/cm^3, with an oil viscosity of 80 mPa•s. The selected well group includes two injection and three production wells, with an inter-well spacing of 100–150 m. The production well T90 is located at the center of injection wells T86 and T93, while production wells T95 and T96 are located at the edge of the field block and are mainly flooded by injection well T93 (Fig. 1).

The concentrations of potential nutrient factors, including crude oil properties, total nitrogen (TN), total phosphorus (TP), and ion concentration of formation brines, are listed in Table 2. The differences in geochemical parameters between crude oil samples from the two blocks are not obvious, indicating similar oil formation characteristics and maturity. The crude oil in both blocks had a higher content of saturates and aromatics, which favor the growth of hydrocarbon-degrading bacteria (HDB), and some anaerobes, such as sulfate-reducing bacteria. The cations and anions of the water samples in the two blocks were similar, with lower nitrogen and phosphorus content, which are essential for the survival and growth of microorganisms. The lack of nitrogen and phosphorus implies a low metabolism level of microorganisms in reservoir.

2.2 Water samples collection and DNA extraction

Based on tracer techniques, the time interval for injected water to flow from an injection well into neighboring production wells was approximately 30–45 days in the sandstone reservoir, and 7–10 days in the conglomerate reservoir (data provided by the Xinjiang Oil Field Company). Injected water from the sandstone reservoir was collected on three occasions every 15 days between October 2012 and November 2012, and the produced water samples (from the reservoir strata) were collected along with the second injected water sample on three occasions at a 30-day interval. All the injected and produced water samples were collected randomly from sampling valves located on the wellhead. In the conglomerate reservoir, the injected water samples were collected in November 2011 from the wellhead and the zone close to downhole (obtained by backflow, that is, the injected water flowed upward through the injection

well) of the injection wells. Seven days later, the produced water samples were collected from neighboring production wells on three occasions at a 7-day interval. The collected water samples were completely filled into 15 L sterilized plastic bottles, which were immediately capped and sealed to avoid contamination and oxygen intrusion.

For DNA extraction, the residual oil was first removed by heating the sample to 60°C for 30 min and by phase separation in sterilized separatory funnels. Microbial cells were then collected from 5 L of each water sample by centrifugation at 4°C for 15 min at 10,000 × g in a high-speed centrifuge (Beckman, CA 92821, USA). The cell deposits collected from the same sampling location were mixed and resuspended with TE buffer (Tris 80 mM, EDTA 40 mM, pH 8.0), and then lysed using a mini bead-beater (BioSpec, Bartlesville, OK 74005, USA) at 200 rpm for 1 min at room temperature with 0.1 mm glass beads. After bead beating, lysozyme was added (final concentration of 1 mg/ml), and the samples were incubated at 37°C for 1 h. Following the lysozyme treatment, 120 μL sodium-dodecyl sulphate (20% SDS, W/V) was added and the samples were incubated at 65°C for 60 min. Total genomic DNA was then extracted from the suspension solution using an AxyPrepTM Genomic DNA miniprep kit (Axygen Biosciences, Tewksbury, MA 01876, USA) according to the manufacturer's instructions and stored at -80°C for subsequent study.

2.3 Pyrosequencing of partial 16S rRNA genes and sequence analysis

Broadly conserved primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 533R (5'-TTA CCG CGG CTG CTG GCA C-3') were used to amplify the bacterial 16S rRNA gene, while primers 344F (5'-ACG GGG YGC AGC AGG CGC GA-3')

and 915R (5'-GTG CTC CCC CGC CAA TTC CT-3') were used to amplify the archaeal 16S rRNA gene. PCR reactions were performed following the protocol described in the Supporting Information (SI). Replicate PCR products of the same sample were mixed in a PCR tube. Then, the amplicons from each reaction mixture were pooled in equimolar ratios based on concentration and subjected to emulsion PCR to generate amplicon libraries. Amplicon pyrosequencing was performed on a Roche Genome Sequencer GS FLX+ platform at Majorbio Bio-Pharm Technology, Shanghai, China.

Sequences generated from pyrosequencing were analyzed using default settings in the open source software package mothur (Schloss et al., 2009). The detailed process is described in the SI. Alpha diversity analyses, including rarefaction and computation of the Shannon, Simpson, Chao1 metric, and phylogenetic diversity (PD), were used to assess biodiversity. The similarity among microbial communities was determined using UniFrac analysis in which weighted and unweighted Principal Coordinate Analysis (PCoA) were performed based on OTUs abundance or phylogenetic relationships. Specific differences in community composition of samples were visualized using heatmaps, ggplot, and Venn diagrams using the R software package.

2.4 Miseq-sequencing of partial 16S rRNA genes and sequence analysis

The bacterial and archaeal 16S rRNA gene V4 region (300–350 bp) were amplified using primer set 515f (GTG CCA GCM GCC GCG GTAA) and 806r (GGA CTA CHV GGG TWT CTA AT) with the protocol described by Caporaso et al. (Caporaso et al., 2011;Caporaso et al., 2012). A composite sample for sequencing was created by combining equimolar ratios of amplicons from the individual samples, followed by

gel purification and ethanol precipitation to remove any remaining contaminants and PCR artifacts. Amplicon sequencing was conducted on an Illumina MiSeq platform at Novogene Co., Beijing, China.

Pairs of reads from the original DNA fragments were merged using FLASH (Magoc and Salzberg, 2011). Sequences were then analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (Caporaso et al., 2010), and the UPARSE pipeline (Edgar, 2013). The detailed process is described in the SI. The similarity among microbial communities was determined using UniFrac analysis in which weighted PCoA was performed based on OTUs composition and phylogenetic relationships. Specific differences in community composition of samples were visualized using heatmaps, ggplot, and Venn diagrams using the R package.

2.5 Quantification of community abundance

Evaluation of community abundance by real-time fluorescent qPCR was performed using the 16S rRNA gene as a molecular marker. Reactions were performed using the FastStart Universal SYBR Green Master PCR mix in a Bio-Rad iQ5 Sequence detection system. The primer set 8F (5′-AGA GTT TGA T(CT)(AC) TGG CTC-3′)/338R (5′-GCT GCC TCC CGT AGG AGT-3′) were used to quantify bacterial community, while 806F (5′-ATT AGA TAC CCS BGT AGT CC-33′)/958R (5′-YCC GGC GTT GAM TCC AAT T-3′) were used to quantify archaeal community (Gittel et al., 2009). Ten-fold serial dilutions of a known copy number of plasmid DNA containing the target gene were subjected to real-time PCR in triplicate to generate an external standard curve. The PCR efficiency and correlation coefficients for the standard curves were higher than 95%, and R^2 values were greater than 0.99

for the curves. The specificity of the PCR amplification was determined by the melting curve. Gene copy numbers in unknown samples were determined based on standard curves.

2.6 Sequence accession numbers

The raw reads were deposited in the National Center for Biotechnology Information (BioProject ID: PRJNA246768, http://www.ncbi.nlm.nih.gov/bioproject/246768).

3 Results

3.1 Microbial community composition in the sandstone reservoir

Up to 4016–5060 bacterial and 2688–2857 archaeal sequences were obtained by pyrosequencing in the sandstone reservoir. These sequences were assigned into 249–538 bacterial and 45–130 archaeal OTUs at a 3% cutoff (Table S1). The individual rarefaction, Shannon, and Phylogenetic diversity curves tended to approach the saturation plateau (Fig. S1). The results of qPCR indicated that the copy number of bacterial 16S rRNA in water samples of the injection well was 8.25×10^6 copies ml^{-1}, while 1.5×10^6 to 2.75×10^6 copies ml^{-1} in the water samples of the production wells. Comparing with the bacteria, the number of archaea was about one percent of the bacterial number, with 3.75×10^4 16S rRNA copies ml^{-1} in injection well and 8.5×10^3 to 5.75×10^4 copies ml^{-1} in the production wells.

Phylogenetic analysis indicated that the injected water (Lu3084) was dominated by *Proteobacteria* (50.43%), *Cyanobacteria* (15.51%), and *Chloroflexi* (9.12%). Among the *Proteobacteria*, *Betaproteobacteria* (20.42%) and *Alphaproteobacteria* (19.63%) were numerically dominant, while a small quantity of *Deltaproteobacteria* (5.49%),

Gamaproteobacteria (4.44%), and *Epsilonproteobacteria* (0.32%) were detected (Fig. 2a I). The produced water from Lu3073 was dominated by *Proteobacteria* (65.35%) *Spirochaetes* (13.38%), and *Bacteroidetes* (12.38%). *Gammaproteobacteria* (23.96%), *Deltaproteobacteria* (22.16%), *Alphaproteobacteria* (13.47%), and *Spirochaetes* (13.38%) dominated at class level (Fig. 2a I). In the produced water from Lu3095, Lu1039, and Lu2180, *Proteobacteria* composed 78.58%–95.75% of the bacterial communities. *Alphaproteobacteria* (15.43%, 26.77%, 53.54%), *Betaproteobacteria* (23.48%, 50.57%, 12.94%), and *Epsilonproteobacteria* (2.79%, 4.38%, 25.54%) were dominant (Fig. 2a I).

To archaeal communities, more than 95% of the archaeal sequences were assigned to *Methanobacteria*, *Methanococci*, and *Methanomicrobia* (Fig. 2a II). In the injected water, 87% sequences were classed into *Methanomicrobia*, and the dominant genera were *Methanosaeta* (42.39%), *Methanomethylovorans* (25.57%), and *Methanolobus* (10.96%). Among them, *Methanosaeta* uses only acetate to produce CH_4. *Methanomicrobia* accounted for 84.03% in the produced water of Lu1039, and *Methanolobus* (83.46%) and *Methanococcus* (11.23%) were the dominant genera. The archaeal communities were much more conserved in the produced water at Lu2180, Lu3073, and Lu3095, with *Methanococcus* accounting for 95.34%, 90.79%, and 86.79%, respectively. The *Methanolobus* and *Methanococcus* are methylotrophic and hydrogenotrophic methanogens.

3.2 Microbial community composition in the conglomerate reservoir

Between 52719 to 129106 16S rRNA gene sequences were analyzed and assigned to 2623 to 3414 genus-level OTUs (Table S2). In combination with the relative

abundance, the number of bacterial and archaeal sequences was calculated, with the number of sequences per sample ranging in size from 51273 to 128980 and 85 to 1445, respectively (Fig. S2). Based on the results of qPCR, the copy number of bacterial bacterial 16S rRNA in the water samples ranged from 1.5×10^{7} to 6.5×10^{7} copies ml^{-1}, while archaeal 16S rRNA ranged from 4.5×10^{5} to 8.5×10^{5} copies ml^{-1}.

In contrast to the sandstone reservoir, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Spirochaetes*, and *Synergistetes* were simultaneously detected in the water samples from the injection and production wells, composing 85.7%–94.1% of all bacterial communities. Similar to the sandstone reservoir, more *Proteobacteria* were detected in water samples from production wells. At the class level, *Gammaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Bacteroidia*, *Bacilli*, and *Clostridia* composed 74.5%–83.7% of the bacterial communities in the water samples from the injection and production wells (Fig. 2b I). The archaea were mainly assigned to *Methanomicrobia*, *Methanococci*, *Methanobacteria*, *Thaumarchaeota*, *Parvarchaea*, and *Thermoplasmata* (Fig. 2b II). Among them, *Methanobacteria*, *Methanococci*, and *Methanomicrobia* were simultaneously detected, and composed 64.3%–94.6% of the archaeal communities in the water samples from the injection and production wells. Compared with the injected water collected from the wellhead of the injection wells (T86-0 and T93-0), more *Methanomicrobia* were detected in the downhole of injection wells (T86-8 and T93-7) and production well T90. At genus level, *Methanocorpusculum*, *Methanococcus*, and *Methanocalculus* were dominant, accounting for 60.3–88.5% of the archaeal communities in the injection wells and production well T90. The three taxa can use H_2 and formate as carbon sources to

produce CH_4.

3.3 Shared microbial populations between injected water and reservoir strata

The shared microbial OTUs and genera between communities in the water samples from the injection and production wells were investigated using Venn diagrams, histograms, and heatmap. Based on the Venn diagrams, 16.3%–32.81% of bacterial OTUs and 13.73%–51.61% of archaeal OTUs were shared between the water samples from the injection well and those of the production wells in the sandstone reservoir (Fig. 3a). These shared bacterial OTUs accounted for 4.6–24.71% of the total bacterial community in water samples from injection well, and 43.23–76.18% in the production wells (Fig. 4a I). Furthermore, only 13 bacterial and 3 archaeal OTUs were shared by both the water samples from the injection and production wells (Fig. 3a). Accordingly, the shared genera only accounted for 2.26% of the bacterial community in the injected water, but dominated each production well (12.02%–36.5%; Fig. 4a II). Similar to the bacteria, three archaeal genera belonging to *Methanobacterium*, *Methanococcus*, and *Methanolobus* were simultaneously detected, comprising 13.58% of the total archaea in the water samples from the injection well, and 90.4%–96.89% in the water samples from the production wells (Fig. 4a III).

In the conglomerate reservoir, most of the OTUs and genera were simultaneously detected in the water samples from the injection and production wells (Fig. 3b and 4b). Similar with the sandstone reservoir, these shared populations accounted for a minor proportion of the communities in the water samples collected from the wellhead of injection wells, but dominated the water samples obtained from the downhole of injection wells and each production well (Fig.3b).

315

316 **3.4 Microbial population distribution in injected water and reservoir strata**

317 Microbial populations were clustered according to injection and production wells to
318 highlight the populations that showed the most variability (Fig. 5). In the sandstone
319 reservoir, more *Sphingomonas* and *Azospirillum* were detected in the water samples
320 from the injection well, while *Arcobacter*, *Marinobacterium*, *Pseudomonas*,
321 *Hyphomonas*, *Novispirillum*, *Proteiniphilum*, *Spirochaeta*, and *Rhizobium* were
322 highly abundant in the water samples from the production wells. In the conglomerate
323 reservoir, higher amounts of *Paracoccus*, *Bacillus*, *Ochrobactrum*, *Parabacteroides*,
324 *Sphaerochaeta*, *Thauera*, *Halomonas*, and *Alcanivorax* were detected in the water
325 samples from the injection well, while *Arcobacter*, *Marinobacterium*, *Pseudomonas*,
326 *Bacteroides*, *Oleibacter*, *Marinobacter*, and *Shewanella* were dominant in the
327 downhole of the injection and production wells. Among them, *Marinobacterium*,
328 *Paracoccus*, *Ochrobactrum*, *Sphingomonas*, *Alcanivorax*, and *Azospirillum* are
329 aerobic bacteria, while *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*,
330 *Spirochaeta*, *Bacillus*, *Thauera*, *Halomonas*, and *Bacteroides* are microaerophilic
331 bacteria, facultative anaerobes, or anaerobes.

332

333 To further investigate the microbial distribution in injected water and reservoir strata,
334 hierarchical clustering and Unifrac PCoA were performed based on microbial OTUs
335 abundance and phylogenetic relationships. In the sandstone reservoir, hierarchical
336 clustering showed that the community in the water samples from the injection well
337 was distinct from those of the production wells (Fig. S3). Weighted PCoA
338 distinguished the bacterial community of the injected water from those of the
339 production wells, while communities of the production wells were placed at a

comparatively decentralized position (Fig. 6a bacteria). Similar to the bacterial communities, hierarchical clustering and PCoA distinguished the archaeal community of the injected water from those of the production wells, whereas production wells were placed at a close proximity (Fig. 6a archaea). In the conglomerate reservoir, communities of water samples collected from the wellhead of injection wells clustered into a group in the PCoA plot, indicating that communities remained unchanged before injected water flowed into the injection wells (Fig. 6b). Communities in the water samples collected from the downhole of injection wells and neighboring production well T90 clustered into one group, while production well T95 and T96 clustered into another (Fig. 6b). This shows that the microbial community reassembled during the process of the injected water flowing into the reservoir strata and each production well.

4 Discussion

MEOR technique is generally classified into exogenous MEOR and indigenous MEOR (Youssef et al., 2009). The former includes injection of exogenous microorganisms and injection of ex-situ produced products into reservoirs to enhance oil recovery (Zobell, 1947). This is an effective way to quickly improve oil recovery. However, because of the sieve effect of strata on microbial cells, the injected microorganisms are generally difficult to migrate into reservoir strata (Youssef et al., 2009;Brown, 2010). Diverse microbial populations inhabit petroleum reservoirs. Among them, hydrocarbon-degrading bacteria (HDB), nitrate-reducing bacteria (NRB), sulfate-reducing bacteria (SRB), and methanogens are the most important functional populations in reservoir ecosystems (Youssef et al., 2009). When injecting

nutrients into reservoir, these microbial populations can be stimulated and produce biosurfactants, organic acids, and gas, which improve oil recovery (Belyaev SS et al., 1998). Comparing with exogenous MEOR, indigenous microorganisms are more adapt to the environmental conditions present in reservoirs. Additionally, because nutrients can be easier to migrate into reservoir strata, indigenous MEOR has higher oil displacement efficiency. Despite the validity in field trial, indigenous MEOR also has some limitations, in particular, the uneven oil displacement efficiency in different production wells in the same reservoir block. The community composition and diversification have been found to have a significant influence on oil displacement efficiency (Li et al., 2014). Therefore, it is needed to investigate the relationship between microbial communities in injected water and reservoir strata, because microbial communities in injected water are expected to flow into oil-bearing strata, and produce a significant influence on those of reservoir strata in long-term water-flooding petroleum reservoirs (Youssef et al., 2009;Dahle et al., 2008).

Molecular methods have been widely used to assess the microbial diversity of petroleum reservoirs. Compared to the traditional 16S rRNA gene clone library and sequencing, high-throughput sequencing has generated hundreds of thousands of short sequences, and significantly improved our ability to compare microbial populations with unprecedented levels of coverage and detail (Caporaso et al., 2012). In the conglomerate reservoir, Miseq-sequencing produced approximately 52719 to 129106 16S rRNA gene sequences. The sequencing reads was approximately 10–20 folds of those (obtained by pyrosequencing) of the sandstone reservoir, and 50–400 folds of

387 the 16S rRNA gene clone library (assuming 300 clones per library). We
388 simultaneously sequenced the bacterial and archaeal V4 region of 16S rRNA gene,
389 obtaining a total of 51273–128980 bacterial sequences per sample, but only 85–1445
390 archaeal sequences. This is consistent with the count result for archaea, which are
391 about one percent of bacterial number. The result implies that deeper sequencing is
392 needed for detecting rare archaeal populations using Miseq-sequencing based on
393 bacterial and archaeal universal primer set 515f and 806r. In contrast, the bacterial and
394 archaeal communities were sequenced independently using pyrosequencing in the
395 sandstone and we obtained 4016–5060 bacterial and 2688–2857 archaeal sequences.
396 The rarefaction curves, Shannon diversity estimates, and observed species, suggesting
397 that this sequencing depth was enough for the investigation of the bacterial and
398 archaeal communities.
399
400 If the microbial populations in the injected water could flow into the reservoir strata
401 and reach the production wells along with the injected water, is the microbial
402 community in the injected water expected to have a similar community composition
403 with those in the production wells? In the homogeneous sandstone reservoir, we found
404 the number of shared bacterial and archaeal populations between the water samples
405 from the injection and production wells was different. As shown in Fig. 3a,
406 16.3%–32.81% of bacterial OTUs and 13.73%–51.61% of archaeal OTUs were
407 shared between the water sample of injection well and those of production wells. It is
408 reasonable to speculate that microbial populations in the injected water produce
409 different levels of impact on those in production wells. Based on the previous
410 research, the main reason may be the sieve effect that can be enhanced by the
411 long inter-well spacing (Ren et al., 2011). Because of this effect on microbial cells

when injected fluid passes through a subsurface formation, it is more difficult for microbial cells to migrate in reservoir strata. In contrast, almost all OTUs and genera detected in the injected water were also observed in downhole of the injection and neighboring production wells in the heterogeneous reservoir. Compared with the sandstone reservoir, this reservoir has a similar permeability, but shorter inter-well spacing. It appears that most microbial populations in the injected water flowed into the oil-bearing strata and reached the production wells during water-flooding process. Additionally, despite lacking for sufficient nutrients, bacterial and archaeal communities in the reservoir strata have high concentrations, which are similar with those in the injected water. This phenomenon implies some correlations of microbial communities in injected water and reservoir strata. However, we appreciate that it is less rigorous to delineate the transport of microbial populations in the reservoir strata simply by detecting the shared microbial populations in the injection and production wells using 16S rRNA sequencing, because this method is not able to demonstrate whether the species detected in the produced water are the same ones as in the injected water. Therefore, labelled strains, such as ones containing green fluorescent protein, may be a suitable way to investigate microbial migration in petroleum reservoirs.

The number of shared microbial populations reflects the influence of microbial communities in injected water on those in reservoir strata to some extent, and show strong association with the unique variation of reservoir environments. Differ with the sandstone reservoir, a large number of microbial populations were shared between the water samples of injection wells and those of production wells in the conglomerate reservoir. However, the community structure, in particular, the abundance of the

437 shared OTUs and genera, exhibited a large difference between the injected water and
438 reservoir strata. The environmental variables, such as salinity, pH, and nutrients, have
439 been supposed to be the primary drivers for the community diversification (Kuang et
440 al., 2013). However, few differences in cations and anions among the injected and
441 produced water samples were observed. Petroleum reservoir represents an anaerobic
442 environment with multiphase fluids of oil, gas and water. Therefore, except for the
443 sieve effect of reservoir strata on microbial migration, dissolved oxygen, which is
444 known to be strongly related to reservoir microbial growth and metabolism (Gao et al.,
445 2013), may be the main factor influencing the community structures. In both the
446 reservoirs, aerobic bacteria, including *Sphingomonas*, *Azospirillum*, *Paracoccus*,
447 *Ochrobactrum*, *Alcanivorax*, and *Hydrogenophilaceae* were more frequently detected
448 in water samples from the injection wells, while microaerophilic bacteria, facultative
449 anaerobes, and anaerobes, including *Pseudomonas*, *Rhizobium*, *Arcobacter*,
450 *Halomonas*, *Spirochaeta*, and *Bacteroides*, were found to have higher relative
451 abundance in reservoir strata (Fig. 5).
452
453 Apart from the dissolved oxygen, crude oil, in particular, the saturated and aromatic
454 hydrocarbon, may also strongly influence the microbial distribution in injected water
455 and reservoir strata. Petroleum reservoirs represent oligotrophic environments.
456 Although diverse microbial populations inhabit the reservoirs, only
457 hydrocarbon-degrading bacteria and some anaerobes, such as sulfate-reducing
458 bacteria, could grow with crude oil as carbon source. This is consistent with the
459 observed results that more hydrocarbon-degrading bacteria, including
460 *Marinobacterium*, *Pseudomonas*, *Rhizobium*, *Halomonas*, and *Oleibacter*, were
461 detected in downhole of injection and production wells.

This study compared the differences in microbial community composition between injected water and reservoir strata using microbial genomes obtained from the aqueous phase. In fact, each component of the reservoir multiphasic fluid, including crude oil, gases, and insoluble particles, may act as an important habitat for microbial growth in addition to the water phase within the petroleum reservoir (Kryachko et al., 2012;Kobayashi et al., 2012). Recent research has also compared microbial communities in aqueous and oil phases of water-flooded petroleum reservoirs, and found that the oil phase also harbored a large number of microorganisms, with large differences in the bacterial community between the aqueous and oil phases of the reservoir fluid (Wang et al., 2014). Therefore, simultaneous analysis of DNA extracted from both aqueous and oil phases may provide a better understanding of the microbial communities in injection and production water samples.

In summary, this study investigated the relationship shared by microbial communities in injected water and reservoir strata in two long-term water-flooding reservoirs. However, the results cannot provide any reliable information on the indigenous microbial community. The indigenous microbial populations may be those in newly drilled wells without water-flooding in the same oil-bearing block. However, the two reservoirs have been water-flooded for decades. Due to the introduction of exogenous microorganisms in injected water and other sources of contaminations by enhanced oil recovery processes, determining whether a microorganism is indigenous to a petroleum reservoir become increasingly difficult. This study implies that the number of shared populations reflects the influence of microbial communities in injected water on those in reservoir strata to some extent, and show strong association with the

unique variation of reservoir environments. However, it cannot make a conclusion on the transport of microbial populations in the reservoir strata by detecting the shared microbial populations in injected water and produced water samples using 16S rRNA sequencing. To further investigate the relationship shared by microbial communities in injection and production wells, injecting labelled strains containing marked gene (e.g., green fluorescent protein coded gene) into reservoirs may bring novel insight and greater predictive power. Therefore, further research on microbial diversification and transferability as injected water flows into reservoir is needed. Solving these problems is significant to guide the application of MEOR approaches based on injecting nutrients or microbial populations into reservoirs.

5 Conclusions

Using high-throughput sequencing, this study revealed the similarities and differences in microbial communities in water samples from injection wells and those from reservoir strata in a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir. The bacterial and archaeal communities in the reservoir strata have high concentrations, which are similar with those in water samples from injection wells. However, microbial community compositions exhibited large differences between the water samples from injection and production wells. The number of shared populations reflects the influence of microbial communities in injected water on those in reservoir strata to some extent, and show strong association with the unique variation of reservoir environments. Additionally, aerobic bacterial populations were more frequently detected in water samples from injection wells, while microaerophilic bacteria, facultative anaerobes, and anaerobes dominated the reservoir strata.

Acknowledgments

This study was supported by the National High Technology Research and Development Program of China (Grant No. 2013AA064402), the National Natural Science Foundation of China (Grant No. 41373074), the Chinese Postdoctoral Science Foundation (Grant No. 2014M561175), and the Key Technology Research and Development Program of Tianjin (Grant No. 14ZCZDSY00016).

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605
606

Figure captions

Fig. 1. Schematic diagram showing (a) the distribution of the injection and production wells of the Liu and Lu field blocks, and (b) the wellhead and downhole of injection and production wells, and the location where the water samples were collected. The injected water samples were collected from the wellhead and the zone close to downhole (obtained by backflow, that is, the injected water flowed upward through the injection well) of the injection wells. The water samples in reservoir strata were collected from the wellhead of production wells. T86, T93 and Lu3084 are the selected injection wells, and T90, T95, T96, Lu1039, Lu2180, Lu3073 and Lu3095 are the production wells.

Fig. 2. Relative proportion of microbial taxa at class level in the injected and produced water samples. (a) Sandstone and (b) conglomerate reservoirs. I: bacterial taxa at class level; II: archaeal taxa at class level; Lu3084, T86-0, and T93-0: water samples from the well head of the injection wells; T86-8 and T93-7: water samples from downhole of the injection wells; T90, T95, T96, Lu1039, Lu2180, Lu3073, and Lu3095: water samples from the well head of the production wells.

Fig. 3. Venn diagrams of the bacterial and archaeal OTUs in the injection and production wells. (a) Sandstone and (b) conglomerate reservoirs. I: bacterial OTUs; II: archaeal OTUs. Venn diagrams indicate the shared microbial OTUs between communities in the injected and produced water samples.

Fig. 4. Comparison of shared microbial genera between the injection and production wells. a-I: pairwise comparison between injection and production wells in the sandstone reservoir; a-II: shared bacterial genera in the injection and production wells; a-III: shared archaeal genera in the injection and production wells; b-I and

631 b-II: comparison between injection and production wells on the conglomerate
632 reservoir; and b-III: dominant shared bacterial genera in the conglomerate reservoir.
633 **Fig. 5.** Genera showing the most variability in the injected water and production wells.
634 (a) Sandstone and (b) conglomerate reservoirs. The black-bordered box indicates the
635 genera most detected in the production wells.
636 **Fig. 6.** Principal coordinate analysis of microbial communities used to investigate the
637 microbial distribution in injected water and reservoir strata. (a) Sandstone and (b)
638 conglomerate reservoirs. Bacteria: bacterial community distribution; Archaea:
639 archaeal community distribution. Sample points that are close together are more
640 similar in community composition than those that are far apart. The arrows in panel (b)
641 indicate the community succession during the process of the injected water flowing
642 into the injection wells and the neighbouring production wells.
643

644 **Table 1** Reservoir characteristics of Lu and Liu field block

Reservoir characteristics	Lu field block	Liu field block
Oil Reservoir		
Formation lithology	Sandstone	Conglomerate
Average depth (m)	1200	1088
Pressure (MPa)	10.2	7.2
Stratal temperature (^{o}C)	37	22.6
Average water content, %	80.8%	86.8%
Interwell distances, m	300-425	100-150
Average permeability, μm^2	522×10^{-3}	362×10^{-3}
Effective porosity, %	29.9	18.96
Water flooding (yr)	13	30
Crude oil properties		
Density (g/cm^3)	0.846	0.912
Viscosity in situ (mPa•s)	18	80.0
Saturates (%)	71.29	61.94
Aromatic (%)	14.85	11.24
Resin (%)	5.94	18.85
Asphalte (%)	5.94	7.97

645

646 **Table 2** Chemical properties of the water samples obtained from Lu and Liu field
647 block (Unit: mg/L)

Samples	Lu field block					Liu field block						
	3084	1039	2180	3073	3095	T86–0	T86–8	T93–0	T93–7	T90	T95	T96
Salinity	10850	11690	11170	10545	11102	10101	11313	11399	13991	13203	8997	9710
Nitrogen	15.1	11.5	10.6	12.7	11.6	7.6	6.8	10.2	11.5	5.7	6.3	8.5
Phosphorus	20.2	19.1	17.5	18.8	19.5	16.8	15.1	22.6	21.2	18.5	16.2	12.1
Na^+ K^+	4525	4803	4565	4309	4487	3364	3630	3802	4349	4014	3097	3139
Mg^{2+}	21.7	32.1	31.6	26.0	28.8	33.1	63.1	28.4	63.1	68.1	17.5	50.2
Ca^{2+}	191.3	281.9	284.7	181.6	216.4	70.2	77.9	72.8	78.0	96.2	86.4	108.7
Cl^-	5640	6125	5820	5160	5850	3010	3630	2922	3453	3099	3816	3406
SO_4^{2-}	116.2	14.04	4.86	23.13	8.86	483.4	89.4	523	362.4	89.9	65.6	165.1
HCO_3^-	356	434	464	846	511	3140	3823	4052	5687	5837	1915	2841

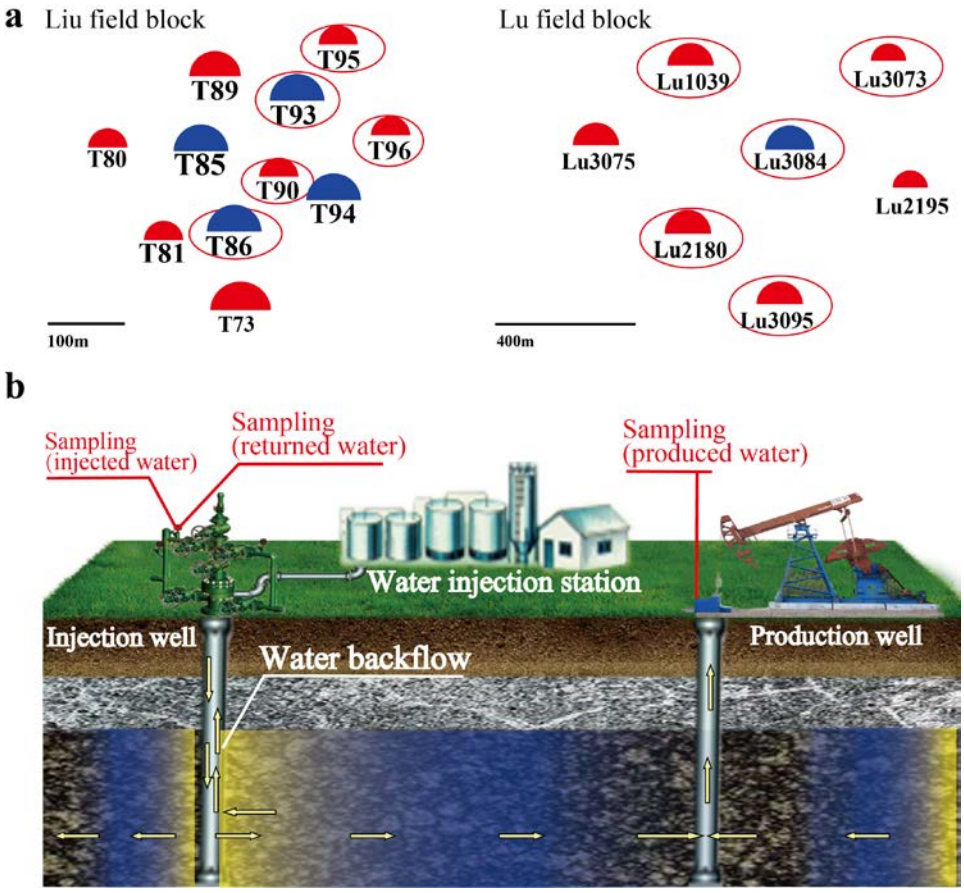

a
Liu field block
T95
T89
T93
T96
T80
T85
T90
T94
T81
T86
T73
100m
Lu field block
Lu1039
Lu3073
Lu3075
Lu3084
Lu2195
Lu2180
Lu3095
400m
b
Sampling
(injected water)
Sampling
(returned water)
Sampling
(produced water)
Water injection station
Injection well
Water backflow
Production well

Fig. 1

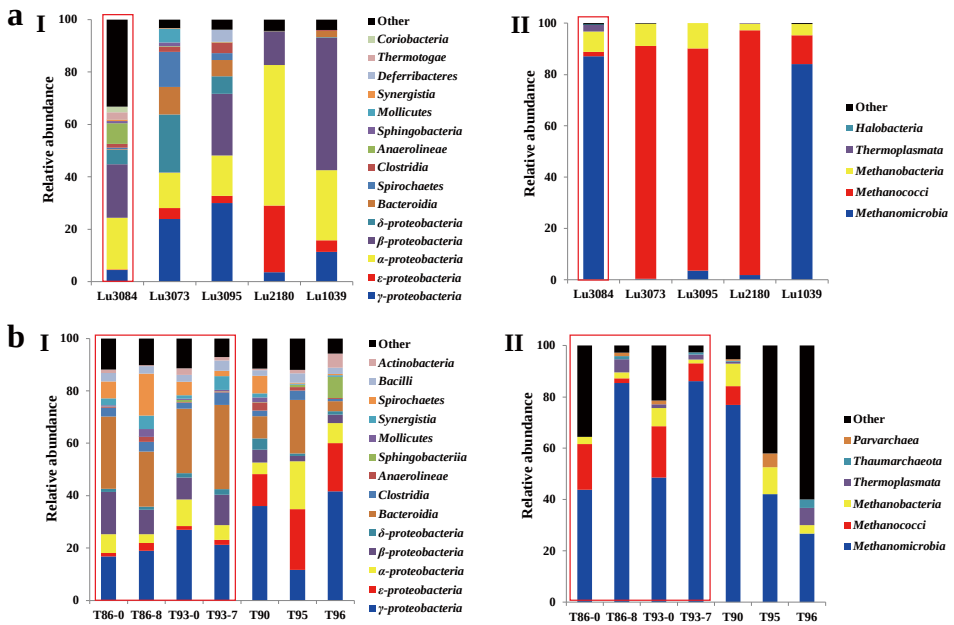

a
I
II
Relative abundance
100
80
60
40
20
0
Lu3084
Lu3073
Lu3095
Lu2180
Lu1039
Other
Coriobacteria
Thermotogae
Deferribacteres
Synergistia
Mollicutes
Sphingobacteria
Anaerolineae
Clostridia
Spirochaetes
Bacteroidia
δ-proteobacteria
β-proteobacteria
α-proteobacteria
ε-proteobacteria
γ-proteobacteria
Other
Halobacteria
Thermoplasmata
Methanobacteria
Methanococci
Methanomicrobia
b
I
II
Relative abundance
T86-0
T86-8
T93-0
T93-7
T90
T95
T96
Other
Actinobacteria
Bacilli
Spirochaetes
Synergistia
Mollicutes
Sphingobacteriia
Anaerolineae
Clostridia
Bacteroidia
δ-proteobacteria
β-proteobacteria
α-proteobacteria
ε-proteobacteria
γ-proteobacteria
Other
Parvarchaea
Thaumarchaeota
Thermoplasmata
Methanobacteria
Methanococci
Methanomicrobia

Fig. 2

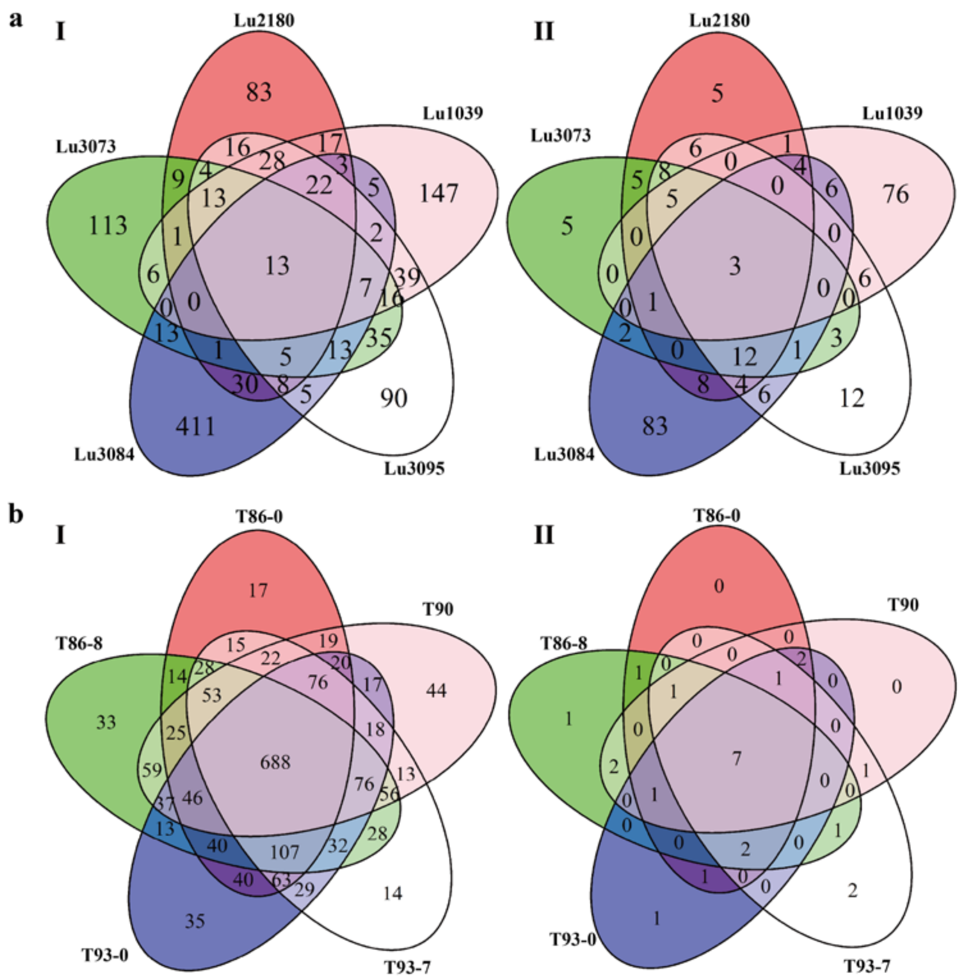
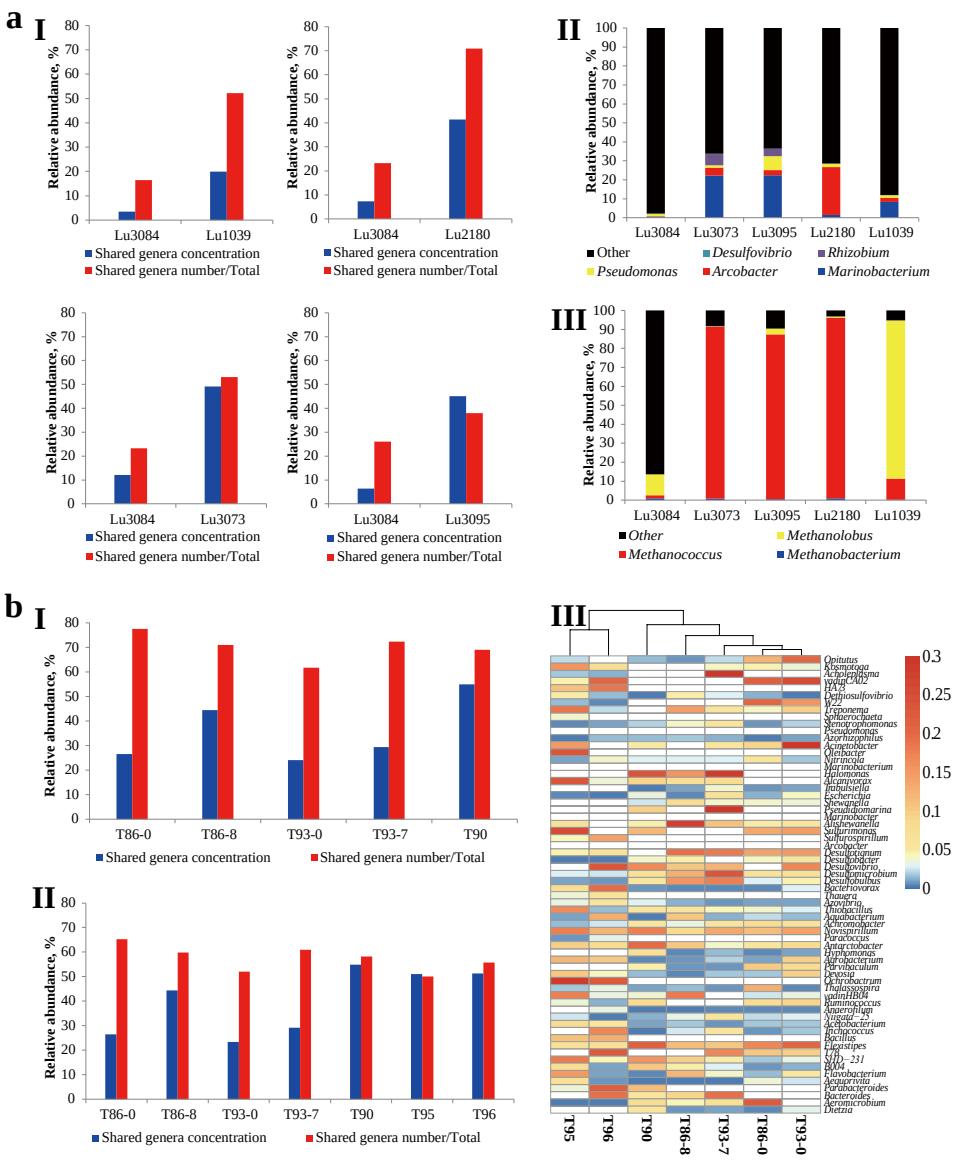

a
I
Lu2180
Lu1039
Lu3073
Lu3084
Lu3095
II
Lu2180
Lu1039
Lu3073
Lu3084
Lu3095
b
I
T86-0
T90
T86-8
T93-0
T93-7
II
T86-0
T90
T86-8
T93-0
T93-7

Fig. 3



657

658 **Fig. 4**

659

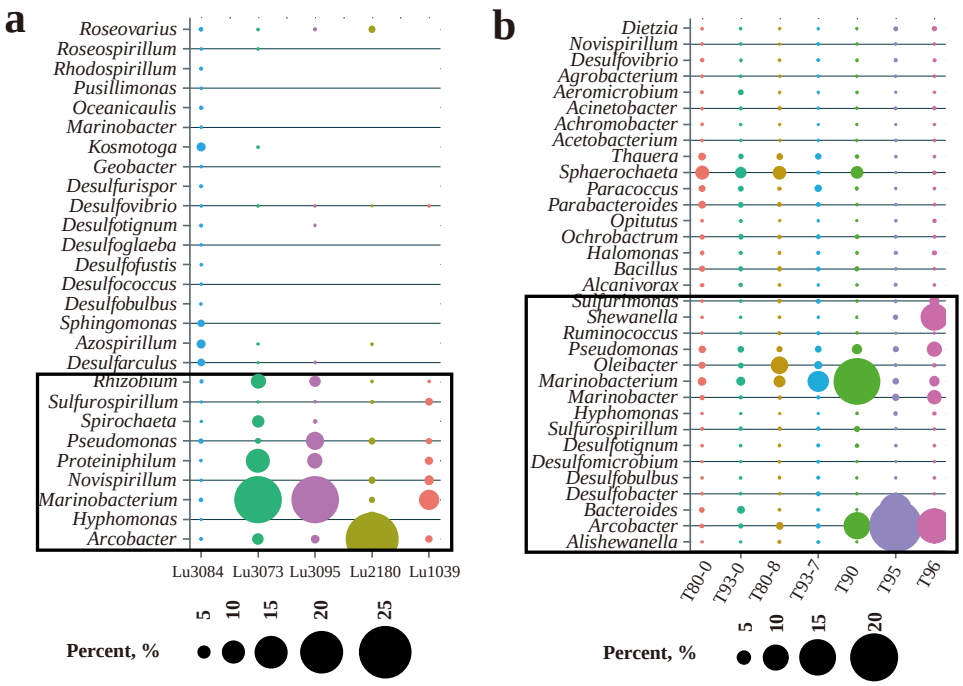
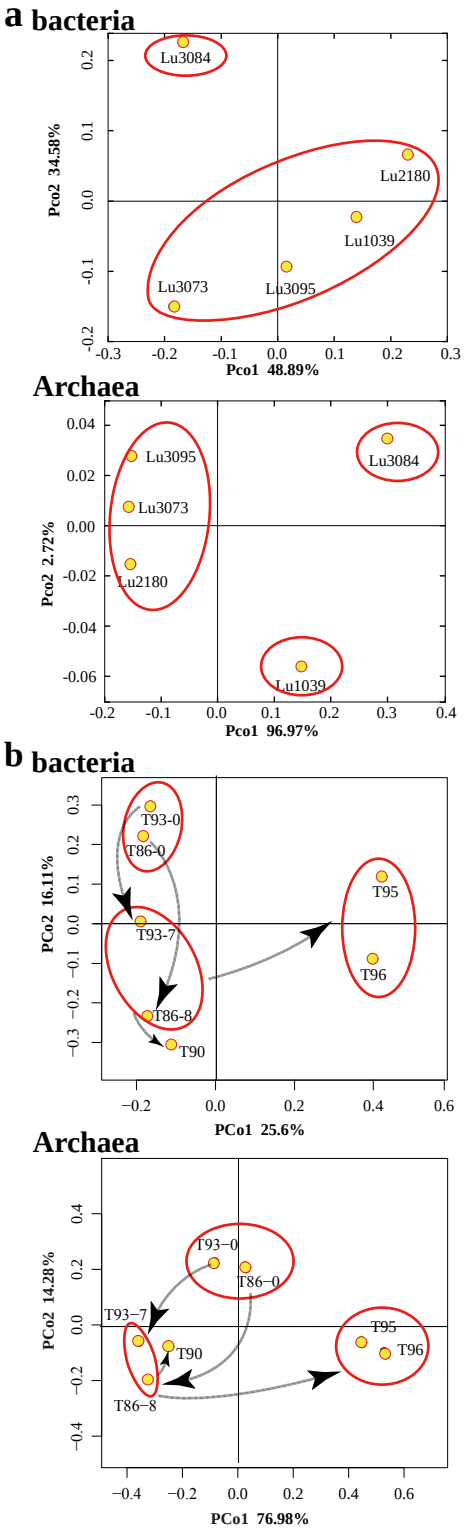

a
Roseovarius
Roseospirillum
Rhodospirillum
Pusillimonas
Oceanicaulis
Marinobacter
Kosmotoga
Geobacter
Desulfurispor
Desulfovibrio
Desulfotignum
Desulfoglaeba
Desulfofustis
Desulfococcus
Desulfobulbus
Sphingomonas
Azospirillum
Desulfarculus
Rhizobium
Sulfurospirillum
Spirochaeta
Pseudomonas
Proteiniphilum
Novispirillum
Marinobacterium
Hyphomonas
Arcobacter
Lu3084 Lu3073 Lu3095 Lu2180 Lu1039
Percent, %
5 10 15 20 25
b
Dietzia
Novispirillum
Desulfovibrio
Agrobacterium
Aeromicrobium
Acinetobacter
Achromobacter
Acetobacterium
Thauera
Sphaerochaeta
Paracoccus
Parabacteroides
Opitutus
Ochrobactrum
Halomonas
Bacillus
Alcanivorax
Sulfurimonas
Shewanella
Ruminococcus
Pseudomonas
Oleibacter
Marinobacterium
Marinobacter
Hyphomonas
Sulfurospirillum
Desulfotignum
Desulfomicrobium
Desulfobulbus
Desulfobacter
Bacteroides
Arcobacter
Alishewanella
T80-0 T93-0 T80-8 T93-7 T90 T95 T96
Percent, %
5 10 15 20

Fig. 5



663

664 **Fig. 6**

批注 [A1]: According to the editor's suggestion, we replaced "I" with "bacteria", and replace "II" with "archaea"