Dear editor and reviewers,

We thank you for your valuable time and constructive comments on our manuscript **bg-2014-521** entitled **"**The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs**"**. The comments were valuable in improving the quality of our paper, as well as guiding our future research.

We have carefully studied the comments, and the manuscript has been revised according to the reviewers' comments. Detailed answers to the comments are given below in the section Authors' Responses to Reviewers Comments and Suggestions. We hope that our revised manuscript will be considered suitable for publication in *Biogeosciences*.

Thank you for your time and efforts.

Sincerely yours,

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Authors' Responses to Reviewers Comments and Suggestions

Anonymous Referee 1

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General comments:

The manuscript entitled "The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs", by P.K. Gao and colleagues, describes the fluctuation of microbial communities along with injected water flowing into reservoir strata and production wells.

Although there are some interesting findings in this manuscript, it is not acceptable in its present form. I think that experimental design needs to be further improved, some of the interpretations should be better qualified and perhaps even modified. Occasionally, the text may be misleading, since the usage of English is sometimes not adequate. Hence, the manuscript should be carefully revised.

Our response: We thank you for the time and thought you put into reading our manuscript and for your helpful suggestions for improvement. Based on the comments and suggestions, we have revised our manuscript in an effort to improve it and address the concerns.

We agree with your comment that the experimental design and interpretations needed to be further improved. We have also made a readjustment to improve the precision of our conclusions based on the data obtained in this study.

Microbial enhanced oil recovery (MEOR) is generally classified into exogenous microbial flooding and indigenous microbial flooding. 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). Indigenous microbial flooding technique improves oil recovery by introducing oxygen and salts through water-based injection to stimulate reservoir microorganisms (Belyaev SS et al., 1998). Despite the validity in field trial, this technology also has some limitations, in particular, instability during microbial

flooding process. Microbial community diversification has been found to have a significant influence on oil displacement efficiency (Li et al., 2014).

Because that reservoir microbial populations and their metabolites play an important role in the enhancement of oil recovery, microbial community composition and distribution in physically and geochemically diverse reservoirs has been extensive studied (Al-Bahry et al., 2013;Kumaraswamy et al., 2011;Lenchi et al., 2013;Okoro et al., 2014). However, the relationship between microbial communities in injection and production wells remains poorly understood. We have therefore compared the differences of microbial community composition between injection and production water samples, and observed the microbial community diversification and succession as the injected water flows into the production wells. The results suggest that the microbial communities have significant differences between the injection and production water samples. Even if most microbial populations were shared, the relative abundance of shared populations exhibited large differences between the injected and produced water samples. Water backflow in the injection wells suggested that the microbial community was reassembled during the process of the injected water flowing into the production wells. As a result, we revised the manuscript title to "Differences in microbial community composition between injection and production water samples of water-flooding petroleum reservoirs".

Major comments:

Question 1*: * English should be significantly improved. This manuscript suffers from grammar errors and poor writing, particularly, in the Results and Discussion section.*

Our response: We have carefully revised the manuscript according to the comments. To improve the quality of the paper, the revised manuscript has been edited by an English Language Editing Service.

Question 2*: * The Title should be reconsidered so that it can directly present the novel findings to the readers.*

Our response: Thanks for your suggestion. The title of the manuscript has been revised to "Differences in microbial community composition between injection and

production water samples of water-flooding petroleum reservoirs".

Question 3: ** Experimental design: the authors chose a sandstone reservoir and a conglomerate reservoir in a Chinese typical oilfield, and analyzed the microbial population composition in injected water and produced water samples by using high-throughput sequencing technology, in order to test whether microbial populations in injected water could pass through oil-bearing strata. Overall the approach is straightforward. However, there are three major shortcomings in this study:*

(i)The lack of control: These two kind of old well groups have water flooded for 13- and 30-years. The negative controls are missing. The indigenous microbial community in the same oil-bearing strata cannot be overlooked. A better way is to determine oilfield water samples from newly drilled well for comparison in the same oil-bearing block.

Our response: We agree with your comment that negative controls, which may be water samples from newly drilled wells without water-flooding in the same oil-bearing block, are necessary to provide background information on indigenous microbial populations. Unfortunately, there are currently no such newly drilled wells in the two petroleum reservoirs. Conversely, because the two reservoirs have been long-term water-flooded, the indigenous microbial community in the subsurface might have been disturbed. Thus, it is difficult to obtain the reliable information on the indigenous microbial community.

We realize that it was less rigorous to delineate the transport of microbial populations in reservoir strata by only detecting the shared microbial populations in both injection and production wells using the 16S rRNA sequencing method, because the method is not able to distinguish whether the species detected in the produced water are the same as those in the injected water. To improve the precision of the conclusions based on the data obtained in this study, we have made a readjustment, which emphasizes the differences in microbial community composition between injection and production water samples. We hope this revision will meet with your approval.

(ii) Some important geological parameters are missing: In this manuscript, by comparison of Lu and Liu field block reservoirs, the authors concluded that injected water can pass through reservoir strata, but the reservoir heterogeneity, sieve effect of strata and dissolved oxygen affect the microbial migration. However, geological parameters such as source rocks and oil sources of Lu and Liu field, the characteristics of crude oil (heavy oil or light oil), reservoir pressures, the depths of Lu and Liu oil-bearing strata are missing. If there are great differences between Lu and Liu field block in these parameters, the comparison does not make sense.

Our response: Thanks for your suggestion. We have added the geological parameters to the manuscript. The reservoir characteristics are listed in Table 1.

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

Well number	**Lu3084**	**Lu1039**	**Lu2180**	**Lu3073**	**Lu3095**	**T86–0**	**T86–8**	**T93–0**	**T93–7**	**T90**	**T95**	**T96**
Well type	Injection well	Production well				Injection well				Production well		
Mineralization	10850	11690	11170	10545	11102	10101	11313	11399	13991	13203	8997	9710
Total nitrogen, mg/L	15.1	11.5	10.6	12.7	11.6	7.6	6.8	10.2	11.5	5.7	6.3	8.5
Total phosphorus, mg/L	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^+	4524.9	4803.1	4565.3	4308.7	4486.9	3364.3	3630.1	3801.6	4348.5	4014.2	3097	3139.8
Mg^{2+}	21.7	32.07	31.55	26.03	28.83	33.1	63.05	28.37	63.05	68.09	17.46	50.2
Ca^{2+}	191.3	281.9	284.7	181.6	216.4	70.18	77.98	72.78	77.98	96.17	86.36	108.66
Cl^-	5640	6125	5820	5160	5850	3010.24	3629.99	2921.7	3452.92	3098.77	3816.21	3405.92
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	434.1	464	846.29	511.4	3140.1	3823	4051.8	5686.9	5836.6	1914.9	2840.6

Although there are some differences in the reservoir characteristics of the Lu and Liu field blocks, the two reservoirs are both located in the Junggar Basin of the Xinjiang Uygur Autonomous Region, Northwest China. The differences in geochemical parameters between crude oil samples from the two blocks are not obvious, indicating similar oil formation characteristics and maturity (Table 1). The crude oil in both blocks has a higher content of saturates and aromatics, which facilitate the growth of hydrocarbon-degrading bacteria. The salinity of Lu block is approximately 11, 000 mg/L, which is similar to the value at Liu block. The cations and anions in the water samples in each block are similar, with a lower total nitrogen and total 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.

(iii) Environmental parameters such as dissolved oxygen (DO) or oxidation-reduction potential (ORP), subsurface temperature, and the composition of organic matter of crude oil (as substrate for fermentative bacteria and methanogenic archaea) have not determined in this study. These parameters are crucial for the fluctuation of microbial communities besides injected water. Therefore, it is also necessary to include these parameters in PCoA analysis. Without these information, the conclusion was untenable.

Our response: According to your suggestion, we have listed the subsurface temperature of the two reservoirs, the component of crude oil, and the concentrations of cations and anions in Table 1.

The subsurface temperatures of the two reservoirs are 37^{o}C and 22.6^{o}C, respectively. The concentration of nutrient characteristics was also measured, including crude oil properties, total nitrogen, total phosphorus, and ion concentration of formation water (Table 1). The ratio of saturates in the two reservoirs are 71.9% and 61.94 %, respectively, while the aromatic content is 14.85% and 11.24%. The resin and asphaltenes content is low. Among them, saturates and aromatics can be used as a carbon source for hydrocarbon-degrading bacteria (HDB), and some anaerobes, such as sulfate-reducing bacteria. The salinity of Lu block was approximately 11, 000 mg/L, which was similar to the value of the Liu block. The cations and anions among

the water samples in each block were similar, with a lower total nitrogen and total phosphorus content, which are essential for the survival and growth of microorganisms. The lack of nitrogen and phosphorus implies a low metabolism level of microorganism. We have provided this information in section “2.1 Sampling locations”.

Unfortunately, in situ oxygen concentrations were not measured at the time. However, microbial populations were clustered to highlight the populations that showed the most variability between the injected and produced water samples. We found that aerobic bacteria, including *Marinobacterium*, *Paracoccus*, *Ochrobactrum*, *Sphingomonas*, *Alcanivorax*, and *Azospirillum*, were detected in higher quantities in the injected water, while *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*, *Spirochaeta*, *Bacillus*, *Thauera*, *Halomonas*, and *Bacteroides* are microaerophilic bacteria, facultative anaerobes or anaerobes, that were dominant downhole of the injection and production wells. We think these data reflect the influence of dissolved-oxygen on microbial community diversification. Furthermore, Unifrac PCoA analysis was performed based on microbial OTUs abundance and phylogenetic relationships to extract and visualize the few highly informative components of variation from complex, multidimensional data. The results suggest the relative similarity and diversification of microbial communities in the injection and production wells. In the biplot, samples with similar community were placed at a close proximity, while samples with different community were placed at a comparatively decentralized position.

Question 4: ** Materials and methods: Amplicon sequencing was performed on two kinds of highthroughput sequencing platform (GS FLX+ and Miseq). Apparently, the outcomes must be different. Did the authors want to make a comparison? I cannot find any clue in this manuscript.*

Our response: Thank you for your suggestion. We have made a relevant discussion in the manuscript. The revised section is as follow: 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 depth was approximately 10–20 folds of pyrosequencing used in the sandstone reservoir, and 50–400 folds of the 16S rRNA gene clone library (assuming 300 clones per library). However, the current sequencing depth is still limited for detecting archaeal populations. As a result, we simultaneously sequenced the bacterial and archaeal V4 region of 16S rRNA gene, obtaining a total of 51273–128980 bacterial sequences per sample, but only 85–1445 archaeal sequences. This is consistent with the count result for archaea, suggesting the need for deeper sequencing for the detection of rare archaeal populations using this sequencing method. In contrast, the bacterial and archaeal communities were sequenced independently using pyrosequencing in the sandstone and we obtained 4016–5060 bacterial and 2688–2857 archaeal sequences. The rarefaction and Shannon curves tended to approach the saturation plateau, suggesting that this sequencing depth was enough for the detection of major bacterial and archaeal communities.

Question 5: ** Discussion: There is no "going home" feeling in this part. Too many hypothesizes were demonstrated. In situ DO in injected and production water and the composition of crude oil should be determined firstly. Then, the content of the true part of the "Discussion" should be carefully revised accompanying with more related new references.*

Our response: The concentrations of nutrient factors including crude oil properties, nitrogen, phosphorus, and ion concentration of formation water, were added in the manuscript (Table 1). The "Discussion" has been carefully revised accompanying with more related new references. The revised Discussion is as follow:

MEOR technique is generally classified into exogenous microbial flooding and indigenous microbial flooding. 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). Indigenous microbial flooding technique improves oil recovery by introducing oxygen and salts through water-based injection to stimulate reservoir microorganisms (Belyaev SS et al., 1998). Despite the validity in field trial, this technology also has some limitations, in particular, instability during microbial flooding process. Microbial community diversification has been found to have a significant influence on oil displacement efficiency (Li et al., 2014). Because that reservoir microbial populations and their metabolites play an important role in the enhancement of oil recovery, microbial community composition and distribution in physically and geochemically diverse reservoirs has been extensive studied (Al-Bahry et al., 2013;Kumaraswamy et al., 2011;Lenchi et al., 2013;Okoro et al., 2014). However, the relationship between microbial communities in injection and production wells remains poorly understood. We have therefore compared the differences of microbial community composition between injection and production water samples, and observed the microbial community diversification and succession as the injected water flows into the production wells.

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 depth was approximately 10–20 folds of pyrosequencing used in the sandstone reservoir, and 50–400 folds of the 16S rRNA gene clone library (assuming 300 clones per library). However, the current sequencing depth is still limited for detecting archaeal populations. As a result, we simultaneously sequenced the bacterial and archaeal V4 region of 16S rRNA gene, obtaining a total of 51273–128980 bacterial sequences per sample, but only 85–1445 archaeal sequences. This is consistent with the count result for archaea, suggesting the need for deeper sequencing for the detection of rare archaeal populations using this sequencing method. In contrast, the bacterial and archaeal communities were sequenced independently using pyrosequencing in the sandstone and we obtained 4016–5060 bacterial and 2688–2857 archaeal sequences. The rarefaction and Shannon curves tended to approach the saturation plateau, suggesting that this sequencing depth was

enough for the detection of major bacterial and archaeal communities.

The community structure exhibited large differences between the injected and produced water samples. Differences in microbial communities may result from a number of different factors. The niche-based processes are supposed to be the primary drivers for the community diversification, and environmental variables such as salinity, pH, nitrogen, and phosphorus identified as the major determinants of microbial community composition (Kuang et al., 2013). However, few differences in cations and anions among the injected and produced water samples were observed. Petroleum reservoirs represent extreme anaerobic environments with multiphase fluids of oil, gas and water. Therefore, the subtle differences in the reservoir strata, in particular, the permeability, porosity, and dissolved oxygen, may exert a significant influence on the microbial communities.

If the microbial populations in the injected water could flow into the reservoir strata and reach the production wells along with the injected water, is the microbial community in the injected water expected to have a similar community composition with those in the production wells? In the homogeneous sandstone reservoir, we found that most microbial populations detected in the injected water were not detected in the production wells. It is reasonable to speculate that the abundant microorganisms in the injected water do not reach the production wells in this sandstone reservoir. Based on previous research, the main reason for this may be the sieve effect that can be enhanced by the 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 the reservoir strata. In contrast, we found that almost all OTUs and genera detected in the injected water were also observed downhole of the injection and neighboring production wells in the heterogeneous reservoir, which has a similar permeability but shorter inter-well spacing, compared with the sandstone reservoir. It appears that most microbial populations in the injected water migrated into the oil-bearing strata and reached the production wells in the conglomerate reservoir. 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. To solve this

issue, labelled strains, such as ones containing green fluorescent protein, may be a suitable way to investigate microbial migration in petroleum reservoirs.

Compared with the sandstone reservoir, a large number of microbial populations were simultaneously detected in the injected and produced water samples in the conglomerate reservoir. However, the shared OTUs and genera accounted for a minor fraction of the injected water in both reservoirs, whereas they dominated the produced water in both reservoirs, suggesting that the microbial community was reassembled as the injected water flowed into the production wells. Dissolved oxygen, which is known to be strongly related to microbial growth and metabolism (Gao et al., 2013), may be the main factor influencing the community structures. Although in situ oxygen concentrations were not recorded in this study, more aerobic bacteria, including *Sphingomonas*, *Azospirillum*, *Paracoccus*, *Ochrobactrum*, *Alcanivorax*, and Hydrogenophilaceae were detected in the injected water, while microaerophilic bacteria, facultative anaerobes, and anaerobes, including Pseudomonas, Rhizobium, *Arcobacter*, *Halomonas*, *Spirochaeta*, and *Bacteroides*, were found to have higher relative abundance in the produced water (Fig. 5). Apart from the dissolved oxygen, another striking factor influencing microbial distribution in the injected water and the production wells may have been the crude oil, in particular, the saturates and aromatic components. Petroleum reservoirs represent oligotrophic environments. Although diverse microbial populations inhabit the reservoirs, only hydrocarbon-degrading bacteria and some anaerobes, such as sulfate-reducing bacteria, could grow with crude oil as carbon source. This is consistent with the observed results that more hydrocarbon-degrading bacteria, including *Marinobacterium*, *Pseudomonas*, *Rhizobium*, *Halomonas*, and *Oleibacter*, were detected downhole of injection and production wells.

This study compared the differences in microbial community composition between injection and production water samples 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.

This study investigated the relationship shared by microbial communities in injection and production water samples, and found the significant differences between microbial communities in the injection and production water samples. However, it is less rigorous to make a conclusion on the transport of microbial populations in the reservoir strata by the current results. To solve the problem, injecting labelled strains containing green fluorescent protein into reservoirs may bring novel insight and greater predictive power to investigate microbial migration in reservoir strata. Therefore, the 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.

Question 6: ** The conclusion is too long and should be carefully rewritten.*

Our response: According to your suggestion, we have carefully rewritten this section. The revised conclusion section is as below: Using high-throughput sequencing, we comprehensively surveyed the relationship shared by microbial communities in injection and production water samples in homogeneous sandstone and heterogeneous conglomerate reservoirs. The results suggest that the microbial communities have significant differences between the injection and production water samples. Even if most microbial populations were shared, the relative abundance of shared populations exhibited large differences between the injected and produced water samples. Water backflow in the injection wells suggested that the microbial community was reassembled during the process of the injected water flowing into the production wells.

Minor comments:

Question 1: ** Page 16774 Line 5-8: "The results indicated that the injected water: : : in this reservoir." This sentence needs to be rephrased.*

Our response: Thanks for your comment. We have made a readjustment to improve the preciseness of our conclusion based on data obtained in this study. The abstract was revised accordingly. The revised abstract is as follow:

The microbial community composition of water-flooding petroleum reservoirs is of great interest because it is strongly related to the enhancement of oil recovery. However, our knowledge about the relationship between microbial communities in injection and production wells is still very limited. The present study investigated the differences in microbial communities in 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. The results indicate that a small number of microbial populations are shared between the injected and produced water samples in the sandstone reservoir, whereas a large number of microbial populations are shared in the conglomerate reservoir. Consistently, the community structure exhibited large differences between the injected and produced water samples, with the shared populations accounting for a minor fraction of the injected water, but dominating the produced water in both reservoirs. This suggests that the community is reassembled as the injected water flows into the production wells. The results imply that microbial communities have significant differences between injection and production wells, in particular, the community composition and the relative abundance, which have a close relationship with the sieve effect of strata and the dissolved oxygen.

Question 2: ** Page 16777 Line 10-12: "All the injected and produced water samples were all collected from the wellhead of injection and production wells by the field personnel of PetroChina." Once the samples were collected, how long would be the genomic DNA extracted?*

Our response: The transportation from Xinjiang Oil Field to Tianjin is seven days. To avoid misunderstanding, we have made correction in the manuscript. The revised section is as below: "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. Following immediate transportation to the laboratory, 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)."

Question 3: ** Page 16779 Line 13-14: why do not quantitate archaeal populations? It would be helpful for the interpretation of methanogenic community later.*

Our response: Thank you for your suggestion. According to your suggestion, we have quantitated the number of archaeal populations using genome DNA preserved.

Question 4: *Page 16780 Line 3-4; Page 16781 Line 2-3: The diversity of the microorganisms in the sandstone reservoir (249-538) is much lower than it in the conglomerate reservoir (51273-128980). Why? In this respect, the geological and environmental condition of Lu and Liu field might be very different from each other.*

Our response: Thank you for your comment. We have made correction in the manuscript. The truth is that 4016–5060 bacterial sequences and 2688–2857 archaeal sequences were obtained by pyrosequencing in the sandstone reservoir, while 52719 to 129106 16S rRNA gene sequences were obtained by miseq-sequencing in the conglomerate reservoir. We have made discussion in the manuscript: 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 depth was approximately 10–20 folds of pyrosequencing used in the sandstone reservoir, and 50–400 folds of the 16S rRNA gene clone library (assuming 300 clones per library). However, the current sequencing depth is still limited for detecting archaeal populations. As a result, we simultaneously sequenced the bacterial and archaeal V4 region of 16S rRNA gene, obtaining a total of 51273–128980 bacterial sequences per sample, but only 85–1445 archaeal sequences. This is consistent with the count result for archaea, suggesting the

need for deeper sequencing for the detection of rare archaeal populations using this sequencing method. In contrast, the bacterial and archaeal communities were sequenced independently using pyrosequencing in the sandstone and we obtained 4016–5060 bacterial and 2688–2857 archaeal sequences. The rarefaction and Shannon curves tended to approach the saturation plateau, suggesting that this sequencing depth was enough for the detection of major bacterial and archaeal communities.

Question 5: ** Page 16781 Line 17: The word of "botained" should be revised to "obtained".*

Our response: Thanks for reminding us. We have made a correction in the manuscript.

Question 6: ** Page 16784 Line 17: "LR, 2010" should be revised to "Brown, 2010".*

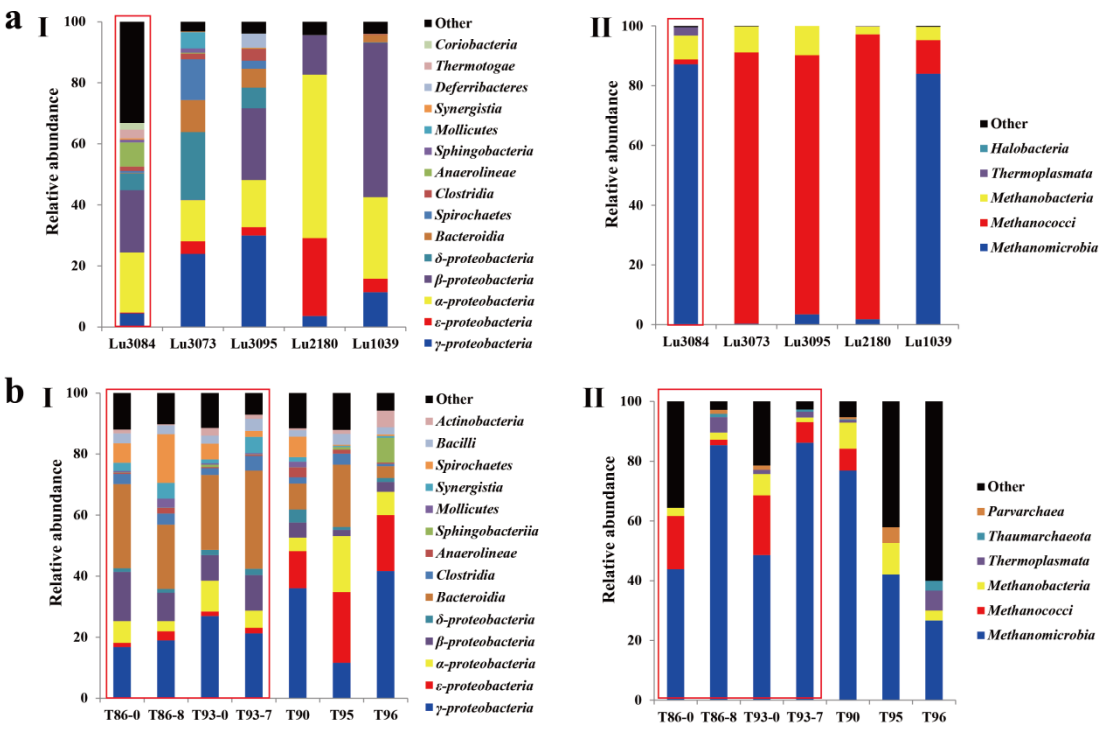
Our response: Thanks for reminding us. We have made correction in the manuscript.

Question 7: ** Page 16791 Table 1: I guess the temperature was the surface temperature. The temperature of oil-bearing strata should also be given.*

Our response: Thank you for your comment. The listed temperature (37 ^{o}C and 22.6 ^{o}C) is the subsurface temperature of the two reservoirs.

Question 8: ** Page 16793 Figure 2: The color representing a designated microbial class should be consistent so that it is easy for comparison. (e.g. The color of Methanococci is blue in Fig. 2aII but red in Fig. 2bII.)*

Our response: Thank you for your suggestion. We have made correction in the revised manuscript. The revised figure is listed below:


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
Halobacteria
Thermoplasmata
Methanobacteria
Methanococci
Methanomicrobia
b
I
II
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
Parvarchaea
Thaumarchaeota
Thermoplasmata
Methanobacteria
Methanococci
Methanomicrobia

Short comments from Prof. Jidong Gu

Received and published: 2 Jan. 2015

General comments:

This published paper on 'The shift of microbial population composition accompanying the injection water flowing in the water-flooding petroleum reservoirs' by Gao et al. certainly shows some descriptive information on the possible transport of microorganisms through oil reservoir subsurface sandstone materials.

Our response: Thank you for reviewing our manuscript, and your constructive comments and suggestions. Based on the comments and suggestions, we have been revising our manuscript in an effort to improve it.

***Question 1**: * I have to say that the title does not fit with the data obtained because the samples did not include a nonintervention control to allow assessment of the indigenous population for a meaningful comparison. Without this critical sample and information, the transport of bacteria is a claim not supported by convincing data*.

Our response: We thank the referee for this constructive advice, which has been important and valuable in improving the manuscript quality.

We agree with your comment that it is less rigorous to delineate the transport of microbial populations in reservoir strata by detecting the shared microbial populations in both injection and production wells using a 16S rRNA sequencing method. Unfortunately, because the two reservoirs have been long-term water-flooded, the indigenous microbial community in the subsurface might have been disturbed. Thus, it is difficult to obtain the reliable information on the indigenous microbial community. We have made a readjustment to improve the precision of our conclusions based on the data obtained in this study.

Because that reservoir microbial populations and their metabolites play an important role in the enhancement of oil recovery, microbial community composition and distribution in physically and geochemically diverse reservoirs has been extensive studied (Al-Bahry et al., 2013;Kumaraswamy et al., 2011;Lenchi et al., 2013;Okoro et al., 2014). However, the relationship between microbial communities in injection and

production wells remains poorly understood. We have therefore compared the differences of microbial community composition between injection and production water samples, and observed the microbial community diversification and succession as the injected water flows into the production wells. The results suggest that the microbial communities have significant differences between the injection and production water samples. Even if most microbial populations were shared, the relative abundance of shared populations exhibited large differences between the injected and produced water samples. Water backflow in the injection wells suggested that the microbial community was reassembled during the process of the injected water flowing into the production wells. As a result, we revised the manuscript title to "Differences in microbial community composition between injection and production water samples of water-flooding petroleum reservoirs".

***Question 2**: * In the text, the differences of detected pyrosequences between injection water and production water were used as the key variables to delineate the transport (migration) of microorganisms, a major shortcoming with this approach is that some microorganisms will not survive the subsurface environmental conditions due to lack of oxygen, nutrients etc. the approach used in this research plan should be reconsidered.*

Our response: Thanks for your suggestion. Since Bastin et al. firstly isolated sulfate-reducing bacteria (SRB) from reservoir in 1926 (Bastin, 1926), culture-independent methodologies, such as fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), quantitative PCR, 16S rRNA clone libraries, and high-throughput sequencing have revealed diverse microbial populations inhabiting petroleum reservoirs. Although these methods could not delineate survive and activity of reservoir microbial populations, culture-dependent methods combination with culture-independent methods have demonstrated the existence and activity of hydrocarbon-degrading bacteria (HDB), nitrate-reducing bacteria (NRB), sulfate-reducing bacteria (SRB), and methanogens in injection and production water samples. On the other hand, even there is no metabolic activity in the subsurface environmental conditions due to lack of oxygen and nutrients, these populations may

lie dormant in reservoir.

To improve the precision of our conclusions based on the data obtained in this study, we have made a readjustment. The study was performed to illustrate the relationship shared by microbial communities in the injection and production water samples. We think that the high-throughput sequencing can distinguish the differences between microbial communities in water samples collected from wellhead or downhole of injection wells, and production wells.

***Question 3**: * First of all, I am sorry to say that the quality of this manuscript writing is low and it is hard to read the text for accurate meaning and the precise information. The writing needs extensive efforts and time to revise to reach to a reasonable level of acceptance. Authors must work hard on this and serious because the results of the information can be compromised seriously when the statements cannot be comprehended well enough by reading*.

Our response: Thanks for your suggestion. We will work hard on writing in this and our future paper. To improve the quality of the paper, the revised manuscript has been edited by an English Language Editing Service.

***Question 4**: *There is little or any disagreement now that oil reservoirs have indigenous population of microorganisms, but non-indigenous microorganisms are introduced into the reservoir systems when water flooding is introduced. It is always a big challenge to obtain the truly indigenous population of microorganisms in the reservoirs because of the difficulties involved in non-contamination sampling of the subsurface environment without any potential contamination. In a similar but different aspect, the physical characteristics of the subsurface materials, either heterogenous or homogenous as stated in this paper is also a term of personal choice here than substance because of their natural origin and heterogeneity no matter called heterogenous or homogenous. Heterogeneity is the true nature of such materials. Therefore, I have concern on the choice of 'homogeneity' and 'hererogeneity' simply based on the average permeability values because this value is an average numerical number, which cannot be used reliably for transportability of*

bacteria. Considering the differences in permeability between the two blocks, there should be no disagreement on bacteria can be transported in both subsurface systems, but the rate of transport may be different. If this is the case, what is the key scientific information that can be extracted from the selection of the 2 blocks in this investigation? If the injection of water had only started with this study, the collected water/oil samples can be of some meaning interpretation, but I do not think such is the case with this set of production wells.

Our response: Thanks for your comment. As you pointed out, the two reservoirs have been water flooded 13- and 30-years, non-indigenous microorganisms might be introduced into the reservoir systems, and the indigenous microbial community in the subsurface might have been disturbed. Thus, it is difficult to obtain the reliable information on indigenous microbial community, even if we obtained water samples from newly drilled well. We planned to delineate the transport of microbial populations in reservoir strata by detecting the shared microbial populations in both injection wells and production wells using a 16S rRNA sequencing method. We now realize that it is less rigorous, because it is not able to demonstrate whether the species detected in produced water are the same ones in the injected water. Based on the data obtained in this study, we have made a readjustment to improve the preciseness of the manuscript. Because the data illustrated the relationship shared by microbial communities in the injection and production water samples. We think it may be better to compare the differences of microbial community composition between injection and production water samples. We hope the revision will meet your approval.

Question 5*: *The 'approximately 30-45 days and 7-10 days, respectively' – I have no way of knowing what do they refer to by the sentence because no designation was offered. I may assume they are associated with the heterogenous and homogenous reservoirs, but such assumption should not be the responsibility of the readers and they must be clearly stated by the authors to avoid any misunderstanding.*

Our response: Thanks for your suggestion. We have revised the sentence as "Tracer technique indicated that the time intervals for injected water from injection well flowing into neighboring production wells are approximately 30–45 days in the sandstone reservoir, while 7–10 days in the conglomerate reservoir."

***Question 6**: *Sampling procedures were inadequately described and I am especially troubled by the statement ': : :by the field personnel of PetroChina.' because the quality of the samples may be compromised for one. In addition, how can the authors interpret the results when they are not involved in the in situ sampling to know the detail steps involved and the effects on the results obtained?*

Our response: Thanks for your suggestion. Actually, we involved in the in-situ sampling, and the sampling process was assisted by the field personnel of Oil Field.

To avoid Misunderstanding, we have made correction in the revised manuscript. The revised section is: "All the injected and produced water samples were collected randomly from sampling valves located on the wellhead. The water samples were completely filled into 15 L sterilized plastic bottles, which were immediately capped and sealed to avoid contamination and oxygen intrusion."

***Question 7**: * Further on the sampling for concentration of bacterial cells, oil/water mixture should separate the oil from the mixture and then concentrate the cells from water phase or both oil and water phases. This detailed information show the understanding of the system you are dealing with and the quality of the cells you would be obtained. Are there any differences in terms of the composition and richness of microbial groups associated with the oil and water phases? Why was the oil phase not treated for extraction of DNA in the similar way as water phase? Actually, recent publication(s) has/have some information on this topic and you should also cited the work here. Were there any quality controls in the extraction of genomic DNA and PCR amplification?*

Our response: This is a good inspiration to our future research in this direction. According to your suggestion, we have looked through the recent publications, which compared the similarities and differences of microbial communities in oil phase and water phase.

We have made a relevant discussion in the manuscript: This study compared the differences in microbial community composition between injection and production water samples 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.

***Question 8**: * 'In the sandstone reservoir' – I do not agree with you to have such a statement and claim simply because there is no strictly control, which did not have any water flooding to show the indigenous population and composition. If the objectives of this study are on migration of microorganisms in subsurface sandstone, I do not think the experimental design can answer the questions effectively. This is a key point in Discussion, I have strong reservation in accepting this. The high-throughput used can be sensitive for detection of microorganisms in samples, but they do not answer the transportability of microorganisms without careful planning, selection of samples (including subsurface) and the analysis involved.*

Our response: We agree with your comment that it is less rigorous to delineate the transport of microbial populations in reservoir strata by detecting the shared microbial populations in both injection wells and production wells using a 16S rRNA sequencing method. To improve the preciseness of the manuscript, we have revised the manuscript title as "Differences of microbial community composition between injection and production water samples of water-flooding petroleum reservoirs". The results suggest that the microbial communities have significant differences between the injection and production water samples. Even if most microbial populations were shared, the relative abundance of shared populations exhibited large differences between the injected and produced water samples. Water backflow in the injection wells suggested that the microbial community was reassembled during the process of the injected water flowing into the production wells.

***Question 9**: * How can you link the microbial groups detected and the possible physiological function in the oil reservoirs? What are the sources of Bcteroides in the production water samples? From the information of archaea detected, which kind of methanogenic metabolism is responsible for CH_4 production?*

Our response: A number of fermentative microorganisms have been isolated from high- and low-temperature oil reservoirs. Many microorganisms in this group possess dual fermentative and respiratory metabolic abilities and could theoretically use both strategies for their in situ growth and survival (Youssef et al. 2009). *Bacteroidetes* include diverse mesophilic fermentative microorganisms. Grabowski et al. first isolated *Petrimonas* within the phylum *Bacteroidetes* in 2005 as the first member of this phylum to be isolated from oil reservoirs (Grabowski et al., 2005). Recently, studies based on 16S rRNA gene-based analysis have also revealed the existence of putatively fermentative members of the genus *Bacteroides* (Grabowski et al., 2005b; Youssef et al. 2009). These fermentative microorganisms may play an important role in reservoir ecosystems, in particular, providing substrates for methanogens to produce methane.

According to your suggestion, we have classified the obtained archaeal taxa based on the reported methyltrophic, acetoclastic, and CO_2-reducing methanogens (Liu, 2008). As reported in previous research (Zhao et al., 2012;Wang et al., 2012), the archaea identified in both reservoirs were overwhelmingly methanogens including methyltrophic, acetoclastic, and CO_2-reducing methanogens. Among them, methyltrophic and CO_2-reducing methanogens dominated both reservoirs.

In the sandstone reservoir, 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%). *Methanomicrobia* made up 84.03% of 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* produce CH_4 when growing with

methylamine as carbon source, while *Methanococcus* use H_2 and formate as carbon sources.

Similarly, *Methanobacteria*, *Methanococci*, and *Methanomicrobia* composed 64.3%–94.6% of the archaeal communities in the conglomerate reservoir. Compared with the injected water collected from the wellhead of the injection wells (T86-0 and T93-0), more *Methanomicrobia* was detected downhole of the 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 use H_2 and formate as carbon sources to produce CH_4.

Question 10*: * The Conclusions is too lengthy and shortening is necessary to show the most significant information of this research if any. References should be updated more extensively to include the current published papers to enrich the information reported here.*

Our response: Thanks for your suggestion. We have carefully rewritten the conclusion: Using high-throughput sequencing, we comprehensively surveyed the relationship shared by microbial communities in injection and production water samples in homogeneous sandstone and heterogeneous conglomerate reservoirs. The results suggest that the microbial communities have significant differences between the injection and production water samples. Even if most microbial populations were shared, the relative abundance of shared populations exhibited large differences between the injected and produced water samples. Water backflow in the injection wells suggested that the microbial community was reassembled during the process of the injected water flowing into the production wells.

The manuscript has been carefully revised referring to more current published papers. To improve the paper quality, the revised manuscript has been edited by English Language Editing Service before resubmission.

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

~~The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs~~

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Conflict of interest

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

Abstract. ~~In water-flooding petroleum reservoir, microbial populations in injected water are expected to migrate into oil-bearing strata and reach production wells. To demonstrate this, we firstly investigated microbial compositions in a homogeneous sandstone reservoir. The results indicated that the injected water harbored more microbial cells than produced water, and the shared populations and their abundance accounted for a minor fraction in injected water, while dominated in produced water, suggesting that most populations in injected water did hardly reach production wells in this reservoir. We further investigated microbial communities in water samples collected from wellhead and downhole of injection wells and production wells in a heterogeneous conglomerate reservoir. The results indicated that, except for the community reconstruction mainly resulted from dissoved oxygen, most populations were simultaneously detected in the wellhead and downhole of injection wells and production wells, suggesting that most microbial populations in injected water reached the production wells. This study suggest that microbial populations in injected water can pass through reservoir strata and reach production wells, but the reservoir heterogeneity, interwell spacing, sieve effect of strata and dissoved oxygen exert significant influence on microbial migration and distribution in reservoirs.~~

The microbial community composition of water-flooding petroleum reservoirs is of great interest because it is strongly related to the enhancement of oil recovery. However, our knowledge about the relationship between microbial communities in injection and production wells is still very limited. The present study investigated the differences in microbial communities in 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. The

results indicate that a small number of microbial populations are shared between the injected and produced water samples in the sandstone reservoir, whereas a large number of microbial populations are shared in the conglomerate reservoir. Consistently, the community structure exhibited large differences between the injected and produced water samples, with the shared populations accounting for a minor fraction of the injected water, but dominating the produced water in both reservoirs. This suggests that the community is reassembled as the injected water flows into the production wells. The results imply that microbial communities have significant differences between injection and production wells, in particular, the community composition and the relative abundance, which have a close relationship with the sieve effect of strata and the dissolved oxygen.

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

1 **Introduction**

Water-flooding is an efficient oil recovery process ~~and~~ that is employed worldwide. After long-term water-flooding, diverse microbial populations inhabit the petroleum reservoirs~~complex ecosystems comprising diverse microorganisms formed in petroleum reservoirs~~. These microbial populations and their metabolites, such as polysaccharide, organic acids and biosurfactants, can improve reservoir properties by blocking preferred water flow paths, lower interfacial tension between brines and oil phase, and decrease oil viscosity. These characteristics have been used to improve oil recovery. ~~Due to the~~With an increasing global energy demand and depletion of oil reserves, microbial enhanced oil recovery (MEOR) is currently studied ~~in~~ intensive~~ly development~~. To date, a large number of laboratory researches and field trials have been performed on stimulating reservoir microbial microorganisms to enhance oil recovery (Abdel-Waly, 1999;Zhang et al., 2012;Bao et al., 2009;Gao et al., 2013;Li et al., 2014).

Microbial populations are important components of reservoir ecosystems, and ~~serve~~ play ~~as~~ an critical role~~s~~ in MEOR process. Recently, culture-dependent and -independent methods, in particular, 16S rRNA-based molecular identification methods, have revealed diverse microorganisms inhabiting petroleum reservoirs (Al-Bahry et al., 2013;Kumaraswamy et al., 2011;Lenchi et al., 2013;Okoro et al., 2014). However, the relationship between microbial communities in injection and production wells remains poorly understood.~~Microbial migration and community succession along with injected water flowing into oil-bearing strata have a direct impact on gathering representative samples before and after MEOR process, and further influence nutrients selection and evaluation of oil displacement efficiency of~~

~~production wells. Microbial migration in reservoir strata also has a direct impact on the application of MEOR approaches based on injecting nutrients or microorganisms into reservoirs. However, these problems have received little attention hitherto, and are thus poorly characterized. After long term water-flooding, microbial populations in injected water are expected to migrate into oil-bearing strata and reach production wells~~. ~~However, using~~Based on 16S rRNA gene clone library method, several studies suggested that despite being flooded by same injected water, each production well harbored specific microbial communities (Tang et al., 2012;Ren et al., 2011). Unfortunately, these studies did not compare the differences of microbial community composition between injection and production water samples~~these studied injected and produced water samples were only selected for once at the same time. Due to neglect the interval that the injected water flows into production wells and the fact that the dissoved oxygen and reservoir pressure will produce significant influence on communities, these obtained results couldn't provide sufficient knowledge about microbial migration and distribution in injection and production wells~~. Moreover, because of low-throughput of clone library method, many infrequent microbial taxa may not be ~~are~~ usually undetected, making it difficult to compare microbial communities in detail.

If microbial populations in injected water ~~could~~ can flow into~~pass through~~ oil-bearing 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?~~? How microbial communities fluctuate along with injected water flowing into reservoir strata and production wells?~~ To

explore these issues, microbial populations and their abundance in injection and production wells in a homogeneous sandstone petroleum reservoir with permeability of 522×10^{-3} μm^2 and interwell spacing of 300–425 m were investigated by 16S rRNA pyrosequencing and real-time fluorescent quantitative PCR (qPCR). At the same time, we investigated microbial communities in water samples collected from wellhead and downhole of injection wells and production wells in a heterogeneous conglomerate water-flooding petroleum reservoir with permeability of 362×10^{-3} μm^2 and interwell spacing of 100–150 m. High-throughput sequencing provides the opportunity to compare microbial populations with unprecedented levels of coverage and detail. 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. The results presented here expand our knowledge on the relationships of microbial communities between injection and production water samples of water-flooding petroleum reservoirs.~~These results presented here will expand our knowledge about microbial migration and community succession in process of injected water flowing into petroleum reservoirs, and guide the application of MEOR.~~

2 Materials and methods

2.1 Sampling locations~~Water samples collection and DNA extraction~~

The Lu and Liu field block reservoirs are located in Xinjiang Oil Field, in the Junggar Basin of Xinjiang Uygur Autonomous Region, northwest China. The Lu field block is a homogeneous sandstone reservoir ~~with an average permeability of 522×10^{-3} μm^2,~~ ~~and~~that has been water flooded since 2001. The depth of the sampling horizon is

approximately 1200 m with a temperature of 37°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 selected well group (an inection well and four production wells), ~~the~~ injection well Lu3084, located ~~at~~ in the center position of the production wells, ~~have~~ has a direct influence on the neighboring producers, with inter-well distances of 300–425 m. The Liu field block is a conglomerate reservoir ~~with an average permeability of 362×10^{-3} μm^2 and interwell spacing of 100–150 m, and~~that has been water flooded for about 30 years. The depth of the block horizon is approximately 1088 m, with a temperature of 22.6°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)~~In the selected well group, including of two injection wlls and three production wells, the production well T90 was located at the center of the injection wells T86 and T93, while production wells T95 and T96 were located at the edge of this field block, and were mainly flooded by the injection well T93 (Fig. 1)~~. Although the injection wells have a direct influence on neighbouring production wells, the conglomerate reservoir heterogeneity is very strong. ~~The detailed reservoir characteristics and physicochemical property the collected water samples were listed in Table 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 1. 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 among 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.

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).~~To the sandstone reservoir and the conglomerate reservoir, tracer technique indicated that the time intervals for injected water flowing into production wells are approximately 30–45 days and 7–10 days, respectively (the data were provided by Xinjiang oilfield company).~~ In consideration of the larger microbial size and time needed for migration, the injected water of the sandstone reservoir was collected on three occasions every~~every~~ 15 days between October 2012 and November 2012,~~for three times since October, 2012~~, and the produced water samples were collected from the neighboring production wells 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) 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. Following immediate transportation to the laboratory, 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.~~2~~ 3 Pyrosequencing of partial 16S rRNA genes and sequences 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.

~~2.3~~2.4 Miseq-sequencing of partial 16S rRNA genes and sequences analysis

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 are merged by using FLASH (Magoc and Salzberg, 2011). Sequences were then analyzed using the QIIME (Caporaso et al., 2010) and UPARSE pipeline (Edgar, 2013). The detailed process is described in SI. The similarity among microbial communities was determined using UniFrac analysis in which weighted ~~principal coordinate analysis (~~PCoA~~)~~ were 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.~~4~~ 5 Quantification of community abundance

Evaluation of community abundance by real-time fluorescent quantitative PCR (qPCR) was performed using the 16S rRNA gene as molecular markers. Reactions were performed using the FastStart Universal SYBR Green Master PCR mix in a Bio-Rad iQ5 Sequence detection system. The primer set is 8F (5′-AGA GTT TGA T(CT)(AC) TGG CTC-3′)/338R (5′-GCT GCC TCC CGT AGG AGT-3′), 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.~~5~~ 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 communities 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. ~~In the sandstone reservoir, 249–538 bacterial OTUs and 45–130 archaeal OTUs were detected~~. The individual rarefaction curves, Shannon curves, and Phylogenetic diversity curves tended to approach saturation plateau (Fig. S1). Based on the results of qPCR, the copy number of bacterial 16S rRNA ranged from 1.5×10^6 to 8.25×10^6 copies ml^{-1}, while archaeal 16S rRNA ranged from 8.5×10^3 to 5.75×10^4 copies ml^{-1} in the water samples. 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).

In the sandstone reservoir, 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%). *Methanomicrobia* accounted for 84.03% in the produced water at 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* produce CH_4 when growing with methylamine as carbon source, while *Methanococcus* use H_2 and formate as carbon sources.

3.2 Microbial communities in the conglomerate reservoir

Between 52719 to 129106 16S rRNA gene sequences were analyzed and assigned to 2623 to 3414 genus-level OTUs. ~~A total of 52719 to 129106 16S rRNA gene sequences were analyzed and assigned to 2623 to 3414 genus-level OTUs.~~ 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). The copy number of 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 both the injected and produced water, composing 85.7%–94.1% of all bacterial communities. Similar to the sandstone reservoir, more *Proteobacteria* were detected in the produced water samples. At the class level, *Gammaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Bacteroidia*, *Bacilli*, and *Clostridia* composed 74.5%–83.7% of the bacterial communities in both the injected and produced water samples (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 in both the injected and produced water, and composed ~~composed~~ 64.3%–94.6% of archaeal comunities. Compared with the injected water botained from the wellhead of injection wells (T86-0 and T93-0), more *Methanomicrobia* were ~~more~~ detected in the downhole of injection wells (T86-8 and T93-7) and production well T90. At genera level, *Methanocorpusculum*, *Methanococcus*, *Methanocalculus* and *Methanosarcinales* were dominant, accounting for 61.6%–89.3%% 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 injection and production wells

The shared microbial OTUs and genera between communities in the injected and produced water samples 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 injected water and each produced water sample in the sandstone reservoir (Fig. 3a). These shared bacterial OTUs accounted for 4.6–24.71% of the total bacterial community in the injected water, and 43.23–76.18% in each produced water sample (Fig. 4a I). Furthermore, only 13 bacterial and 3 archaeal OTUs were shared by both the injected and produced water samples (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 detected in the injected and produced water samples, comprising 13.58% of the total archaea in the injected water, and 90.4%–96.89% in each of the produced waters (Fig. 4a III).

In the conglomerate reservoir, most of the OTUs and genera were simultaneously detected in the injected and produced water samples (Fig. 3b and 4b). 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).

3.4 Microbial populations distribution in injection and produciton wells

Microbial populations were clustered according to injection and production wells to highlight the populations that showed the most variability (Fig. 5). In the sandstone

reservoir, *Sphingomonas* and *Azospirillum* were found more detected in injected water, while *Arcobacter*, *Marinobacterium*, *Pseudomonas*, *Hyphomonas*, *Novispirillum*, *Proteiniphilum*, *Spirochaeta* and *Rhizobium* were highly abundant in produced water. In the conglomerate reservoir, *Paracoccus*, *Bacillus*, *Ochrobactrum*, *Parabacteroides*, *Sphaerochaeta*, *Thauera*, *Halomonas* and *Alcanivorax* were more detected in injected water, while *Arcobacter*, *Marinobacterium*, *Pseudomonas*, *Bacteroides*, *Oleibacter*, *Marinobacter* and *Shewanella* were dominant in the downhole of the injection wells and production wells. Among them, *Marinobacterium*, *Paracoccus*, *Ochrobactrum*, *Sphingomonas*, *Alcanivorax* and *Azospirillum* are aerobic bacteria, while *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*, *Spirochaeta*, *Bacillus*, *Thauera*, *Halomonas*, *Bacteroides* are microaerophilic bacteria, facultative anaerobe or anaerobe.

To further investigate microbial distribution in injection and produciton wells, hierarchical clustering and Unifrac PCoA were performed based on microbial OTUs aboundance and phylogenetic relationships. To the sandstone reservoir, hierarchical clustering showed that the community in injected water was distinct from those of produced water (Fig. S3). Weighted PCoA distinguished baterial community of the injected water from those of the production wells, while communities of the production wells were placed at a comparatively decentralized position (Fig. 6a I). Similar with the baterial communities, hierarchical clustering and PCoA distinguished archaeal community of the injected water from those of the production wells, wheras production wells were placed at a close proximity position (Fig. 6a II). In the conglomerate reservoir, communities of water samples obtained from the wellhead of injection wells were clustered into a group in PCoA plot, indicating that communities

remained unchanged before injected water flowing into the injection wells (Fig. 6b). Communities of water samples obtained from the downhole of injection wells and neighbouring production well T90 were clustered into a group, while production well T95 and T96 were clustered into another group (Fig. 6b). The phenomenon indicatied that microbial community reconstructed in the process of injected water flowing into reservoir strata and each production wells.

4 Discussion

MEOR technique is generally classified into exogenous microbial flooding and indigenous microbial flooding. 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). Indigenous microbial flooding technique improves oil recovery by introducing oxygen and salts through water-based injection to stimulate reservoir microorganisms (Belyaev SS et al., 1998). Despite the validity in field trial, this technology also has some limitations, in particular, instability during microbial flooding process. Microbial community diversification has been found to have a significant influence on oil displacement efficiency (Li et al., 2014). Because that reservoir microbial populations and their metabolites play an important role in the enhancement of oil recovery, microbial community composition and distribution in physically and geochemically diverse reservoirs has been extensive studied (Al-Bahry et al., 2013;Kumaraswamy et al., 2011;Lenchi et al., 2013;Okoro et al., 2014). However, the relationship between microbial communities in injection and production

wells remains poorly understood. We have therefore compared the differences of microbial community composition between injection and production water samples, and observed the microbial community diversification and succession as the injected water flows into the production wells.

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 depth was approximately 10–20 folds of pyrosequencing used in the sandstone reservoir, and 50–400 folds of the 16S rRNA gene clone library (assuming 300 clones per library). However, the current sequencing depth is still limited for detecting archaeal populations. As a result, we simultaneously sequenced the bacterial and archaeal V4 region of 16S rRNA gene, obtaining a total of 51273–128980 bacterial sequences per sample, but only 85–1445 archaeal sequences. This is consistent with the count result for archaea, suggesting the need for deeper sequencing for the detection of rare archaeal populations using this sequencing method. In contrast, the bacterial and archaeal communities were sequenced independently using pyrosequencing in the sandstone and we obtained 4016–5060 bacterial and 2688–2857 archaeal sequences. The rarefaction and Shannon curves tended to approach the saturation plateau, suggesting that this sequencing depth was enough for the detection of major bacterial and archaeal communities.

The community structure exhibited large differences between the injected and produced water samples. Differences in microbial communities may result from a number of different factors. The niche-based processes are supposed to be the primary drivers for the community diversification, and environmental variables such as salinity, pH, nitrogen, and phosphorus identified as the major determinants of microbial community composition (Kuang et al., 2013). However, few differences in cations and anions among the injected and produced water samples were observed. Petroleum reservoirs represent extreme anaerobic environments with multiphase fluids of oil, gas and water. Therefore, the subtle differences in the reservoir strata, in particular, the permeability, porosity, and dissolved oxygen, may exert a significant influence on the microbial communities.

If the microbial populations in the injected water could flow into the reservoir strata and reach the production wells along with the injected water, is the microbial community in the injected water expected to have a similar community composition with those in the production wells? In the homogeneous sandstone reservoir, we found that most microbial populations detected in the injected water were not detected in the production wells. It is reasonable to speculate that the abundant microorganisms in the injected water do not reach the production wells in this sandstone reservoir. Based on previous research, the main reason for this may be the sieve effect that can be enhanced by the 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 the reservoir strata. In contrast, we found that almost all OTUs and genera detected in the injected water were also observed downhole of the injection and neighboring production wells in the heterogeneous

reservoir, which has a similar permeability but shorter inter-well spacing, compared with the sandstone reservoir. It appears that most microbial populations in the injected water migrated into the oil-bearing strata and reached the production wells in the conglomerate reservoir. 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. To solve this issue, labelled strains, such as ones containing green fluorescent protein, may be a suitable way to investigate microbial migration in petroleum reservoirs.

Compared with the sandstone reservoir, a large number of microbial populations were simultaneously detected in the injected and produced water samples in the conglomerate reservoir. However, the shared OTUs and genera accounted for a minor fraction of the injected water in both reservoirs, whereas they dominated the produced water in both reservoirs, suggesting that the microbial community was reassembled as the injected water flowed into the production wells. Dissolved oxygen, which is known to be strongly related to microbial growth and metabolism (Gao et al., 2013), may be the main factor influencing the community structures. Although in situ oxygen concentrations were not recorded in this study, more aerobic bacteria, including *Sphingomonas*, *Azospirillum*, *Paracoccus*, *Ochrobactrum*, *Alcanivorax*, and *Hydrogenophilaceae* were detected in the injected water, while microaerophilic bacteria, facultative anaerobes, and anaerobes, including *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*, *Spirochaeta*, and *Bacteroides*, were found to have higher relative abundance in the produced water (Fig. 5). Apart from the dissolved oxygen,

another striking factor influencing microbial distribution in the injected water and the production wells may have been the crude oil, in particular, the saturates and aromatic components. Petroleum reservoirs represent oligotrophic environments. Although diverse microbial populations inhabit the reservoirs, only hydrocarbon-degrading bacteria and some anaerobes, such as sulfate-reducing bacteria, could grow with crude oil as carbon source. This is consistent with the observed results that more hydrocarbon-degrading bacteria, including *Marinobacterium*, *Pseudomonas*, *Rhizobium*, *Halomonas*, and *Oleibacter*, were detected downhole of injection and production wells.

This study compared the differences in microbial community composition between injection and production water samples 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.

This study investigated the relationship shared by microbial communities in injection and production water samples, and found the significant differences between

microbial communities in the injection and production water samples. However, it is less rigorous to make a conclusion on the transport of microbial populations in the reservoir strata by the current results. To solve the problem, injecting labelled strains containing green fluorescent protein into reservoirs may bring novel insight and greater predictive power to investigate microbial migration in reservoir strata. Therefore, the 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~~we comprehensively surveyed microbial migration in process of injected water flowing into reservoir strata of a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir. The results ~~indicated~~ suggest that the microbial communities have significant differences between the injection and production water samples. Even if most microbial populations were shared, the relative abundance of shared populations exhibited large differences between the injected and produced water samples. Water backflow in the injection wells suggested that the microbial community was reassembled during the process of the injected water flowing into the production wells. ~~microbial populations in injected water did hardly reach production wells in the homogeneous sandstone reservoir, while most populations in injected water passed through reservoir strata and reached production wells in the heterogeneous conglomerate reservoir. The results demonstrate that microbial populations in injected water can pass through reservoir strata and reach production wells, but the reservoir heterogeneity, interwell spacing~~

~~and sieve effect of strata might exert significant influence on microbial migration in reservoirs. Aerobic bacteria, including *Sphingomonas*, *Azospirillum*, *Paracoccus*, *Ochrobactrum*, *Alcanivorax* and *Hydrogenophilaceae*_uncultured bacteria were more detected in injected water, while microaerophilic bacteria, facultative anaerobe and anaerobe, including *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*, *Spirochaeta* and *Bacteroides* were found have higher relative abundance in produced water. In addition, hydrocarbon-degrading bacteria, including of *Marinobacterium*, *Rhizobium*, *Halomonas* and *Oleibacter* were more detected in downhole of injection wells and the production wells. These data imply that the dissolved oxygen and crude oil have an observed influence on microbial population composition in the process of injected water flowing into production wells. Our results expand the knowledge about microbial migration and distribution in process of injected water flowing into petroleum reservoirs, and provide guides for gathering representative samples before and after MEOR process and for the application of MEOR approaches based on injecting nutrients or microorganisms into reservoirs.~~

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Figure captions

Fig. 1. ~~The s~~Schematic diagram showing~~revealing~~ (a) the distribution of the injection and production wells of the Liu and Lu field blocks, and (b) the wellhead and downhole of injection ~~wells~~ and production wells, and the location~~place~~ where the water samples were collected. ~~The selected~~ T86, T93 and Lu3084 are the selected injection wells, and ~~while the selected~~ T90, T95, T96, Lu1039, Lu2180, Lu3073 and Lu3095 the~~are~~ production wells.

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

Fig. 4. Comparison of shared microbial genera between the injection and production wells. a-I:~~,~~ ~~represents~~ pairwise comparison between injection and production wells in the sandstone reservoir; a-II:~~,~~ ~~shows the~~ shared bacterial genera in ~~both of~~ the injection and production wells; a-III:~~,~~ ~~shows the~~ shared archaeal genera in ~~both of~~ the injection and production wells; b-I and b-II:~~,~~ ~~represents~~ comparison between injection and production wells on the conglomerate reservoir; and b-III:~~,~~ ~~shows the~~ dominant shared bacterial genera in the conglomerate reservoir.

Fig. 5. ~~The g~~Genera showing the most variability in the injected water and production wells. (a)~~,~~ ~~represents the s~~Sandstone and~~reservoir;~~ (b)~~,~~ ~~represents the~~ conglomerate reservoirs. The black-bordered box ~~in the layout~~ indicates ~~that~~ the genera ~~were~~ most~~more~~ detected in the production wells.

Fig. 6. Principal coordinate analysis ~~(PCoA)~~ of microbial communities. (a)~~,~~ ~~represents the s~~Sandstone and~~reservoir;~~ (b)~~, represents the~~ conglomerate reservoirs.~~;~~ I:~~,~~ ~~represents~~ bacterial communi~~ty~~~~ties~~ distribution; II:~~, represents~~ archaeal communi~~ty~~~~ies~~ distribution. Sample points that are close together are more similar in community composition than those that are far apart. ~~In panel b,~~ The arrows in panel (b) indicate~~show that~~ the community succession during the~~in~~ process of the injected water flowing~~–~~ into the injection wells and the neighbouring ~~–~~production wells.