Competition and cooperation of sulfate reducing bacteria and five other bacteria during oil production

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1.Introduction

Sulfate-reducing bacteria (SRB) are widely distributed in the oil extraction facilities (Barton and Fauque, 2009). The activities of SRB in the reservoir produce a quantity of by-product hydrogen sulfide (H_2S), which can increase formation pressure, dissolve carbonate layers, promote the release of crude oil and increase the permeability of the formation (Gibson, 2010). Moderate concentrations of SRB strains can also degrade heavy fractions in petroleum and improve the fluidity and recovery of crude oil (Cord-Ruwisch et al., 1987). However, excessive concentrations of SRB may cause multiple problems, including metal corrosion, plugging of pumping wells, contamination of crude oil, and souring of oil reservoirs (Gerard and Stams, 2008; Javaherdashti, 2011). Furthermore, the product of SRB, H_2S, is a toxic gas, which is harmful to the safety and health of oilfield employees (Myhr et al., 2002). In the oil and gas industry, the loss caused by SRB is estimated to be hundreds of millions of dollars annually in the USA (Beech and Sunner, 2007) (excluding costs of lost revenues and necessary remediation treatments). Due to these economic losses and threat to human health, SRB needs to be strictly controlled during oil production.

Currently, several methods are used to control of SRB during oil production, including physical sterilization, chemical sterilization, and bio-competitive exclusion technology. Physical sterilization equipment is expensive to operate and maintain and are ineffective for SRB biofilms, failing to achieve the desired effects (Kaur et al., 2009). Chemical sterilization usually requires the addition of chemical fungicides to inhibit the reproduction of SRB in the petroleum industry. This is simple and effective, but long-term use of chemical fungicides may lead to bacterial resistance and fungicides residues in the environment (Cusack et al., 1988). Additionally, some chemical fungicides can cause corrosion to pipelines and may also pose a threat to the

environment (Javaherdashti, 2011).

Bio-competitive exclusion, utilizing the competition and cooperation among microorganisms, can control SRB economically and is environmentally friendly (Rongjun et al., 2004; Zhao et al., 2016). Previous studies have reported competition and cooperation between SRB and other microbes in a variety of environments (Gerard and Stams, 2008; Gibson, 2010; Kaster et al., 2007). In laboratory simulation experiments, as sulfate reduction and biomass of SRB increase, methane yield and biomass of methanogenic bacteria (MGB) gradually decrease, indicating an effective inhibition of MGB by SRB (Chou et al., 2008). MGB has the advantage of competing with SRB under a particular condition, namely, when the ratio of electron donor to sulfate is high or sulfide is formed (Dar et al., 2008). Competitive interactions in anaerobic environments with a low redox potential are also observed between SRB and zymophyte bacteria (ZPB), proton-reducing acetogenic bacteria, and homoacetogens (Gerard and Stams, 2008). Furthermore, SRB and green sulphur bacteria form a co-culture in the presence of sulfides in a marine coastal environment (Gibson, 2010). A symbiotic relationship between SRB and sulfur oxidizing bacteria was found to promote the circulation of sulfur in a littoral salt marsh wetland ecosystem (Lee et al., 1999).

A large number of functional microorganisms, including MGB, saprophytic bacteria (SPB), iron bacteria (IB), and ZPB, co-habit and coexist with SRB in oilfield systems (Wei et al., 2010; Tuccar et al., 2019), and may have beneficial impacts on oil production, but probably cause detrimental effects of corrosion and blockage of pipelines (Eduok et al., 2019; Varjani and Gnansounou, 2017). The presence of these bacterial groups in oilfields may alter SRB activity in different ways (Tuccar et al., 2019). However, there is little information available about the competition and

cooperation between SRB and other functional microorganisms during oil production. In addition, temperature, pH, ammonia, oxidation reduction potential, dissolved oxygen, and total phosphorus are important environmental variables affecting the activity of microbial organisms, including SRB (Ahmadun et al., 2009), denitrifying bacteria (DNB) (Kuba et al., 1996), and MGB (Liu and Whitman, 2008). However, the response of SRB to the environmental variables of an oilfield remain largely unexplored.

In our study, we continuously monitored the dynamics of SRB, MGB, DNB, SPB, IB, and ZPB, as well as seventeen environmental variables (including temperature, pH, nitrogen-containing compounds, oxidation reduction potential, dissolved oxygen, total phosphorus) of produced water in the oilfield production wells located in the Shengli oilfield region of China, from 2017 to 2018. We then analysed the response of SRB to oilfield environmental variables and the synergy or competitive relationship between SRB and other oilfield functional microorganisms, to provide insights to our understanding of SRB activities and provide important information and new strategies for *in-situ* SRB bio-competitive inhibition during oil production.

2 Materials and Methods

2.1 Sampling of produced water

Oilfield produced water (PW) was sampled from eight oil producing wells (A - J) in the Shengli oilfield region, located in Hekou district, Dongying city of Shandong Province, China. These oil wells are distributed in four areas (Chengdong (A, 118.643°E 38.037°N; B, 118.643°E 38.033°N; H, 118.643°E 38.039°N), Bonan (C, 118.592°E 37.898°N; D, 118.590°E 37.896°N; I, 118.592°E 37.891°N), Da-81 (F, 118.458°E 37.924°N) and Zhan-3 (J, 118.379°E 37.873°N)) of the Shengli oilfield

89 (Fig. 1). The PW samples were collected in sterilized 500 mL serum bottles sealed
90 with a rubber cork and an aluminium cap to minimise external microbial
91 contamination. The samples were immediately transported to the laboratory on ice
92 under aseptic storage conditions, and then stored at 4°C before analysis.

93 ***2.2 Microbiological analyses***

94 Microbial concentrations of SRB and five other functional microorganisms (SPB,
95 IB, DNB, ZPB, and MGB) were determined by the most-probable-number (MPN)
96 analysis. Briefly, 1 mL of serial 10-fold diluted samples were inoculated in a sterile
97 culture medium using disposable sterilized syringes for each MPN analysis. Different
98 culture media were implemented for the six microorganisms.

99 Commercial bacterial test bottles (manufactured according to an industrial
100 standard: examination of bacteria and algae in industrial circulating cooling water
101 developed by the Standardization Administration of the People's Republic of China
102 (GB/T 14643, 2009)) were purchased from China National Petroleum Corporation
103 and used as the culture medium for SRB, SPB, and IB in this study. The culture
104 medium for DNB contained 2.0 g/L KNO_3, 5.0 g/L $Na_3C_6H_5O_7 \cdot 2H_2O$, 0.5 g/L
105 KH_2PO_4, 1.0 g/L K_2HPO_4, and 0.2 g/L $MgSO_4 \cdot 7H_2O$, pH was then adjusted to 7.2
106 and autoclaved at 1.1 atm for 20 min (Gevertz et al., 2000). The culture medium for
107 ZPB contained 4 g/L peptone, 10.0 g/L glucose, 2.0 g/L Na_2SO_4, 1.0 g/L
108 $MgSO_4 \cdot 7H_2O$, and 50.0 mg/L $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, pH was adjusted to 7.0 with 5%
109 $NaHCO_3$, and then autoclaved at 0.5 atm for 15 min. The culture medium for MGB
110 was prepared according to the composition described in Table 1. First, a mixture of
111 KH_2PO_4 (0.75 g/L), K_2HPO_4 (1.45 g/L), NH_4Cl (0.9 g/L), $MgCl_2$ (0.2 g/L), and NaCl
112 (1 g/L) were prepared and autoclaved at 1.1 atm for 20 min. Membrane-sterilized
113 trace elements and vitamins and ultraviolet-sterilized yeast extract (0.75 g) and

$FeSO_4 \cdot 7H_2O$ (0.2 g) were then added to the cool sterile mixture. Third, 5% $NaHCO_3$ was used to adjust the pH of the culture mixture to 7.2. Last, $Na_2S \cdot 9H_2O$ (5%) were added as a reducing agents to create an anaerobic environment for MGB culture and Resazurin (0.001 $g \cdot L^{-1}$) was added as a redox indicator. The inoculated cultures were incubated at 35℃ in an incubator for one week. Microbial content of these microorganisms was then determined. There were three replicates (n=3) for all samples.

2.3 Measurement of water quality variables

Seventeen water quality variabilities (temperature (T), pH, dissolved oxygen (DO), total dissolved solids (TDS), oxidation reduction potential (ORP), conductivity, biochemical oxygen demand (BOD_5), ammonia nitrogen (NH_4^+), nitrite nitrogen (NO_2^-), nitrate nitrogen (NO_3^-), total phosphorus (TP), sulfate (SO_4^{2-}), salinity (Sa), turbidity, chroma (Ch), mixed liquid suspended solids (MLSS), chemical oxygen demand (CODcr)) of the PW samples were analysed. Temperature was measured *in-situ* using a mercury thermometer, and pH, DO, TDS, ORP, and conductivity were measured *in-situ* using a HACH HQ30d spectrophotometer. NH_4^+, NO_2^-, NO_3^-, SO_4^{2-}, TP, Sc, Sa, turbidity, Ch, and COD_{Cr} were determined using the methods for the wastewater quality analysis using a HACH DR2800 spectrophotometer (Sapkota et al., 2018). The HACH HQ30d spectrophotometer was routinely and carefully cleaned using sterilized ultra-pure water before each measurement. BOD_5 of PW was analysed based on dissolved oxygen changes across five days using a HACH HQ30d dissolved oxygen meter (Sapkota et al., 2018). MLSS concentration of the PW was analysed following a standard analytical method, as described by Martínpascual et al. (2015).

2.4. Statistical analyses

Statistical analyses were performed by SPSS 16.0 software. A one-way ANOVA analysis with Tukey's test was applied to analyse water quality and microbe differences between the PW samples, with a significance difference of $p<0.05$ for all comparisons. Data were expressed as the mean ± standard deviation. Species and concentration of microorganisms in the PW samples were compared based on a principal coordinate analysis (PCA) to investigate the cooperation and competition associations among the studied microorganisms, using Canoco5.

3. Results and discussion

3.1 Environmental quality of Produced water

Yearly means of seventeen water quality indices of the oilfield produced water (PW) samples from the studied oil wells are shown in Table 2. The results showed that NH_4^+, COD_{Cr}, BOD_5, TP, SO_4^{2+}, DO, and MLSS concentrations, and T, Conductivity, Ch and Turbidity differed significantly among different wells. Annual average oilwell temperature fluctuated between 58.37 and 80.57℃, which is higher than that of Daqing Oilfield, China (38.7~39.2℃) (Zhang et al., 2020) and a Southern Algerian oilfield (35~50℃) (Gana et al., 2011). Little difference in temperature was found for the same oilwell throughout the year. The annual average concentrations of NH_4^+, COD_{Cr}, BOD_5, DO, and MLSS concentrations differed significantly, ranging from 4.52 to 41.06, from 216.85 to 1014.1, from 1.84 to 4.81, from 4.79 to 8.83, and from 61.78 to 252.04 $mg \cdot L^{-1}$ for PW samples, respectively. The MLSS concentrations were similar with that in the oilwells (88.5~139.8 $mg \cdot L^{-1}$) of Daqing Oilfield, China (Zhang et al., 2020). High TP and SO_4^{2+} concentrations were observed in some oilwells whereas others contained no detectable TP or SO_4^{2+}. The SO_4^{2+} concentrations (29.47~87.21 $mg \cdot L^{-1}$) was much higher than that in the oilwells (3.0~3.4 $mg \cdot L^{-1}$) of Daqing Oilfield, China (Zhang et al., 2020). The average Ch and

turbidity of PW samples from the sampled oilwells were 16.06~688.26 and 3.26~57.29, respectively. However, no significant differences were found for NO_3^-, NO_2^-, pH, Sa, TDS, and ORP in the tested water quality parameters among the sampled wells. Stable pH (8.18~8.71) was observed for the produced water in this study, lower than that in the oilwells of Daqing Oilfield, China (10.87) (Zhang et al., 2020), but the pH was significantly higher than a Southern Algerian oilfield (6.90~7.00) (Gana et al., 2011).

Our water quality values for NH_4^+, COD_{Cr}, TP, and MLSS exceeded the effluent levels for the petroleum refining industry developed by the Standardization Administration of the People's Republic of China (GB 31570, 2015) by 2.2~2.7-, 2.1~10.1-, 2.2~4.4- and 1.2~2.5-fold, respectively. COD, TP, SS of the effluent met the third grade discharge standard, and NH_4^+ and Ch met the first and second grade discharge standard, respectively, according to the Integrated wastewater discharge standard developed by the Standardization Administration of the People's Republic of China (GB 8978, 1996), However, BOD_5 and pH of all the PW samples did not exceed the Integrated wastewater discharge standard.

Nitrogen-containing compounds of PW samples showed significant monthly variations (Figs. 2 – 4). The monthly NH_4^+ concentration of PW samples from these wells varied from 0.37 to 72.73 $mg \cdot L^{-1}$. The NH_4^+ pollution of PW might have been derived from the oilfield injection water. The concentration of NH_4^+ was the lowest in December for all sampling wells. The variation of NH_4^+ concentration in oilwell A, B, H and I (0.37~11.80 $mg \cdot L^{-1}$) was lower than the remaining oilwells (2.77~72.73 $mg \cdot L^{-1}$) (Fig. 2). The monthly average concentration of NO_2^- and NO_3^- was lower than that of NH_4^+, with an average concentration of 0.02 and 1.42 $mg \cdot L^{-1}$. Except oilwell H, the NO_2^- concentration was always low in January compared to other months. The

188 NO_3^- concentration was the lowest, but the NH_4^+ concentration was relatively higher,
189 in March and April for all wells, probably due to weak nitrification by nitrifying
190 bacteria and strong denitrification by denitrifying bacteria under the anoxic conditions
191 of oilwells. Nitrifying bacteria are aerobic bacteria which do not thrive in the
192 anaerobic environment experienced during oilfield production (Ahmadun et al.,
193 2009).

194 ***3.2 Dynamic changes of the microbial quantity in produced water***

195 The microbial concentration from different sampling wells varied widely across
196 microbial species. The concentration of SRB and IB was significantly lower than that
197 of other microorganisms, followed by DNB and MGB. The highest concentration was
198 observed for SPB and ZPB. The concentration of these microorganisms changed
199 significantly with sampling time and demonstrated a similar trend over time for the
200 same microorganisms. For example, the concentration of SRB at each point was
201 highest in April, while the concentration of DNB was highest in March and May. This
202 phenomenon indicates that the concentration of these functional microorganisms
203 changed frequently in the oilfield.

204 ***SRB and DNB***

205 Dynamic changes in concentrations of SRB and DNB are shown in Fig. 5 and
206 Fig. S1. The average concentration of SRB at different sampling points ranged from
207 2.57 to 126.3 cells·mL^{-1}, among which the SRB concentration at point B was the
208 lowest, and at Point J was the highest. Except for few months, the concentration of
209 SRB was always lower than 30 cells·mL^{-1} all year. The peak concentration of SRB
210 was always much higher at one of these months for all sampling sites. Thus, SRB
211 reached a maximum concentration in Bonan, Da-81, and Zhan-3 during April,
212 indicating that SRB may respond to a range of environmental variables in these

months. The concentration of SRB of the Shengli Oilfield in this study was slightly lower than reported in the Daqing Oilfield (641~897 cells·mL^{-1}) (Zhang et al., 2020).

The average concentration of DNB varied greatly among different sampling points, fluctuating from a low concentration of 75.4 cells·mL^{-1} to a high of 21222.4 cells·mL^{-1}. The concentration of DNB changed gradually and was maximal in March for wells A and H of Chengdong, but in May for wells C, D, I, and J of Bonan and Zhan-3, respectively, similar to SRB, and differing by several orders of magnitude between adjacent months. These unusual changes indicated that the concentration of DNB might have been affected by other environmental variables, including NO_3^- and NO_2^- contents during oilfield production (Rivett et al., 2008). In general, there was less change for the endogenous nitrogen concentration in the oilfield environment (Van Hamme et al., 2003). However, the injected water during oilfield production might bring exogenous nitrogen contamination (Gieg et al., 2011), because the commonly used injected seawater in Shengli Oilfield has suffered from nitrogen contamination due to local aquaculture sewage discharge (Penuelas et al., 2013).

MGB and SPB

The changes in concentrations of MGB and SPB are shown in Figs. S2 and S3. The concentration of MGB and SPB varied greatly among different sampling wells. The average concentration of MGB ranged between 0.57~42857 cells·mL^{-1}. However, the concentration of SPB was high (10192~84835.5 cells·mL^{-1}) in most sampling wells. In comparison, the average concentrations of SPB in wells A, B, and J were relatively lower. The concentration of MGB was the lowest in well A, but the highest in the wells D and I (a maximum concentration of 90000 cells·mL^{-1}). Variations in concentration of MGB and SPB were time-dependent and differed significantly over time. The peak concentration of MGB occurred in November and January at wells B,

H, and J of Chengdong and Zhan-3 areas, but occurred in December and March at wells C and D of the Bonan area. Similar phenomena were also found in SPB. The concentration of SPB exhibited a large increase in December in wells A, B, F and H of Chengdong and Da-8 areas but was maximal in April in wells C and D of the Bonan area. These results showed similar trends over time for oil wells of the same region, which might be due to the similarity of the environmental conditions. Previous investigations of microbial functional genes has indicated that the concentration of MGB increased with oil contamination (Yang et al., 2018). For the same oil well, the peak MGB concentration was much higher than that in the remaining months, indicating that the oilfield had undergone large changes in this month, resulting in a significant impact on the concentration of MGB.

IB and ZPB

The changes in concentrations of IB and ZPB are shown in Figs. S4, S5. The concentration of IB was significantly lower than that of other microorganisms, which was much lower than previously reported in the Daqing Oilfield (Zhang et al., 2020). Excluding four sampling wells (A, B, D, and I) with the highest concentrations, the average concentration of IB was usually below 50 cells·mL^{-1}, significantly lower than the average concentration of other microorganisms. At sampling wells A, B, D, and I, the peak IB concentration was much higher than the concentration during the remaining months, which might be affected by the changes of environmental variables. In contrast, the concentration of ZPB was the highest among all microorganisms, and the average concentration ranged from 83,762 to 366,891 cells·mL^{-1}. The concentration of ZPB also changed significantly with time, with a low concentration in January in all sampling wells, possibly resulting from the large change to low temperatures in this month.

3.3 Competition and cooperation between the oilfield microorganisms

The utilization of the bio-competitive exclusion technique mainly considers the use of microorganisms that are metabolically similar to SRB, and then controls SRB through competitive exclusion between microorganisms. Such an approach is economical and environmentally friendly (Gieg et al., 2011). The effects of bio-competitive exclusion technology have been reported in many simulation tests (Hubert and Voordouw, 2007; Bodtker et al., 2008), including industrial applications. For example, previous studies have demonstrated that DNB and SRB compete for available carbon nutrients (Garcia de Lomas et al., 2006; Zhao et al., 2016), which inhibits SRB growth and prevents the production of H_2S. However, there is little information available about competitive and cooperative interactions between SRB and other functional microorganisms during oilfield production.

In this study, a PCA analysis was applied to elucidate the competition and cooperation correlations among oilfield microbial communities, including SRB, DNB, SPB, MGB, ZPB, and IB (Fig. 6). The PCA results demonstrated that samples from different sites, but collected in the same months, exhibited a clear aggregation (for example, samples in April and June were concentrated in the first and second quadrant, samples in January were focused in the second and third quadrant, while samples collected in March were clustered in the third quadrant). This phenomenon, that the microbial concentrations and structure of the PW samples from different sampling sites were similar in the same sampling month, demonstrated that sampling time was an important factor affecting the microbial dynamics of these microorganisms in the Shengli oilfield region. SRB and SPB were the dominant microbes in April and June (Fig. 6), consistent with the results of the average concentration of SRB in Fig. 5. However, MGB, ZPB, and DNB were the dominant

288 microbes in colder months, including November, January, and March. These obtained
289 results demonstrated that the oilfield environment in April and June is most suitable
290 for the growth of SRB and SPB. In contrast, the oilfield environment in November,
291 January, and March, is most suitable for the growth of MGB, ZPB, and DNB.

292 The PCA results also demonstrated cooperation correlations among the
293 microorganisms examined. A positive association for SRB with SPB and IB indicated
294 a cooperation correlation between them, probably because the activity of SPB and IB
295 in the oilfield created favourable growing conditions for SRB (Van Hamme et al.,
296 2003). In an oilfield system, SPB refers to the sum of various aerobic heterotrophic
297 bacteria which produce a large amount of viscous substances, leading to an increase
298 of the viscosity and a reduction of oxygen of the PW, and thus creating a local
299 anaerobic environment suitable for SRB growth. IB forms iron hydroxide precipitates
300 during metabolism, and its colonies and products can inhibit the formation of a local
301 anaerobic environment conducive to SRB growth (Barton and Fauque, 2009). Similar
302 cooperation phenomenon between SRB and IB was also identified in the heap
303 bioleaching residues (Phyo et al., 2020). A similar cooperation association was found
304 for ZPB, MGB, and DNB, mainly because of the important role of ZPB. In the
305 oilfield system, ZPB is widely distributed in the anaerobic zone of the formation and
306 produces a mass of active products, including organic acids, carbon dioxide, and
307 hydrogen (Staff, 1998), which are the reaction substrates of MGB and DNB (Ferry,
308 2010).

309 A competition correlation was also observed for SRB with other microorganisms.
310 A significant negative correlation was found for SRB, especially with ZPB, followed
311 by MGB and DNB in the PCA analysis. The species composition and abundance of
312 SRB were frequently reported to be significantly changed by the presence of DNB in

oilfield production water (Zhang et al., 2014; Zhao et al., 2016). Previous studies have reported that MGB and DNB share and compete for electron acceptors (including acetic acid, propionic acid and butyric acid, and hydrogen) with SRB, which might result in their competitive correlation (Garcia de Lomas et al., 2006; Gerard and Stams, 2008). ZPB can produce a series of active products and reaction substrates for SRB (Barton and Fauque, 2009), which may result in a positive correlation with SRB. However, there was a strong negative correlation between ZPB and SRB, probably resulting from the great promotion of MGB and DNB growth facilitated by ZPB.

In an oilfield system, the possibility of sulfate reduction under conditions of electron donor saturation is not excluded. This is the case when nitrate and nitrite are consumed in near-well environments, or in the microenvironment within the reservoir matrix, so that SRB can still be active in the deeper reservoir (Voordouw et al., 2009). Therefore, to ensure the inhibition of SRB activity, saturated nitrate must be added to the injection fluids (Engelbrektson et al., 2014), which is not feasible during petroleum production.

3.4 Microbial Response to the oilfield environmental variables

The correlation coefficients between the functional microorganisms and the water variables examined were calculated to investigate microbial responses to oilfield variables (Table 3). The results indicated that these functional microorganisms mainly responded to water quality indicators, including NO_3^-, NH_4^+, TP, TDS, T, ORP, conductivity, and turbidity.

SRB was significantly and negatively correlated with NO_3^- and ORP in all the collected samples, consistent with previous studies under laboratory anaerobic conditions (Fan et al., 2020; Zhang et al., 2014). However, there were no correlations with other environmental indicators. In our study, redox potential had a stronger

inhibitory effect on SRB ($P<0.01$) than NO_3^- ($P<0.05$). SRB is a strict anaerobic bacterium which cannot survive in an environment with a high oxygen content (Tate, 1985), and high NO_3^- content is not beneficial for the growth of SRB (Garcia de Lomas et al., 2006), consistent with our results.

Correlation results demonstrated that DNB was positively correlated with TP ($P<0.01$), which might be primarily affected by the high concentration of denitrifying phosphorus-removing bacteria (DPB) in the oilfield (Kuba et al., 1996). DPB is one kind of the DNB which can ingest stored polyphosphate in cells and releases the phosphorus in the form of soluble phosphate when growth conditions are unfavourable (Kuba et al., 1996). Limited availability of P was previously reported to be responsible for the decreased bacterial growth and activities in highly contaminated oilfield soils (Qian et al., 2014). No significant correlation was observed between DNB and NO_3^-, which may be due to the complexity of the oilfield environment, so that the concentration of DNB was greatly influenced by other environmental variables.

MGB and SPB in all samples were clearly affected by many oilfield environmental variables. MGB concentrations were significantly and positively correlated with NH_4^+, TP and TDS ($P<0.05$), and SPB was significantly and positively correlated with TDS ($P<0.05$), T ($P<0.05$), and turbidity ($P<0.001$) (Table 3). MGB was mostly influenced by TDS, followed by TP and NH_4^+. MGB and SPB are heterotrophic bacteria in oilfields which require and consume a large amount of nutrients during growth. Previous investigations have shown that TDS content is an indicator of dissolved ions, organic and inorganic compounds, and turbidity is an indicator of suspended matters, including organic matter and microorganisms. A high concentration of these variables indicate that the samples contain a large amount of

363 important nutrients required for microbial growth. Total phosphorus and NH_4^+,
364 exhibited positive correlations with MGB and SPB, which are essential elements for
365 their growth (Liu and Whitman, 2008), resulting in the positive relationship of MGB
366 and SPB with these environmental variables. pH is a sensitive influencer of SRB and
367 MGB activities in laboratory anaerobic conditions (Gutierrez et al., 2009), whereas no
368 significant effect was observed in this study, which might be attributed to small
369 changes in pH of oilfield produced waters among these oilwells.

370 ZPB in all samples was significantly and negatively correlated with ORP
371 ($P<0.001$) and TDS ($P<0.01$). The anaerobic environment, with lower ORP, is
372 beneficial for the growth of ZPB in the environment, as reported in many studies
373 (Wang et al., 2012). TDS concentration is an indicator of soluble organic
374 hydrocarbons, including phenols, benzene, and organic acids, and therein high
375 concentration of organic acids could substantially inhibit the fermentation of ZPB
376 (Wang et al., 2012). The negative correlation between ZPB and TDS might be
377 attributed to the high organic acids content in the oilfield PW samples. Conductivity is
378 a measure of the concentration of soluble ions in the PW, which are nutrients required
379 for the growth of ZPB, so that a positively correlation of ZPB with conductivity
380 ($P<0.05$) was observed in our study.

381 A negative correlation was observed for SRB with DNB, MGB, and ZPB,
382 whereas a positive correlation was found for SRB with SPB and IB. The increased
383 NO_3^- concentrations and ORP directly inhibited SRB growth. A high concentration of
384 TP and NH_4^+ are suggested to inhibit and control SRB through the promotion of
385 growth of DNB and MGB. However, low TDS content, turbidity, and temperature and
386 high conductivity were recommended for the prevention and control of SRB through
387 the promotion of ZPB growth and inhibition of SPB growth.

4. Conclusions

(1) Functional microorganisms in oilfield production water (PW), including SRB, DNB, MGB, SPB, and ZPB, presented strong responses to a wide range of oilfield environmental conditions. The increase of NO_3^- concentrations and ORP directly inhibited SRB growth. High TDS concentrations and ORP, and low conductivity inhibited the growth of ZPB. However, TP promoted the growth of DNB. An increase of NH_4^+, TP, and TDS promoted the growth of MGB while higher T, TDS, and turbidity concentrations promoted the growth of SPB.

(2) The functional microorganisms examined here presented significant cooperative and competitive relationships with SRB in the oilfield produced water. A competitive relationship was observed for SRB with DNB, MGB, and ZPB, whereas a cooperative relationship was found for SRB with SPB and IB.

(3) During oilfield production, DNB exhibited a symbiotic relationship with SPB and ZPB, while SPB exhibited a competitive inhibition relationship with MGB.

(4) Higher concentrations of TP and NH_4^+ are suggested to facilitate the prevention and control of SRB through the promotion of the growth of DNB and MGB. However, low TDS content, turbidity, and temperature, and high conductivity were recommended for the prevention and control of SRB through the promotion of ZPB growth and inhibition of SPB growth.

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6. Compliance with Ethical Standards

The authors declare that we have no conflicts of interest. The manuscript is approved by all authors and has not been submitted to more than one journal for simultaneous consideration. This manuscript has not been previously published. The submitted work has not received any financial support from any third party, and there is no financial relationship with any entities. All of the financial organizations associated with this work have been disclosed. There is no patent, planned, pending or issued, broadly relevant to the submitted work.

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589

Trace elements		Vitamins	
Ingredient	**Concentration (**g/L)	**Ingredient**	**Concentration (**mg/L**)**
$MgSO_4 \cdot 7H_2O$	3.0	Biotin	2
$MnSO_4 \cdot 2H_2O$	0.5	Folic acid	2
NaCl	10	Pyridoxine	10
$FeSO_4 \cdot 7H_2O$	0.1	Thiamine	5
CoCl	0.1	Lactoflavin (B_2)	5
H_3BO_3	0.01	Lipoic acid	5
$ZnSO_4$	0.1	Para aminobenzoic	5
$CuSO_4 \cdot 5H_2O$	0.01		
$AlK(SO_4)_2$	0.01		
$CaCl_2$	0.1		
$NaMoO_4 \cdot 2H_2O$	0.01		

590 **Table 1**

591 The composition of trace elements and vitamins in MGB culture medium.

592

	A	B	C	D	F	H	I	J
NO_3^-(mg/L)	1.13±0.62	1.34±0.80	1.45±0.98	1.69±1.22	1.3±0.72	1.31±0.72	1.32±0.74	1.83±1.18
NO_2^-(mg/L)	0.04±0.03	0.03±0.01	0.02±0.01	0.02±0.01	0.01±0.01	0.04±0.02	0.02±0.02	0.03±0.03
NH_4^+(mg/L)	6.35±3.19	5.88±3.25	41.06±21.40	37.76±22.65	33.73±20.50	4.52±2.31	26.27±13.44	7.78±2.06
COD_{Cr}(mg/L)	216.86±107.67	614.02±336.44	413.95±111.88	492.9±132	1014.1±208.66	315.1±158.94	426.17±160.78	343.22±227.51
BOD_5(mg/L)	1.92±2.36	1.84±1.98	4.81±2.96	3.23±2.08	4.09±2.37	2.79±2.6	5.71±2.70	2.99±2.20
TP(mg/L)	0.00±0.00	0.00±0.00	2.21±1.51	4.41±1.50	0.04±0.06	0.00±0.01	0.23±0.07	0.00±0.00
SO_4^{2-}(mg/L)	0.00±0.00	0.00±0.00	0.00±0.00	29.47±30.5	87.21±29.68	0.00±0.00	3.68±9.02	83.86±57.98
pH	8.35±0.3	8.18±0.35	8.69±0.36	8.71±0.35	8.57±0.28	8.32±0.30	8.69±0.37	8.28±0.37
Sa (%)	0.76±0.05	0.75±0.04	1.01±0.09	1.16±0.08	1.12±0.09	0.75±0.06	1.00±0.03	0.98±0.06
TDS(ppm)	4417.71±882.82	4511.71±1112.76	5857.67±1195.25	5999.33±1215.02	5902.14±1342.81	4853.73±622.03	5808.22±922.30	5025.22±1168.02
T (℃)	59.54±3.28	59.14±2.94	60.46±7.51	58.64±2.74	80.57±2.71	60.04±1.30	65.65±2.76	58.37±2.96
ORP（mv）	104.67±50.40	105.42±43.51	101.92±32.21	103.55±36.85	91.68±34.02	103.75±37.24	93.62±44.28	105.61±48.87
Conductivity(ms/cm)	28.09±13.12	27.63±13.36	37.38±23.38	37.65±23.25	36.3±21.45	21.98±11.29	30.95±19.88	34.03±20.78
DO(mg/L)	8.61±0.60	8.69±0.36	6.57±2.51	6.17±2.53	4.79±2.05	8.83±0.36	7.36±1.57	7.38±1.91
Ch (PCU)	54.58±48.29	688.26±459.56	74.67±41.45	63.21±41.68	134.67±74.92	235.87±427.70	157.98±87.87	16.06±18.79
Turbidity(NTU)	5.90±3.94	54.63±27.84	5.52±4.76	3.79±2.60	57.29±132.76	22.51±38.40	10.96±7.39	3.26±3.38
MLSS(mg/L)	70.37±79.77	252.04±371.01	61.78±71.55	77.83±73.96	62.40±67.86	96.20±103.33	149.59±180.3	126.53±125.38

593 **Table 2.**

594 Environmental quality of PW from different sampling points (annual mean ± standard deviation).

595

	MGB	DNB	ZPB	SPB	IB	SRB
NO_3^-	0.0221	0.0096	0.0087	0.0044	0.0360	0.0817 $^{*\downarrow}$
NO_2^-	0.0159	0.0165	0.0323	0.0306	0.0008	0.0018
NH_4^+	0.0808$^{*\uparrow}$	0.0570	0.0155	0.0053	0.0143	0.0044
COD_{Cr}	0.0005	0.0184	0.0274	0.0979	0.0066	0.0279
BOD_5	0.0575	0.0033	0.0249	0.0006	0.0260	0.0013
TP	0.1029$^{*\uparrow}$	0.1759 $^{**\uparrow}$	0.0180	0.0031	0.0013	0.0012
Sc	0.0040	0.0001	0.0573	0.0077	0.0038	0.0054
pH	0.0244	0.0493	0.0674	0.0186	0.0061	0.0336
Sa	0.0644	0.0646	0.0231	0.0548	0.0170	0.0021
TDS	0.147 $^{**\uparrow}$	0.0009	0.1280 $^{**\downarrow}$	0.0763$^{*\uparrow}$	0.0668	0.0163
T	0.0034	0.0139	0.0019	0.0935 $^{*\uparrow}$	0.0125	0.0019
ORP	0.0052	0.0095	0.3602$^{***\downarrow}$	0.0004	0.0002	0.1749 $^{**\downarrow}$
Conductivity	0.0684	0.0303	0.0846$^{*\uparrow}$	0.0007	0.0163	0.0703
DO	0.0017	0.0003	0.0111	0.0541	0.0038	0.0014
Ch	0.0149	0.0023	0.0000	0.0000	0.0021	0.0001
Turbidity	0.0132	0.0032	0.0026	0.7321 $^{***\uparrow}$	0.0031	0.0019
MLSS	0.0577	0.0144	0.0444	0.0083	0.0022	0.0227

596 *Significant at $p<0.05$.

597 ** Significant at $p<0.01$.

598 ***Significant at $p<0.001$.

599 $^{\downarrow}$ Negative correlation.

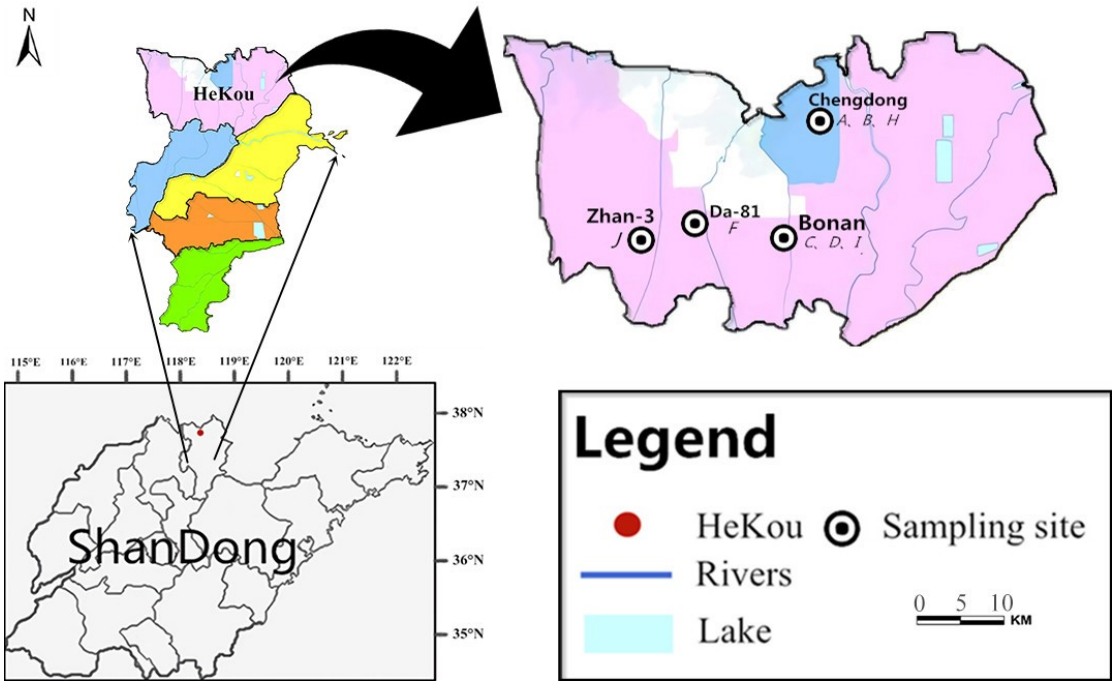
600 $^{\uparrow}$ Positive correlation.

601

602 **Table 3**

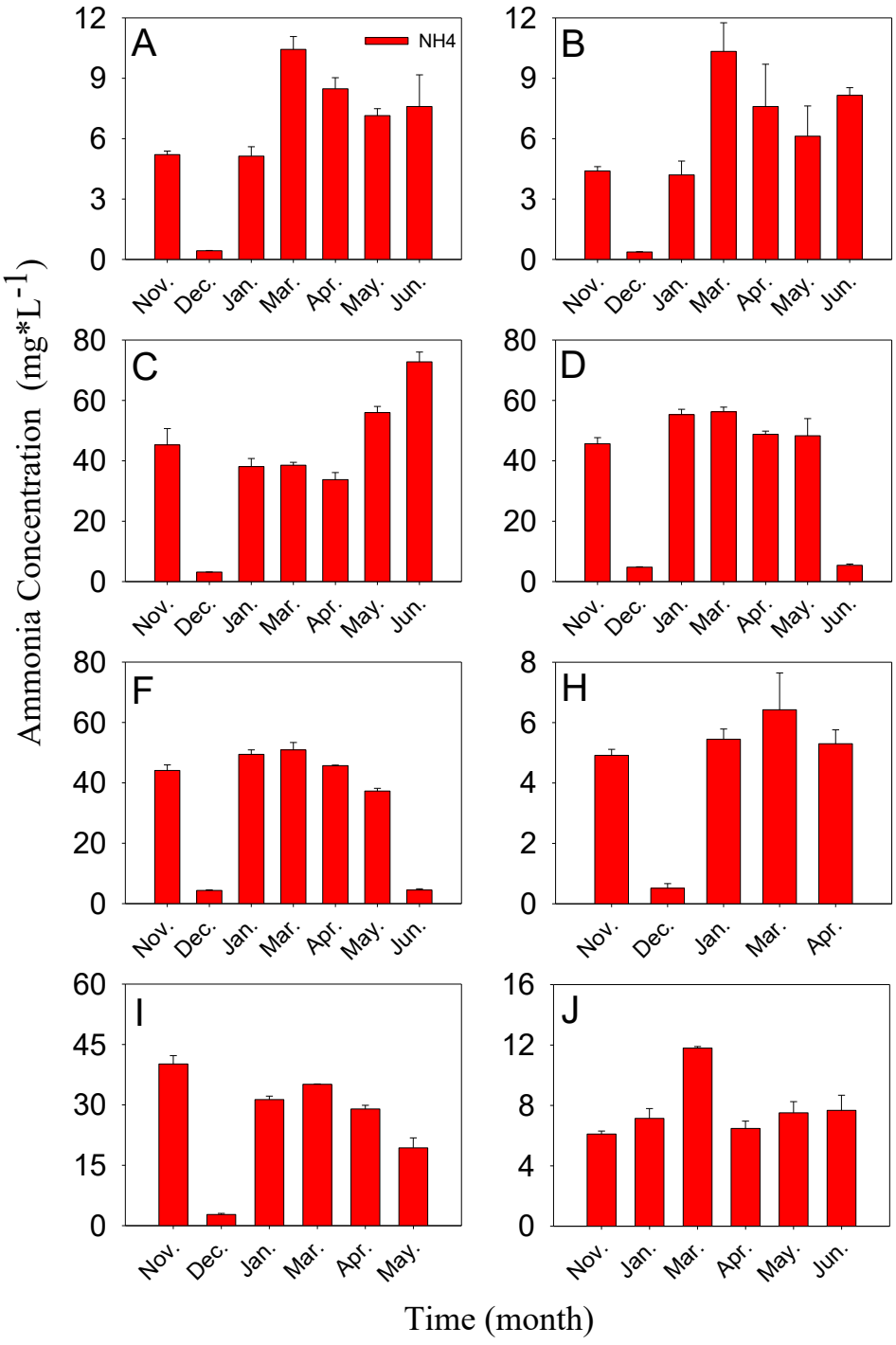
603 Correlation coefficients between the functional microorganisms and environmental

604 variables.



605 Note: The eight sampling wells were located in Chengdong (A, 118.643°E 38.037°N;
606 B, 118.643°E 38.033°N; H, 118.643°E 38.039°N), Bonan (C, 118.592°E 37.898°N; D,
607 118.590°E 37.896°N; I, 118.592°E 37.891°N;), Da-81 (F, 118.458°E 37.924°N) and
608 Zhan-3 (J, 118.379°E 37.873°N) areas.

609 **Fig. 1.** Geographical information of sampling oil wells in the Shengli oil field
610 (Shandong, China).

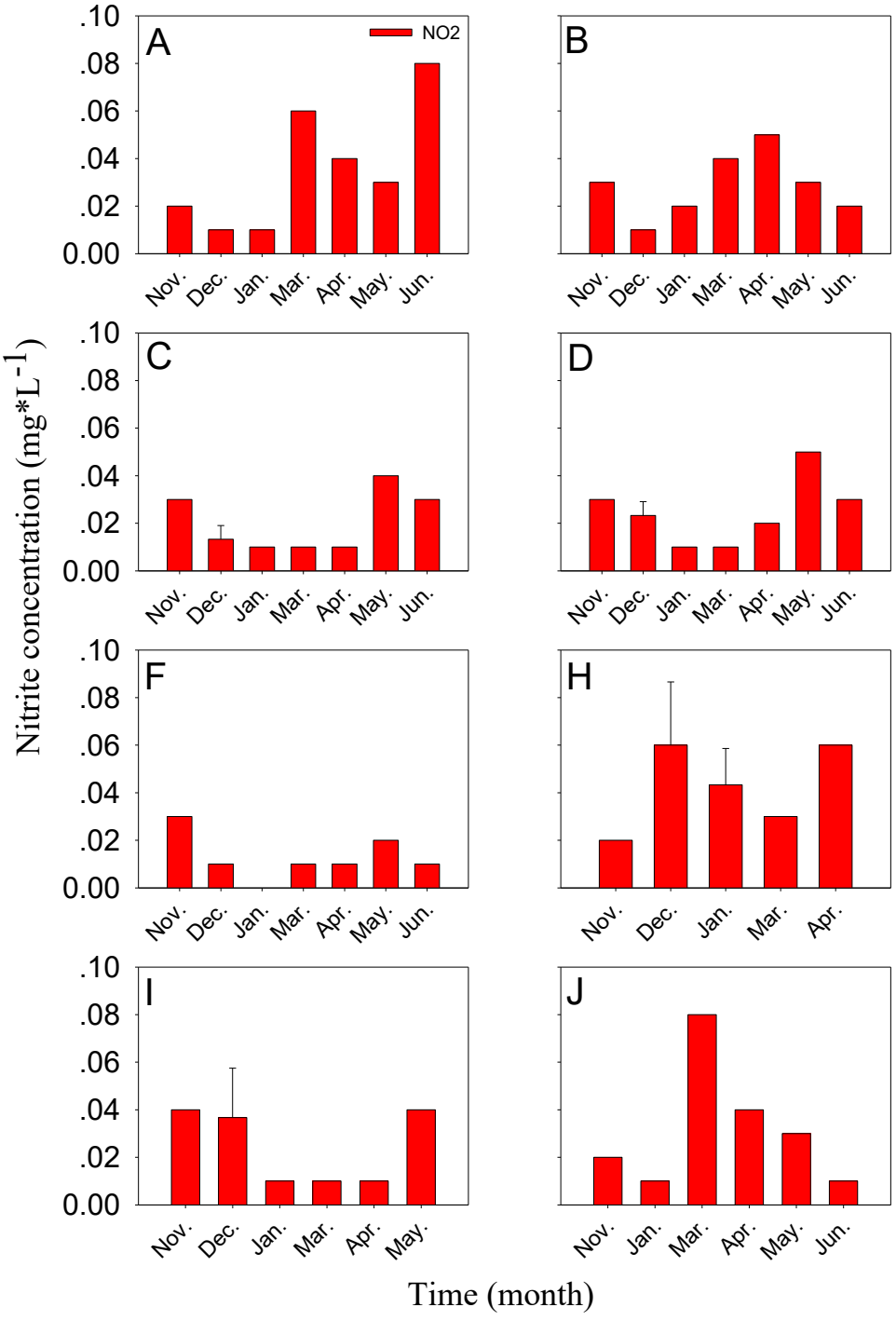


611

612 **Fig. 2.** Monthly ammonia concentration variations in produced water, from November

613 2017 to June 2018.

614

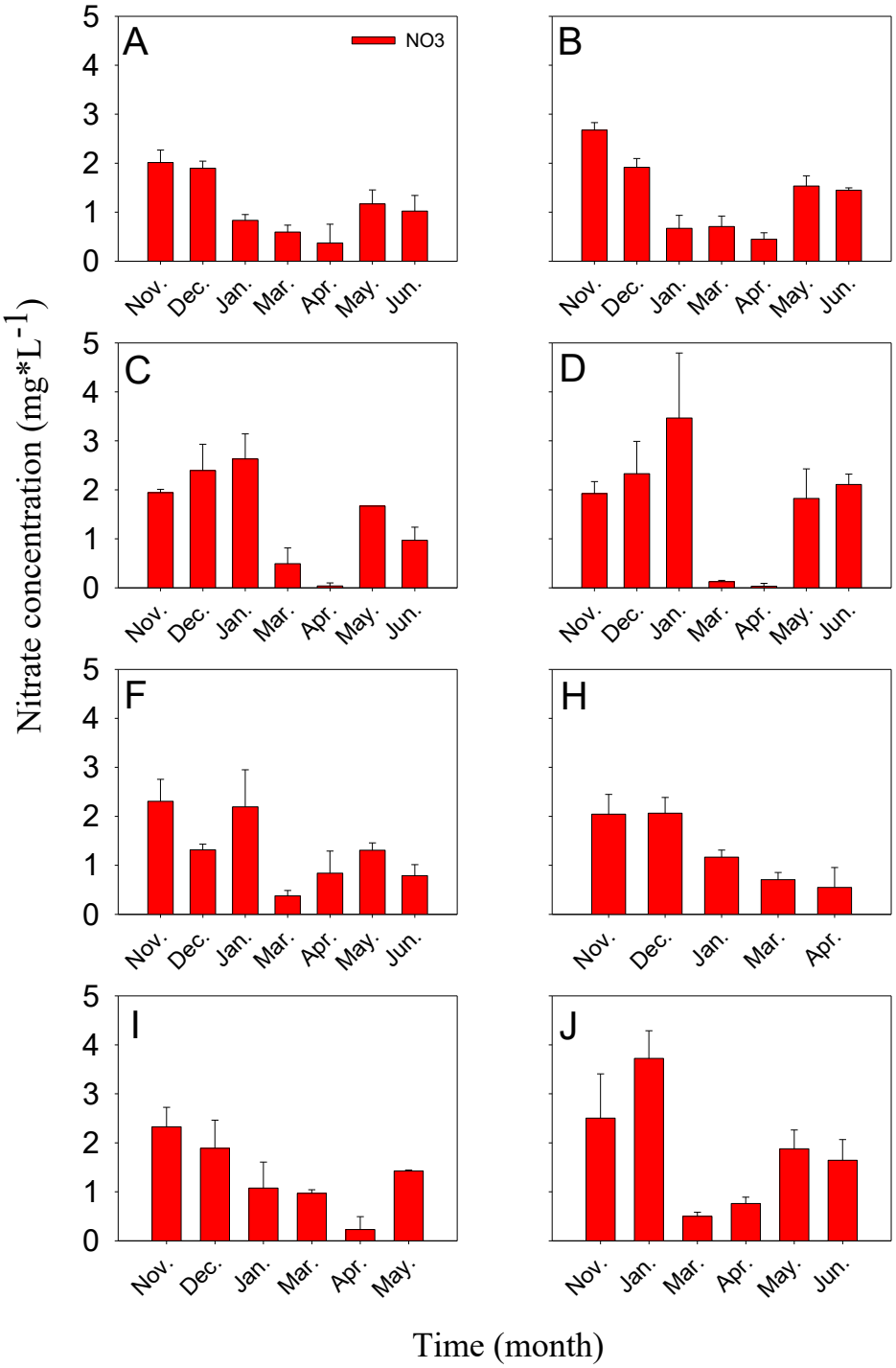


615

616 **Fig. 3.** Monthly nitrite concentration variations in produced water, from November

617 2017 to June 2018.

618

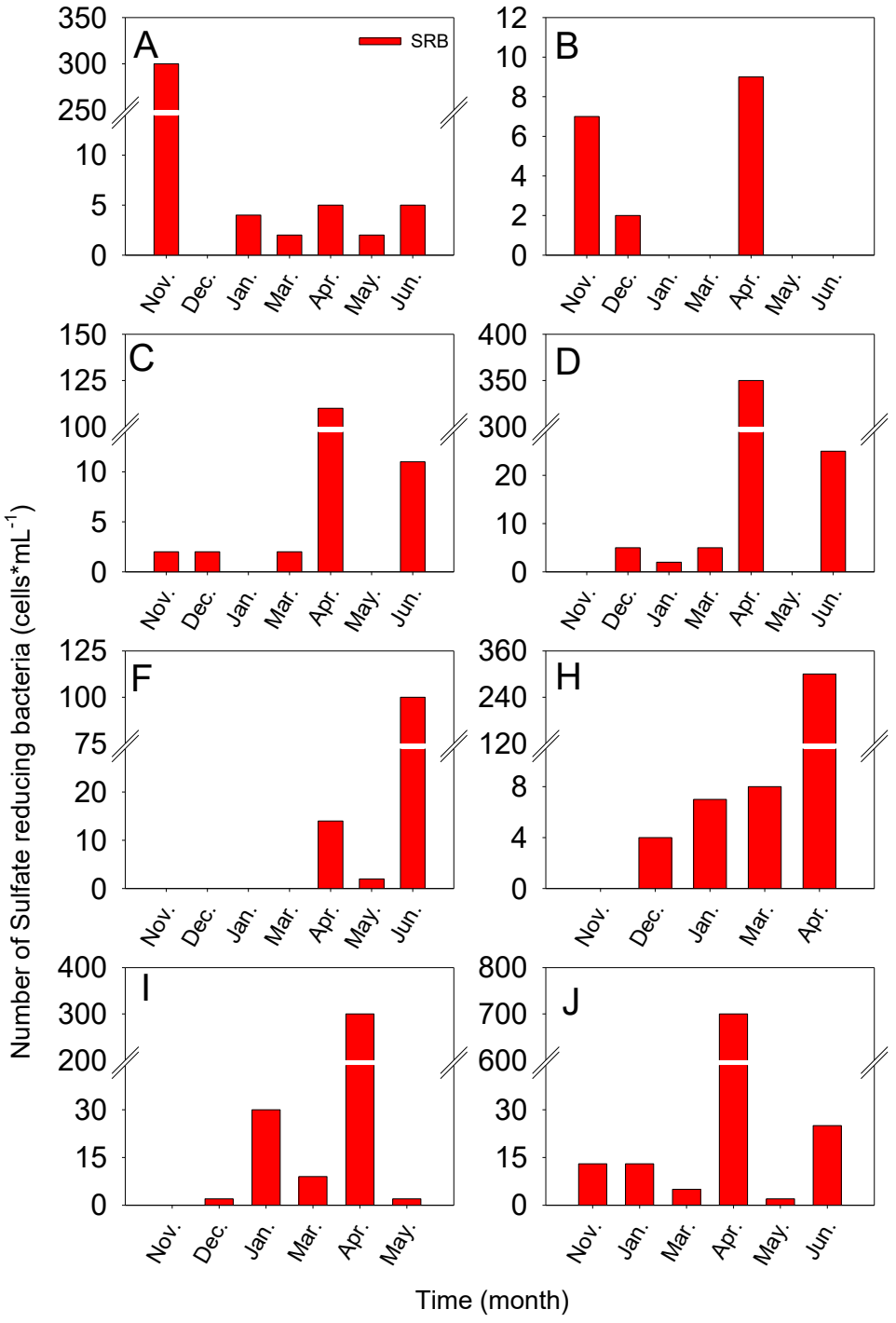


619

620 **Fig. 4.** Monthly nitrate concentration variations in produced water, from November

621 2017 to June 2018.

622



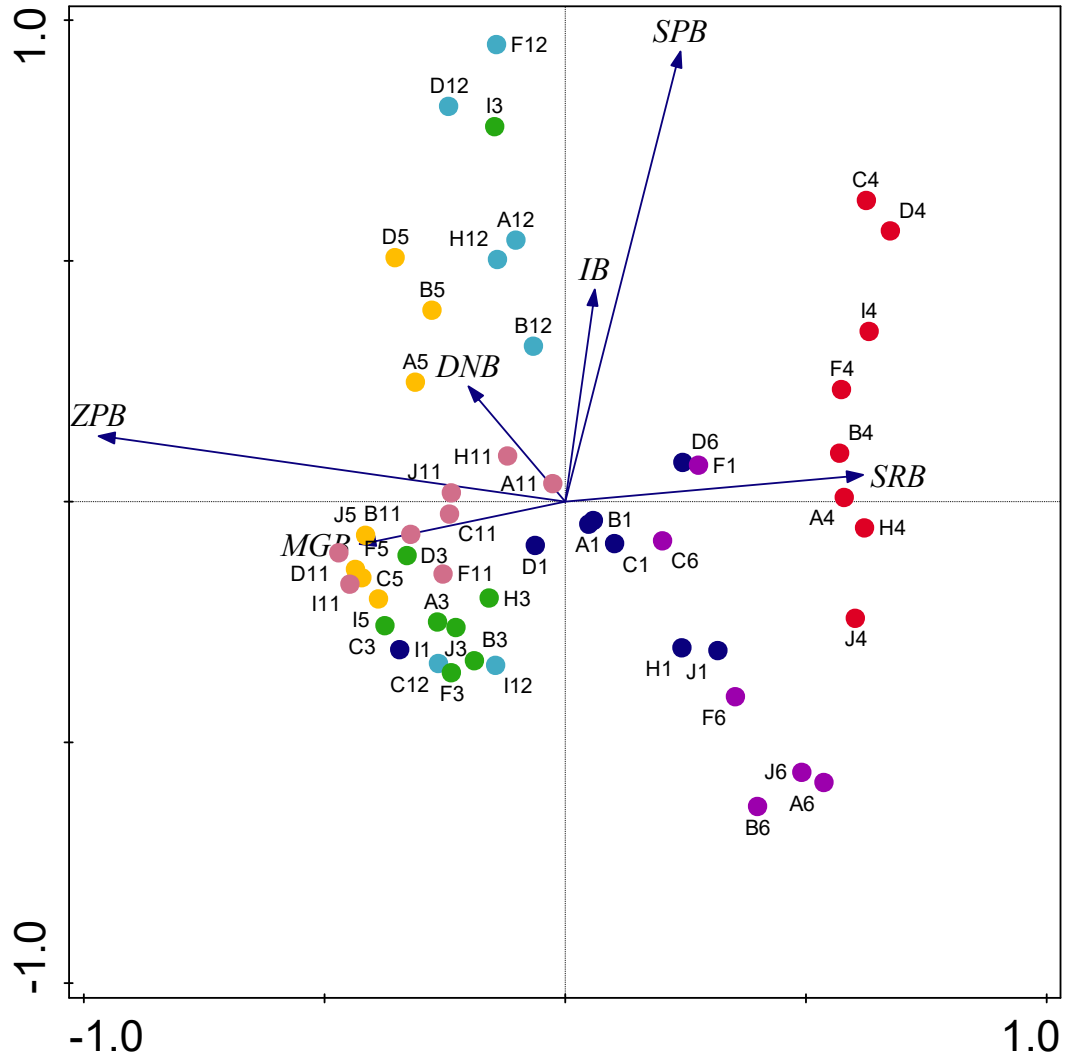
623

624 **Fig. 5.** Monthly SRB concentration variations in produced water, from November

625 2017 to June 2018.

626

627



628 The points P_n indicate the microbial concentration in the sampling oil well of P
629 collected during month n (n= 1, January; 3, March; 4, April; 5, May; 6, June; 11,
630 November; 12, December). The data points from all sampling oil wells collected at
631 the same month are presented with the same colour.

632 **Fig. 6.** PCA analysis biplot between oilfield microbial communities (SRB, DNB, SPB,
633 MGB , ZPB and IB).