Primary Studies on the Effect of Microbially Coalbed Methane Enhancement in Suit in the Qinshui Basin

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Abstract

Methods used to yield bio-methane with coal to increase coalbed methane reserves had researched, thus providing a means for improving gas drainage efficiency. One such method utilized to convert coal into gas involves coal biodegradation technology. In order to confirm the practical application of this technology, the experiments were conducted in wells, Z-159, Z-163, Z-167, and Z-7H, in the Qinshui Basin in China, and the duration of the experiments was 32 months. Cl^- ion tracer, number changes of *Methanogen sp.*, and coal bed biome evolution indicated that the culture medium

diffused in the Z-159 and Z-7H wells. These wells resumed gas production separately. Gasification of coal lasted 635 and 799 days, and yielded 74817 m^3 and 251754 m^3 coalbed methane in Z-159 and Z-7H wells, respectively. Results demonstrate that coalbed methane enhancement with biogasification of coal is a potential technical to achieve the productivity improvement of coalbed methane wells.

Keywords: coal bed methane; biogasification; bioenergy; coal; *methanogen sp.*; biome.

Abbreviations:

CBM, Coal bed methane; TCD, Thermal Conductivity Detector.

1. INTRODUCTION

Coal bed methane (CBM) is a type of associated gas resulting from coal production. The main component of this gas is methane. CBM is a valuable energy resource, meanwhile, it is a dangerous source for mining [1]. Gas drainage represents a widely used technique that not only controls CBM operations but improves the comprehensive utilization efficiency of fossil energy. Therefore, research involving gas drainage mechanisms, applications and coal gasification methods, which service to increase CBM reserves and improve gas well productivity has gradually deepened. [2–4]. Biogasification of coal is one of the methods of coal gasification [5]. The coal components are complex. It contains organic compounds and inorganic components. The methanogenic bacteria could degrade a part of biodegradable organics to convert coal into methane. Activating characteristics of *Methanogen sp.* in coal of the Qinshui basin, and the biodegradability of coal have been preliminary studied in previous

43 research [5]. The in-situ efficiency of this technical requires further research.

44 The Qinshui basin, located in the southern region of the Shanxi province, has an
45 abundance of CBM and constitutes one of the most important CBM reservoirs and gas
46 development regions in China (Figure. 1). The CBM reserves are estimated at 3.95
47 trillion m^3 within this 27137 Km^2 area [6]. As defined by the Chinese petroleum and
48 natural gas reserves standard, this basin represents a massive gas field. The geologic
49 structure of the Qinshui basin is comprised of the compound syncline, which warps the
50 seams of the southern and northern basins and supplies the fundamental symmetry-
51 bearing portion of the eastern and western regions. The geologic structure in the middle
52 of this basin is flat and contains few faults. The Fm $3^{\#}$, Fm $9^{\#}$ and Fm $15^{\#}$ coal beds are
53 the main gas bearing layers with good permeability conditions [7].

54

55 Figure 1 here

56 Figure 1. The Qinshui Basin geographical location map. The Qinshui Basin is located
57 in the southern part of Shanxi Province. Neighboring provinces of Herbei and Henan.
58 Anze, Changzhi, Qinshui, Gaoping, and Jincheng are the main coal bed gas
59 development areas. The test location was in Qinshui, where marked with a pentagram
60 symbol in the figure.

61

62 Methane productivity of some wells is quite low, and even did not produce gas in
63 a part wells, which like Z-7H, Z-159, and Z-163 wells in experiment. Nitrogen foam

fracturing have been used in these wells to expansion crack and improve coal permeability. However, the effect was not satisfying. In this case, the CBM development cost was prohibitive[1]. In previous research, methanogenic bacteria, which include hydrolytic fermentation bacteria, fermenting bacteria, and *Methanogen sp.*, etc. had been found in Fm $3^{\#}$, Fm $9^{\#}$ coal bed in Qinshui basin, and the microbe culture method had been studied also[5,8]. If the coal biogasification technology can be applied on site, it would prove a potential technical to improve gas wells production efficiency and control the cost of CBM development.

2. Materials and methods

2.1 Experiment Wells and Control Wells Survey

This experiment was carried out in a horizontal multiple branching well and a vertical well. The horizontal well identified as Z-7H (GPS coordinates: 38.716117, 106.475351) and Z-159(GPS coordinates: 35.710277, 112.472500). Z-163, Z-167wells, which were located beside the test wells, were selected as control wells.

Total drill footage of Z-7H well was 5472 m, pure drilling footage in coal was 4405 m and a well control area consisted of 0.371 Km^2. The connection point depth was 797.5 m, and the deflection point depth was 508 m. The well diameter was 6 inches. The Z-7H well has 2 main well bores and 9 branches. The gently sloping coal seam provides for a favorable geographical condition (Figure. 2). The well was completed on October 15, 2012. The desorption pressure gradually increased and achieved a value of 2.5 MPa and began to product gas on February 23, 2013. However, Z-7H stopped gas

85 production after 644 m^3 of gas were produced from February to March. The average
86 gas production over the production period was only 64.37 m^3/day from its initial
87 commissioning until the end of 2014.

88 Z-159, Z-163, Z-167 wells were open vertical boreholes, located adjacent to the
89 horizontal pinnate branch well of Z-7H (Figure. 2) and had similar geological
90 conditions to that of Z-7H. The wells diameter were 6 inches, were completed on
91 September to December, 2012, and drilling depth of each borehole were 572.5 m, 591.9
92 m, 607.9 m respectively. No gas was obtained in Z-159 and Z-163 wells before the
93 experiment performed. Many nitrogen foam fracturing and flushing operations were
94 carried out in Z-7H, Z-159 and Z-163 wells to expansion crack and improve coal
95 permeability, but failed. Well Z-167 had gas productivity of 460 m^3/day on average
96 before the experiment performed.

97 Figure 2 here

98 Figure 2. Well Z-7H trajectory stereogram and contour map. Relative geographic
99 locations next to Z-7H well. Wells Z-159, Z-163, and Z-167 are located in south of Z-
100 7H well.

101

102 **2.2 Condition of the Fm 3# Coal Seam**

103 Fm 3# coal in Qinshui is a high rank coal with a value of $R_{o,max}$>3.0. This coal
104 seam is formed of semibright coal and glance coal, and a small amount of semidull coal.
105 The porosity of FM 3# coal averages 4.88–6.14 %. The main structure in this coal seam

106 is formed by an initial fissure; fissure nondevelopment. The fissure number is 6-11 per
107 5.0 cm, length is 0.5-10.0 cm, and width is 2.0-120.0 μm. The connectivity of these
108 fissures is low, and is influenced by a calcite film in the coal. The average permeability
109 of Fm $3^{\#}$ coal is 0.02 mD, which was tested using the injection-falloff method in this
110 zone. The reservoir pressure for coal bed gas is 5.0-7.0 MPa, the fluid pressure gradient
111 is 0.07-0.09 MPa/m, and the desorption pressure is 2.0-2.5 MPa. Blockages form easily
112 in this coal seam with a nondevelopment fissure.

113 **2.3 Medium Preparation and Injection**

114 A standard medium was used to provide acclimation and culture the *Methanogen*
115 *sp.* in the coal bed. The final concentrations of the compounds (kg/m^3) were: yeast
116 extract, 0.50; $NaHCO_3$, 0.05; NH_4Cl, 2.30; KH_2PO_4, 1.30; K_2HPO_4, 0.70; NaCl, 0.05;
117 $MgSO_4 \cdot 7H_2O$, 0.20; $CaCl_2 \cdot 2H_2O$, 0.05. The final pH was 6.80, and the Cl^- ion
118 concentration was 44.59 mmol/L [9].

119 100 m^3 medium were prepared for Z-7H well, and 30 m^3 medium was prepared
120 for Z-159 well. The medium prepared with a 50 m^3 tank car, and injection performed
121 with a fracturing truck (4150 8X8, Benz, Germany). The injection pressure was
122 adjusted and maintained at less than 4.00 MPa. The Z-159 well and Z-7H well were
123 sealed in March 11 and 26, 2015. And the wellhead pressure was monitored no less
124 than 2 months (Figure 3).

125 **2.4 Medium Diffusion Monitors**

126 Medium diffusion in coal seam was monitored by Cl^- concentration changes

127 within the underground water [9]. Concentrations of Cl^- were identified using an ICS-
128 1100 ion chromatography system (Thermo Scientific Dionex, Bannockburn, United
129 States). The underground water sample was collected in wells Z-159, Z-163, Z-167 and
130 Z-7H and used aseptic, anaerobic 50 ml tubes. High-speed multifunction centrifugation
131 (J2-MC, Beckman Instruments, Fullerton, United States) and 0.22um filter membrane
132 were utilized to separate the suspended particles and microbes in underground water
133 samples. Purified samples were stored at -80 °C in a refrigerator (DW-86L728J, Haier,
134 Qingdao, China).

135 **2.5 Microbial observation**

136 Observations of microbe formation, fluorescence detection and microbial counting
137 were performed using an Olympus BX41 (Olympus, Tokyo, Japan) fluorescence
138 microscope at 400× and 1000× magnification with a blood cell counting plate. The
139 F420 fluorescence method was used to test for the presence of methanogenic bacteria
140 (McInerney and Beaty, 1988,).

141 **2.6 CBM wells productivity monitor**

142 CBM production; methane, carbon dioxide, and nitrogen concentrations were the
143 parameters used to analyze gas productivity and identify the effects of biological coal
144 gasification. Gas production rates in each well were monitored using a gas roots flow
145 meter (model: FLLQ, Fuma, Wenzhou, China). Measurement accuracy of the FLLQ
146 gas roots flow meter was class 1.0, and flow range of this meter was 0.6-400 m^3/h.

147 Methane and carbon dioxide analyses were performced using Agilent 7890A gas

chromatograph (Agilent, Tokyo, Japan). The nitrogen (carrier gas) flow rate was fixed at 1 ml/min. The injection port was maintained at 150 ℃, the oven temperature was 25 ℃ and the TCD was operated at 200 ℃. Retention time for methane was 3.76 minutes and 5.0 minutes for carbon dioxide. Calibration standards consisting of 40% methane, 20% carbon dioxide, 10% hydrogen and 30% nitrogen were injected at atmospheric pressure to provide the calibration plot.

2.7 DNA extraction and PCR

100 mL of underground water in the Z-7H well was collected at the initial and termination stages of this experiment. Bacteria was concentrated to 1 mL by centrifugation (J2-MC, Beckman Instruments, Fullerton, United States) and stored in cryovials at -80 ℃ (refrigerator type: DW-86L728J, Haier, Qingdao, China) until DNA was extracted. The centrifugal force was set to 13000 x g, and centrifuged for 10 minutes. Total genomic DNA was extracted from 1 mL concentrated underground water samples using E.A.N.A. Soil DNA Kit (OMEGA, Georgia, GA, USA) following the manufacturer's instructions.

The V4 region of 16S rRNA gene was amplified with polymerase chain reaction (PCR) using primers 515F (5'- GTG CCA GCM GCC GCG GTAA - 3') and 806R (5'- GGA CTA CHV GGG TWT CTA AT - 3') [11]. The primer pair was reported to generate an optimal community clustering with the sequence length in the V4 region [12]. Each 20 μL PCR reaction was composed of 2 ng of template DNA, 0.2 μM primers, 0.2 mM dNTP, 2 μL 10×Pfu Buffer with $MgSO_4$ (Applied Thermo, Califonia, CA,

169 USA), with H_2O up to 20 μL. The DNA amplification was performed under the
170 following cycling conditions: 1 cycle of 2 min at 95 ºC, followed by 30 cycles with 30
171 s at 95 ºC, 30 s at 55 ºC and 1 min at 72 ºC, then a final extension period of 5 min at 72
172 ºC. Before sequencing on the Illumina Miseq sequencing platform, we would amplify
173 the V4 region by adding sample-specific 10-base barcodes and universal sequencing
174 tags by Sample-Specific PCR protocol. The PCR procedure was as following: 1 cycle
175 of 95 ºC at 2 min, 15 cycles of 95 ºC at 15 s, 60 ºC at 30 s, 68 ºC at 1 min, the final step
176 was 1 cycle of 68 ºC at 3 min. Equal volume of each barcoded product was pooled into
177 amplicon libraries and purified using Agencourt AMPure XP system (Beckman Coulter,
178 CA, USA) then examined on Agilent Bioanalyzer 2100 for product size distribution.
179 The purified libraries were quantified with Qubit® dsDNA HS Assay Kit (Life
180 Technologies, CA, USA) and used for sequencing.

181 **2.8 Sequencing and Data Analysis**

182 16S rRNA gene libraries were sequenced using an Illumina MiSeq (San Diego,
183 CA, USA) platform and the sequencing data were base-called and demultiplexed using
184 MiSeq Reporter v.1.8.1 (Illumina, SanDiego, CA, USA) with default parameters. The
185 adapter sequences and low quality reads were trimmed away from the raw reads with
186 Trimmomatic v.0.32 [13]. Then the clean reads were analyzed using the Uparse [14]
187 and Mothur pipeline [15] to generate operational taxonomic units (OTUs). The OTUs
188 were picked out at 97% similarity [16]. The resulting representative sequence set was
189 aligned against the core sequence database of the SILVA 123 release with the Mothur

190 script (www.mothur.org/) and given a taxonomic classification using RDP at the 80%
191 confidence level [17]. The richness estimators (ACE and Chao1) and diversity indices
192 (Shannon and Simpson) were calculated using the Mothur program. OTUs'
193 comparisons were performed using the Venn diagram package. Neighbor-joining
194 phylogenetic tree was used to investigate the similarity of species abundance using the
195 Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering method
196 [18].

197 # 3. Results and Discussion

198 ## 3.1 Medium Diffusion in Coal Bed

199 The key factor needed to enhance the biodegradation of coal is nutrient distribution
200 in coal. Z-159 and Z-7H were the medium injection wells in the experiments. And the
201 medium was injected on March 10 and 25, 2015 respectively. Meanwhile, the Z-163
202 and Z-167 wells were the contrast wells. The coal bed water samples were collected
203 before the medium was injected, and on 7 February, 2016, after the Z-159 and Z-7H
204 wells resumed gas production. The change in of Cl^- ion concentration was served as an
205 indicator to assess whether the medium had diffused in surrounding wells field.

206 Chloride, in the form of the Cl^- ion, constitutes one of the major inorganic anions
207 in the underground water [19,20]. It originates from the dissociation of salts, such as
208 sodium chloride or calcium chloride, in underground water [21]. These salts, and their
209 resulting chloride ions, come from coal seam minerals. Medium diffusion in coal
210 changed the Cl^- ion concentration in the underground water, and as a result of

anthropogenic impacts [22]. The concentration of Cl^- in the underground water of wells Z-7H and Z-159 was altered following nutrition medium injection, showing significant variations in Cl^- concentrations due to the effects of nutrition distribution. Cl^- concentrations would increase in other wells if there was nutrition seepage flow into the control area of neighboring wells.

Baseline Cl^- concentrations for the Z-159, Z-163, Z-167, and Z-7H wells were considered to be stable at 100~110 mg/L. This value increased two to three times in 70 days, after the experiments were performed for the Z-159 and Z-7H wells. In contrast, the Cl^- concentration within the Z-163 and Z-167 wells was maintained at about the same level over the experimental period (shown in Table 1). These findings confirmed that the distribution of the culture medium and the coal biogasification were active in the Z-159 and Z-7H wells, and did not spread to the Z-163 and Z-167 wells.

Table 1 here

Table 1. Cl^- concentration, *Methanogen sp.* number, and gas concentrations changes, before and during the experiments.

3.2 Changes in the number of *Methanogen sp.*

The biogasification of coal is performed through the collaboration of different microorganisms. They include hydrolytic fermentation bacteria, fermenting bacteria, and *Methanogen sp.*, etc [19]. And these bacteria had been found in Fm $3^{\#}$, Fm $9^{\#}$ coal

bed in Qinshui basin [5,8]. As the terminal bacteria of coal biogasification process, *Methanogen sp.* can indicate the development of methanogenic consortia for coal biodegradation in coal bed [10]. The number of *Methanogen sp.* in the sample could be observed by fluorescence microscopy, because of *Methanogen sp.* has a fluorescent effect in 420 nm light[5,20]. On this condition, it has a positive correction between biogasification of coal with the number of *Methanogen sp.*. Quantitative analyses of *Methanogen sp.* were performed before the medium was injected, and on 7 February, 2016 when the Z-159 and Z-7H wells resumed gas production. Initial levels of *Methanogen sp.* were lower than 1.25×10^5 per ml on average in the experimental and contrast wells. The *Methanogen sp.* numbers increased from less than 1.25×10^5 to 5.60×10^7 and 7.60×10^7 per ml in the Z-159 and Z-7H wells with the medium diffusing in the coal bed (shown in Table 1). Meanwhile, the number was stable at the original level in the Z-163 and Z-167 wells. These findings confirmed that the methanogenic consortia developed with intervention, and created biological conditions for biogasification of coal.

3.3 Effect Analysis of biogasification of coal

Biogasification of coal was performed through the bio-fermentation of organic compounds in the coal. 100 m^3 and 30 m^3 of medium were injected into the Z-7H and Z-159 wells, and were used to provide main nutrients for microbial growth in the coal. The Z-159 well resumed gas production on May 23, 2015 and terminated on February 16, 2017. And the Z-7H wells resumed gas production on June 7, 2015 and terminated

253 on August 14, 2017. The gasification of coal lasted 635 and 799 days, and yielded
254 74817 m^3 and 251754 m^3 CBM in Z-159 and Z-7H wells, respectively. The gas
255 production of contrast wells maintained the original characteristics during the
256 experiment (Figure 3). Especially, the Z-163 well maintained at non-gas productive
257 state during gas productivity has resumed in experimental wells.
258
259 Figure 3 here
260 Figure 3. Gas production (Qg) changes in experiment wells and in contrast wells
261 during experiment. The Z-7H and Z-159 were experiment wells. And Z-163 and Z-
262 167 were contrast wells. The Z-159 well resumed gas production on May 23, 2015
263 and terminated on February 16, 2017. And the Z-7H wells resumed gas production on
264 June 7, 2015 and terminated on August 14, 2017.
265
266 Ion tracers indicate that the nutrition diffused in the Z-159 and Z-7H wells in
267 experiments. With the effect of nutrition control, *Methanogen sp.* numbers increased,
268 from lower than 1.25×10^5 to more than 5.60×10^7 per ml, in nutrition injected wells.
269 These data were found to have a positive correlation with the changes in gas
270 productivity in certain wells.
271 In this experiment, gas concentrations showed certain changes, which were
272 influenced by the bio fermentation of coal. The yield of carbon dioxide, which as by-
273 products of coal biogasification, would influenced the CBM composition. Therefore,

the increase in the CO_2 content of the CBM could be another indication of the biogasification phenomenon of the coal (indicated in Table 1). The gas components of CBM in Z-167, which without influence of medium injection, were maintained at a stable level.

3.4 Analyses of changes in microbial community structure

Figure 4 here

Figure 4 Coalbed microbial community structures before and after experiment. SHR is microbial in row coal bed before experiment. And SHC is coal bed microbial after cultured.

Different microbial populations and coal constitute a coal bed ecological community. Microbial community ecology evolution is closely related to changes of coal bed environment factors. These factors include gas composition, nutrition, and specific surface area of coal [8]. Biodiversity testing before and after the experiment in Z-7H well had confirmed this evolution. Data indicated that microbial species have significant changes with medium injection in coal.

There were 105 strains detected in high-throughput sequencing of the original coal bed microbial community before the experiment. High relatively abundant genus was as follows: *Propionibacterium sp., Balneimonas sp., Anoxybacillus sp., Cupriavidus sp., Schlegelella sp., Clostridium sp., Clostridium sp., Pseudoalteromonas sp.,*

295 *Pseudoalteromonas sp.* etc. After cultured, meanwhile, the detected strains value in
296 High-throughput sequencing of the Z-7H well biome decreased to 85. And compare the
297 microbial community structure before and after experiment, only 11 species were the
298 common specie. And the number of common species with a relative abundance higher
299 than 0.5% is only four, which include: *Anoxybacillus_kestanbolensis sp.,*
300 *Escherichia_coli sp., Cupriavidus sp., Schlegelella sp.*. Analysis of the response of coal
301 bed biome to environmental changes and community evolution showed that two groups
302 of microorganisms were the most obvious. First of all was *Methanoculleus sp.* and
303 *Methanosarcina sp.*, both of them belong to the family *Methanomicrobia sp.*. Most
304 *methanogen sp.* make methane from CO_2 and H_2. Others utilize acetate in the
305 acetoclastic pathway. In addition to these two pathways, species of *Methanosarcina sp.*
306 can also metabolize methylated one-carbon compounds through methylotrophic
307 methanogenesis. Such one-carbon compounds include methylamines, methanol, and
308 methyl thiols [21]. Only *Methanosarcina sp.* species possess all three known pathways
309 for methanogenesis, and are capable of utilizing no less than nine methanogenic
310 substrates, including acetate [22]. *Methanoculleus sp.* is differ from other methanogens,
311 it can use ethanol and some secondary alcohols as electron donors as they produce
312 methane [23]. And followed by *Anaerolinea sp.* and some species from *Clostridiales*
313 *sp.*. *Anaerolinea sp.* has a fermentative metabolism, utilizing carbohydrates as well as
314 proteinaceous carbon sources [24]. It produces acetate, lactate and hydrogen as by-
315 products of glucose fermentation [25]. For *Clostridiales sp., Peptococcaceae sp.* and

316 *Clostridiales sp.*, they could produce butyric acid with organic fermentation. Varying
317 concentrations of acetic acid, lactic acid and/or ethanol, propanol or butanol are also
318 formed as fermentation products [26]. They provided nutrients for methanogens and
319 played an important roles in the new biome. The relative abundance of these microbe
320 was very low in original coal bed microbial community. The evolution of this coal bed
321 ecological community demonstrates the critical role of this experimental methods in
322 improving the gas production capacity in situ.

323 ## 4. Discussion

324 Results from this research have demonstrated that biogasification of coal could be
325 utilized to enhance gas well productivity. The factor of Cl^- concentration changes had
326 been tested to assess the medium diffusion in coal. The baseline of this factor was stable
327 at 100-110 mg/L in original coal seam. This value increased two to three times after
328 experiment performed for 70 days in both experimental wells. This conclusion
329 confirmed that the injected medium was maintained within the experimental wells and
330 not diffused to the periphery. Furthermore, the range of nutrition influence was
331 controlled in the scope of Z-159 and Z-7H wells.

332 Quantitative analyses of *Methanogen sp.* were performed before and after
333 experiment with 420 nm fluorescence counting. The *Methanogen sp.* numbers
334 increased from less than 1.25×10^5 to 5.60×10^7 and 7.60×10^7 per ml in the Z-159 and Z-
335 7H wells with medium diffusing in the coal bed. The number changes of *methanogen*
336 *sp.* confirmed that the methanogenic consortia developed with intervention, and created

337 biological conditions for biogasification of coal. The resumption of gas production in
338 wells Z-159 and Z-7H confirmed the effect of CBM enhancement with biogasification
339 of coal. The gasification of coal lasted 635 and 799 days, and yielded 74817 m^3 and
340 251754 m^3 CBM in Z-159 and Z-7H wells, respectively.

341 High-throughput sequencing had been used to analysis the coalbed ecological
342 community evolution in experiment. The proliferation of *Methanomicrobia sp.*,
343 *Anaerolinea sp. and Clostridiales sp.* reflected the coalbed microbial community
344 changes with medium diffusion in coal. And the biome indicated that the
345 methanogenesis mechanism of the Sihe coalbed had a composite character.

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429

Table

Table 1. Cl^- concentration, *Methanogen sp.* number, and gas concentrations changes, before and during the experiments.

	Well	Cl^- Concentration mmol/L	*Methanogen sp.* Number Per ml	Gas Concentrations % Vol CH_4	CO_2	N_2
Before the experiment	Z-159	1.92	$<1.25 \times 10^5$	97.21%	2.65%	0.14%
	Z-163	1.98	$<1.25 \times 10^5$	98.02%	1.95%	0.03%
	Z-167	1.96	$<1.25 \times 10^5$	97.52%	2.36%	0.12%
	Z-7H	2.03	$<1.25 \times 10^5$	97.82%	2.11%	0.07%
After the experiment	Z-159	5.40	5.60×10^7	87.00%	12.88%	0.12%
	Z-163	2.01	$<1.25 \times 10^5$	97.45%	2.44%	0.11%
	Z-167	2.04	$<1.25 \times 10^5$	97.82%	2.03%	0.14%
	Z-7H	4.01	7.60×10^7	79.95%	19.97%	0.08%

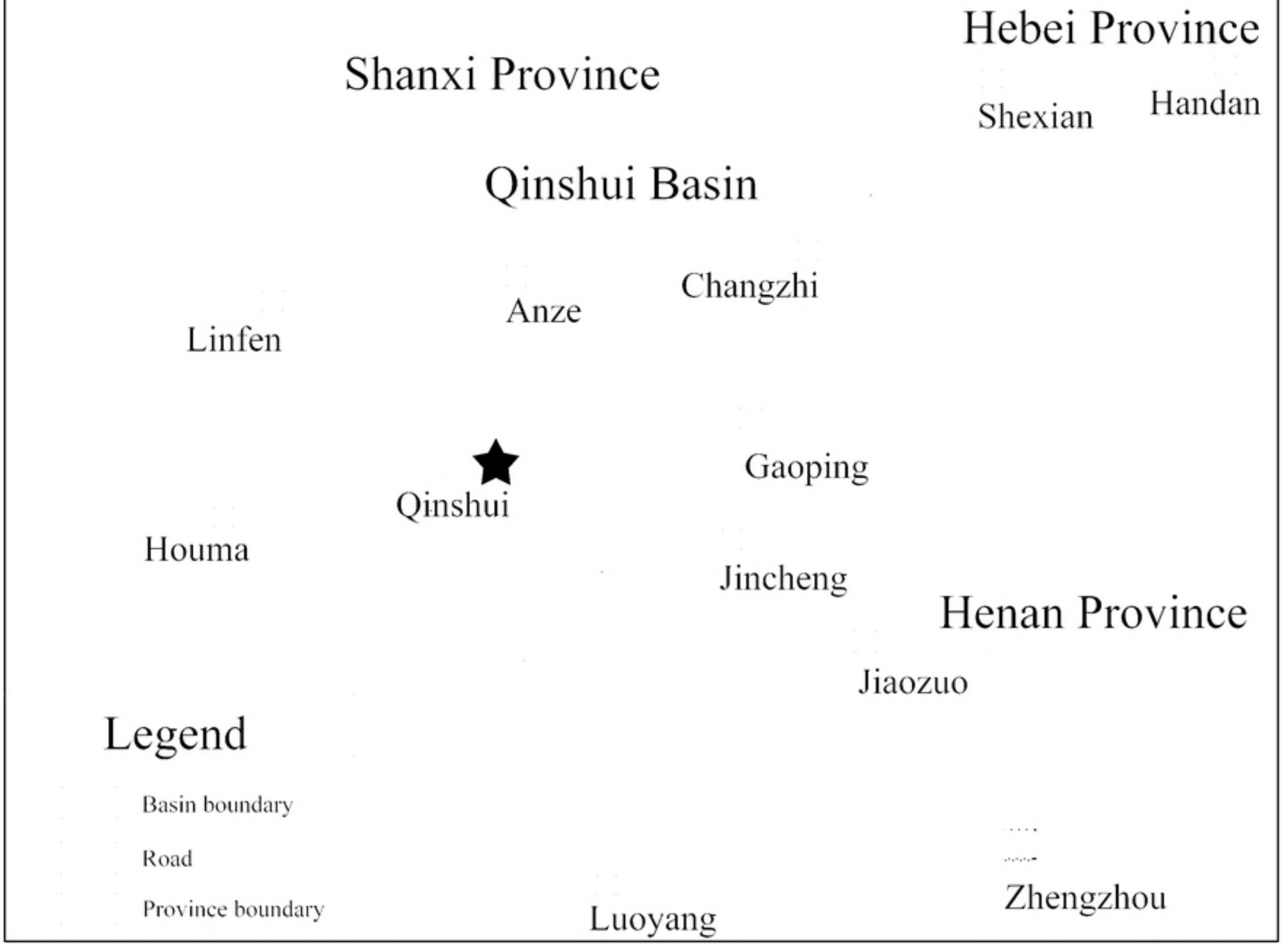
Hebei Province
Shanxi Province
Shexian
Handan
Qinshui Basin
Changzhi
Anze
Linfen
Gaoping
Qinshui
Houma
Jincheng
Henan Province
Jiaozuo
Legend
Basin boundary
Road
Province boundary
Zhengzhou
Luoyang

Figure 1

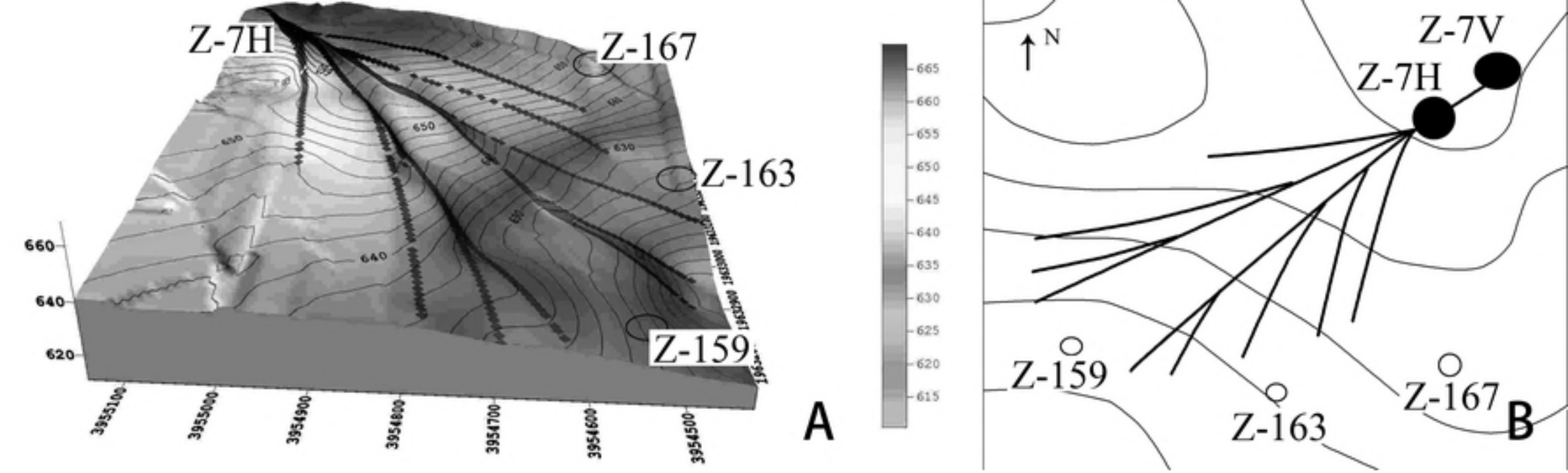
Z-7H
Z-167
Z-163
Z-159
660
640
620
650
630
640
3955100
3955000
3954900
3954800
3954700
3954600
3954500
A
665
660
655
650
645
640
635
630
625
620
615
N
Z-7V
Z-7H
Z-159
Z-163
Z-167
B

Figure 2

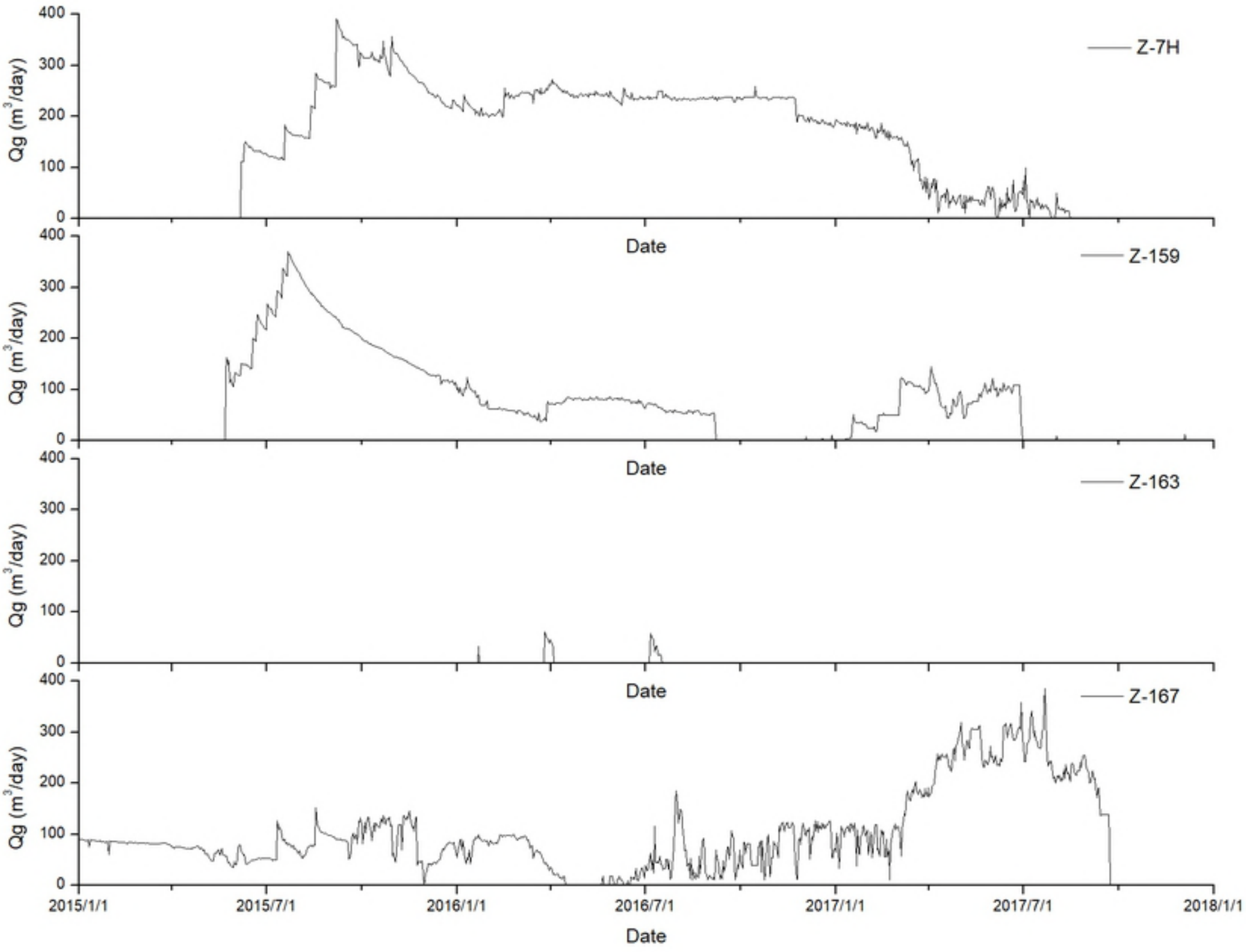
Z-7H
Z-159
Z-163
Z-167
Qg (m³/day)
0
100
200
300
400
Date
2015/1/1
2015/7/1
2016/1/1
2016/7/1
2017/1/1
2017/7/1
2018/1/1

Figure 3

	Source	Relative abundance(%)	Species annotation	
OTU203	SHC	0.567	*Not assigned*	**Firmicutes**
OTU90	SHC	3.270	*Not assigned*	
OTU61	SHR	0.185	*Clostridium butyricum*	
OTU143	SHC	0.727	*Tepidimicrobium sp.*	
OTU121	SHC	0.831	*Oscillospira sp.*	
OTU15	**SHC**	12.543	*Not assigned*	
OTU7	**SHC**	34.536	*Pelotomaculum sp.*	
OTU126	SHC	1.328	*Not assigned*	
OTU41	SHC	3.560	*Not assigned*	
OTU124	SHC	0.704	*Not assigned*	
OTU8	**SHR/SHC**	33.295/0.010	*Anoxybacillus kestanbolensis*	
OTU40	SHR	2.605	*Staphylococcus sp.*	
OTU177	SHC	0.571	*Cellulosimicrobium sp.*	**Actinobacteria**
OTU53	SHR	1.759	*Propionibacterium acnes*	
OTU116	SHR	0.779	*Arthrospira platensis*	**Cyanobacteria**
OTU12	**SHC**	14.498	*Anaerolinea sp.*	**Chloroflexi**
OTU2495	SHC	0.771	*Not assigned*	
OTU73	SHR	2.494	*Not assigned*	**Proteobacteria**
OTU11	**SHR**	17.887	*Not assigned*	
OTU68	SHR	1.569	*Acidovorax defluvii*	
OTU14	**SHR/SHC**	13.876/0.003	*Schlegelella sp.*	
OTU25	SHR/SHC	6.839/0.003	*Cupriavidus sp.*	
OTU89	SHR/SHC	1.584/0.013	*Escherichia coli*	
OTU117	SHR	0.943	*Not assigned*	
OTU112	SHR	0.749	*Oceanisphaera sp.*	
OTU39	SHR	2.754	*Pseudoalteromonas sp.*	
OTU37	SHR	2.572	*Balneimonas sp.*	
OTU114	SHR	0.876	*Rhizobium sp.*	
OTU32	SHR	3.235	*Sediminibacterium sp.*	**Bacteroidetes**
OTU22	SHC	6.427	*Methanosarcina sp.*	**Euryarchaeota**
OTU3426	SHC	0.924	*Methanoculleus sp.*	
OTU10	**SHC**	18.713	*Methanoculleus sp.*	

Figure 4