

1 **Effects of Xiantao sewage treatment on microbial**
2 **community structure of shallow groundwater in**
3 **wetland**

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13 **Abstract**

14 In order to solve the problem of urban domestic sewage treated by semi natural
15 wetland method with high connectivity between surface water and underground water
16 in the flood diversion channel, Whether there is a blank study on the pollution of
17 shallow groundwater. The community structure and abundances of COD_{Cr}, total
18 nitrogen, total phosphorus and microorganism were added to the surface water.
19 Environmental factors such as COD_{Cr}, TN, TP, NH₄⁺-N and microbial community and
20 abundance indices were monitored through surface water and groundwater. In this
21 study, physical and chemical indicators, microbial diversity and community structure

22 of 12 water samples from Xiantao artificial wetland were studied by using the
23 Illumina Miseq sequencing technique and the resulting microbial data were
24 statistically analyzed in combination with environmental variables. The results
25 showed that COD_{Cr} concentration had a very significant positive correlation with total
26 microbial communities ($r=0.530$, $P<0.01$), NH₄⁺-N and TN were significantly
27 positively correlated ($r=0.337$ and 0.325 , $P<0.05$). In addition, COD_{Cr} concentration
28 was highly positively correlated with abundant groups ($r=0.520$, $P<0.05$), NH₄⁺-N and
29 TN were significantly positively correlated ($r=0.325$ and 0.304 , $P<0.05$). For rare taxa,
30 they might be more sensitive to the environment than their abundant groups. The
31 relative abundance of the rare group is 0 at the depth of 10m, so we should carefully
32 evaluate microbial reaction (rare group) environmental conditions in the future.

33 **Key world:** Wetland method, shallow groundwater, microorganism, diversity

34 **Introduce**

35 In recent years, the crisis in China's groundwater has been highly concerned by the
36 international community. In July 15, 2010, the News edition of the Nature¹ magazine
37 covered the whole page of the groundwater crisis in China (Qiu, 2010). In some areas,
38 the storage of groundwater is decreasing at an alarming rate, and 90% of the water in
39 the country has been polluted in varying degrees, of which 60% are seriously
40 polluted.

41 According to the survey, in addition to landfill and gas station, urban sewage,
42 industrial wastewater and agricultural wastewater from some large farms are also
43 polluting in China's urban groundwater (Xu, 2012). Some industrial and mining
44 enterprises and large farms, driven by economic benefits, try to drain the sewage into
45 the underground and think that the polluted water is "safe". In fact, shallow

46 groundwater and deep groundwater are only a relative concept, the distribution of
47 groundwater is not uniform, and the absolute aquifers are not. The shallow
48 groundwater and deep water are interconnected in many places, so the shallow
49 groundwater will naturally flow into the deep layer.

50 Artificial wetland is an ecological wastewater treatment technology a new type of
51 environmental protection (Yin, 2007), It has been successfully applied to storm flood,
52 domestic sewage and eutrophication water purification (Kobayashi, *et al.*, 2009;
53 Iasur-Kruh, *et al.*, 2010; Peralta, *et al.*, 2012; Sánchez-Carrillo, *et al.*, 2014).

54 The first example is to use the unused spillway semi natural wetland for urban
55 sewage treatment, and the Xiantao wetland wastewater treatment plant system is made
56 using the high water level surface water and the high connectivity water system of the
57 groundwater. There are few studies on the influence of sewage treatment on
58 groundwater by artificial wetland. The community and abundance of microbes in
59 shallow groundwater under this condition, especially the effect of the cumulative
60 results of pollutants on the groundwater under the long-term treatment conditions is
61 almost not. The comprehensive study on the utilization of unused spillways, the
62 treatment of domestic sewage by semi natural wetland, wetland method, the
63 community and abundance of microbes in groundwater, the contamination of
64 groundwater, the community and abundance of microbes in groundwater and the
65 environmental factors of groundwater are the blank. The microbial diversity of
66 surface water is one of the most important branches of water environment (Fu, *et al.*,
67 2017; Guo, 2010). The physical and chemical properties of surface water vary with the
68 conditions of space and light. However, the distribution of microorganisms in shallow
69 groundwater under different depths is also a very important research topic. In this

70 study, the samples not only have a wide range of samples, but also select three depths
71 of 0.2m, 5M and 10m in the vertical direction. The community structure and
72 abundance index of COD_{Cr}, total nitrogen, total phosphorus and microbes are added to
73 the surface water, and the environmental factors of surface water and groundwater
74 (such as COD_{Cr}, TN, TP, NH₄⁺-N) and high throughput sequencing are monitored.
75 Methods the microbial diversity was analyzed, and the structure and composition of
76 microbial community in the shallow groundwater of domestic sewage, the
77 relationship between the microbial community and the environmental factors were
78 carried out by the wetland method. It can reflect the influence of the microbial
79 community in the shallow groundwater during the operation of the wetland, and the
80 correlation between the microbial community and the abundance change and the
81 pollution level of the organic matter in the groundwater can provide the follow-up
82 workers with the corresponding theoretical and practical support.

83 **1. Materials and Methods**

84 **1.1 Introduction of Xiantao wetland method for domestic sewage treatment test**

85 The Xiantao wetland experimental base is introduced in detail and the sampling
86 azimuth reference Song (Song *et al.*, 2015).

87 **1.2 Determination of sampling and environmental factors of water samples**

88 Methods of sampling and environmental factor determination refer to Song (Song *et*
89 *al.*, 2015).

90 **1.3 Sequencing analyses**

91 The processing method of raw data is obtained by referring to Song (Song *et al.*,
92 2015).

93 2. Results

94 2.1 Analysis of physical and chemical indexes of wastewater.

95 According to the state regulations, the treated sewage should reach the standard of
96 pollutant discharge of GB 18918-2002 «municipal wastewater treatment plant»,
97 M1(400m) and M4(600m) were high concentration($\text{COD}_{\text{Cr}} > 120\text{mg/L}$); M7(3000m)
98 had achieved the two level standard of «municipal wastewater treatment plant»(100
99 $\text{mg/L} < \text{COD}_{\text{Cr}} < 120 \text{mg/L}$); and M2, M3, M5, M6, M8 and M9 were reach the first
100 level standard ($\text{COD}_{\text{Cr}} < 50 \text{g/L}$). The TP of these samples were $0.02 - 5.14\text{mg/L}$, very
101 significant positive correlation with COD_{Cr} ($r=0.984$, $P<0.0001$). Samples $\text{NH}_4^+\text{-N}$
102 contents were $0.49-8.81\text{mg/L}$, very significant positive correlation with COD_{Cr} (r
103 $=0.987$, $P<0.0001$). Samples TN contents were $1.13-19.17\text{mg/L}$, very significant
104 positive correlation with COD_{Cr} ($r =0.983$, $P<0.0001$) (**Table 1**). As can be seen from
105 table 1, along with the level of wetland before treatment, the environmental factors
106 gradually decreased. There is no phosphorus in the groundwater quality standard
107 (GB/T 14848-2017). It is generally believed that the concentration of phosphorus in
108 the groundwater is very low, but the phosphorus in the groundwater is present, and the
109 amount of the phosphorus is large, and the pollution of phosphorus can not be ignored.
110 The characteristics of the highly connected water system of surface water and
111 groundwater are fully reflected here. From the surface water to the vertical direction
112 of groundwater, that is, from surface water, groundwater depth of 5 meters, and buried
113 depth of 10 meters, the environmental factors index is also gradually decreasing.
114 But from the horizontal direction of the wetland in the depth of 5 meters and 10
115 meters, the reduction is different.

116 2.2. Microbial diversity of the researched constructed wetland

117 A total of 80524 high-quality sequences with 5755-24785 sequences (mean=13454)
118 and 726.9-1077.1 OTUs (mean=855) for twelve water samples were obtained. the
119 diversity index, including Shannon (4.9-6.0), phylogenetic distance of a whole
120 tree(48.1-68.5), and Chao 1 (2243.8–3291.9). All the calculated diversity indices in
121 this study decreased with geographical position variation of azimuth of the studied
122 samples (**Table 2**). The dominant phyla (the relative abundance of more than 1%) in
123 the studied samples were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*,
124 *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes*, *Acidobacteria*, *Chloroflex* and
125 *Euryarchaeota* (**Figure 1**). *Proteobacteria* is the most abundant phylum (more than
126 84% of total sequence reads).

127 Among the retrieved OTUs, a total of 8-20 OTUs were classified as abundant
128 OTUs. These abundant OTUs accounted for 5.63-15.15% of total OTUs and
129 represented 64.66-80.85% relative abundance of sequence reads in the studied
130 samples. In contrast, a total of 0-139 rare species were identified and they accounted
131 for 0-64.19% of total OTUs and 0-6.98% relative abundance of sequence reads in the
132 studied samples (**Table 3**). Most abundant OTUs belonged to *Proteobacteria* and
133 *Bacteroidetes*, which the abundant OTUs accounted for 29.61-72.66% and 0-38.5% of
134 total sequence reads in the studied samples (**Table 4**).

135 The studied shows that the water samples with similar physical and chemical tests
136 have similar patterns of microbial structure. For example, cluster analysis revealed
137 that the 12 samples are aggregated into two large clusters, of which the wetland
138 surface water samples (M1, M4 and M7) are clustered into one cluster. The 5m deep
139 and 10m deep water samples are divided into one cluster (except M9) (**Figure 2**). The
140 horizontal direction Surface water of groundwater in Constructed Wetlands (MC1, M1,
141 M4 and M7) were dominated by sequences affiliated with *Betaproteobacteria*,

142 *Gammaproteobacteria*, *Epsilonproteobacteria*, *Actinobacteria*. The depth is 5 meters
143 (MC2, M2, M5, and M8) were dominated by sequences affiliated with
144 *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, *Melainabacteria*,
145 *Sphingobacteriia*. The depth is 10 meters (MC3, M3, M6 and M9) were dominated by
146 *Betaproteobacteria*, *Bacilli*, *Gammaproteobacteria*, *Alphaproteobacteria*,
147 *Flavobacteriia*, *Sphingobacteriia*. the vertical of 400m (M1, M2 and M3) were
148 dominated by sequences affiliated with *Betaproteobacteria*. 600m (M4, M5 and M6)
149 were dominated by sequences affiliated with *Betaproteobacteria*, *Bacilli*,
150 *Gammaproteobacteria*, *Alphaproteobacteria*, *Sphingobacteriia*. 3000m(M7, M8 and
151 M9)were dominated by sequences affiliated with *Betaproteobacteria*,
152 *Gammaproteobacteria*, *Flavobacteriia*, *Epsilonproteobacteria*, *Melainabacteria*,
153 *Sphingobacteriia*. The contrast samples(MC1, MC2 and MC3) were dominated by
154 *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, *Actinobacteria* (**Table 5**).

155 **2.3 Statistical analyses.**

156 Statistical analyses further corroborated the influence of different positions on the
157 structure of community in the studied samples. Mantel test showed that structure of
158 community of the treatment of surface water from domestic sewage by wetland
159 method was correlated ($P<0.01$) with COD_{cr} ($r=0.520$) (**Table 6**). Furthermore,
160 Bray-Curtis similarity of abundant microbial communities were significantly
161 correlated ($r=0.530$) with treatment of surface water from domestic sewage by
162 wetland method (**Figure 3**). Likewise, Mantel test indicated that the abundant
163 structure of community were great significantly correlated to COD_{cr} ($r=0.530$)(**Table**
164 **6**). In addition, Mantel tests also showed that three other indicators the total and
165 abundant structure of community were significantly correlated with NH_4^+-N ($r=0.325$

166 and $r=0.337$), TN($r=0.304$ and $r= 0.325$) (**Table 6**).

167 **3. Discussion**

168 The interaction of surface and groundwater covering almost in the process of water
169 circulation, rainfall (Vidal, *et al.*, 2008), interception (Schellekens, 2000), evaporation
170 (Zhang, *et al.*, 2008), infiltration (Yimer, *et al.*, 2008), mathematical simulation and
171 physical processes, and filtration (Fukada, *et al.*, 2008), pollutants migration
172 (Willhelm, *et al.*, 1996) and other chemical processes, biological processes and the
173 main contents of the study appear in the review article or some aspects of
174 bioremediation for pollution.

175 In constructed wetlands, the removal of pollutants is mainly done through the
176 physical, chemical and biological synergies of the matrix, microbes and plants in the
177 wetland (Babatunde, *et al.*, 2008). It is generally believed that biological action is the
178 core factor of sewage purification in artificial wetland (Liang, *et al.*, 2003). Some
179 scholars through soil column simulation test, the effect of underground infiltration
180 system on pollutant removal in sewage has been studied (Lance, *et al.*, 1980). The
181 results also confirm that the main removal effect in underground percolation system is
182 biological action.

183 Combined with artificial wetland, the changes of microbial community in shallow
184 groundwater in artificial wetland in the process of habitat restoration were compared
185 and analyzed. It is found that geographical location is an important factor affecting the
186 microbial diversity and community structure of the lake surface sediments. This
187 finding is consistent with recent studies. Some scholars found that the number of
188 microbes in fine sand decreased with the increase of sand depth (Liu, 2014). Some
189 scholars found that the number of bacteria and fungi on the surface of the system was

190 significantly higher than that of the lower layer. As the vertical height of the system
191 deepened, the number of fungi decreased gradually (Zhou, *et al.*, 2009). The total
192 trend of bacteria and nitrite in the vertical direction is decreasing, and the number of
193 microbes in the upper layer is more than that in the lower layer. The overall trend of
194 bacteria and nitrite in the direction of water flow is also decreasing, that is, the front
195 part is more than the middle and posterior parts (Fu, *et al.*, 2005). Some scholars
196 pointed out that in the same vertical subsurface flow wetland system, planting
197 different plants also had an impact on the microbial community in the wetland, but
198 they only had a significant impact on the 0~10 cm surface of the system, and the
199 deeper microbial communities were basically similar (Sleytr, *et al.*, 2009). In addition,
200 in this study, experiments showed that the total microbial community structure was
201 also significantly related to the $\text{NH}_4^+\text{-N}$ and TN values of wetland, but lower than the
202 correlation coefficient of COD_{cr} ($r = 0.337, 0.325$ VS $R = 0.530$, Table 6).

203 It is noteworthy that M9 and wetland surface water samples (M1, M4, M7), M6 and
204 M8, M2, M3 and M5 three groups of samples gathered separately (**Figure 2**),
205 indicating that some similar microorganisms may be shared between polluted area and
206 water purification area. The reason for this phenomenon is that the concentration of
207 pollutants in the surface water samples is too high to show the eutrophication state.
208 Therefore, the number of microbes is large, and the concentration of pollutants
209 decreases with the depth and direction of flow, and the nutrients are less, so the
210 number of microbes is less.

211 It is also notable that the relative abundance of rare and abundant species of high
212 surface water in the surface water treated in this study is less than that in the target
213 area (**Table 3 and 4**), The rare communities showed more obvious reaction than the
214 rich communities (which were proved by larger relative abundance), which indicated

215 that rare taxa might have more restrictive distribution than the rich groups. Abundant
216 groups can make use of abundant resources, thus having a very low probability of
217 extinction and high probability of propagation. In addition, rare taxa may have less
218 suitable niches in the 10m buried area, which are more vulnerable to environmental
219 conditions than abundant taxa. Therefore, rare taxa may be responsive more
220 sensitively to other environmental conditions than other rich taxa.

221 From the above research results, it can be seen that the geographical location is the
222 most important factor affecting the microbial diversity and structure, whether it is a
223 rich or rare community in the fairy peach artificial wetland. Rare groups are more
224 sensitive to geographical location (possibly including other environmental conditions)
225 than their abundant species.

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317 **Table legends**

318 **Table 1. Detection results of surface water and groundwater in Constructed Wetland.**

Sample		Location	COD _{Cr} (mg/L)	TP (mg/L)	NH ₄ ⁺ -N (mg/L)	TN (mg/L)
MC1	-2000	surface water	38.59	0.04	1.96	2.03
MC2	-2000	deep of 5m	2.89	0.03	1.73	1.28
MC3	-2000	deep of 10m	2.37	0.02	0.49	1.13
M1	400m	surface water	182.53	5.14	8.81	19.17
M2	400m	deep of 5m	3.67	0.18	1.85	2.94
M3	400m	deep of 10m	3.00	0.05	1.14	1.93
M4	600m	surface water	151.66	4.98	8.43	16.51
M5	600m	deep of 5m	2.94	0.05	1.33	2.87
M6	600m	deep of 10m	2.36	0.04	1.08	1.66
M7	3000m	surface water	111.16	3.45	6.55	14.21
M8	3000m	deep of 5m	2.92	0.04	0.96	1.77
M9	3000m	deep of 10m	2.25	0.03	0.91	1.39

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336 **Table 2. Alpha-diversity of the surface water and groundwater in the studied Xiantao**

337 **constructed wetland.**

sample	Total reads	Observed OTUs	Simpson	Shannon_ Wiener	PD_whole _tree	Good's Coverage	Chao1
MC1	18976	802.6	0.994	8.7	53.5	0.7	2393.2
MC2	21167	735.1	0.972	7.8	50.9	0.7	2306.8
MC3	16403	763.7	0.99	8.2	57.0	0.7	2336.8
M1	8237	966.7	0.987	8.7	59.3	0.6	3055.5
M2	11825	1077.1	0.992	9.2	68.5	0.6	3165.8
M3	7857	1046.8	0.991	9.0	66.6	0.6	3291.9
M4	11839	937	0.989	8.7	60.8	0.6	2638.8

M5	20513	897	0.993	8.8	55.3	0.6	3029.1
M6	7066	848.2	0.98	8.2	55.2	0.6	2898.5
M7	24785	730.8	0.975	7.6	57.6	0.7	2535.6
M8	7030	730.4	0.977	7.7	51.7	0.7	2419.5
M9	5755	726.9	0.985	8.0	48.1	0.7	2243.8

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357 **Table 3. Abundance estimates of the abundant and rare OTUs in the studied surface water**
358 **and groundwater samples in this study.**

Sample	Abundant OTUs		Rare OTUs	
	(Percentage of abundant OTUs/ total OTUs in each sample)	Abundant OTU relative abundance (%)	(Percentage of rare OTUs/ total OTUs in each sample)	Rare OTU relative abundance (%)
MC1	20 (10.05%)	67.96	92 (46.23%)	5.27
MC2	17 (7.91%)	77.93	138 (64.19%)	6.98
MC3	16 (9.58%)	79.76	96 (57.49%)	5.75
M1	15 (10.42%)	64.79	0 (0.00%)	0
M2	8 (5.63%)	66.82	36 (25.35%)	3.27
M3	9 (7.69%)	71.97	0 (0%)	0
M4	17 (9.29%)	64.66	37 (20.22%)	2.47
M5	12 (6.45%)	73.03	93 (50.00%)	5.03
M6	13 (11.11%)	74.34	0 (0.00%)	0
M7	14 (6.01%)	74.05	139 (59.66%)	6.49
M8	12 (12.37%)	80.85	0 (0.00%)	0
M9	15 (15.15%)	78.77	0 (0.00%)	0

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376 **Table 4. Relative abundance of abundant OTUs within different phyla across the studied**
377 **samples in this study.**

Phylum	Proteobacteria	Bacteroidetes	Actinobacteria	Cyanobacteria	Firmicutes	Verrucomicrobia	Chloroflexi	Euryarchaeota
MC1	29.61	5.95	19.71	5.95	1.06	4.61	1.06	0.00
MC2	63.30	14.64	0.00	0.00	0.00	0.00	0.00	0.00
MC3	72.66	2.15	0.00	0.00	1.29	0.00	3.66	0.00
M1	58.21	5.05	0.00	0.00	1.53	0.00	0.00	0.00
M2	65.37	0.00	0.00	0.00	1.45	0.00	0.00	0.00
M3	69.71	0.00	0.00	0.00	1.16	0.00	0.00	1.10
M4	54.80	8.39	0.00	0.00	1.47	0.00	0.00	0.00

M5	63.21	8.76	0.00	0.00	1.06	0.00	0.00	0.00
M6	66.31	0.00	0.00	0.00	8.03	0.00	0.00	0.00
M7	66.40	6.14	0.00	0.00	1.52	0.00	0.00	0.00
M8	68.14	0.00	0.00	11.18	1.53	0.00	0.00	0.00
M9	40.24	38.53	0.00	0.00	0.00	0.00	0.00	0.00

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396 **Table 5. Relative abundance (%) of top 10 classes in the surface water and groundwater of**
397 **the studied Xiantao constructed wetland.**

Top10 classes	<i>Betaproteobacteria</i>	Bacilli	Gamma proteobacteria	Alphaproteobacteria	Flavobacteriia	Epsilon proteobacteria	Bacteroidia	Melainabacteria
MC1	32.58	3.16	2.66	5.95	5.17	0.11	0.13	0.13
MC2	60.24	1.24	7.68	1.42	0.56	0.13	13.16	0.04
MC3	44.14	3.53	34.38	1.91	0.51	0.09	3.46	0.00
M1	60.19	2.56	9.71	2.78	6.25	4.94	2.67	0.00
M2	74.35	1.95	4.54	2.72	0.86	0.32	0.00	0.23
M3	76.05	2.48	5.67	2.04	0.83	0.33	0.77	0.50
M4	54.30	2.41	10.33	2.54	7.99	6.02	4.95	0.07
M5	36.31	2.55	37.74	1.90	0.65	0.09	0.75	0.95
M6	27.25	11.42	12.55	37.70	1.64	0.34	0.79	0.23
M7	57.47	1.17	3.56	1.59	5.70	15.50	3.85	0.05
M8	59.76	3.22	13.55	4.59	0.21	0.21	0.00	11.18
M9	36.13	0.63	10.23	2.34	31.65	0.19	2.21	0.00

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415 **Table 6. Mantel test showing the correlation between structure of community similarity and**
416 **environment parameters of the studied surface water and groundwater in this study.**

	Abundant OTUs	All OTUs
COD	0.520**	0.530**
TP	0.206	0.232
NH ₄ ⁺ -N	0.325*	0.337*
TN	0.304*	0.325*

417 The Pearson's coefficients were calculated and their significances were tested based on 999 permutations.

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* $P < 0.05$ and ** $P < 0.01$.

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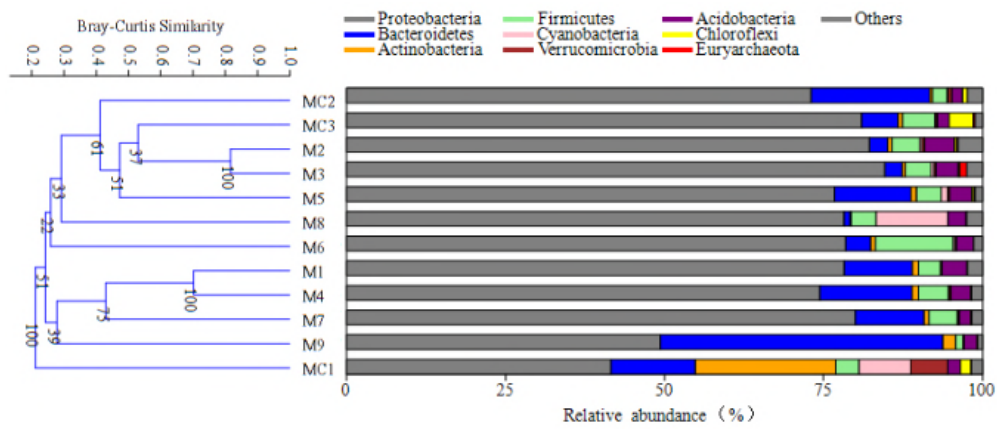
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443 **Figure legends**

444 **Figure 1. Bray-Curtis similarity-based cluster analysis (left) of structure of community in the studied**

445 samples and schematic figures (right) showing the frequencies of OTUs affiliated with major phyla in this
446 study.



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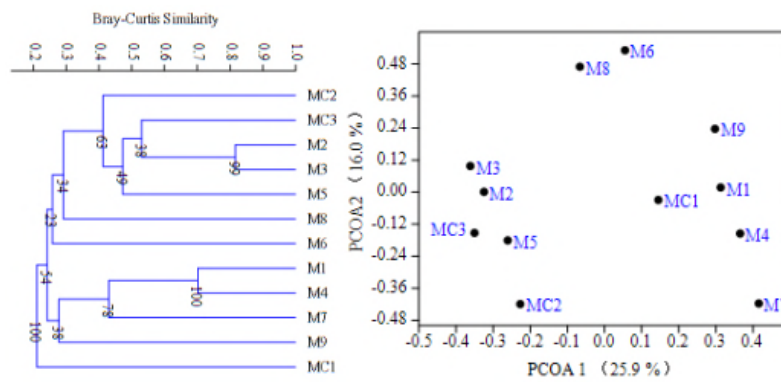
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474 **Figure 2. Clustering and principal coordinates analysis of total MCC among the studied samples based on**

475 **Bray-Curtis similarity.**



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506 **Figure 3. Cluster analyses and principal coordinates analyses of abundant structure of community among**

507 **the studied samples based on Bray-Curtis similarity.**

