

Effects of Xiantao sewage treatment on microbial community structure of shallow groundwater in wetland

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Abstract

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

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

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

Introduce

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

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

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

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

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

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

1. Materials and Methods

1.1 Introduction of Xiantao wetland method for domestic sewage treatment test

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

1.2 Determination of sampling and environmental factors of water samples

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

1.3 Sequencing analyses

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

2. Results

2.1 Analysis of physical and chemical indexes of wastewater.

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

But from the horizontal direction of the wetland in the depth of 5 meters and 10 meters, the reduction is different.

2.2. Microbial diversity of the researched constructed wetland

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

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

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

Gammaproteobacteria, *Epsilonproteobacteria*, *Actinobacteria*. The depth is 5 meters (MC2, M2, M5, and M8) were dominated by sequences affiliated with *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, *Melainabacteria*, *Sphingobacteriia*. The depth is 10 meters (MC3, M3, M6 and M9) were dominated by *Betaproteobacteria*, *Bacilli*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Flavobacteriia*, *Sphingobacteriia*. the vertical of 400m (M1, M2 and M3) were dominated by sequences affiliated with *Betaproteobacteria*. 600m (M4, M5 and M6) were dominated by sequences affiliated with *Betaproteobacteria*, *Bacilli*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Sphingobacteriia*. 3000m(M7, M8 and M9)were dominated by sequences affiliated with *Betaproteobacteria*, *Gammaproteobacteria*, *Flavobacteriia*, *Epsilonproteobacteria*, *Melainabacteria*, *Sphingobacteriia*. The contrast samples(MC1, MC2 and MC3) were dominated by *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, *Actinobacteria* (**Table 5**).

2.3 Statistical analyses.

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

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

3. Discussion

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

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

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

significantly higher than that of the lower layer. As the vertical height of the system deepened, the number of fungi decreased gradually (Zhou, *et al.*, 2009). The total trend of bacteria and nitrite in the vertical direction is decreasing, and the number of microbes in the upper layer is more than that in the lower layer. The overall trend of bacteria and nitrite in the direction of water flow is also decreasing, that is, the front part is more than the middle and posterior parts (Fu, *et al.*, 2005). Some scholars pointed out that in the same vertical subsurface flow wetland system, planting different plants also had an impact on the microbial community in the wetland, but they only had a significant impact on the 0~10 cm surface of the system, and the deeper microbial communities were basically similar (Sleytr, *et al.*, 2009). In addition, in this study, experiments showed that the total microbial community structure was also significantly related to the NH_4^+ -N and TN values of wetland, but lower than the correlation coefficient of COD_{Cr} ($r = 0.337, 0.325$ VS $R = 0.530$, Table 6).

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

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

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

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

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References

- Babatunde, A.O., Zhao, Y.Q., O'Neill, M. and O'Sullivan B.** 2008. Constructed wetlands for environmental pollution control: a review of developments, research and practice in Ireland. *Environ. Int.* **34**, 116-126.
- Fu, R.B., Yang, H.Z., Gu, G.W. and Zhang, Z.** 2005. Analysis of substrate microorganisms status in constructed wetlands and their correlation with pollutants removal for wastewater treatment. *Res. Environ. Sci.* **18**, 44-49.

- 239 **Fu, X.H., Liu, G.N., He, J.L., Ma, Y.Q., Song, X.K., Meng, L. and Jia, Q.M.** 2017.
240 Analysis of microbial community diversity in the Bohai Sea marine protected areas of
241 the Shandong Province. *Mar. Sci.* **41**, 39-47.
- 242 **Fukada, T., Hiscock, K.M., Dennis, P.F. and Grischek, T.** 2003. A dual isotope
243 approach to identify denitrification in groundwater at a river-bank infiltration site.
244 *Water. Res.* **37**, 3070-3078.
- 245 **Guo, C.** 2010. Thesis. Nanjing Agricultural University, Jiangsu, Nanjing, China.
- 246 **Iasur-Kruh, L., Hadar, Y., Milstein, D., Gasith, A. and Minz, D.** 2010. Microbial
247 population and activity in wetland microcosms constructed for improving treated
248 municipal wastewater. *Microb. Ecol.* **59**, 700-709.
- 249 **Kobayashi, T., Ryder, D.S., Gordon, G., Shannon, I., Ingleton, T., Carpenter, M.**
250 **and Jacobs, S.J.** 2009. Short-term response of nutrients, carbon and planktonic
251 microbial communities to floodplain wetland inundation. *Aquat. Ecol.* **43**, 843-858.
- 252 **Lance, J.C., Rice, R.C. and Gilbert, R.G.** 1980. Renovation of wastewater by soil
253 columns flooded with Primary effluent. *Water. Pollut. Control. Federation.* **52**,
254 381-388.
- 255 **Liang, W., Wu, Z.B., Cheng, S.P., Zhou, Q.H. and Hu, H.Y.** 2003. Roles of
256 substrate microorganisms and urease activities in wastewater purification in a
257 constructed wetland system. *Ecol. Eng.* **21**, 191-195.
- 258 **Liu, L.L.** 2014. Thesis. Xi'an University of Architecture and Technology, Shanxi,
259 Xi'an, China.
- 260 **Peralta, A.L., Matthews, J.W., Flanagan, D.N. and Kent, A.D.** 2012.
261 Environmental factors at dissimilar spatial scales influence plant and microbial
262 communities in restored wetlands. *Wetlands.* **32**, 1125-1134.
- 263 **Qiu, Jane.** 2010. China faces up to groundwater crisis. *Nature.* **466**, 308-308.
- 264 **Sánchez-Carrillo, S., Reddy, K.R., Inglett, K.S. Álvarez-Cobelas, M. and**
265 **Sánchez-Andrés R.** 2014. Biogeochemical indicators of nutrient enrichments in

266 wetlands: the microbial response as a sensitive indicator of wetland eutrophication, pp.
 267 203-222. Abid A. Ansari, Eutrophication: Causes, Consequences and Control-2014,
 268 Springer Science+Business Media Dordrecht, Netherlands, Germany.

269 **Schellekens J.** 2000. The interception and runoff generating processes in the bisle
 270 catchment,luquillo experimental forest, Puerto Rico. *Phys. Chem. Earth. Part. B.*
 271 *Hydrol. Oceans. Atmos.* **25**, 659-664.

272 **Sleytr, K., Tietz, A., Langergraber. G., Haberl, R. and Sessitsch, A.** 2009.
 273 Diversity of abundant bacteria in subsurface vertical flow constructed wetlands. *Ecol.*
 274 *Eng.* **35**, 1021-1025.

275 **Song, S.F., Yang, F., Ma,L.A. and Gao, X.F.** 2018. Effects of sewage treatment on
 276 microbial community structure of surface water in Xiantao wetland. *OALib.* **5**, e4583.

277 **Vidal, J.P. and Wade, S.D.** 2008. Multimodel projections of catchment-scale
 278 precipitation regime. *J. Hydrol.* **353**, 143-158.

279 **Willhelm, S.R., Schiff, S.L. and Robertson, W.D.** 1996. Biochemical evolution of
 280 domestic waste water in septic systems: 2.Application of conceptual model in sandy
 281 aquifers. *Ground. Water.* **34**, 853-864.

282 **Xu, Z.Z.** 2012. Thesis. Shanghai Jiao Tong University, Shanghai, Shanghai, China.

283 **Yang, J., Ma, L.A., Jiang, H.C., Wu, G. and Dong, H.** 2016. Salinity shapes
 284 microbial diversity and community structure in surface sediments of the
 285 Qinghai-Tibetan Lakes. *Sci. Rep.* **6**, 25078.

286 **Yimer, F., Messing, I., Ledin, S. and Abdelkadir, A.** 2008. Effects of different land
 287 use types on infiltration capacity in a catchment in the highlands of Ethiopia. *Soil. Use.*
 288 *Manage.* **24**, 344-349.

289 **Yin, J., Wen, Y. and Zhou, Q.** 2007. Progress of microbial ecology in constructed
 290 wetlands. *Environ. Sci. Technol.* **30**, 108-110.

291 **Zhang, Y.Q., Chiew, F.H.S., Zhang, L., Leuning, R. and Cleugh, H.A.** 2008.
 292 Estimating catchment evaporation and runoff using MODIS leaf area index and the

293 Penman-Monteith Equation. *Water. Resour. Res.* **44**, 2183-2188.

294 **Zhou, Q.H., He, F., Zhang, L.P., Wang, Y.F. and Wu, Z.B.** 2009. Characteristics of
295 the microbial communities in the integrated vertical-flow constructed wetlands. *J.*
296 *Environ. Sci.* 21, 1261-1267.

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Table legends

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

Sample	Abundant OTUs	Abundant OTU relative abundance (%)	Rare OTUs	Rare OTU relative abundance (%)
	(Percentage of abundant OTUs/ total OTUs in each sample)		(Percentage of rare OTUs/ total OTUs in each sample)	
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	Gammaproteobacteria	Alphaproteobacteria	Flavobacteriia	Epsilonproteobacteria	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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Table 6. Mantel test showing the correlation between structure of community similarity and 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*

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

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

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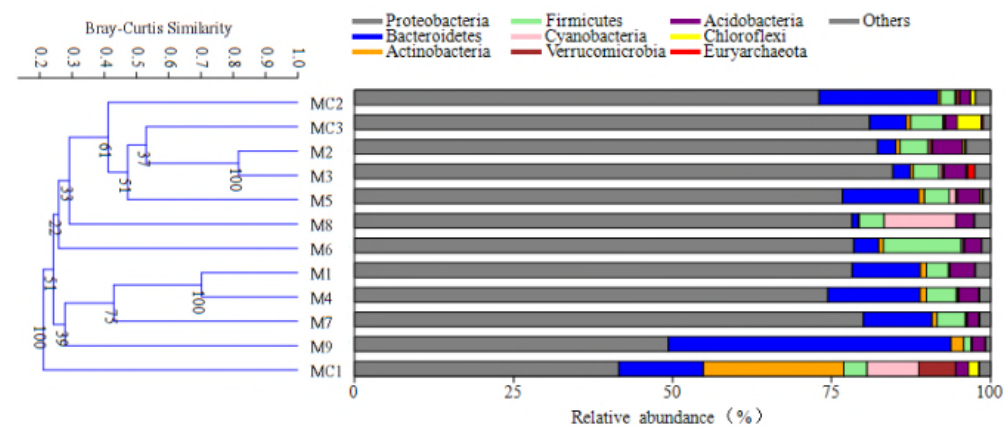
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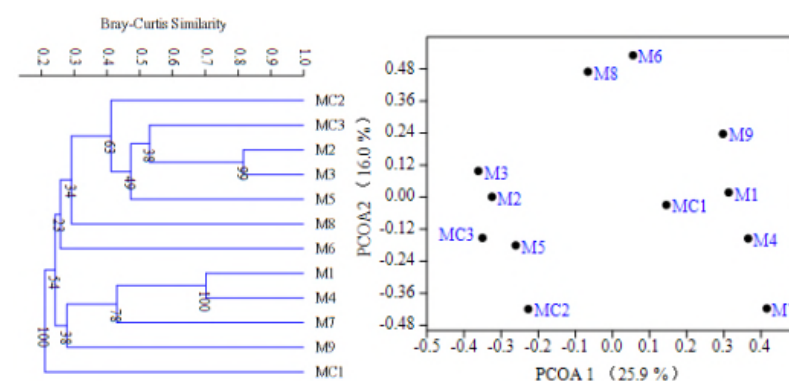
443 **Figure legends**

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

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



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Figure 3. Cluster analyses and principal coordinates analyses of abundant structure of community among the studied samples based on Bray-Curtis similarity.

