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Microbial community structure in confluence region of Huang, Wei and Luo rivers in Guanzhong plain in central China

Yalin Li*, Xiaoxing Ye and Qingqing Wang

Department of Biological Science, College of Environmental and Life Science, Weinan Normal University, Weinan, 714099, China.

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To investigating microbial abundance and community in confluence region of Huang, Wei and Luo rivers in Guanzhong plain in central China, plates culture were used to visualize quantity of soil microorganism colony, polymerase chain reaction and Denaturing Gradient Gel Electrophoresis (DGGE) were used to detect microorganism abundance and community. The results show that microorganism reduce in winter with the temperature lowering but increase in summer. River water can protect microorganism in sediment to repress quantity of microorganism reduce in winter. Microorganism is more abundance in vegetation surface than bench and sediment. In different three types of wetland microorganism species are also different, but proteobacteria is always a dominant bacteria in any time and any wetland. From these finding we can conclude that vegetation and soil organic matter affect the heterogeneity of microbial distribution. The presence of vegetation improves the soil alkaline environment. Microbiological composition in this area will provide a basis for construction of wetlands ecosystems.

Key words: wetland, bacteria, microorganism, microbial community.

INTRODUCTION

The wetland in the confluence area of Yellow river, Wei river and Luo river is a typical river wetland that is nature ecological environment and protected by local government. It is located in central China in Tongguan county, Weinan city, Shaanxi province (Li, 2008). Soil microorganisms are an important part of the wetland system, in which they play a role in the maintenance of wetland biodiversity, ecological balance. It is also as decomposers in degrading pollutants, resisting drought, regulating climate, preventing and delaying pollutants into the ocean, and also are important indicators for the evaluation of the wetland pollutant purification capacity (Yuan et al., 2016). Microorganism is also an indispensable component in wetland ecosystem and plays a very important role in material circulation, energy flow and other activities (Liu et al., 2013).

At present, researches from domestic and foreign scholars on wetland soil microorganism is still in the initial

*Corresponding author. E-mail: lyal1222@126.com.

Figure 1. Study area and sample sites in confluence region of Huang, Wei and Luo River in China (34° 61′N, 110° 25′E; elevation, 326 m).

stage, and most of the researches focus on the study of artificial wetland microorganism (Chen et al., 2017; Amaral et al., 2013). There are few reports on natural wetland microorganism. Microorganism community in natural wetland, the confluence area of Yellow, Wei and Luo river is always curious to us. This research is first time that we investigated the microorganism structure in this protected original ecological region. Microbial detection of this wetland has great significance to the development and utilization of microbial resources, the protection of wetland environment and the construction of wetlands.

MATERIALS AND METHODS

Samples collection

Sludge samples were collected from 3 spots within 1 to 5 cm surface: reed swamp vegetation, bench land and river sediments from conservancy area of confluence region of Huang, Wei and Luo river in China (34° 61′N, 110° 25′E; elevation, 326 m Figure 1). Enough sludge for each spots were collected in different season (April 3rd, July 5th, October 3rd and January 2nd). Sludge samples were placed in plastic Ziploc bags and stored in an icebox. All the samples were carried into laboratory and separated to three aliquot for three different using: soil characteristics analysis, major microorganism detecting and DNA extraction. Samples were used within 24 h from collecting.

Analysis of physicochemical parameters

Sample was spin and supernatant was using for pH and chemical elements detecting. 5 g sample was placed in 60°C constant temperature overnight for water content detection (water content% =(original soil weight-dry soil weight)/original soil weight*100%). Total nitrogen was detected by Kjedahl test. Total phosphorus was detected by H_2SO_4 – $HClO_4$ decoction and molybdenum blue colorimetry with ultraviolet spectrophotometer. Total potassium was detected with Atomic Absorption Spectrometry. Total organic carbon was detected by concentrated sulfuric acid - potassium dichromate H_2SO_4-K_2Cr_2O） heating.

Microorganism detecting

The quantity of Bacteria, Fungi and Actinomycetes was determined by plate culture method with beef extract peptone medium, Martin medium and Gause 1 medium respectively. Soil samples were diluted with sterile water by 3 ratios 1:10, 1:100 and 1:1000 to smear to plate medium. Each sample smeared three repeat plates. Then these plates were cultivated in 37°C for four days. The quantity of Bacteria, Fungi and Actinomycetes was estimated by counting colonies number.

DNA extraction

We investigated the microbial species further with molecular biological method. Total DNA for soil microorganism was extracted from 2 g soil sample using a Powersoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, United States) according to the protocol. The extracted DNA was dissolved with 50 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at 4°C until further use (within one week). DNA yields were measured with an ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Polymerase chain reaction (PCR) amplification

The general bacterial primers GC341f (5-CGC CCG CCG CGC GCG GCG GGC GGG GGG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3) and 534r (5-ATT ACC GCG GCT GCT

Table 1. Characteristics of soil in different type of wetland.

Place	Season	pH	H_2O	N	P	K	C
			%	g/kg	g/kg	g/kg	g/kg
Vegetation	Spring	7.23ng.31	57.4±2.5	2.74±2.	1.12±2.51	0.8322.51n/	57.622.5
	Summer	7.19er.51	52.6er.5	2.56er.	0.97er.51	0.825r.51n/	54.35r.5
	Autumn	7.14mn.51	55.4mn.5	2.84mn.	0.84mn.51	0.827n.51n/	55.87n.5
	Winter	7.21er.51	58.7±2.8	2.47±2.	1.03±2.81	0.8192.81n/	56.792.8
Bench	Spring	7.29ng.81	45.6ng.8	1.9±0.3	0.950.381	0.794.381n/	43.24.38
	Summer	7.32er381	38.4±2.7	2.14±2.	0.87±2.71	0.8102.71n/	45.702.7
	Autumn	7.24mn.71	42.3mn.7	1.83mn3	0.92mn371	0.807n371n/	48.67n37
	Winter	7.36er371	40.5er37	1.95er3	0.97er371	0.789r371n/	47.29r37
Sediment	Spring	7.32ngnt1	65.4ngnt	1.5±0.4	0.720.4t1	0.734.4t1n/	41.84.4t
	Summer	7.24er4t1	63.7er4t	1.3±0.2	0.680.2t1	0.750.2t1n/	43.60.2t
	Autumn	7.35mn2t1	68.9mn2t	1.69mn2	0.78mn2t1	0.783n2t1n/	43.73n2t
	Winter	7.31er2t1	66.1±4.0	1.51±4.	0.74±4.01	0.7414.01n/	42.514.0

GG-3) were used to get amplicon for 16S rRNA V3 hypervariable regions of the bacteria (Yu and Morrison, 2004). PCR was carried out in 50 µl reaction volume with template DNA (2 µl), dNTP(2.5 mM, 4 µl), Taq (2.5 U/µl, 1 µl), 10 × Taq buffer (5 µl), each primer (0.25 mmol/ L,1 µl) and sterile ddH_2O. PCR amplification was set as follows: 94°C for 5 min; 35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 30 s); 72°C for 10 min (Boon et al., 2002). PCR products was checked for the expected size on 2% agarose gel with ethidium bromide under UV light, and DNA extraction was carried with Qiagen MinElute Gel Extraction kit.

DGGE analysis

Denaturing gradient gel electrophoresis was used to detect bacteria communities (Liu et al., 2009). In this study, the 16S rDNA hypervariable V3 region was amplified by PCR with the bacterial primers 341f and −534r. PCR products were loaded onto 8% polyacrylamide gel with 40-60% denaturing gradient, which contains 7M urea and 40% formamide in 100% denaturation. The electrophoresis was run for 6 h at 60°C and 150V with 1 × TAE. After electrophoresis, the gel was stained to be visible with a Bio-Rad Gel imaging system, and DNA was extracted with Gel Extraction kit. Denaturing gradient gel electrophoresis (DGGE) bands were analysed using Quantity One software (Version 4.1.0, Biorad, USA). Extracted DNA sample then was send to do sequences analysis. Basic Local Alignment Search Tool (BLAST) was used to do homology analysis.

Data analysis

Values were presented as mean±SD of three independent samples. Data calculations and analysis were performed using the SPSS program (SPSS Inc., Chicago, IL, USA; Version 12.0). Duncan's test was performed to detect the statistical significance of differences ($p > 0.05$) between different groups.

RESULTS

Characteristics of soil

Physicochemical parameters for wetland soil are shown in Table 1. It shows no obvious difference in four season, but it really has some changes in different place. pH is slightly alkaline at all three places and the value is bigger at bench soil and sediment soil than vegetation soil. No difference in pH change in four season. Water content is sediment>vegetation>bench. Content of N, P, K and C is all higher in vegetation surface, but that is lower in sediment.

Microbial culture

For plate culture, bacteria and fungi grew out on the second day after smearing, but actinomyces appeared later on the third day. The number of bacteria, fungi and actinomycetes increased on the fourth day. It shows that the number of bacteria was the largest, followed by the fungi and actinomycetes. Bacteria amount is the most in summer and least in winter in vegetation and bench. In sediment, bacteria is just little less in winter but no big difference in other three season. Fungi is the most in autumn followed by summer and less in spring and winter in vegetation and sediment, while it is the most in summer followed by autumn, spring and winter in bench. Actinomycetes have the same trend with bacteria in vegetation and bench but it is just a little much in summer and no big difference in other three seasons in sediment (Figure 2).

Microbial community structure

DGGE experiments shows that there are different microbial community structure in three different spots as well as four seasons (Figure 3 and 4). In vegetation surface, the largest 6 species microorganism in proportion are Proteobacteria> Acidobacteria>

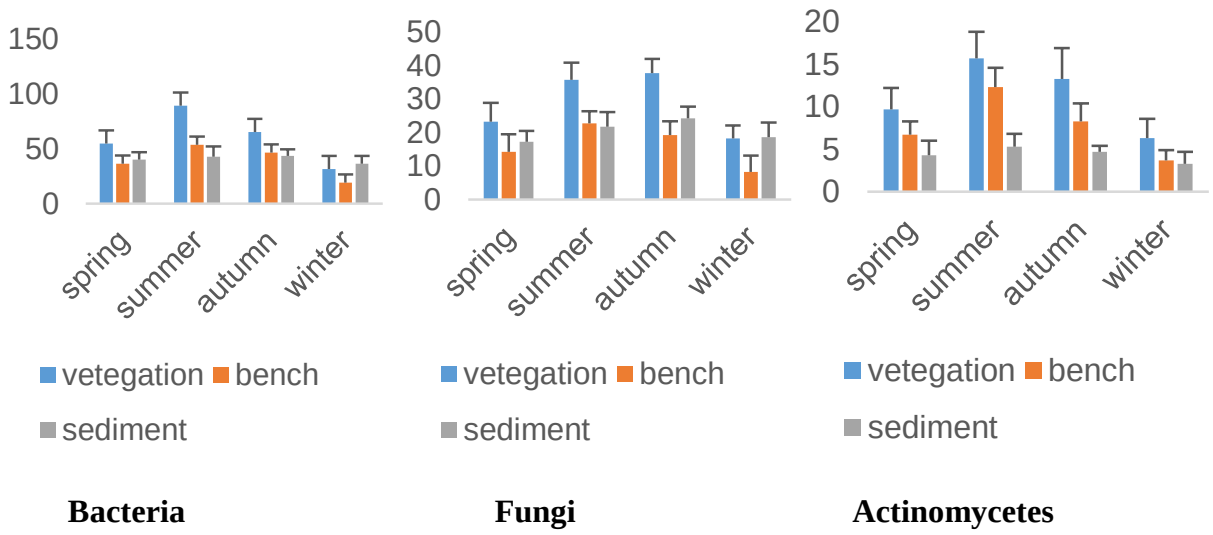


Figure 2. The relative abundance of bacteria, fungi and actinomycetes in different type of wetland.

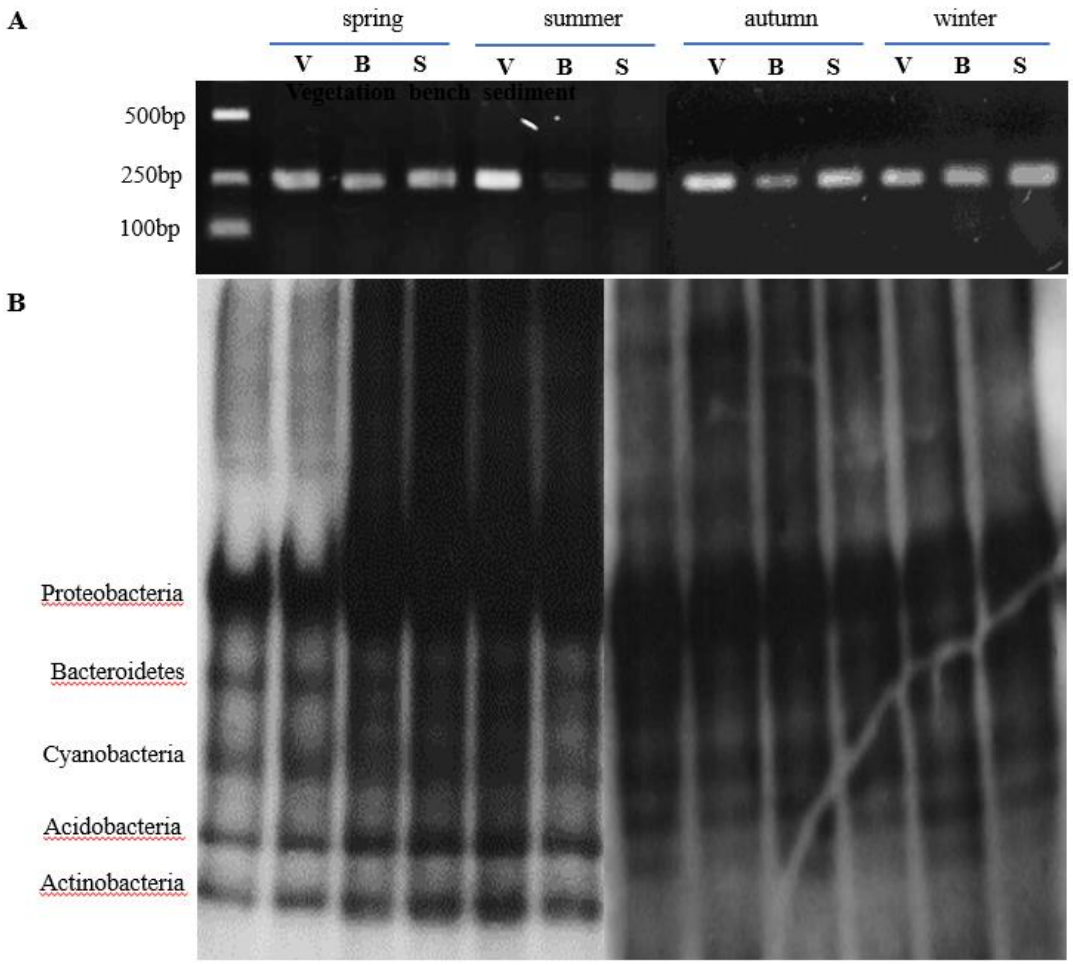
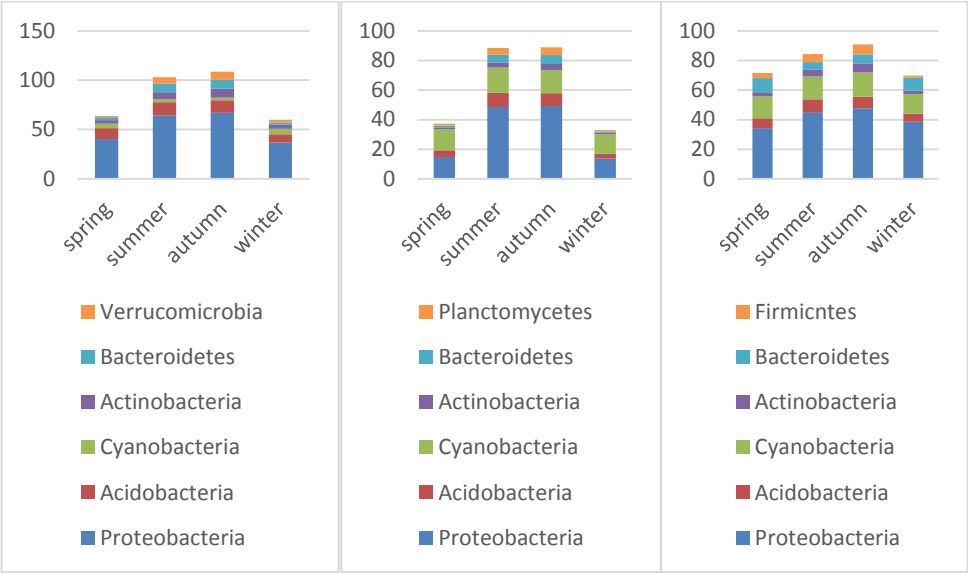


Figure 3. Different microorganism in four seasons and different types wetland. A: PCR results of different wetlands soil sample; B: different kinds of microorganism separated by DGGE. V, B and S represent vegetation, bench and sediment respectively.



(A) Vegetation (B) Bench (C) Sediment

Figure 4. Relative abundance of microorganism bacterial in phylum level in four seasons and different types wetland.

Bacteroidetes>Actinobacteria>Verrucomicrobia>Cyanobacteria in summer and autumn, but these microorganism reduce in spring and winter except cyanobacteria increasing. In bench surface it shows different microbial structure. Cyanobacteria amount was much higher than other microbial amount except proteobacteria in four seasons. Microbial community in sediment was similar to bench surface, but microbial amount in spring and winter was just a little less than that in summer and autumn. Proteobacteria was absolutely dominant species in any spots and any seasons.

DISCUSSION

The confluence region of Huang river, Wei river and Luo river belongs to temperature inland monsoon climate with distinct four seasons and limited rainfall. It is original ecological pollution-free protected area where there are three different types of wetland. The microbial abundance and community structure in this area have never been investigated. In this study we combined traditional methods and molecular biological methods to detect wetland microorganism constituent and seasonal variation in three different sorts wetland.

Soil property and temperature are two major factors for microorganism structure. In confluence region of three rivers the soil pH is slightly alkaline. The total content of N, P, K and C elements is primary influence factor to soil nutrient and presence of plant, which can change soil structure and increase soil nutrient (Singh and Singh, 2018). In the present research, it shows that content of N, P, K and C is all higher in vegetation surface, leading to more microbial amount in vegetation surface than bench and sediment. Results disclose that wetland microorganism is positive correlation with soil nutrient. The conclusion is consistent with the report by Xiao et al. (2015). In vegetation surface, pH value is smaller and nutritive element content is higher than these in bench and sediment. This finding shows that vegetation increase soil nutrient and adjust soil acid-base property. Environment temperature is also a major factor to influence microbial population and structure. The present study shows that microbial biomass is more abundant in summer and autumn than that in spring and winter, which is consistent with Wang et al. (2016).

Bacteria, fungi and actinomycetes were cultured with special different medium. The finding shows that bacteria is absolutely dominant species in any spots and any time, it followed by fungi then actinomycetes. The content of three microorganisms are related with temperature and follow the order summer>autumn>spring>winter in vegetation and bench. But in sediment microbial biomass is just little less in winter than summer. It suggest that presence of water can reduce temperature declining in sediment, so that microorganism is relatively more in winter than vegetation and bench, but totally microbial biomass in sediment is much less than vegetation and beach. The result shows soil microorganism in water-flooded layer reduce probably because soil respiration weaken and microorganism proliferation is repressed.

Microorganisms, soil and vegetation are composed of wetland ecosystem. The vegetation significantly affects the characteristics of the microbial community. Vegetation

provide oxygen, litters, root exudates and create a different soil environment that is benefit for presence of microorganism. Plant rhizosphere secretes organic substances to increase microbial density, degree of diversity and activity as Rhizosphere effect (Neubauer et al., 2002).

In order to investigate the microorganism community, we used bacteria general primes to get amplicon and sequencing it. The results reveal in phylum level, proteobacteria is dominate microorganism species in all three different spots. Acidobacteria is the secondly major species in vegetation, while in bench and sediment cyanobacteria is second, one followed by Acidobacteria. This finding shows there are different microorganism community in different types wetland. Same to plate culture results, all these microorganism content is relative with temperature. The order is autumn>summer> spring>winter in vegetation and sediment, but in bench the order is summer > autumn >spring>winter. Microbial biomass is relative higher in winter in sediment than that in vegetation and bench, this result is consistent with microorganism plate culture.

Our investigation firstly shows microbial diversity in confluence region of Huang river, Wei river and Luo river. The finding shows there is spatial variation in different types of wetland in this region. Microorganisms, soil and vegetation form a micro wetland ecosystem, and existence of plants can obviously increase soil nutrition and improve the living environment of microorganisms. Microbial coexist with vegetation also keeps soil nutrition element like N, P, K, C and improves the function of wetland ecosystem (Baptista et al., 2003). Microbiological composition in this region will provide a basis for construction of wetlands ecosystems, it implies that people should enlarge the area of aquatic plants artificially to improve beach condition and increase the function of wetland ecosystem, as well as develop and utilize wetland resources appropriately.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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