

Microbial diversity in lake–river ecotone of Poyang Lake, China

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Received: 24 July 2015 / Accepted: 17 February 2016 / Published online: 4 June 2016
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Abstract The ecological environment of lake–river ecotone is complex and changeable, it is an important water purification area and pollution buffer zone, where the microorganisms play an important role in the mediation of material cycle, nutrition transfer, and element transformation. Microbial diversity is sensitive to environmental changes. In this study, 12 water samples from the lake–river ecotone of the Poyang Lake in China were collected to determine microbial gene sequences employing next-generation DNA sequencing techniques, and to evaluate total nitrogen (TN) and total phosphorus (TP) using UV spectrophotometric method. The results showed the major Poyang Lake has the largest microbial population, followed by Yao Lake, Ganjiang River and Raohe River. Based on the Shannon and

Simpson Index, major Poyang Lake has the largest biodiversity of microbial communities, followed by Ganjiang River, Yao Lake, and Raohe River. Microbial characteristics vary with the TN and TP concentration, for instance, the nitrifying bacteria were relatively rich in Yao Lake and Ganjiang River ecotone, and the polyphosphate-accumulating organisms (PAO) in Raohe River were richer than those in Ganjiang River. The tag numbers of Euryarchaeota, Acidobacteria, Chlamydiae and Proteobacteria in Ganjiang River were more than in Raohe River, while significant differences were found between the major Poyang Lake and other lake–river ecotone, the operational taxonomic units (OTU) number (removing singletons) of major Poyang Lake have 3047, which was far more than the others. The study indicates that the Poyang Lake serves as a huge microbial gene pool, including a large number of DNA from ammonia oxidizing bacteria to ammonia oxidizing archaea.

This article is part of a Topical Collection in Environmental Earth Sciences on “Environment and Health in China II”, guest edited by Tian-Xiang Yue, Cui Chen, Bing Xu and Olaf Kolditz.

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Keywords Poyang Lake · Lake–river ecotone · Microbial groups · Diversity

Introduction

The environment of the lake–river ecotone is influenced by human activities, natural disaster, biogeochemical cycles and interaction between Lake and Rivers. Launched by IGBP/IHDP/WCRP (World Climate Research Programme)/DIVERSITAS (Integrating biodiversity science for human well-being), the global water system project (GWSP) has paid attention to the ecological environment problems in lake–river ecotone. Compared with the adjacent river ecological system or lake ecosystems, the boundary ecosystems may have a unique blend of characteristics and ecological communities (Patrick 2014), it is a more important water

purification zone and buffer zone of pollution, for its abundant species diversity, high primary productivity and secondary production, leading to a special place for the energy and material flow, the transitional zone have special characteristics that must be taken into account for conservation of biodiversity (Valdovinos et al. 2012). The microorganisms play an extremely important role in the energy conversion, material recycling, nutrients and element speciation transformation, accumulation and migration. So far, some microecological in the River and Lake ecotone is still unknown because of the limits of the traditional technology. Due to the rapid development of molecular biology technology which is based on the system of 16S rRNA gene (Pace et al. 1986; Winter et al. 2007), it is feasible to analyze microbial community comprehensively in different habitats (Venter et al. 2004; Lai et al. 2006; Agogue et al. 2005). Compared with the ordinary sequencing, the next-generation sequencing technology has the advantage of high flux, short test period, and is low-cost and repeatable (Mardis 2008; Ansorge 2009) and has been widely used in all kinds of environments, such as soil (Woo et al. 2014; Hansel et al. 2008), ocean (Nakajima et al. 2014), hot spring (Egas et al. 2014), lakes and rivers (Pascault et al. 2014; Liu et al. 2011), it promotes the study of uncultured microorganisms and trace amount bacteria in the environment, opening up a new research upsurge for the study of environmental microbial diversity. This study aimed to identify the microbial diversity in different lake–river ecotone, and to explore the evolution and adaptation of the microbial population to changing environmental conditions. This is the first report of the variation of microbial communities in pristine habitats of lake–river ecotone from China. Based on the analysis of the characteristics of the microbial distribution, this work is helpful to enhance understanding between microbes' population and environmental variations, and provide scientific basis for water environment protection and water ecological security.

Materials and methods

Study area

Poyang Lake is China's largest freshwater Lake, located at $28^{\circ} 22' N$ to $29^{\circ} 45' N$, $115^{\circ} 47' E$ to $116^{\circ} 45' E$, on the southern bank of the middle and lower reaches of the Yangtze River in the northern Jiangxi Province. Poyang Lake swallows a large quantity of water coming from five tributaries, including Xiushui River, Ganjiang River, Fu River, Xinjiang River and Raohe River. Due to the unique hydrological regime and special geographical conditions, the water level of Poyang Lake is controlled by both its tributaries and the Yangtze River and has a great seasonal variation (Wang et al. 2015a, b), producing wetlands over

3000 km^2 . Poyang Lake wetland is famous for its abundant biodiversity and has been registered in the UN as one of the world's important wetlands since 1992. Previous studies observed the microbial diversity in traditional Lakes and Rivers, found a significant difference in microbial diversity from northern to southern Poyang Lake (Wu et al. 2012), and more, examined the differences in microbial diversity at different seasons and locations across the Three Gorges Dam of the Yangtze River (Wang et al. 2012a, b), other studies compared the levels of bacterial diversity in freshwater, Intertidal wetland, and marine sediments (Wang et al. 2012a, b), it could provide the basis and reference for microbial diversity in lake–river ecotone. This study takes the lake–river ecotone of Poyang Lake as a case, sets the sampling sites in Poyang Lake and Raohe River ecotone (hx13 and hx11), Yao Lake and the Ganjiang River ecotone (hu1 and he2) (Fig. 1), the former is mainly affected by the agricultural activities (Zhang et al. 2015), the latter is connected with Poyang Lake but relatively independent and located at the edge of the city, mainly affected by domestic waste. Sampling sites are located in

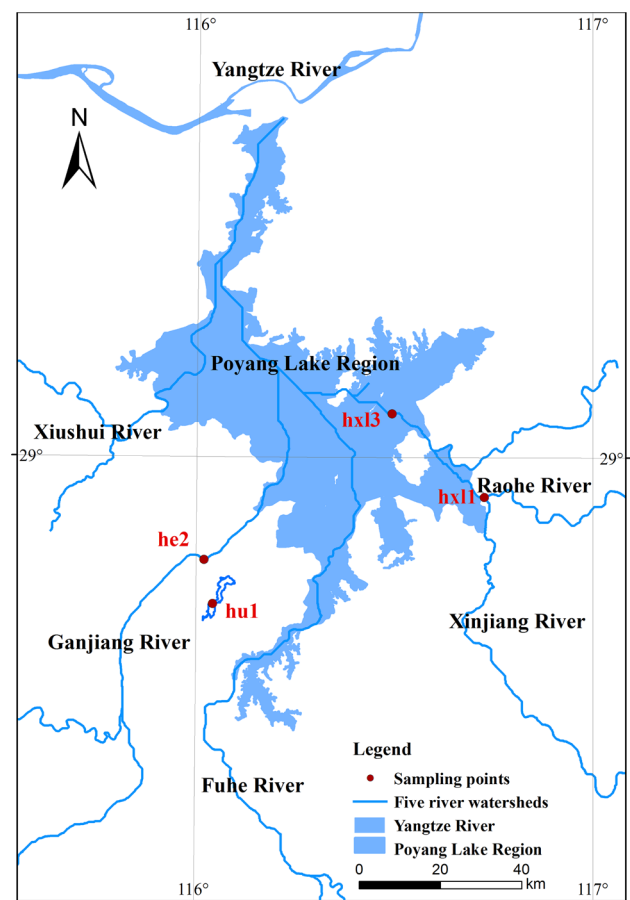


Fig. 1 Water sampling sites in lake–river ecotone (hx13 Poyang Lake, hx11 Raohe River, hu1 Yao Lake, he2 Ganjiang River, three sampling points are set up for each)

the Poyang Lake wetland, its surrounding Rivers merge in the Poyang Lake, which is multi-resources lacustrine sediment.

Sample analysis

Bacterial genomic DNA was extracted from the water samples of Yao Lake, Ganjiang River, Raohe River and Poyang Lake, respectively. The molecular weight of genomic DNA extracted from these water samples was approximately 23 kb (Zhang et al. 2012), which was in agreement with the expected value (Huang et al. 2013).

The TN concentrations were determined using an alkaline potassium persulfate digestion-UV spectrophotometric method at 275 and 220 nm with 1-cm light path cuvette (Yang et al. 2015). Determination of total phosphorus was done using ammonium molybdate spectrophotometric method (GB 11893-89).

Next-generation sequencing

The DNA was sequenced through the Illumina platform (Miseq) for paired-end sequencing (Mao et al. 2013), and the low-quality reads were removed under the machine data. The statistical data are shown in Table 1.

In order to obtain high-quality tags, the splicing tags sequence was processed. After processing all the samples, a total of 1142790 high-quality tags were obtained, and the average sample was 285697 ± 41785 . At the 0.97 level of clustering similarity for species classification of operational taxonomic units (OTU), a total of 8657 OTU were obtained for the 4 samples. The Singletons OTU (i.e., abundance was 1 OTU) number was 3155, and the non-Singletons OTU number was 5502. Singletons OTU may

be caused by sequencing errors, which was ignored and not included in the later analysis. Statistical results of sample OTU are in Table 2.

Results and discussion

Analysis of OTU Venn diagram

The Venn diagram, a visual display of overlap between OTU samples, was used to display a plurality of samples and their unique OTU number, which was obtained in 0.97 of the similarity of each sample, by the OTU, which represents species, we can find out the core of microorganisms in different environments (Fig. 2).

Different color graphics represent different samples or different groups. The overlapping part of different color graphics represents the common OTU number between two samples or two groups. Similarly, among a plurality of color graphics overlapping part number represents the number of OTU between multiple samples or groups have similarity. From the OTU Venn chart analysis, the OTU number of 205 is shared by Ganjiang River, Yao Lake, Raohe River and Poyang Lake samplers. The common OTU number of Ganjiang River and Yao Lake is 640, and the common OTU number from Poyang Lake and Raohe River is 339. The number of unique OTU from Ganjiang River is 601, making up 10.92 % of the total OUT, the number of unique OTU from Yao Lake is 782, making up 14.21 percent of the total OUT, the number of unique OTU from Raohe River is 433, making up 7.87 % of the total OUT, and the number of unique OTU from Poyang Lake is 2477, making up 45.01 percent of the total OUT. Out of all of these water samples, Poyang Lake had the largest

Table 1 Sample sequencing data statistics

Sampling sites	Reads length (bp)	Raw data (Mbp)	Adapter (%)	N base (%)	Ploy base (%)	Low quality (%)	Clean data (Mbp)	Data utilization ratio (%)
Ganjiang River	250:250	251.23	0	0	0.017	6.438	225.22	89.65
Yao Lake	250:250	192.11	0	0	0.009	8.307	166.66	86.75
Raohe River	250:250	227.56	0	0	0.015	17.255	158.38	69.60
Poyang Lake	250:250	167.94	0	0	0.014	5.146	153.64	91.48

Table 2 Sample OTU statistics

Sampling sites	Tag number	OTU number	OTU number (removing singletons)	Non-singletons OTU ratio (%)
Ganjiang River	353,270	1851	1493	80.66
Yao Lake	283,182	2813	1681	59.76
Raohe River	265,326	1491	1169	78.40
Poyang Lake	241,012	4390	3047	69.41

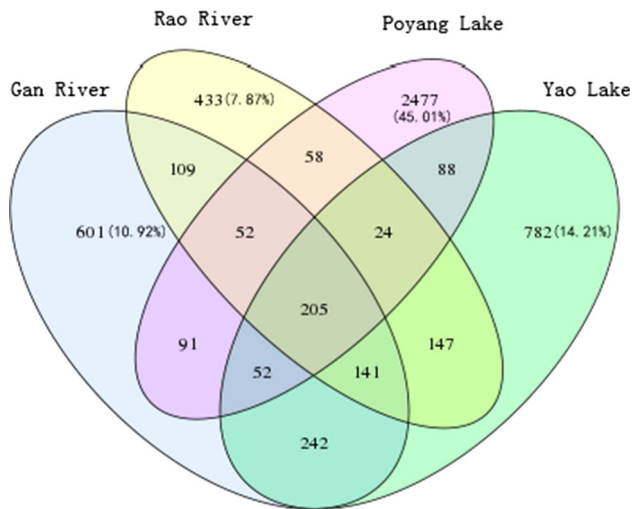


Fig. 2 Venn diagram (different *color graphics* represent different groups among a plurality of *color graphics* overlapping part number represents the number of OTU between multiple groups have similarity)

number of unique microbial population, followed by Yao Lake and Ganjiang River orderly, and Raohe River had the least.

Comparison of the sequences similarity, we took more than 97 % cloned sequence similarity to the same operational taxonomic unit (OTU), the microbial diversity index were analyzed with MOTHUR in microbial communities from the four samples.

Diversity indices analyses

Alpha diversity including Observed species index, Chao index, Shannon index and Simpson index, etc., we calculated Alpha diversity value by mothur (v1.31.2) software, and made the corresponding dilution curve by R (v2.15.3) software.

Observed species index and Chao index reflect the species richness in a sample, representing the number of species in a community, not the abundance of each species in the community. Dilution curves can reflect the adequacy of sample sequencing. If the curve tends to be flattening, the sequencing depth has been basically covered by all species in sample. Figure 3a, b shows that the number of sequencing had covered all species in sampling sites.

Greater Shannon value represents higher community diversity, while greater Simpson index value represents lower community diversity (Fig. 3c, d). When species richness is the same, community with greater species evenness has more diversity. Results showed that the microbial community diversity was abundant in Poyang Lake, followed successively by the Ganjiang River, the Yao Lake, and the Raohe River.

The microbial diversity and community composition are varying with the surrounding environment. The microbial communities were compared by phylogenetic information, and obvious evolutionary differences exist between Poyang Lake water samples and the other samples. Due to the effect of Yangtze River and five Rivers (Ganjiang River, Fu River, Xinjiang River, Raohe River, Xiushui River), microbial resources in Poyang Lake is rich and diverse.

Microbial variation with the environment

The dominant population in samplers is defined as that account for more than 5 % (Huang et al. 2012) of the total number of similar microbial sequencing (Table 3).

Quantitative and qualitative analysis shows that, the most abundant microbial populations is Proteobacteria, followed by Bacteroidetes, then Actinobacteria, Acidobacteria, Verrucomicrobia, Firmicutes, Planctomycetes, etc. From the Table 3, Planctomycetes in Yao Lake and the Ganjiang River ecotone are more abundant compared to Raohe River ecotone, some bacteria from Planctomycetes could use nitrite under hypoxia (NO_2^-) oxidation of ammonium ion (NH_4^+) generated nitrogen to obtain energy, It has the vital significance to the global nitrogen cycle, and also important in wastewater treatment (Meckenstock et al. 2002). A number of 2974 Crenarchaeota was detected from Poyang Lake, and a little Crenarchaeota in Raohe River. America scholars (Könneke et al. 2005) successfully isolated a strain of Crenarchaeota *Nitrosopumilus maritimus* from the sea for the first time in 2005, which contained ammonia oxidation that had Archaea ammonia monooxygenase (*amoA*) genes. It can oxidize ammonia nitrogen to obtain the energy assimilation of inorganic carbon growth. *Nitrospira* were relatively rich in Poyang Lake, it has an effect on nitrification and nitrite oxidation (Wang et al. 2014), and mainly distributed in the environment that is affected by human activities. The *Chlamydiae* was found in Ganjiang River water flowing through the Nanchang City, which is a kind of special bacteria that can only survive in the cytoplasm, and mainly includes two kinds of bacteria, *Chlamydia trachomatis* and *Chlamydia psittaci*. *C. trachomatis* usually only infects humans, while *Chlamydia psittaci* can infect many kinds of animal and birds, and lead to respiratory diseases, abortion, and arthritis. Archaea in Yao Lake were rare, and Fusobacteria was most abundant in the Ganjiang River water. Studies (Kostic et al. 2013; Rubinstein et al. 2013) have found out that Fusobacterium is unusually active in colon cancer cells, and seems to coexist with tumor malignant degree. On the whole, microbial components in different geographic space were both related and varied with different environments.

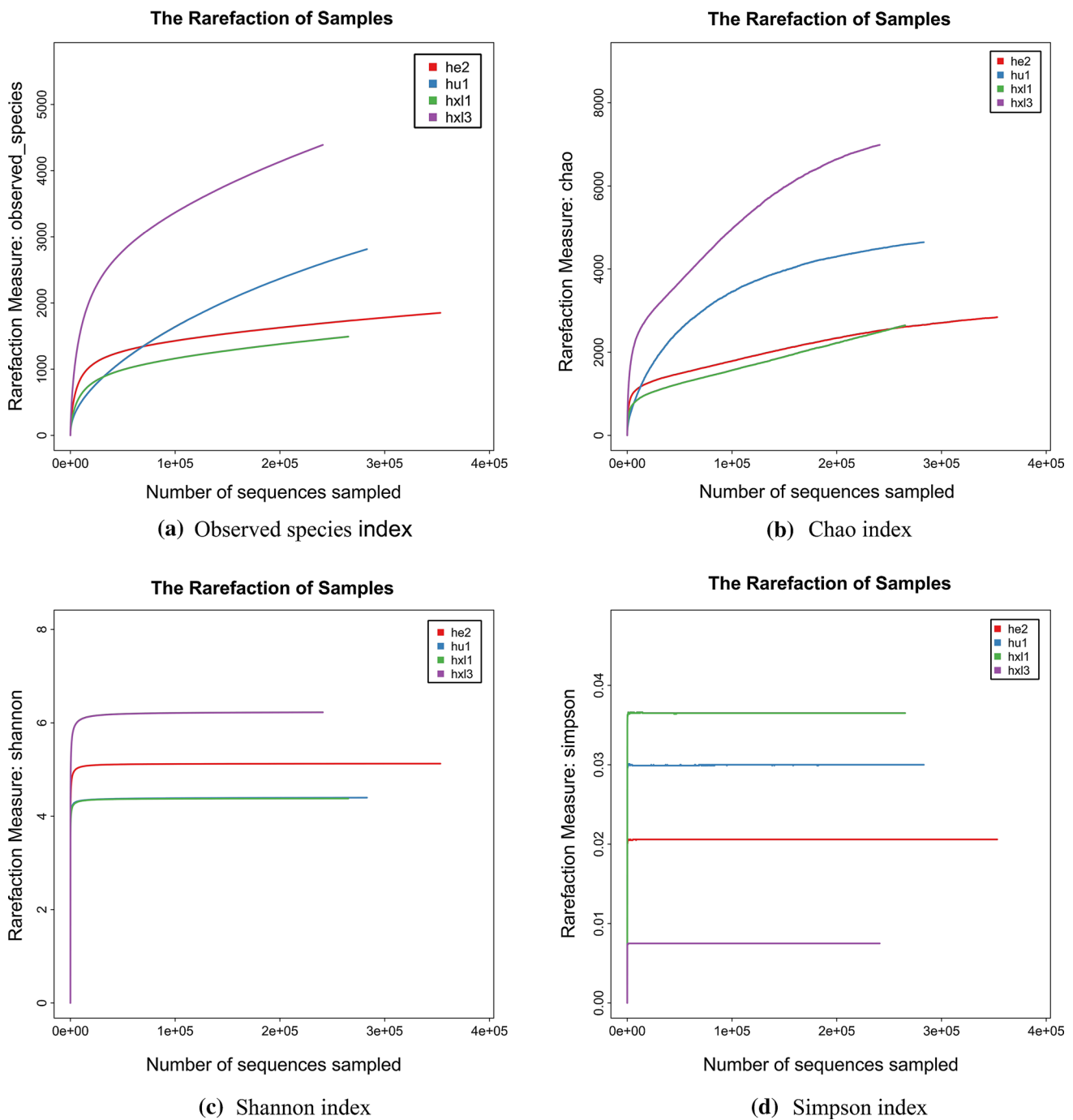


Fig. 3 The richness/diversity estimators from samples (*hxl3* Poyang Lake, *hxl1*: Raohe River, *hu1* Yao Lake, *he2* Ganjiang River)

Due to the changes of the surrounding environment (Wang et al. 2015a, b; Xiang et al. 2015), the microbial diversity and community composition also show constantly collaborative response. Raohe River was mainly affected by the agricultural activities and copper/phosphorite mines, etc., which could cause the high-concentration value of TP that occurred in the river mouth area of Poyang Lake (Table 4), and the PAO (such as *Pseudomonas*, *Acinetobacter*) were richer than Ganjiang River. Yao Lake and

Ganjiang River were mainly affected by the city residents living pollution, as a result of population increase, the amount of TN discharged into the Ganjiang River and Yao Lake have increased in recent years (Yang et al. 2015), which caused a richness of nitrifying bacteria (Table 4). Affected by domestic sewage and industrial activities of the city, *Chlamydiae* and *Fusobacteria* in Ganjiang River are more abundant compared to other samples. Poyang Lake is the confluence of these flowing, by both the

Table 3 Abundance of microorganisms in each sampling point (Tags number)

Taxon	Ganjiang River	Ganjiang River (%)	Yao Lake	Yao Lake (%)	Raohe River	Raohe River (%)	Poyang Lake	Poyang Lake (%)
Crenarchaeota	0	0.00	0	0.00	3	0.00	2974	1.24
Euryarchaeota	443	0.13	2	0.00	162	0.06	1	0.00
Other Archaea	103	0.03	2	0.00	70	0.03	7272	3.03
Acidobacteria	1265	0.36	35	0.01	400	0.15	57,158	23.85
Actinobacteria	31,728	8.99	50,758	18.00	62,200	23.47	13,525	5.64
Armatimonadetes	529	0.15	4	0.00	3437	1.30	1018	0.42
Bacteroidetes	63,464	17.98	69,527	24.65	42,588	16.07	28,077	11.71
Chlamydiae	459	0.13	31	0.01	219	0.08	30	0.01
Chlorobi	40	0.01	0	0.00	0	0.00	0	0.00
Chloroflexi	324	0.09	0	0.00	335	0.13	687	0.29
Deferribacteres	0	0.00	0	0.00	46	0.02	0	0.00
Deinococcus-Thermus	1175	0.33	2	0.00	64	0.02	0	0.00
Fibrobacteres	50	0.01	4	0.00	0	0.00	0	0.00
Fusobacteria	1596	0.45	50	0.02	153	0.06	0	0.00
Gemmatimonadetes	437	0.12	532	0.19	97	0.04	266	0.11
Nitrospira	26	0.01	0	0.00	20	0.01	462	0.19
Planctomycetes	3980	1.13	5178	1.84	1519	0.57	3949	1.65
Proteobacteria	189,505	53.70	132,484	46.97	107,727	40.65	80,613	33.64
Spirochaetes	221	0.06	7	0.00	114	0.04	1	0.00
Synergistetes	20	0.01	16	0.01	0	0.00	0	0.00
Tenericutes	40	0.01	0	0.00	142	0.05	0	0.00
Verrucomicrobia	22,941	6.50	14,143	5.01	20,069	7.57	8332	3.48
Firmicutes	8832	2.50	1049	0.37	1974	0.74	2551	1.06
OD1	339	0.10	19	0.01	279	0.11	52	0.02
SR1	50	0.01	9	0.00	0	0.00	0	0.00
TM7	9	0.00	0	0.00	0	0.00	0	0.00
WS3	0	0.00	2	0.00	0	0.00	93	0.04
Other	25,336	7.18	8196	2.91	23,386	8.82	32,608	13.61

Table 4 TN, TP and some responsive microorganisms

Sampling sites	Ph	TN (mg L ⁻¹)	TP (mg L ⁻¹)	Nitrifying bacteria (%)	Pseudomonas (%)	Acinetobacter (%)
Ganjiang River	7.0	3.062365	0.04698	0.49	0.07	0.15
Yao Lake	7.3	4.073491	0.04242	0.96	0.31	0.57
Raohe River	7.2	2.682573	0.32635	0.45	0.43	1.21
Poyang Lake	7.0	2.022573	0.19635	0.31	0.44	0.77

Yangtze River and five River, Poyang Lake has more frequent substance and energy exchange, more hydrostatic and stream habitat interaction, so that Poyang Lake has rich microbial resources. Poyang Lake is an enormous gene pool, including a large number of DNA sequences of ammonia oxidizing bacteria and ammonia oxidizing archaea. Research data show that it is possible to isolate functional microbes from Poyang Lake, which play an important role in nitrogen cycle of the earth.

Acknowledgments This work was Supported by the National Natural Science Foundation of China (No. 41561002; No. 41331174), the Collaborative Innovation Center for Major Ecological Security Issues of Jiangxi Province and Monitoring Implementation (No. JXS-EW-00), the Opening Foundation of Key Laboratory of Environment Change and Resources Use in Beibu Gulf (Guangxi Teachers Education University), Ministry of Education, China (No. 2014BGERLKF02).

Sincere thanks go to Professor Caiying Ni, Hongmei Zhao, Ph.D. Tao Zhang, Wenjing Yang, Guihua Liu, and Hua Zhang for field sampling of this research.

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