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Ecosystem functions of rivers have been greatly impaired due to the negative effects of human actions on river structures in different regions of the Earth during the last decades. It is an urgent task to understand the complete structures and ecosystem functions of a natural rive for restoration of the impaired river ecosystems. A natural river is composed of the repeatedly occurring rapid-pool-benchland system, i.e., a basic structural unit. We respectively selected three of the rapid-pool-benchland systems in the upper, middle and lower reaches of the Duliu River, a natural river in China as materials and tested the abundance of microorganisms in the sediments of these systems with plate counting method and a MPN (most-probable-number) method and the activity of enzymes with titration or colorimetric methods. Results indicated that the number of bacteria was far more than ammonifiers, actinomycetes, fungus and denitrifying bacteria in the sediments of all these rapid-pool-benchland systems. In each of the rapid-pool-benchland systems, all the microbial abundance in the sediments of the pools was always highest, and that of the rapids was lowest. In the upper reach, the microbial abundance was more than the middle and lower reaches. Catalase activity in the sediments of the benchlands was higher than the rapids and pools but phosphatase activity in the pools was higher than the rapids and benchlands. Phosphatas and urease activities were significantly correlated to the abundance of the bacteria, ammonifiers, actinomycetes, fungus and denitrifying bacteria in the sediments. The rapid-pool-benchland systems in a river obviously affect the microbial abundance and catalase and phosphatase activities related to biochemical functions in the sediments. However, the microbial abundance only indicated a middle-level correlations (r ranges from 0.434 to 0.836, $p<0.05$) with the activity of four extracellular enzymes. The study clarifies if the repeatedly occurring rapid-pool-benchland systems, a kind of habitat heterogeneity in a river have an effect on the microbial

abundance and enzyme activity in the sediments, and further the relationships between the microbial abundance and enzyme activity.

Abundance of microorganisms and enzyme activity in the rapid-pool-benchland systems in the natural Duliu River of China

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Abstract Ecosystem functions of rivers have been greatly impaired due to the negative effects of human actions on river structures in different regions of the Earth during the last decades. It is an urgent task to understand the complete structures and ecosystem functions of a natural rive for restoration of the impaired river ecosystems. A natural river is composed of the repeatedly occurring rapid-pool-benchland system, i.e., a basic structural unit. We respectively selected three of the rapid-pool-benchland systems in the upper, middle and lower reaches of the Duliu River, a natural river in China as materials and tested the abundance of microorganisms in the sediments of these systems with plate counting method and a MPN (most-probable-number) method and the activity of enzymes with titration or colorimetric methods. Results indicated that the number of bacteria was far more than ammonifiers, actinomycetes, fungus and denitrifying bacteria in the sediments of all these rapid-pool-benchland systems. In each of the rapid-pool-benchland systems, all the microbial abundance in the sediments of the pools was always highest, and that of the rapids was lowest. In the upper reach, the microbial abundance was more than the middle and lower reaches. Catalase activity in the sediments of the benchlands was higher than the rapids and pools but phosphatase activity in the pools was higher than the rapids and benchlands. Phosphatas and urease activities were significantly correlated to the abundance of the bacteria, ammonifiers, actinomycetes, fungus and denitrifying bacteria in the sediments. The rapid-pool-benchland systems in a river obviously affect the microbial abundance and catalase and phosphatase activities related to biochemical functions in the sediments. However, the microbial abundance only indicated a middle-level correlations (r ranges from 0.434 to 0.836, $p<0.05$) with the activity of four extracellular enzymes. The study clarifies if the repeatedly occurring rapid-pool-benchland systems, a kind of habitat heterogeneity in a river have an effect on the microbial abundance and enzyme activity in the sediments, and further the relationships between the microbial abundance and enzyme activity.

Key word: Natural rivers, rapids, pools, benchlands, microorganisms, enzyme activity, sediments, the Duliu River o China

INTRODUCITON

A river is a corridor connecting terrestrial environments to the ocean realm and it plays an important role in the supply of water resources for maintenance of life systems, shipping and purification of polluted water (Wu, Chen, Zhang et al.，2008; Padmalal and Maya, 2014). Almost all the immemorial population assembly and the following ancient civilization, such as Indian civilization in the Ganges River basin, Egyptian civilization in the Nile River basin, mesopotamian civilization between the Tigris River and the Euphrates River and Chinese civilization in the Yellow River basin, origin on the coasts of rivers, and many

modern cities are also built along rivers even today (Wang, Wu, Zhang, et al., 2013). Arguably, a river is a footstone of the human civilization. However, human disturbance have imposed tremendous pressures on rivers, and consequently most of the rivers, especially the small rivers, have been altered to levels, often beyond their natural resilience capability in structures and ecosystem functions (Dong, Sun, Peng, 2009; Padmalal and Maya, 2014). These disturbances include many aspects. For example, riverway is occupied due to urbanization; riverway is changed into a straight form leading to the disappearances of a meandering form; the natural riverbanks are replaced by concrete riverbanks for slope stability (Dong, Sun, Peng, 2009; Padmalal and Maya, 2014). As a result, habitat heterogeneity in a river significantly declines, which results in the decreases of biodiversity, self-purification capacity of a waterbody, landscape values and affinity between humans and the waterbody (Wang, Wu, Zhang, et al., 2013).

Habitat heterogeneity in a river deals with many aspects, including a diversity of river forms, river structures, river materials, or river slopes. In river forms, scientists suggest that rivers generally present straight forms, bifurcation forms, middle-bar forms, wandering forms and braiding forms (Niezgoda and Johnson, 2005; Yin, Li, Liu et al., 2012). These forms are products of the long-term interactions between water flow and a variety of environmental factors. The river structures can be reflected with a spatial variation of riverbed, waterflow, organisms and river banks along a transverse or a longitudinal direction of a river (Gregory, Benito, Downs, 2005). The river materials, i.e., river-bed materials consist of sediments, different sizes, forms and sources of stones and organic matter, and each of these materials in types and characteristics is also diverse (Asai，Takasaki，Muraoka et al., 2013). The river slope is variable along a longitudinal direction of a river, which is mainly related to landform and geological conditions. Clearly, a river is a complex system, but the system is becoming drab with a decline of habitat heterogeneity.

In previous studies, we found a new river structure, the rapid-pool-benchland systems in natural rivers based on a great number of field investigations (Fig 1 and Supplemental file 1) (Ma, Wang, Chen, 2014; Wu, Wang, Cheng, 2014; Chen, Ma, Wang, 2015; Wang, Wang, Liu, et al., 2015). The system continually occurs from the upper to lower reaches along a natural river, or a rapid-pool-benchland system in an upper reach in a river directly links another rapid-pool-benchland system in the lower reach. In the system, there are also waterflow, organisms, riverbed (sediment and pebbles) and riverbank (dry lands during most of time) that make up a complete river from a traditional perspective of habitat heterogeneity and the different river-bed materials (Gregory, Benito, Downs, 2005; Asai，Takasaki，Muraoka et al., 2013). However, the rapid-pool-benchland system explains a complete river from a new perspective of habitat heterogeneity. Specifically, in the rapid section in a rapid-pool-benchland system, there is a greater

Figure 1 A rapid-pool-benchland system in the upper reach of Yangtz River in China. The square symbol: pool; the triangular symbol: rapid; the circle symbol: benchland.

slope than the pool and benchland section, which generates a relatively great potential energy to make a rapid water flow. Moreover, there are bigger pebbles, stones or ratchel and few plants and sediments due to scouring effects. In addition, the rapid often occurs in the middle section of a river (Fig 2 and Supplemental file 1). The pool section in a rapid-pool-benchland system is generated by the long-term scouting effects of the water flow from the rapid section. In the pool, there are many fine-particle sediments and even sludge deposited from the slow water flow, relatively rich plants and deeper water body than the rapid section. The pool section generally occurs close to the river bank in a river (Fig 2 and Supplemental file 1). The benchland section is often formed by transportation and sedimentation of a variety of the river materials in floods. Some benchlands are directly riverbanks or link to the riverbank. A significant characteristic of the banchland is that there is frequent alternation of wetting and drying habitats because of a change of water levels during a year. As a result, many hydrophytes and xerophytes, and amphibious animals and insects are distributed in the benchland. In a natural river, there are significant relationships in areas and perimeters among the rapids, pools and benchlands, and the proportions of the areas or perimeters of the rapids to the pools or to the benchlands almost equal to a constant. Further studies indicate that the rapid-pool-benchland system is shaped by the long-term dynamical effects of the water flow and is a basic unite of the structure of a natural and healthy river. In such a river, there is a dynamic equilibrium among the rapids, pools and benchlands.

However, it remains unclear if abundance of microorganisms and the enzyme activity in the sediments of rapid, pool and benchland in a river will indicate different. The abundance of microorganisms and the enzyme activity in the sediments are important ecological functions in a river and referents of the river health. The microorganisms (bacteria, fungi, etc) process most of the organic wastes from a variety of sources and they oxidize, reduce and precipitate mineral ions, thereby controlling the geochemical cycles in the river (Winiarski, Bedell, Delolme et al., 2006). The enzyme activity is an interesting parameter to measure the biological quality of soils or sediments because a number of studies have shown it to be one of the most sensitive parameters for evaluating the toxicity of certain pollutants in the soils or

sediments (Garcia Hernandez, Costa, 1997; Tam, 1998; Ellis, Neish, Trett et al., 2001; Irha, Slet, Petersell, 2003; Benitez, Melgar, Nogales et al., 2004). Some recent studies have reported that enzymes in wetland systems play very important roles in matter transformation (Reboreda and Cacador, 2008). For example, there is a positive correlation between dehydrogenase activity and CO^2 emission, carbon cycle and biomass, and dehydrogenase activity is also known to be sensitive to pollutants such as heavy metals (Garcia, Hernandez, Costa, 1994; Neto Ohannessian, Delolme et al., 2007). Ecologists have also found that there are significantly positive correlations between removal of total nitrogen and the number of soil microbes and activities of soil enzymes in wetlands (Huang, Gao, Liu et al., 2012). In a lake, the differences in waterbody pollution can be well denoted by the number of microbes and activities of enzymes in sediments (Deng, Huang,Hu et al., 2009). The enzyme activity in sediments is also related to the anthropogenic nutrients and carbon loading input from the upper reaches of the river (Hill and Moffett, 2010; Millar, Payne, Ochs et al., 2015).

In the study, we selected three successively connecting rapid-pool-benchland systems respectively in the upper, middle and lower reaches of the Duliu River, a typical natural river in Yungui platau of China and collected the sediment samples in the rapids, pools and benchlands. Then, these sediment samples were delivered to laboratory to test the abundance of the microorganisms and the activity of four important extracellular enzymes. We assumed that the rapid-pool-benchland systems continually occurred to create habitat heterogeneity along the river, and consequently which would result in differences in the abundance of the microorganisms in the sediments of the rapids, pools and benchlands. Correspondingly, the enzyme activity in the sediments of the rapids, pools and benchlands also changed and further affected the biochemical functions. Therefore, we hope to answer the following questions: 1) if the rapid-pool-benchaland system as a type of habitat heterogeneity in a river directly influences the abundance of the microorganisms in the sediments, and further the enzyme activity? 2) If it does, what degree of the correlations between the abundance of microorganisms and the activity of the four extracellular enzymes primarily released by the microorganisms will occur in the rapid-pool-benchland systems? 3)What ecological processes or functions have been denoted by the results of the abundance of the microorganisms and the enzyme activity?

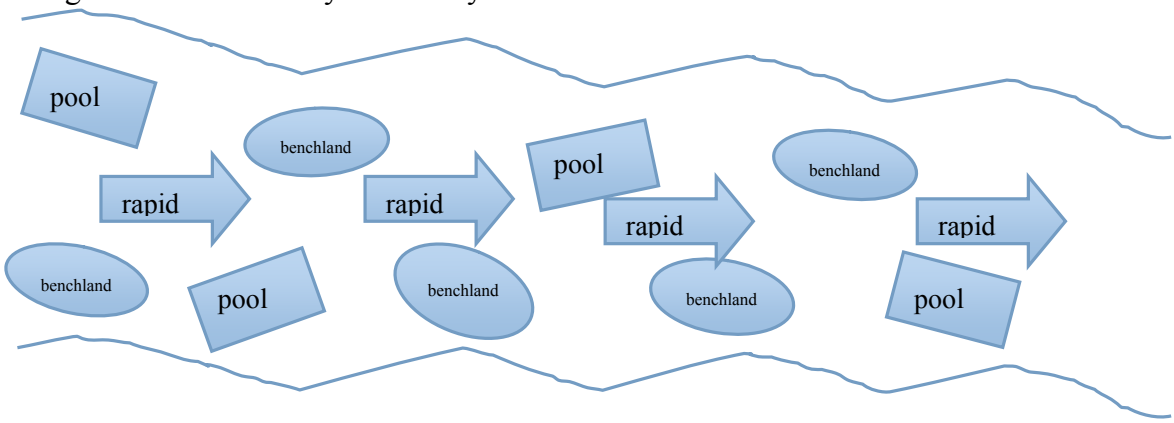

Fig.2 The typical model of the rapid-pool-benchland system

MATERIALS AND METHODS

Site descriptions

The Duliu River is a primary tributary of the Pearl River, the third greatest river in China. The Duliu River origins from Dushan county, flows through four counties in Guizhou Province, and lastly joins the Xi River, the upper reach of Pearl River. Total length of the

Duliu River is 310 km and the drop of the trunk stream is 84.5 m. The catchment area is 11,326 km^2. Annual mean rainfall ranges from 1350-1500 mm. The average annual flow is 145m^3/s. The forest coverage is 74.1% in the Duliu River watershed. There are not any dams and artificial riverbanks along the river, so the Duliu River is a natural river. We selected an upper reach of the Duliu River in Puan town (26°04′ N, 107°48′ E), a middle reach in Sandu county (25°58′N, 107°53′ E) and a lower reach in Rongjiang county (25°89′N, 108°50′ E), respectively as materials. In each river reach, three rapid-pool-benchland systems successively connect with each other. From the three rapid-pool-benchland systems in each river reach to their neighboring upper or lower reaches, the rapid-pool-benchland systems connecting with each other can still be seen. The parameters of the three river reaches are showed in Table 1.

Table 1 The parameters of the river reaches

River reaches	Longitudinal ratio	Bending coefficient	Rapid area (m^2)	Pool area (m^2)	Benchland area (m^2)
UR*	0.05±0.04	1.16±0.00	791.16±262.61	872.12±294	796.58±473.63
MR	0.02±0.01	1.05±0.00	1991.16±2262.41	1322.15±2041.96	1492.06±1686.82
LR	0.01±0.00	1.20±0.00	3128.52±714.4	3691.54±2994.8	12261.48±12307.98

*UR: the upper reach; MR: the middle reach; LR: the lower reach.

Field sampling

In each set of the rapid-pool-benchland systems in the upper, middle or lower reach of the Duliu River, we collected sediments in the rapids, pools and benchlands, respectively. Specifically, ten sampling sites were firstly even set up in the rapids, pools or benchlands. Then, we used a small shovel with a long handle to collect about 300 g of the sediments at each site. The sediments from the ten sites were fully mixed and kept at 4 ℃. The sediments in the rapids and pools were collected from the bottom of water. The total weight of the mixed samples of the sediments respectively collected from a rapid, pool or benchland was about 3kg. The number of the mixed samples was 27.

A test of abundance of microorganisms

Bacteria, fungus and actinomycetes: we adopted plate counting method to test the number of the bacteria, fungus and actinomycetes. We firstly made up the beef-protein mediums, Martin mediums and the No.1 medium of Gaos. After sterilization, these mediums were respectively sub-packed on three culture dishes that had been sterilized. Then, we weighed 10g of the fresh sediments and put them in a triangular flask with 100 ml of aqua sterilisata. Subsequently, the dilution of the soil solutions in the triangular flask was conducted in a sterile working chamber. The dilution proportions were respectively 10^{-6}, 10^{-7} and 10^{-8} for bacteria, and 10^{-4}, 10^{-5} and 10^{-6} for fungus and actinomycetes, respectively. We further used pipettes to transfer the diluted soil solutions to other culture dishes. Then, the culture dishes were incubated at 28℃. Each of the dilution proportions was replicated three times.

Ammonifying bacteria and denitrifying bacteria: we adopted a MPN (most-probable-number) method to test the number of the two types of bacteria. Firstly, the peptone agar mediums and denitrifying bacteria mediums were respectively made up and were sub-packed into cuvettes. Each cuvette was filled with 5 ml, plugged using silica gel stopple and sterilized. A reversed Durham's fermentation tube was put in each of these filled cuvettes to test the generation of gases. Then, we weighed 10g of the fresh sediments and put it in a triangular flask with 100 ml of aqua sterilisata. The triangular flask was oscillated for 30 min to change the mixtures in the cuvettes into suspension. Subsequently, the suspension was diluted into four concentration levels, i.e. the 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} times of the suspension. We used sterile pipettes to transfer these diluted solutions to the cuvettes filled

with the mediums, and these cuvettes were incubate at 30℃. Each concentration level was replicated three times. After three and five days, we checked the turbidity of the mediums for ammonifying bacteria. When the incubation continued till the seventh day, we used Nessler reagents to test if ammonia had been generated. For denitrifying bacteria, we observed if bubbles had occurred in the cuvvettes and if the solutions in the cuvettes had become turbid after 14 days, and used Nessler reagents to test if ammonia had been generated. Based on the growth of the bacteria, we contrasted a MPN table to determine the number of the living bacteria per ml of the suspension before the dilution.

Tests of the enzyme activity

Catalase: we adopted permanganate titration to test the catalase activity. Specifically, we weighed 5g of the fresh sediments and put them in a triangular flask. 25ml of H_2O_2 solutions and a little distilled water was decanted into the triangular flask, and then the triangular flask was continually oscillated for 30 minutes. The chemical reactions occurring in the triangular flask were terminated with addition of H_2SO_4. The mixtures in the triangular flask were filtrated and the filtrates were titrated with $KMnO_4$. When the filtrates changed into red, the titration was stopped. The consumption volume (ml/g) of $KMnO_4$ solution represented the catalase activity.

Alkaline phosphatase: the activity of alkaline phosphatase was tested with the phosphatase two sodium colorimetric methods. We firstly weighed 5 g of fresh sediments and put them in a triangular flask. Then, toluene-disodium phenyl phosphate-borate buffer was decanted into the triangular flask. The triangular flask was continually incubated 24 h and then the mixtures in the triangular flask were filtrated. Chromogenic agents were added to the filtrates. Finally, the filtrates were transferred to a volumetric flask, and diluted into 750ml of constant volume to test absorbance.

Urease: the activity of the urease was tested by the phenol sodium hypochlorite colorimetric method. We firstly weighed 5g of fresh sediments and put them in a 50 ml volumetric flask. Then, we added 1ml of toluene to the volumetric flask, tightly plugged the volumetric flask and gently shook it about 15 minutes. 5ml of 10% solutions of urease and 10ml of citrate buffer (pH=7.6) were added to the volumetric flask and the soil solution in the volumetric flask must be completely mixed by shaking. After the volumetric flask had been incubated at 37℃ for 24 h, we used the distilled water (at 38 ℃) to dilute the solution to constant volume. This moment, the toluene should be floated above the solution. After the solution was further shook to be evenly mixed, soliquoid was filtrated. Subsequently, 1ml of the filtrates was taken to put in a 50ml volumetric flask and 4 ml of sodium phenate solutions and 3 ml of chloros was added to the 50 ml volumetric flask. After coloration, the solutions were distilled to constant volume. The absorbance of the solutions was tested after 1 h.

Dehydrogenase: we adopted (chloride three phenyl tetrazole) colorimetric method to test the activity of the dehydrogenase. We firstly weighed 5g of the sediments and put them in a triangular flask. Then, we added 2 ml of 1% triphenyltetrazolium chloride (TTC) solutions, glucose solutions, Tris-HCl buffer, and Na_2S solutions to the triangular flask. The triangular flask was oscillated for 2h, and then the oxygen-free water and the sodium sulfite solutions were added to the triangular flask. After the solutions had been fully mixed, we tested the absorbance of the solutions. The enzyme activity was indicated with the yielding quantity of three phenyl formazen per unit soil and time.

RESULTS

Abundance of microorganisms

The abundance of individual bacteria in the sediments collected from the rapids, pools or benchlands in these river reaches was highest among five types of microorganisms (Table 2). The abundance of individual ammonifying bacteria, actinomycetes, fungus and denitrifying bacteria was respectively ranked as from the second to fourth. One of the important causes why bacteria were more than actinomycetes and fungus was because the bacteria existed in the sediments with smaller individuals than the actinomycetes and fungus. In natural ecosystems, there is generally a law, i.e., the individual size of organisms is negatively related to their individual numbers. Secondly, the abundance of these microorganisms in the pools in different reaches obviously exceeded the rapids and benchlands. In the pools, there was more nutrient accumulation in the sediments because of a slower water flow than the rapids, which was beneficial to the growth and reproduction of the microorganisms. In the benchlands, floods often resulted in an alternate occurrence of waterflooding and dry environments and the nutrient delivery and consequently which promoted the growth and reproduction of the aerobic and anaerobic microorganism. In the rapids, greater loss of nutrients and disturbance of water flows might refrain the growth and reproduction of the microorganisms than the pools and benchlands. Thus, the abundance of these microorganisms in the rapids was relatively less than the pools and benchlands. Thirdly, the abundance of these microorganisms was higher in the upper reaches than the lower reaches because there were more COD input to the river in the upper reaches. In addition, results also indicated that the individual number of the bacteria, fungus, actinomycetes, ammonifying bacteria and denitrifying bacteria in all the rapids, pools or benchlands respectively occupied 60%−99.07%, 60%−99.07%, 0.28%−23.21%, 5%−31.89% and 0.009%−0.027% in total number of microorganisms.

Enzyme activity

Catalase activity in the sediments averaged 3.638 ml/g in all rapids, pools and benchlands in the three river reaches in Duliu River and ranged from 1.74-5.465ml/g. The activity of the enzyme was relatively higher on average in the lower reaches than the upper and middle reaches (P<0.01, Fig 3A). However, the activity of the enzyme was lowest in the pools of all three river reaches and highest in the benchlands of the upper and middle reaches （P<0.01）. In the rapids of the upper and middle reaches, the activity of the enzyme in the sediments presented moderate levels. These results occurred possibly because in the benchlands, there were high oxgen contents in the sediments because of an alternate waterflooding condition with a change of water levels in the river, which promoted the

Table 2 The number of the microorganisms in the sediments of the rapid-pool-benchland system

River reaches	Systems	Bacteria ($\times10^6\cdot g^{-1}$)	Actinomycetes ($\times10^4\cdot g^{-1}$)	Fungus ($\times10^4\cdot g^{-1}$)	Ammonifying bacteria($\times10^5\cdot g^{-1}$)	Denitrifying bacteria($\times10^2\cdot g^{-1}$)	Total ($\times10^6\cdot g^{-1}$)
Upper reaches	Pool	5.50±0.32	18.20±1.39	11.00±0.71	21.44±1.72	14.47±1.05	7.84±0.53
	Rapid	2.22±0.15	0.71±0.06	1.68±0.11	3.21±0.15	5.32±0.23	2.55±0.12
	Benchland	2.77±0.12	15.60±1.22	1.76±0.15	5.17±0.18	9.29±0.38	3.45±0.16
± Middel reaches	Pool	2.14±0.17	10.01±1.07	4.60±0.21	5.21±0.20	4.31±0.17	2.68±0.13
	Rapid	0.32±0.02	0.61±0.03	1.08±0.05	0.33±0.01	1.22±0.01	0.66±0.02
	Benchland	0.96±0.08	8.55±0.57	1.12±0.04	1.46±0.08	1.79±0.01	1.20±0.04
Lower reaches	Pool	3.79±0.21	12.30±0.82	6.10±0.34	18.34±1.20	8.89±0.49	5.75±0.24
	Rapid	0.14±0.08	9.12±0.47	0.18±0.01	2.45±0.09	1.35±0.01	1.50±0.04
	Benchland	1.26±0.09	10.40±0.66	0.21±0.01	3.23±0.10	3.79±0.22	1.57±0.04

catalysis of the enzyme in decomposition of organic matter and correspondingly the activity of the enzyme. Similarly, the activity of the enzyme was also higher in the rapids than the pools because there was relatively richer dissolved oxygen in the rapids.

Phosphatase activity in the sediments averaged 3.066 ml/g in all rapids, pools and benchlands in the three river reaches and ranged from 0.357-8.889mg/g ml/g. The activity of the enzyme was relatively higher on average in the upper reaches than the middle and lower reaches (P<0.01, Fig 3B), as just opposed to the catalase activity. However, the activity of the enzyme was lowest in the benchlands of all three river reaches and highest in the pools（P<0.01), as also opposed to the catalase activity. In the pools in the upper reaches, the activity of the enzyme was about 2 times higher than the rapids in the middle reaches and 3 times higher than the benchlands in the lower reaches. Moreover, the differences of the activity of the enzyme were also pretty obvious among the rapids, pools and benchlands in the upper, middle or lower reaches. Generally, the activity of the enzyme in the pools was 5 time higher than the benchlands. However, a difference of the activity of enzymes between the pools and rapids was relatively small because of habitat similarity. These interesting results might be caused by the relatively high pH in the waterbody in the upper reaches and the pools.

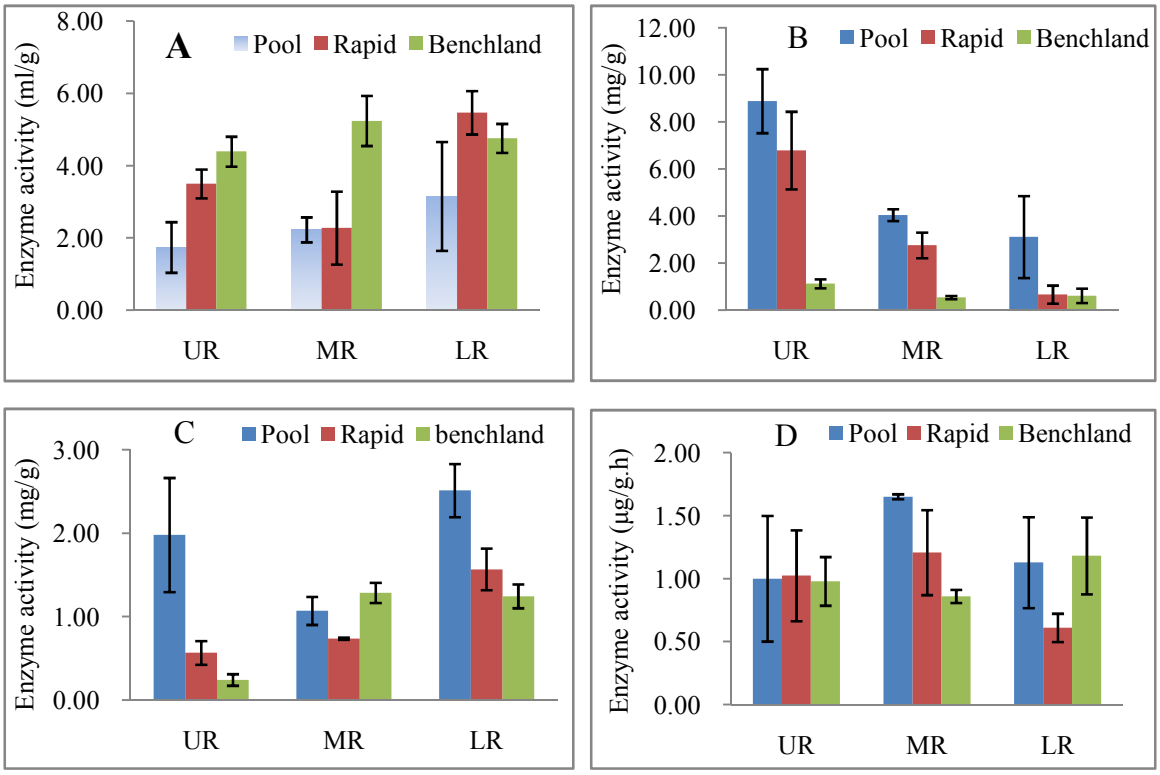

Figure 3 The enzyme activities in the sediments of the rapid-pool-benchland systems in the upper, middle and lower reaches in Duliu River. A: catalase; B: phosphatase; C: urease; D: dehydrogenase.

Urease activity in the sediments averaged 1.245mg/g·in all rapids, pools and benchlands in the three river reaches and ranged from 0.243-2.511mg/g. The activity of the enzyme did not present a regular change from the upper to lower reaches as the catalase and phosphatase activities (Fig 3C). Overall, the activity of the enzyme in the rapids, pools and benchlands in the lower reaches was higher than the upper and middle reaches. The differences of the activity of the enzyme among the rapids, pools and benchlands in the upper and lower reaches were very significant. However, there was not a significant difference in the activity of the enzyme among the rapids, pools and benchlands in the middle reaches（P>0.01）. In the

middle reaches, the benchlands got the highest activity of the enzyme, and the pools and rapids were ranked as the second and third, respectively, as was fully different from the results in the activity of the enzyme in the upper and lower reaches. The result in the middle reaches might have been affected by some stochastic factors.

Dehydrogenase activity in the sediments averaged 1.071μg/g·h·in all rapids, pools and benchlands in the three river reaches and ranged from 0.61-1.65μg/g·h, lower than the three enzymes as above. The activity of the enzyme did not also present a regular change from the upper to lower reaches as the catalase and phosphatase (Fig 3D). Moreover, the activity of the enzyme did not indicated a significant difference among the lower, middle and lower reaches (P>0.05). In the upper reaches, the activity of the enzyme was almost fully identical in the rapids, pools and benchlands. However, the differences and regularity of the activity of the enzyme were very significant among the rapids, pools and benchlands in the middle reaches. In the lower reach, the activity of the enzyme in the pools and benchalands was very similar but which was far greater than that in the rapids.

Relationships between the activity of the enzyme and the abundance of microorganisms

There were significant relationships between the abundance of all microorganisms and the activities of the urease and phosphatas in the sediments in the rapids, pools and benchlands in different river reaches (Table 3). However, statistical analysis only indicated a significant relationship of the catalase activity with two types of microorganisms, i.e., actinomycetes and bacteria. Similarly, the dehydrogenase activity was only related to the bacteria and ammonifying bacteria as well. These high-significant relationships indicated that enzymes and the corresponding microorganisms releasing the enzymes were important to the generation of the resultants catalyzed by these enzymes. Conversely, these low relationships indicated that the enzymes and the corresponding microorganisms releasing these enzymes did not play a key role in the formation of the resultants generated by the catalysis of these enzymes. The enzymes in the sediments come from a release of the cells of microorganisms and the abundant microorganisms in the sediments must produce a great number of different enzymes, effectively catalyzing reactants to generate resultants, and consequently being tested high enzyme activity. However, the generation of some resultants is not fully dependent on the enzyme catalysis and other factors also affect the formation of the resultants. For example, the hydrogen peroxide is catalyzed by the catalase to generate oxygen and water, but Mn and Fe also catalyze the hydrogen peroxide to generate the oxygen and water. Thus, low relationships between the enzyme activity and the microorganisms might have indicated that the effects of other factors exceeded the catalysis effects of the enzymes from microorganisms.

Table 3 Correlation coefficients between the enzyme activity in the sediments and the abundance of microbes

Microorganisms	Catalase	Phosphatas	Urease	Dehydrogenase
Bacteria	0.424*	0.836**	0.651*	0.619*
Fungus	0.308	0.758*	0.498*	0.092
Actinomycetes	0.612*	0.712*	0.677*	0.128
Ammonifying bacteria	0.232	0.653*	0.812**	0.372*
Denitrifying bacteria	0.077	0.689*	0.791*	0.108

Asterisk ** and * represent a significance at 0.95 and 0.99 confidence levels, respectively; N=27.

DISCUSSION

Many previous studies on abundance of microorganisms and activity of enzymes in the sediments have mainly focused on the city riverway, wetlands and lakes under the pollution conditions to cope with the challenges of water pollution and less data has been collected

from a natural river (Winiarski, Bedell, Delolme et al., 2006; Neto, Ohannessian, Delolme et al., 2007; Huang，Gao，Liu et al., 2012). In a natural river, the rapid-pool-benchland system as a basic unit of the river structure can be repeatedly seen and consequently the river structure is complete (Ma, Wang, Chen, 2014; Wu, Wang, Cheng, 2014; Chen, Ma, Wang, 2015; Wang, Wang, Liu et al., 2015, Fig.1 and Supplemental file 1). The complete river structure maintains ecosystem functions such as biodiversity, self-purification of a waterbody, landscape values and affinity between humans and the waterbody (Padmalal and Maya, 2014). The repeated occurrences of the rapid-pool-benchland systems in a river also indicates habitat heterogeneity in the river (Ma, Wang, Chen, 2014; Wu, Wang, Cheng, 2014; Chen, Ma, Wang, 2015; Wang, Wang, Liu et al., 2015). Therefore, we selected the rapid-pool-benchland systems in natural Duliu River as materials and tested abundance of microorganisms and activity of enzymes in the sediments to clarify the effects of habitat heterogeneity in a river on the two ecological processes, and the relationships between the abundance of microorganisms and the activity of enzymes closely related to biochemical functions. The final goal of the study is to understand the significance of the maintenance and restoration of a natural river structure.

In each of the rapid-pool-benchland systems in the Duliu River, the abundance of the microorganisms (including bacteria, fungus, actinomycetes, ammonifying bacteria and denitrifying bacteria) in the sediments of the pools was significantly higher than the rapids and benchlands. To compare the rapids with the banclands, the abundance of microorganisms in the benchlands is relatively high. These results indicate that the repeated occurrences of the rapid-pool-benchland systems, indicating habitat heterogeneity in the river, surely results in the differences of the abundances of microorganisms, which verifies our assumption. However, the abundance of the bacteria was always highest in the sediments of the pools, rapids or benchlands in different river reaches in Duliu River, and the ammonifying bacteria, denitrifying bacteria, actinomycetes and fungus were respectively ranked from the second to fifth (Table 1). The order of the abundance of these types of microorganisms in the pools, rapids or benchlands in the river is similar to that in the artificial wetland (Du, Huang, Gao, et al., 2013) and in a calcareous purple paddy soil (Gu YF, Yun X, Zhang XP, et al., 2008). These results indicate that habitat heterogeneity do not significantly affect relative abundance among different types of microorganisms. The study also indicated that the microorganisms were more abundant in the rapid-pool-benchland systems in the upper reaches, where there were greater loads of organic pollution than the middle and lower reaches. This agrees with the previous results that some pollution matter in the river can induce the growth of microorganisms (Hill and Moffett, 2010; Millar, Payne, Ochs et al., 2015).

The enzyme activity in the sediments can reflect an efficiency and the ability that organic pollution mater is discomposed (Garcia et al. 1994; Neto Ohannessian, Delolme et al., 2007; Reboreda and Cacador, 2008). The enzyme activity is also one way of describing the general condition of soil microbial populations (Margesin, Zimmerbauer, Schinner, 2008). In the rapid-pool-benchland systems, the activity of catalase and phosphatase presented a significantly difference among the sediments of the rapids, pools and benchlands, indicating habitat heterogeneity greatly induces variation of the activity of catalase and phosphatase. The catalase plays a great role in the decomposition of hydrogen peroxide and in the prevention of soil microorganisms, plant roots and soils from harmfulness of hydrogen peroxide in sediments (Huang, Gao, Liu et al., 2012). The phosphatase catalyzes dephosphorylation of substrate in sediments to promote the decomposition of organic matter and produce molecular information to regulate biochemical functions. In the sediments of the rapids and pools in the rapid-pool-benchland systems in the

different reaches of Duliu River, there is a higher activity of catalase and phosphatase, respectively (Fig.3). This suggests that a natural river maintains the repeated occurrences of the rapid-pool-benchland systems, a kind of habitat heterogeneity, and further affect divergent biochemical functions. Therefore, habitat heterogeneity in a river structure, which can be denoted by a basic unit of a river, i.e., the rapid-pool-benchland systems, can impose different effects on ecosystem functions in a river. In addition, in the different river reaches in Duliu River, the activity of all enzymes was lower than that in the sediments in the lake (Liu, Xie, Li, 2013）. This is mainly because the nutrients in the Duliu River were relatively poorer than those in the lake.

The biochemical functions in the sediments in different waterbodies are dependent on the catalysis of enzymes and the enzymes come from the secretion of microorganisms (Gardner and White, 2010）. Logically, the abundance of microorganisms should be positively correlated to the activity of enzymes. However, the analysis of the relationships elucidates that there are positively and negatively linear relationships between the canonical variables of the abundance of microbes and the activity of catalase, urease and dehydrogenase in artificial wetlands possibly due to the interactions of many factors (Huang, Gao, Liu et al., 2012). In the study, the correlations between the abundance of all types of microorganisms and the phosphatas and urease activities were very significant but dehydrogenase and catalase were only related to the abundance of two types of microorganisms, respectively. In a study of forest soils, although there is also a correlation between the abundance of soil microorganisms and the enzyme activity, but a close correlation occurs between the abundance of microorganism and physical and chemical properties (Hu，Zhang，Gao et al., 2002). These results indicate that the enzyme activity in the sediments to affect the biochemical functions is decided by many factors including the abundance of microorganism, although the rapid-pool-benchland systems also regulate the abundance of microorganism and the enzyme activity in the sediments.

CONCLUSIONS

The enzyme activity is crucial to biochemical functions in the sediments in a river. The microorganism abundance partially affects the enzyme activity and further the biochemical functions. The rapid-pool-benchland systems, a basic unit of a river, reflect the habitat heterogeneity in a river, and directly regulate the abundance of microorganism and the enzyme activity in the sediments in different degree. The restoration of the river structures changed by humans towards the rapid-pool-benchland systems is beneficial to a complete function of the river ecosystem.

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