Abundance of microorganisms and enzyme activity in the rapids-pool-benchland systems in natural Douliu River of China

Zhenhong Wang[1*], Zhenhong Wang[2], Chen Chen[3], Qing Wu[3]

[1]School of Environmental Science and Engineering, Chang`an University, Xi`an, China
[2]Key Laboratory of Subsurface Hydrology and Ecological Effects in Arid Region,Ministry of Education, Chang`an University, Xi`an, China
[3]College of Life Science,Guizhou University, Guiyang, China.
*Correpondence author: w_zhenhong@126.com

Abstract River structures and their ecosystem funtciotns have been greatly destructed due to the human disturbance during the last decades. It is a pressing task to understand the complete structures and ecosystem functions of a natural river to restore the impired river ecosystems. We found a basic structure of a natural river, i.e., the rapid-pool-benchland system, in the previous studies, so we selected the rapid-pool-benchland systems in the upper, middle and lower reaches of Duliu River, a natural river in China and tested the abundance of microorganisms and the enzyme activity in the sediments in these systems. Results indicated that the number of bacteria was far more than ammonifiers, actinomycetes, fungus and denitrifying bacteria in the sediments of all river reaches. In the upper reach, the microbial abundance in the sediments was more than the middle and lower reaches. In each of the rapid-pool-benchland system, the microbial abundance in the sediemnets of the pool was always highest, and that of the rapid was lowest. The catalase activity in the sediments of the benchland was higher than the rapid and pool but the phosphatase activity in the pool was higher than the rapid and benchland. The 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 system in a river obviously regulates the microbial abundance and the catalase and phosphatase activities in the sediments. However, the abuncance of microorganisms only indicated a 0.434-0.836 level of significant correlatioships with the activity of four enzymes, and further influences biochemical functions.
Key word: Natural rivers, rapids, pools, benchlands, microorganisms, enzyme activity, sediments, Duliu River

INTRODUCITON

A river is a corridor connecting the terrestrial environment to the ocean realm and it plays an important role in the supply of water resources for sustenance 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 origin on the coasts of rivers, and even to this day, many modern cities are also built along rivers (Wang, Wu, Zhang, et al., 2013). Arguably, a river is a footstone of the human civilization. However, human interventions, especially in developming countries, have imposed tremendous pressure on rivers, and consequently most of the rivers, especially the small rivers, have been altered to levels, often beyond their natural resilience capability (Dong, Sun, Peng, 2009; Padmalal and Maya, 2014). These interventions include many aspects. For example, rive way is occupied due to the urbanization; river way is changed into a straight form leading to the disappearances of the meandering form; the natural riverbanks are replaced by concrete riverbanks for slope stability (Dong, Sun, Peng, 2009; Padmalal and Maya, 2014). As a result, the habitat heterogeneity of a river significantly declines, which results in the changes of biodiversity, self-purification capacity, landscape values and affinity

between humans and waterbody (Wang, Wu, Zhang, et al., 2013).

The habitat heterogeneity in rivers 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, braiding forms and so on (Niezgoda and Johnson, 2005; Yin, Li, Liu et al., 2012). These forms are products of the long-term interations between water flow and a veriety of environmental factors. The river structures can be reflected with a spatial variation of riverbed, waterflow, organisms, river banks along a transverse and a longitudinal direction of a river (Gregory, Benito, Downs, 2005). The river-bed materials consist of water, sediments, different sizes and forms of stones, organic matters, ions in water and organisms, and each of these materials in types and traits 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 condtions. Clearly, a river is a complex system, but the system is becoming drab with a decline of the habitat heterogeneity.

In previous studies, we found a new river structure, the rapid-pool-benchland system in natural rivers by a great amount of field investigation (Fig 1 and Supplemenatal 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, i.e., a rapid-pool-benchland system in the upper reach in a river directly links another rapid-pool-benchland system in the lower reach. In the system, there are also waterflow, organism, riverbed (sediment and pebbles) and river bank (dry land during most of time) that make up a complete river from a perspective of the different material compositons. However, the rapid-pool-benchland system explains a complete river in structure from a perspective of the habitat difference. 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 pool and benchland, which generates a relatively great potential energy and consequently results in 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). The pool 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, even sludge, disposed from the slow water flow, relatively rich plants and deeper water body than the rapid section. The pool genrally occurs close to the river bank in a river (Fig 2). The benchland is often formed by transportation and sedimentation of floods and mainly consists of gravels and sediments. Some benchlands are directly river banks or links to the river bank. The most significant characteristic of the banchland is that there is alternation of wetting and drying 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 significantly relationships in areas and perimeters among the rapids, pools and benchlands, and the propotions 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 structrue of a natural and healthy river. In such a river, there is a dynamic equilium among the rapids, pools and benchlands.

However, it remains unclear if abundance of microorganisms and the enzyme acitivty in the sediments of rapid, pool and benchland in a river will inidicate different. The abundance of microorganisms and the enzyme activity in the sediments are important ecological fcuntions in the rivers and referents of the river health. Microorganisms (bacteria, fungi, etc) process most of the organic waste from a variety of sources and they oxidize, reduce and precipitate mineral ions, thereby controlling the geochemical cycles in the rivers (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 eample, there is a positive correlation between dehydrogenase activity and CO^2 emission, carbon cycle and biomass, and dehydrogenase activity is 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 the 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 expressed by the number of microbes and activities of enzymes in sediments (Deng, Huang,Hu et al., 2009). The enzyme activity in river sedments is closely 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 succesivly connecting rapid-pool-benchland systems respectively in the upper, middle and lower reachs of 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 latoratory to test the abundance of the microorganisms and the activity of several important enzymes. We assumed that the rapid-pool-benchland systems continually occurred to create habitat heterogeneity in the river, and consequently, which resulted in differences in the abundance of the microorganisms in the sediments of the rapid, pool and benchland systems. Correspondingly, the enzyme acitivty in the sediments of the rapid, pool and benchland systems 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 environmental heterogeneity

in a river directly influences the abundance of the microorganisms in the sediments, and further the enzyme activity? 2) If it does, to what degree can the correlationships between the abundance of the microorganisms and the enzyme activity be explainded by the rapid-pool-benchland system?

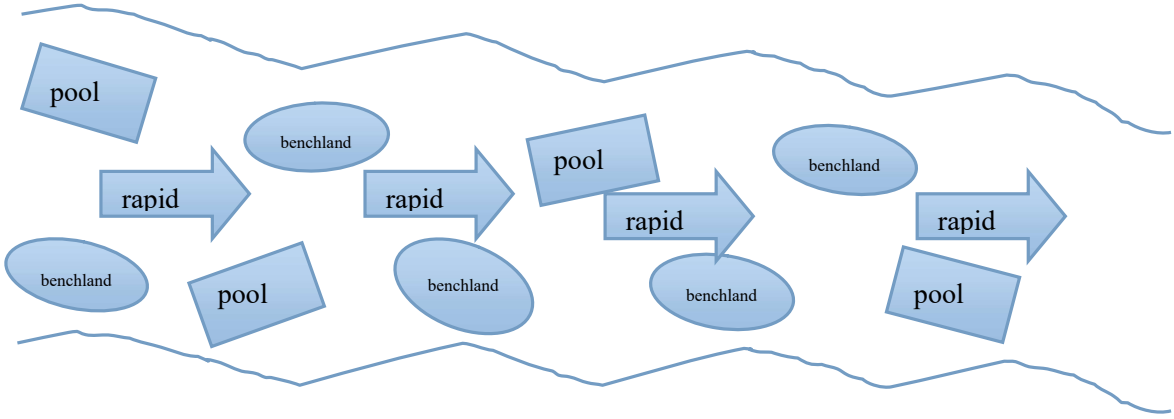

Fig.2 The typical model of the rapid-pool-benchland system

MATERIALS AND METHODS

Site decripitons

Duliu River is a primary tributary of Pearl River, the third greatest river in China. Duliu River orgins from Dushan county, flows through four counties in Guizhou Province, and lastly joins Xi River, the upper reach of Pearl River. Total length of Duliu River is 310 km and the natural 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 145145m^3/s. The forest coverage is 74.1% in Dului River watershed. There are not dams and artificial riverbanks along the river, so Duliu River is a natural river. We selected the upper Puan (26°04′ N, 107°48′ E), middle Sandu (25°58′N, 107°53′ E) and lower Rongjiang (25°89′N, 108°50′ E) river reaches, respectively as research objects. 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 the upper or lower reaches, the rapid-pool-benchland systems connecting with each other are still 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

*URR: the upper reach; MR: the middle reach; LRR: the lower reach.

Field samling

In each set of the rapid-pool-benchland systems in the upper, middle or lower reach of Duliu River, we sampled the sediments in the rapid, pool and benchlands, respectively. Specifically, ten sampling sites were firstly even set up in the rapid, pool or benchland. 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 repid and pool 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. In the upper, middle and lower reaches, 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 triagulare flask was conducted in a sterile working chamber. The dilution propotions were respectively 10^{-6}, 10^{-7} and 10^{-8} for bacteria, and 10^{-4}, 10^{-5} and 10^{-6} for fungus and actinomycetes. We furhter used pipettes to transfer the diluted soil solutions to the culture dishes. The culture dishes were incubated at 28℃. Each of the dilution propotions was replicated three times.

Amonifying 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 the denitrifying bacteria mediums were respectively made up and were sub-pacged into cuvettes. Each cuvette was filled with 5 ml, pluged using silica gel stopple and sterilized. In each of these filled cuvettes, a reversed Durham's fermentation tube was put to test the generaton 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 30min to make suspension. Subsequently, the suppernsion was diluted into four levels of concentrations, i.e. the 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} times of the mother solutions. We used sterile pipettes to transfer these diluted solutions to the cuvettes filled with the mediums, and these cuvettes were incubate at 20-30℃. Eeach level of the concentrations was replicated three times. After three and five days, we checked the turbidity of the mediums for the amonifying bacteria. When the incubation continued till the seventh day, we used Nessler reagents to test if ammonia had been generated. For the denitrifying bacteria, we observed if bubbles had occurred in the cuvvettes and if the solutions 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 mother suspension.

Tests of the enzyme activity

Catalase: we adoped permanganate titration to test the catalase activity. Specifically, we weighed 5g of the fresh sediments and put them in in a triangular flask. 25ml of H_2O_2 solutions and a little distilled water were decanted into the triangular flask, and then the triangular flask was continually oscillated for 30 minutes. The chemical reations occurring in the the triangular flask were terminated with addition of H_2SO_4. The mixtures in the triangular flask were filtrated and the fitrates were titrated with $KMnO_4$. When the fitrates changed ino red, the titration was stopped. The consumption volume (ml/g) of the KMnO4 solution represented the catalase activity.

Alkaline phosphatase: the activity of alkaline phosphatase was tested with the phosphatase two sodium colorimetric method. We firstly weighed 5 g of the fresh sediments and put them in a triangular flask. Then, the toluene, disodium phenyl phosphate and borate buffer solution were decanted into the triangular flask. The triangular flask was continually incubated 24 h and then the mixtures in the triangular flask were filtrated. Potassium ferricyanide and 4-amino safety were used as chromogenic agents and were added to the filtrates. Finally, the filtrates were transferred to a volumetric flask, diluted to 750ml of constant volume for absorbance measurement.

Urease: the activity of the urease was tested by the phenol sodium hypochlorite

colorimetric method. We firstly weighed 5g of the fresh sediments and put them in a 50 ml volumetric flask. Then, we added 1ml of toluene to the volumetric flask, tightly pluged the volumetric flask and slightly waved it about 15 minutes. Succesively, 5ml of 10% solutions of urease and 10ml of citrate buffer sulution (pH=7.6) were added to the volumetric flask and the soil solution in the volumetric flask must be fully mixed by waving. After the volumetric flask had been incubated at 37℃ for 24 h, we used the distilled water at 38 ℃ to dilute the solution in the volumetric flask to constant volume. This moment, the toluene should be floated above the solution in the volumetric flask. After the solution in the volumetric flask was further waved 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 were added to the 50 ml volumetric flask. After coloration of the solutions in the volumetric flask, 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 solutions, 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 futher added to the triangulare flask. After the solutions in the triangular flask had been fully mixed, we took the solutions to test the absorbance of the solutions. The enzyme activity was indicated with the quantity of three phenyl formazen per unit soil and time.

RESULTS

Abundance of microorganics

The abundance in individual bacteria in the sediments collected from the rapids, pools or benchlands in these river reaches was highest among all types of microorganisms (Table 2). The abundance in individual amonifying bacteria, actinomycetes, fngus and denitrifying bacteria was respectively ranked second to fouth. One of the important causes why bacteria were more than actinomycetes and fngus was because the bacteria existed in the sediments with smaller individual than the actinomycetes and fngus. In natural ecosystems, there is generally a law, i.e., the individual size of organisms is negatively related to their invidual numbers. Secondly, the abuncance of these microorganisms in the pool 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 in the rapids, which was banificial to the growth and reproduction of the microoganims. In the benchlands, floods often resulted in the alternate occurrence of waterflooding and dry environments and the delivary of the nutrients and consequently which was benificial to the growth and reproduction of the aerobic and anaerobic microorganism. Greater loss of nutrients and disturbance of water flows in the rapids might refrain the growth and reproduction of the microorganisms than the pools and benchlands. Thus, the abundance of these microorganisms in the rapids presented relatively less than the pools and benchlands. Thirtly, the abudance of these microorganisms were higher in the upper reaches than the lower reaches because there were more COD input to the river in the upper reaches.

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 in the upper and middle reaches

（P<0.01）. In the rapids in the upper and middle reaches, the acitivty of the enzyme in the sediments presented moderate levels. These results occurred possibly because in the benchlands, there were high oxigen contents in the sediments under an alterative floodingwater condtions with a change of water levels in the river, which promoted the 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 becase there was relatively richer dissolved oxygen in the rapids.

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 ($\times10^4\cdot g^{-1}$)	Amonifying 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

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 acitivty 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, the difference between the pools and rapids was relatively small because of the habitat similarity. These interesting results might be caused by the relatively high pH in the waterbody in the upper reaches and pools.

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 were very significant among the rapids, pools and benchlands in the upper and lower reaches. 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 higherst activity of the enzyme, and the pools and rapids were ranked second and third, respectivly, 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 had been affected by some stachastical factors.

The dehydrogenase activity in the sediments averaged 1.071μg/g·h·in all rapids, pools

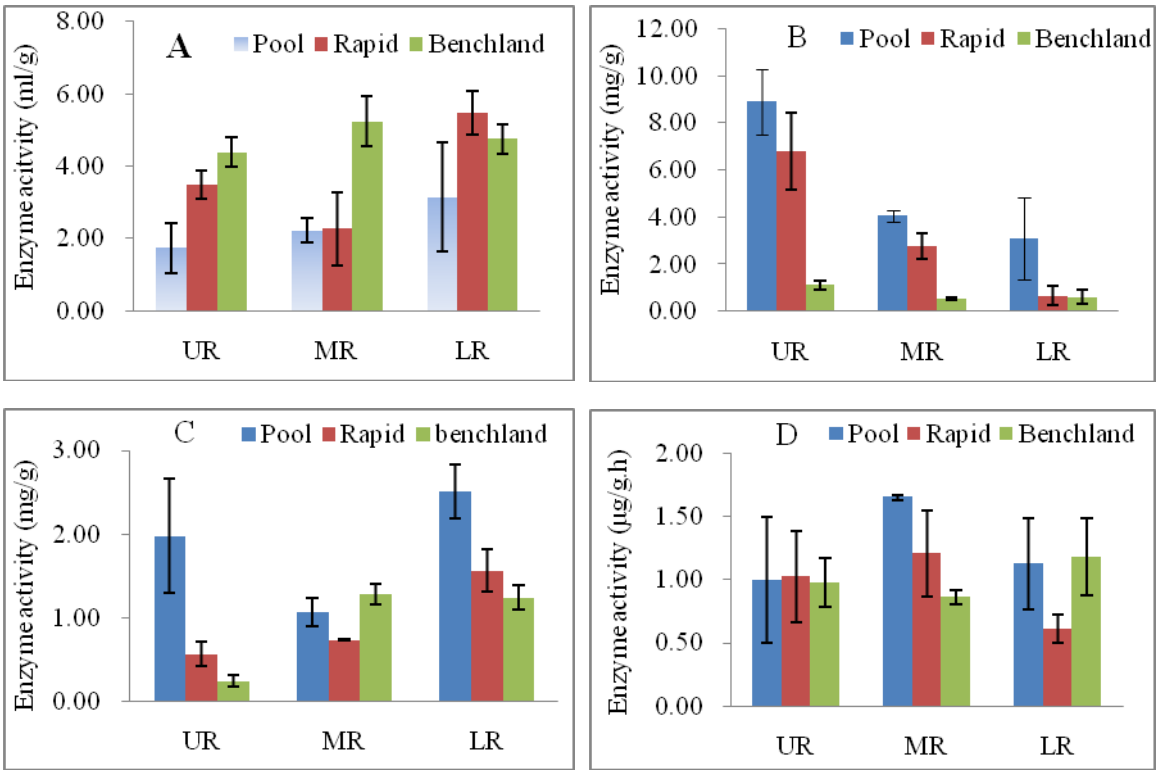

Figure 3 The enzyme activities in the sediments of the rapid-pool-benchland in the upper, middle and lower reaches in Duliu River. A: catalase; B: phosphatase; C: urease; D: dehydrogenase.

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 also 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. The differences and regularity of the activity of the enzyme were very significant among the rapids, pools and benchlands in the middle reaches. 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 type of mincroorganisms, i.e., actinomycetes and bacteria among all tested microorganisms. Similarly, the dehydrogenase activity was only related to the bacteria and amonifying bacteria as well. These high-significant relationships indicated that the 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 enzymess and the corresponding microorganisms releasing these enzymes did not play a key role in the formation of the resustants generated by the catalysis of these enzymes. The enzymes in the sediments come from a realese of the cells of microorganisms and the abundant microorganisms in the sediments must produce a great amount of different enzymes, effectively catalyzing reactants to generate resultans, and consequently being tested high enzyme activity. However, the generation of some resultans is not fully dependent on the enzyme catalysis and other factors also affect the formation of the

resustants. For example, the hydrogen peroxide is catalyzed by the catalase to geneate oxygen and water, but Mn and Fe also catalyze the hydrogen peroxide to generate the oxygen and water. Thus, no relationships between the enzyme activity and the microorganisms might have indicated that the effects of other factors perhaps exceedd the catalysis effects of the enzymes from low abundant 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*
Fugus	0.308	0.758*	0.498*	0.092
Actinomycetes	0.612*	0.712*	0.677*	0.128
Amonifying bacteria	0.232	0.653*	0.812**	0.372*
Denitrifying bacteria	0.077	0.689*	0.791*	0.108

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

DISCUSSION

Previous much studies on abundance of microorganisms and activity of enzymes in the sediments mainly focused on the city riverway, wetlands and lakes under the pollution conditions to compe with the challenges of water pollution problems 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 river structure is complete and consequently ecosystem functions of the riverway such as biodiverisy, self-purificaiton of waterbody, landscape values and affinity between humans and waterbody are also complete (Padmalal and Maya, 2014).The rapid-pool-benchland system is a basic unit in river structure in a natural river based on our previous studies (Ma, Wang, Chen, 2014; Wu, Wang, Cheng, 2014; Chen, Ma, Wang, 2015; Wang, Wang, Liu et al., 2015). Therefore, we seclected the rapid-pool-benchland system as a representive of a complete structure in Duliu River and tested the abundance of microorganisms and activity of enzies in the sediments to clariy the effects of the river structure on the microorganism patterns, and the relationships bwtween the abundance of the microorganism and the biochemical functions. The final goal of the study is to clarify the important significance of the maintenance and restroaiton of a natural river structure.

In each of the rapid-pool-benchland systems, the abuncance of the microorganisms (including bacteria, fugus, actinomycetes, amonifying bacteria and denitrifying bacteria) in the sedimetns of the pools in different reaches is significantly higher than the rapids and benchlands. Comparisively, the abuncance of the microorganisms in the benchlands is also higher than the rapids. These results indicate that the hatitant hetoregenity surely results in the differences of the abuncance of the microorganisms and verify our assumpption. However, the abuncance of the bacteria, fugus, actinomycetes, amonifying bacteria and denitrifying bacteria in sediments respectively occupied 60%−99.07%, 0.012-0.172%, 0.28%−23.21%, 5.00%−31.89%, 0.009%−0.027% in total abundance of microorganisms regardless of the habitat difference in the rapid-pool-benchland systems and these propotions are similar to those 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). This indicates that the hatitat difference within the rapid-pool-benchland systems do not significantly affect the relative abundance among different groups of microorganism. The study results also indicated that the microoragnims was 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 habitats can induce the growth of microorganism (Hill and Moffett, 2010; Millar, Payne, Ochs et al., 2015).

The enzyme activity in the sediments can reflect the efficiency in matter transformation, and the ability that organic pollution mater may be 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 in the sediments presented signficanlty different among the rapids, pools and benchlands, indicating the habitat difference 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 prevetion of soil microorganisism, 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 decompsotion of organic matter or produce the information moleculars to regualte biochemical functions. In the sediments of the rapids and pools, there is a higher acitivity of catalase and phosphatase, respectively (Fig.3), which suggests that the rapid-pool-benchland systems shape different environemtns for divergent biochemical functions in a river. Therefore, a habitat heroterosity in a river structure, which can be denoted by a basic unit of a river, i.e., the rapid-pool-benchland systems, imposed different effects on ecosystem fucnitons in a river. In addition, in the different river reaches in Duliu River, the activity of all enzynes 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 differne waterbodies are dependent on the catalysis of enzymes and the enzymes come from the sectation of microorganisms (Gardner and White, 2010）. Thus, the abundance of mirocroorganism shoud be positively correlated to the acitivty of enzymes. However, the analysis of the relationship suggests that there are positively and negatively linear relationships between the canonical variables of the microbes and the activity of catalase, urease and dehydrogenase in artificial wetlands possibliy due to the interactions of these variables (Huang, Gao, Liu et al., 2012). In the study, the correlationships between abundance of all types of microorganisms and the phosphatas and urease activities were very significant but dehydrogenase and catalase were only realeted to the abundance of two types of microorganisms, respectively. In the study of forest soils, although there is also a correlation between soil microorganisms and the enzyme activity, but a close correlation occurs between abundcance of microorganism and physical and chemical property (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 abundcance of microorganism, athough the rapid-pool-benchland systems also regulate the abundcance of microorganism and the enzyme activity in the sediments.

CONCLUSIONS

The enzme activity is crucial to biochemical functions in the sediments in a river. The microorganism abundance particialy affects the enzyme activity and further the biochemical functions. The rapid-pool-benchland systems, a basic unit of a river, reflect the habitat hetoregeinty in a river, and directly regulate the abundance of microorganism and the enzyme activity in the sediments. The resotoration of the river structures disturbed by huanms towards the rapid-pool-benchland systems is benifical to a complete function of the river ecosystem.

REFERENCES

1. Asai K，Takasaki A，Muraoka K，Yorozu H. 2013. Study on river bed material change due to flood Occed on JULY 2009 in Saba River and long term rived bed variation. *Journal of Japan Society of Civil Engineers* 69(4): 997-1002.
2. Background for progress towards a palaeohydrology protocol. *Geo-morphology* 98(1-2): 153-172.
3. Benitez E, Melgar R, Nogales R. 2004. Estimating soil resilience to a toxic organic waste by measuring enzyme activities. *Soil Biology and Biochemistry* 36: 1615–1623.
4. Chen C，Wang ZH，Ma Z. 2015. Evaluations on the difference of water quality of the riffle-deep pool-benchland systems of watercourses in Duliujiang River. *Environmental Science and Technology* (China)，38（3）：182-188.
5. Deng JJ，Huang XF， Hu JW，Li CX，Yi Y, Long J. 2009. Distribution of several microorganisms and activity of alkaline phosphatase in sediments from Baihua Lake. *Asia-Pacific Journal of Chemical Engineering* 4(5):711–716.
6. Dong ZR, Sun DY, Peng J. 2009. Theories and practices of river eco-restoration. *Water resources and hydropower engineering* 40(1):4-9.
7. Du G, Huang L, Gao X, Lu YY, Liu M, Guo JS. 2013. Number of microbe and relationship between it and removal of pollutants in constructed wetlands. *Wetland science* 11(1):13-17.
8. Ellis RJ, Neish B, Trett MW, Best JG, Weightman AJ, Morgan P, Fry JC. 2001. Comparison of microbial and meiofaunal community analyses for determining impact of heavy metal contamination. *Journal Microbiol Methods* 45: 171–185.
9. Garcia C, Hernandez T, Costa F. 1994. Microbial activity in soils under Mediterranean environmental conditions. *Soil Biology and Biochemistry* 26: 1185–1191.
10. Garcia C, Hernandez T, Costa F. 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. *Communications in Soils Science and Plant Analysis* 28: 123–134.
11. Gregory K J, Benito G, Downs P W. 2008. Applying fluvial geomorphology to river channel management:
12. Grenouillet G, Pont D and Seip KL. 2002. Abundance and species richness as a function of food resources and vegetation structure: juvenile fish assemblages in rivers. *Ecography* 25(6) 641-650.
13. Gu YF, Yun X, Zhang XP, Tu SH, Sun XF, Kristina L. 2008. Effect of different fertilizer treatments on soil microbes and ammonium oxidizing bacterial community in a calcareous purple paddy Soil. *Scientia Agricultura Sinica* 41(12):4119-4126.
14. Hu H，Zhang J，Gao Z，Chen S，Zang T. 2002. Study on quantitative distribution of soil microorganism and relationship with enzyme activity and physical, chemical property of shelter-forest in rocky coastal area. *Forest Research* 15(1):88-95.
15. Irha N, Slet J, Petersell V. 2003. Effect of heavy metals and PAH on soil assessed via dehydrogenase assay. *Environment International* 28: 779–782.
16. Huang L，Gao X，Liu M，Du G，Guo J. 2012. Correlation among soil microorganisms, soil enzyme activities, and removal rates of pollutants in three constructed wetlands purifying micro-polluted river water. *Ecological Engineering* 46(3):98-106.
17. Gardner LM, White JR. 2010. Denitrifcation enzyme activity as an indicator of nitrate movement through a diversion wetland. *Wetland Soils* 74(3):1037-1047.
18. Liu XW, Xie DP, Li KM, Zhou WJ, Wang HJ, Jia Y. 2013. Effects of variation of DO on the enzyme

activity and microbial diversity in sediments. *Environmental Science and Technology* (China) 36(6):6-10.

19. Ma Z, Wang ZH, Chen C. 2014. Investigation in river channel parameters of riffle-deep pool-sand bank system in Duliujiang River. *Journal of Mountain Agriculture and Biology* 33(1): 049-052.
20. Margesin R, Zimmerbauer A, Schinner F. 2000. Monitoring of bioremediation by soil biological activities. *Chemosphere* 40: 339–346.
21. Millar JJ, Payne JT, Ochs CA, Jackson CR. 2015. Particle-associated and cell-free extracellular enzyme activity in relation to nutrient status of large tributaries of the Lower Mississippi River. *Biogeochemistry* 124: 255–271.
22. Neto M, Ohannessian A, Delolme C, Bedell JP. 2007. Towards an Optimized Protocol for Measuring Global Dehydrogenase Activity in Storm-Water Sediments. *Journal of Soils Sediments* 7 (2): 101–110.
23. Niezgoda SL, Johnson PA. 2005. Improving the urban stream restoration effort: Identifying critical form and processes relationships. *Environmental Management* 35(5):579-592.
24. Padmalal D，Maya K. 2014. Rivers-structure and functions. In: Padmalal D，Maya K ed *Sand Mining*, Berlin: Springer Verlag, 9-22.
25. Reboreda R, Cacador I. 2008. Enzymatic activity in the rhizosphere of Spartina maritima: potential contribution for phytoremediation of metals. *Marine Environment Research* 65: 77–84.
26. Tam NFY. 1998. Effects of wastewater discharge on microbial populations and enzyme activities in mangrove soils. *Environmental Pollution* 102: 233–242.
27. Wang QH, Wang ZH, Liu LB, Guo CX. 2015. Influence of typical structures of a natural river on self-purification of water. *Journal of Mountain Agriculture and Biology* 34(6):63-67.
28. Wang ZH, Wu YG, Zhang CY, Liu HY, Zhou YC, Ying XL, Xu CM, Zhang MJ. 2013. Typical terrestrial ecosystems in Yungui plateau (II) Typical watershed ecosystems, water ecological processes and non-point source pollution control. Beijng: Science.
29. Winiarski T, Bedell JP, Delolme C, Ghidini M, Perrodin Y. 2006. Study of spatial distribution of physico-chemical and biological parameters along a soil vertical profile in a stormwater infiltration basin in the Lyon area. *Journal of Hydrogeology* 14 (7): 1244–1251.
30. Wu EN, Chen Y, Zhang HW, Yang K. 2008. Urban river regulation: past, present and prospects. *China Water and Wastewater* 24(4):13-18
31. Wu Q, Wang ZH, Cheng C. 2014. Evaluation of heavy metal pollution on riffle-deep pools-sand bank systems in different section Duliu River. *Journal of Mountain Agriculture and Biology* 33(6) :066-071.
32. Yin XL, Li GC, Liu K, Zhong YJ. 2012. Progress of urban stream transformation of critical forms and stability relationships. *Progress in Geography* 31(7): 837-845.