

## Evaluating the effectiveness of ecological restoration of hard bank rivers: a case study from Shedu river port, China

Van Tai Tang, Dafang Fu, Rajendra Prasad Singh, Eldon R. Rene, Tran Ngoc Binh and Anil Kumar Sharma

### ABSTRACT

The main aim of this study was to evaluate the performance of ecological restoration of hard bank rivers at the Shedu river port in Yixing city, China. The pre-fabricated porous concrete templates were covered on the hard bank surface in order to reduce the negative impact of hard bank on the river water quality. The results showed that the microbial community, population, and the water purification ability of biofilm were affected by the seasonal changes. The biofilm growing on the surface of the porous concrete templates showed the highest population ( $3.27 \times 10^7$  cell  $\text{cm}^{-2}$ ) and a healthy microbial community structure during the autumn season. The chemical oxygen demand (COD),  $\text{NH}_3\text{-N}$ ,  $\text{NO}_3\text{-N}$ , total nitrogen (TN), and total phosphorus (TP) removal during the autumn season was 48.6, 39.8, 40.2, 42.6 and 35.8%, respectively. During the one-year experimental period, biomass ( $0.3794 \pm 0.0040$  g) of *Barnyard* grass grew on the porous concrete templates. A total of seven insects and two faunal species were also observed on the porous concrete templates. The good results from this study showed that the pre-fabricated porous concrete templates are very efficient for river water purification.

**Key words** | ecological restoration, hard bank rivers, porous concrete, water purification, Yixing-Shedu port

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### INTRODUCTION

Since 1986, the economy of the Taihu Lake region (China) has developed rapidly. However, measures concerning the protection of the environment are progressing at a relatively slow pace. The water pollution from the river tributary has also affected the water quality in Taihu Lake. In order to meet the safety requirements, hard concrete bank systems were constructed to ensure flood control, bank protection, and waterway traffic. Besides these benefits, the construction of the hard concrete bank system also caused negative effects on the river ecosystem. Hard bank construction has not only destroyed the aquatic and amphibian habitats, but also deteriorated the river's ecological and natural

landscapes (Liu *et al.* 2014). Hence, ecological engineering-based methods were proposed by researchers to reconstruct or transform the hard concrete bank systems.

Chen & Ho (2013) used image spectrum for investigating the stone revetment construction at the Nan-Shi-Ken stream, Taiwan and suggested possible changes on the stream bank landscape and the recovery of lost vegetation using stone revetments. Liu *et al.* (2014) proposed combining chain-typed concrete with ash concrete to construct the ecological revetment on Shikui river. Shi *et al.* (2009) investigated the structures in Hunhe river and proposed a decision-making method for evaluating and prioritizing the

eco-revetment projects. Most of the literature on this topic has focused on studying the growth of plant, faunal, and microbial communities to prove ecological restoration effect. However, these previous research works did not evaluate the effect of seasonal climate on the growth of plants and microorganisms on ecological revetments, and its water purification capacity.

The main aim of this study was to promote the use of porous concrete templates to cover the river hard bank works in Shedu river and enhance its ecological restoration effect. Under different seasons, the microbial community variations and structure of biofilm on the surface porous concrete templates were studied to evaluate their influence on the water purification ability. The growth of floral and faunal species on the pre-fabricated porous concrete templates was also ascertained to demonstrate the ecological restoration ability.

## MATERIAL AND METHODS

### Site description

Shedu river is located in the west of Taihu Lake basin, Yixing city, China. The river flows through Yixing city of Gaocheng and Qiting town and finally flows into the Wuyi canal. The length of Shedu river is 9.3 km and the width varies from 10 to 30 m. The river bank is protected by vertical hard concrete revetments. The study site was located in the Shedu port area (31°24' latitude and 119°25' longitude) of the Shedu river in China (Figure 1).

The river flow velocity ranged between 0.48 and 0.63 m/min. The surroundings of Shedu port include residential buildings, farmlands, and animal fields, which produce large amounts of domestic sewage and garbage waste every day. The poor garbage collection and sewage treatment systems adversely affect the deterioration of the river water quality. 10,000 porous concrete templates were used to cover 3 km of vertical hard bank (Figure 2). The covering of porous concrete templates work was carried out from September 25th, 2015 to October 18th, 2015. After a period of two months, experimental work on the porous concrete templates installed on the hard bank was initiated.

### Preparation of the porous concretes

In order to ensure good river water purification ability and ease of installation, the porous concrete template was modular and prepared into volumes of  $60 \times 30 \times 8 \text{ cm}^3$  each. Two small holes were perforated on each template in order to attach them to the hard bank surface using steel bars (Figure 2). In this study, cement with P52.5 label was used as the cementing material (Park & Tia 2004). To increase the pollutant adsorption ability of the porous concrete template, burnt ceramic grains (diameter: 10–15 mm) were used as the coarse aggregate material during preparation. For producing the porous concrete volume of  $1 \text{ m}^3$  with a porosity of 30%, a mixture of ceramsite:cement:water was mixed in a weight ratio of 366:369:111 (kg). The porous concrete templates were cured for 28 days to ensure adequate strength of the material.

### Determination of microbial biodiversity

To analyze the growth of microorganisms on the porous concrete during the four seasons, biofilm samples were collected from the porous concrete templates in the months of March (spring), June (summer), September (autumn), and December (winter) of 2016, respectively. The biofilm samples from the spring, summer, autumn, and winter seasons are indicated as sample T1, T2, T3, and T4, respectively. The biofilm samples were immediately transferred to Yixing Yongxin Biological Co. Ltd in order to determine the microbial diversity. The high throughput DNA sequencing technology was used to explore the microbial community profiles in the mud samples (Ma *et al.* 2013b).

### Extracellular polymeric substances and determination of microbial population

Total extracellular polymeric substance (EPS) yields are represented by the polysaccharide and protein content since they are the main components of EPS (Gao *et al.* 2008). Protein concentration was determined using the bicinchoninic acid (BCA) method (Smith *et al.* 1985), while the polysaccharide content was determined by the phenol/sulfuric acid method (Dubois *et al.* 1956). After straining the cells,

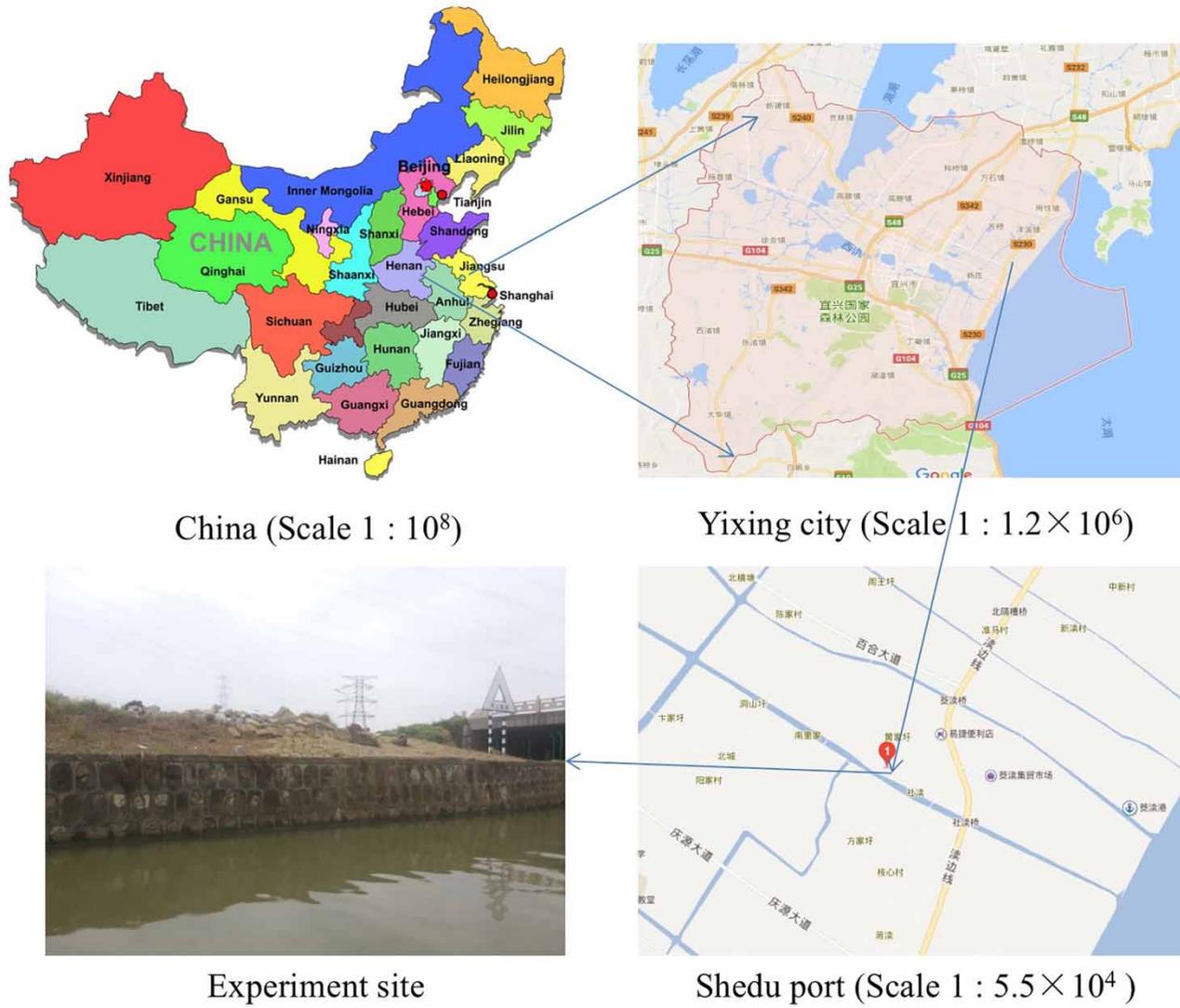


Figure 1 | Location of the experimental site at the Shedu port area, China.

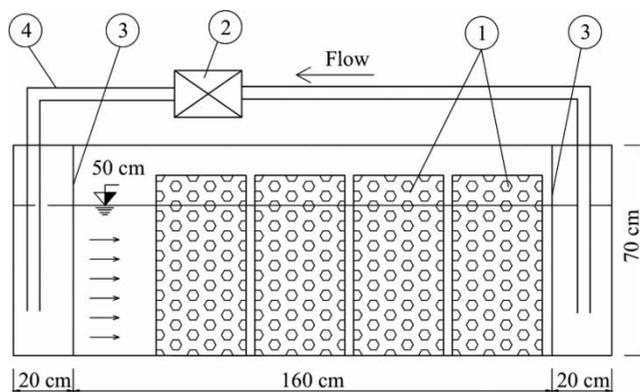


Figure 2 | (a) Porous concrete templates and (b) porous concretes installed vertically on the hard bank.

the total microbial population was estimated according to the procedure outlined by Zimmermann *et al.* (1978). The EPS of the biofilm and the microorganisms' population index was determined by Yixing Yongxin Biological Co. Ltd, China.

### Water quality improvement tests

The water purification effect is not significantly reflected in the Shedu river due to its realistic volume. In addition, the pollutants from agricultural lands, residential areas, and industrial facilities located on both sides of the river could also be discharged into the river water. In order to ascertain the real water quality improvement in the river water, long-term studies should be carried out by accounting for all the inflows. River simulator devices of volume  $2 \times 1 \times 0.7 = 1.4 \text{ m}^3$  (1,400 L), made using plastic containers, were used to measure the water purification ability of porous concrete templates. The use of such river simulator devices helps to determine the river water improvement effect under controlled experimental conditions. In addition, the influence of external environmental factors such as rainfall and non-point pollution sources that could reduce the reliability of the results can also be avoided. During each season, the porous concrete templates were removed and placed within the river simulator device (Figure 3). About 1,000 L of fresh river water was added into the device in order to maintain the water level at  $\sim 50 \text{ cm}$ . The water flow velocity was set at 0.5–0.6 m/min using a pump and the hydraulic retention time (HRT) was 5 days.



**Figure 3** | Schematic of the river simulator device: 1. porous concrete templates; 2. pump; 3. plate designed with multiple holes for proper water distribution; 4. water supply pipe.

### Status of plant growth

The status of plant growth was measured once every month. During these measurements, 20 plant samples were selected to determine the plant biomass in different concrete templates. The biomass of plant samples and the acid-insoluble lignin content were determined according to the chemical analysis protocols specified in the TAPPI-T222 standard test methods (Loelovich 2015). In brief, the samples were pre-hydrolyzed with 72% (w/w) sulfuric acid, at 20 °C for 2 hours; thereafter, the acid was diluted with water and the sample was hydrolyzed with 3% (w/w) sulfuric acid, at boiling temperature for 4 hours. After 24 hours, the acidic dispersion of lignin was filtered through a glass-filter having an average pore diameter of 10  $\mu\text{m}$ . The sediment slurry of lignin present in the filter was washed with hot water to reach a pH value of  $\sim 7.0$ , and then dried at 105 °C to constant weight.

### Determination of faunal species

During the period of one year, samples for estimating the floral and faunal species growing on the porous concrete templates were collected on a weekly basis, as well as visually inspected. The samples were collected along the experimental hard bank area in polyethylene bags. With the help of visual observations, the various faunal and floral species growing on the porous concrete templates were listed.

## RESULTS AND DISCUSSION

### Microbial community diversity

The seasonal changes that also correspond to a change in temperature had a direct impact on the microbial community profiles and the water purification effects. As seen from Figure 4, the microbial community abundance (%) was obviously different under different seasonal conditions. *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*, *Acinobacteria*, *Cyanobacteria*, and *Firmicutes* were detected in the biofilm samples. *Cyanobacteria* (%) in the biofilm during the spring, summer, autumn, and winter seasons was 11.8, 20.7, 4.4, and 1.5%, respectively. The *Proteobacteria*

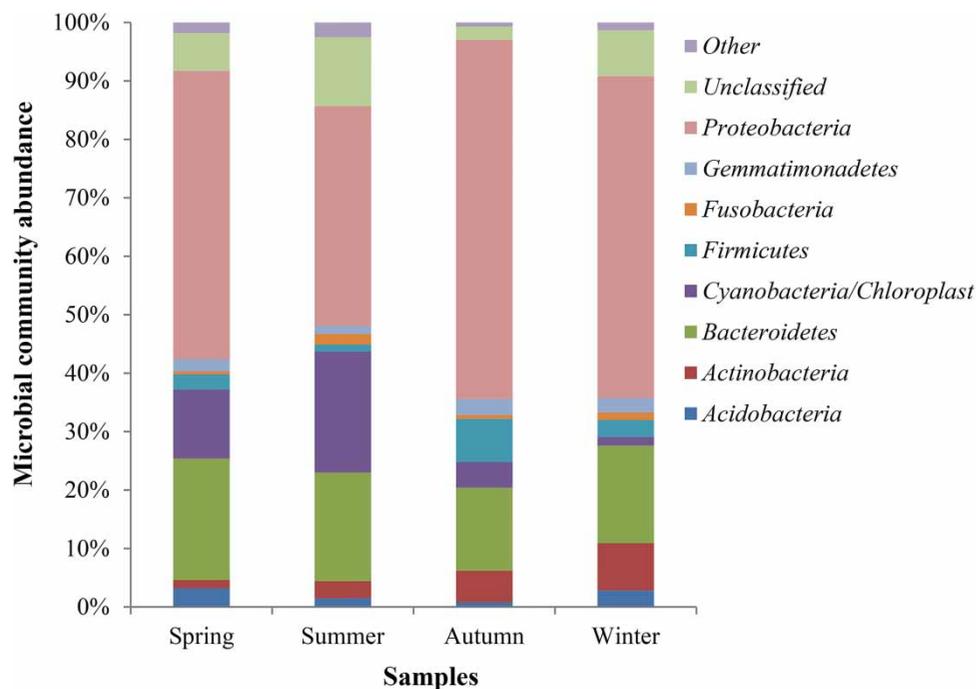


Figure 4 | Microbial community abundance in the biofilm during different seasons.

and *Firmicutes* (%) composition in the biofilm during the spring, summer, autumn, and winter seasons were 49.3%, 37.6%, 61.4%, 55.1% and 2.6%, 1.2%, 7.4%, 2.9%, respectively. These results showed that *Cyanobacteria* was dominantly present during the summer and spring seasons, while their abundance was low during the autumn and winter seasons. *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Gemmatimonadetes* were also highly dominant during the autumn season. Ma et al. (2013b) studied the effect of temperature on the microbial community structure in a membrane bioreactor and reported that, at lower temperatures (<20 °C),  $\alpha$ -*Proteobacteria* and some filamentous bacteria were relatively rich in composition. O'Neil et al. (2012) reported that as the temperatures exceeded values >20 °C, the growth rates of eukaryotic phytoplankton were generally stable; however, the growth rates decreased when the growth rate of *Cyanobacteria* increased in the environment.

### Biofilm composition

The amount of biomass and the total EPS content of the biofilm during the different seasons are shown in Table 1. During the spring season, i.e., 9–18 °C, the amount of

Table 1 | Amount of biomass and total EPS in the biofilm

Season	Biomass (cells cm <sup>-2</sup> )	Total EPS (mg g <sup>-1</sup> VSS)
Spring	$2.39 \times 10^7$	27.5
Summer	$1.52 \times 10^7$	15.4
Autumn	$3.27 \times 10^7$	36.2
Winter	$2.17 \times 10^7$	42.8

EPS, extracellular polymeric substances; VSS, volatile suspended solids.

biomass in the biofilm and the total EPS index were  $2.39 \times 10^7$  cell cm<sup>-2</sup> and 27.5 mg g<sup>-1</sup> VSS, respectively. According to O'Neil et al. (2012) and Wang et al. (2014), the increasing of temperature during the spring season usually provided good conditions for the development of *Cyanobacteria* in river water. Thus, under flow conditions, *Cyanobacteria* is transported to the porous concrete templates and becomes attached to the biofilm. Thereafter, *Cyanobacteria* becomes an integral part of the biofilm structure and brings native effects to the growth and development of the biofilm. As well, many genera of *Cyanobacteria* can produce a wide variety of toxins and anti-microbial bioactive compounds, which also add to the native effect of the biofilm (Peperzak 2003; Santhakumari et al. 2016).

During the summer season with high temperature conditions (26–34 °C), the microorganisms' abundance index ( $1.52 \times 10^7$  cell cm<sup>-2</sup>) and the total EPS index (15.4 mg g<sup>-1</sup> VSS) were the lowest among the four seasons. The high temperatures and light intensity during the summer season causes a strong abundance of *Cyanobacteria* in the river water (Figueiredo et al. 2006; Ma et al. 2013a). Similar to the spring season, a large number of *Cyanobacteria* was also present in the biofilm during the summer season (20.7%) (Qu et al. 2012). Santhakumari et al. (2016) showed that some *Cyanobacteria* and *Synechococcus* sp. have anti-biofilm and quorum sensor inhibitory potential characteristics, and they are capable of producing bioactive compounds with medicinal properties. Many research studies have shown that the prevalence of high *Cyanobacteria* composition in the biofilm is the main reason for the decreased growth of other microorganisms and poor development of the biofilm structure.

However, during the autumn season (18–27 °C), the microorganisms' abundance index ( $3.27 \times 10^7$  cell cm<sup>-2</sup>) was the highest, while the total EPS index (36.2 mg g<sup>-1</sup> VSS) was second among the four seasons. The decrease in *Cyanobacteria* population during this season caused the rapid growth and reproduction of other microorganisms such as *Proteobacteria*, *Firmicutes*, *Acinobacteria*, *Gemmonasdetes*, etc., that also helped in improving the water purification capacity of biofilm. During the winter season, with an average temperature of -1–9 °C, the microorganisms' abundance index was low ( $2.17 \times 10^7$  cell cm<sup>-2</sup>), but the total EPS index was the highest (42.8 mg g<sup>-1</sup> VSS). Although the total EPS index reached the highest value, it did not provide good conditions for the

development of the biofilm, leading to low water purification effects. Ma et al. (2013b) studied the effect of temperature variations on membrane fouling and microbial community structure in a membrane bioreactor treating wastewater and showed that the concentration of EPS ranged from 22.3 mg g<sup>-1</sup> MLSS (mixed liquor suspended solids) at 9.2 °C to 5.9 mg g<sup>-1</sup> MLSS at ~20 °C. These results proved that the microorganisms were triggered/stimulated to secrete more EPS as a survival and protection strategy against the prevailing low surrounding temperatures.

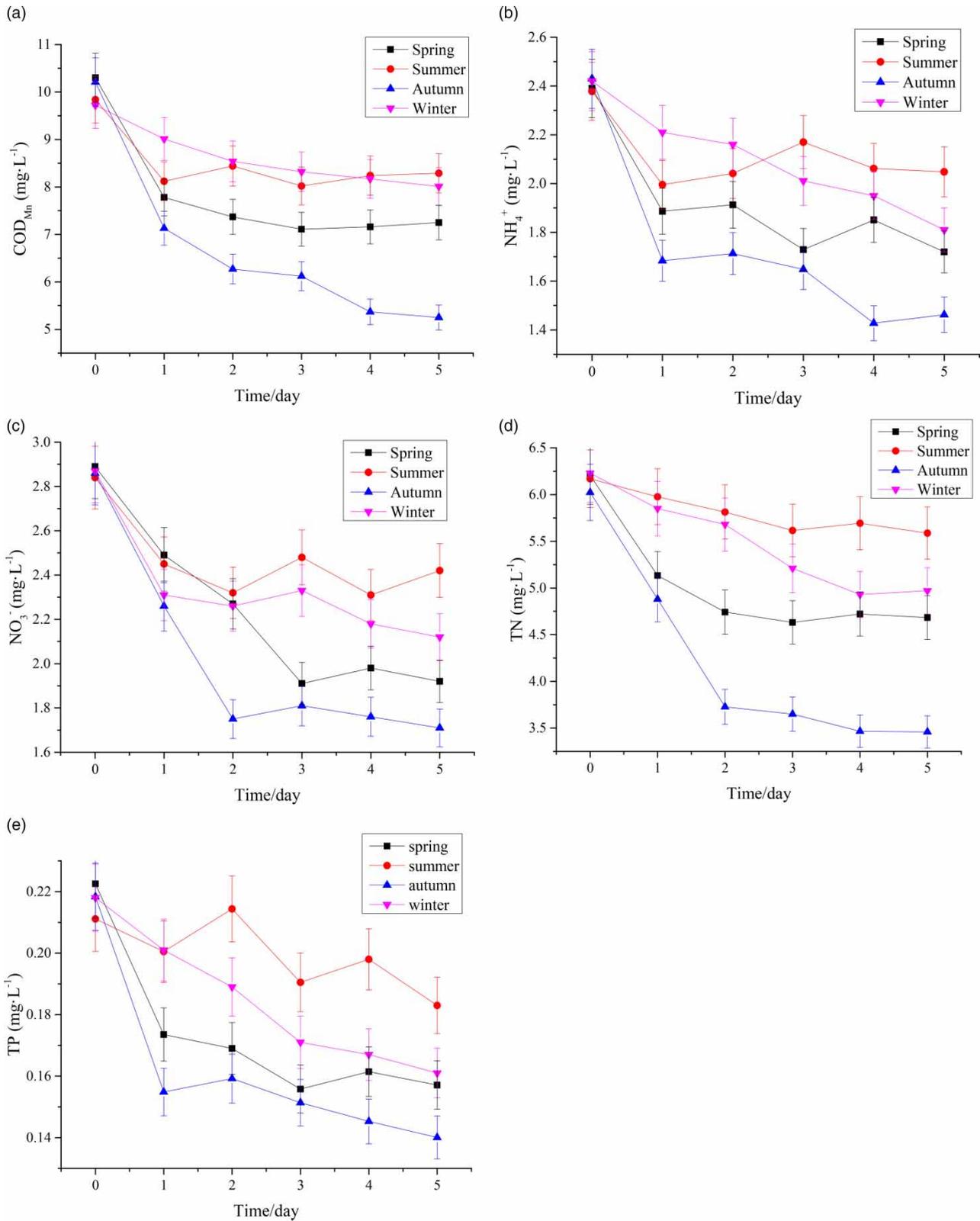
### Water quality improvement experiment in the simulated river device

The water purification effects were clearly evident depending on the change of biofilm structure and composition during the four seasons (Table 2 and Figure 5). At a HRT of 5 days, the chemical oxygen demand (COD), NH<sub>3</sub>-N, NO<sub>3</sub>-N, total nitrogen (TN), and total phosphorus (TP) removal during the spring season of the biofilm structure (T1) was 29.6, 28.0, 33.6, 24.5, and 29.6%, respectively. The number of microorganisms present in the biofilm had a direct effect on the water purification effects during the four seasons. The number of microorganisms during the spring season is ranked second among the four seasons, and hence the COD, TN, and TP removals by the T1 biofilm (spring) were higher than the T2 (summer) and T3 (winter), but were lower than the removals by the T4 (autumn) biofilm. NH<sub>3</sub>-N removal by the T1 biofilm (spring) was found to be lower than both T2 (summer) and T3 (winter) biofilms, respectively (Table 2).

**Table 2** | Seasonal water quality improvement effects

Type of biofilm	Water quality improvement effect							
	T1		T2		T3		T4	
	Spring		Summer		Autumn		Winter	
HRT	0 d	5 d	0 d	5 d	0 d	5 d	0 d	5 d
COD <sub>Mn</sub> (mg·L <sup>-1</sup> )	10.30 ± 0.52	7.25 ± 0.36	9.84 ± 0.49	8.29 ± 0.41	10.21 ± 0.51	5.25 ± 0.28	9.72 ± 0.49	8.01 ± 0.40
NH <sub>3</sub> -N (mg·L <sup>-1</sup> )	2.39 ± 0.12	1.72 ± 0.09	2.38 ± 0.12	2.05 ± 0.10	2.43 ± 0.12	1.46 ± 0.07	2.42 ± 0.12	1.81 ± 0.09
NO <sub>3</sub> -N (mg·L <sup>-1</sup> )	2.89 ± 0.14	1.92 ± 0.10	2.84 ± 0.14	2.42 ± 0.12	2.86 ± 0.14	1.71 ± 0.09	2.87 ± 0.14	2.12 ± 0.11
TN (mg·L <sup>-1</sup> )	6.21 ± 0.31	4.69 ± 0.23	6.17 ± 0.30	5.59 ± 0.28	6.02 ± 0.30	3.46 ± 0.17	6.23 ± 0.31	4.97 ± 0.25
TP (mg·L <sup>-1</sup> )	0.223 ± 0.011	0.157 ± .008	0.211 ± 0.011	0.183 ± 0.009	0.218 ± 0.011	0.140 ± 0.007	0.218 ± 0.011	0.161 ± 0.008

COD, chemical oxygen demand; NH<sub>3</sub>-N, ammoniacal nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; TN, total nitrogen; TP, total phosphorus.



**Figure 5** | Changes in water quality indicators in the simulated river device: (a)  $\text{COD}_{\text{Mn}}$ , (b)  $\text{NH}_3\text{-N}$ , (c)  $\text{NO}_3\text{-N}$ , (d) TN, and (e) TP.

During the summer season, the COD, NH<sub>3</sub>-N, NO<sub>3</sub>-N, TN, and TP removal ratio of biofilm structure T1 was 15.8, 13.9, 14.8, 9.4, and 13.3%, respectively. The prevalence of an active biofilm on the concrete structures can facilitate the occurrence of a metabolic bioprocess comprising adsorption, absorption, biotransformation, bioaccumulation, and biodegradation of the pollutants, thereby improving the water quality of the river (Shang *et al.* 2002). As explained previously, the large amount of *Cyanobacteria* during the summer season provided the native effects for the biofilm (Peperzak 2003); however, due to the lower amount of microorganisms, the pollutant decomposition and transformation ability was also low during the summer. It has been reported that some *Cyanobacteria* strains can secrete toxins that can be a threat to other dominant species in the biofilm, even leading to the detachment of the biofilm from the surface of concrete structures (Figueiredo *et al.* 2006; Ma *et al.* 2013a).

In addition, the other negative impacts on the biofilm include biofilm fouling, decreased biofilm permeability, less uptake of nutrients from river water, decreased activity of other metabolic processes, and subsequently, the degradation of the biofilm structure (Qu *et al.* 2012). The decomposition of dead *Cyanobacterial* cells and microorganisms can release a large amount of internal nutrient load to the water bodies (Ma *et al.* 2013a).

During the autumn season, the COD, NH<sub>3</sub>-N, NO<sub>3</sub>-N, TN, and TP removal ratio of biofilm structure T3 was 48.6, 39.8, 40.2, 42.6, and 35.8%, respectively. The increasing composition of *Proteobacteria* (61.4%), *Firmicutes* (7.4%), and a high microorganisms' abundance index ( $3.27 \times 10^7$  cell cm<sup>-2</sup>) is beneficial for removing the pollutants in

river water (Ma *et al.* 2013a, 2013b). In another study, Yoo *et al.* (1999) showed that temperature in the range of 22–27 °C could induce maximum bacteria nitrification activity in the simultaneous nitrification and denitrification process.

On the other hand, during the winter season, the COD, NH<sub>3</sub>-N, NO<sub>3</sub>-N, TN, and TP removal ratios of biofilm structure T4 were 17.6, 25.2, 25.9, 20.2, and 26.1%, respectively. The low temperatures (–1–9 °C) caused a decrease in metabolic and cellular functions of the biofilm and led to reduced pollutant removal ability (Chiemchaisri & Yamamoto 1993). Under low temperature conditions, a large number of microorganisms secreted EPS that caused biofilm fouling and decreased the microbial metabolism capacity (Qu *et al.* 2012). Shang *et al.* (2002) revealed that the ammonia oxidation rate and the specific denitrification rate were 4.49 and 2.91 times higher, respectively, at 26 °C than at 10 °C. Chiemchaisri & Yamamoto (1993) studied biological nitrogen removal in a membrane separation bioreactor and reported that the nitrogen removal decreased from greater than 90% at 25 °C to 20% at 5 °C.

### Plant growth and faunal diversity analysis

One month after installing the porous concrete templates on the hard bank surface, the *Barnyard* grass had grown on the surface (Figure 6(a)). *Barnyard* grass is a native weed, which is highly durable, widely distributed, and vigorously developed. It is usually grown in swamps, farmland, riverbanks, ditches, and wetland (Bhowmik & Reddy 1988). The biomass growth profile of *Barnyard* grass is shown in Figure 7.



**Figure 6** | (a) *Barnyard* grass growing on the concrete templates and (b) worm living in the porous concrete surface.

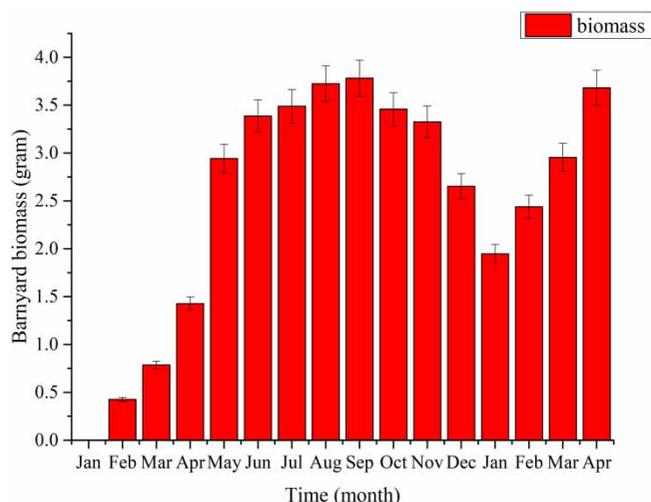


Figure 7 | Profile of the growth of *Barnyard* grass biomass on the concrete templates.

In the spring (February to April) season, the average amount of biomass from *Barnyard* grass was  $0.1385 \pm 0.1361$  g. The average height and biomass reached their highest value in the summer and early autumn (May to October) season and were found to be  $0.3794 \pm 0.0040$  g. Under high humid and temperature conditions, i.e., the summer season, the *Barnyard* grass could grow rapidly and reproduce by utilizing the nutrients as well as the pollutants from the river water. During the late autumn and winter (November to next year January) months, the biomass value was  $0.2144 \pm 0.044$  g. The *Barnyard* grass continued to reproduce in the follow year. According to Xu et al. (2016), the plant root system of *Barnyard* grass provides a growth habitat for microbial attachment and growth, absorbs nutrients and uptakes metal ions, and transforms complex organic substances to new simple inorganic substances.

Concerning the faunal diversity, one year after installing the porous concrete templates on the hard bank surface, a total of seven insects and two faunal species were detected by visual observation on the porous concrete templates. The insects identified on the porous concrete templates were as follows: *Acrida chinensis*, *Pheidole megacephala*, *Gryllus campestris*, *Bicyclus anynana*, *Anoplophora glabripennis*, *Leptocorisa varicornis* and *Tenodera sinensis*. The results also revealed that the Phylum *Nemathelminthes* and centipede insect species were also detected in the *Barnyard* grass root system (Figure 6(b)). Some small

fauna such as snails and lizards were also visibly noticed at the site.

The good results from this study clearly show that the growth of plants on the porous concrete surfaces will provide an ideal habitat for the survival of a variety of insects and small fauna. Some mollusks such as snails and mussels feed on algae and organic debris, and at the same time, they secrete flocculating substances that can remove the suspended matter in water (Dillon 2001). In such a spontaneously evolved ecosystem, the hard bank ecological transformation is caused by the increasing faunal and floral species and the development of a beneficial biofilm that helps in river water purification.

## CONCLUSIONS

The porous concrete templates provided a good habitat for biofilm attachment with the highest microorganisms' abundance index of  $3.27 \times 10^7$  cell  $\text{cm}^{-2}$  and the highest water purification capacity during the autumn season. However, during the winter season, the microbial population was low while the amount of EPS secreted was high, causing a decline in the water purification effect. The porous templates were also able to provide a habitat for the growth and reproduction of microbial, floral, and faunal species.

## ACKNOWLEDGEMENTS

This study was financially supported by Department of Science and Technology of Jiangsu Province (Project No. BE2015356-2). We also thank IHE-Delft for providing infrastructural and staff time support to collaborate with Southeast University.

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First received 13 July 2018; accepted in revised form 10 October 2018. Available online 13 November 2018