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Enzyme activities in pilot-scale constructed wetlands for treating urban runoff in China: temporal and spatial variations

Guo Weijie[a,c], Li Zhu[a,c], Cheng Shuiping[b,*], Liang Wei[a], Wu Zhenbin[a,*]

[a] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China, Tel./Fax: +86 27 68780675; emails: guoweijie1986@163.com (G. Weijie), lizhu1985@foxmail.com (L. Zhu), Tel./Fax: +86 27 68780951; email: wliang@ihb.ac.cn (L. Wei), Tel./Fax: +86 27 68780675; email: wuzb@ihb.ac.cn (W. Zhenbin)*
[b] *Key Laboratory of Yangtze River Water Environment, Ministry of Education, Tongji University, Shanghai 200092, China, Tel./Fax: +86 21 65980763; email: shpcheng@tongji.edu.cn (C. Shuiping)*
[c] *University of Chinese Academy of Sciences, Beijing 100049, China*

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ABSTRACT

Two pilot-scale integrated constructed wetland (ICW) systems were constructed to assess the feasibility of treating urban runoff in Hefei, P.R. China. Each ICW consisted of a down-flow chamber (50 m^2, planted with *Canna indica*), an up-flow chamber (50 m^2, planted with *Iris pseudacorus*), and a horizontal subsurface-flow chamber (50 m^2, planted with *Acorus calamus*) in series. Substrate enzyme activities, growth of vegetation, and contaminant removal efficiencies were monitored during a one-year period. These two systems, achieved an average efficiency of 64.8% for total phosphorus (TP), 59.6% for total nitrogen (TN), 52.7% for ammonium (NH_4^+-N), and 72.7% for chemical oxygen demand (COD). There were significant correlations between the phosphatase (PP) activity and the removal efficiencies of TP and COD, as well as between the urease (UR) activity and TN removal. The activities of both nitrate reductase (NR) and PP were the highest in down-flow chamber (DFC), and then in up-flow chamber (UFC) and horizontal subsurface-flow chamber (HFC), successively. Meanwhile, the maximum enzyme activities of DFC and UFC occurred in summer or autumn when plants were in the vigorous growing stage. Furthermore, the enzyme activities of both PP and UR were significantly correlated with all growth parameters of *C. indica* in DFC. Significant correlations existed between the root number of *I. pseudacorus*, and PP and UR activities in UFC. In HFC, there were no significant correlations between the enzyme activities (PP, UR, and NR) and all growth parameters of *A. calamus*.

Keywords: Pilot-scale constructed wetland; Plant growth; Removal efficiency; Substrate enzyme activity; Urban runoff

1. Introduction

Urban runoff, with nutrients, suspended solids, and heavy metals typically found, is a source of diffuse pollution, and potentially a serious risk to receiving watercourses around the world [1,2]. The urban runoff pollution, being a potential and main source of non-point pollution to contribute to water eutrophication and degradation, must be given much

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*Corresponding authors.

attention, and some effective measures should be taken to relieve or control it.

Constructed wetlands (CWs), as an effective, economical, and ecologically sustainable alternative to conventional treatment, have been widely used to treat different categories of wastewater, including domestic wastewater, industrial drainage, landfill leachate, non-point source pollution, and organic wastewater [3–6]. The treatment performance of CWs is mainly attributed to the combined interactions of substrates, plants, and associated microbial assemblages through a series of physical, chemical, and biological processes [7]. Just like soil, substrates in CWs are also a living dynamic system including many free enzymes, immobilized extra-cellular enzymes, and enzymes within microbial cells [8]. These intra-cellular or extra-cellular enzymes catalyze the decomposition of macro-molecular pollutants into assimilable low-molecular moieties [9,10], and some enzymes, such as protease, urease, nitrate reductase (NR), and phosphatase (PP), are widely distributed and related to nitrogen and phosphorus cycles in CWs [8]. Enzyme activity is proposed to be one of the pivotal determinants for water quality amelioration in wetland systems [11,12]. In CWs, it is affected by many factors, including biological factors (microbial populations and wetland plant and fauna), substrate factors (pH, texture, organic matter content, depth profiles, etc.), and climate factors [10,13]. However, many research studies on enzyme activities in CWs have been implemented in small-scale and simulated systems fed with artificial wastewater or performed in a short-term period [12,14,15]. Spatial and temporal variations of enzyme activities in a long-term period are yet to be fully comprehended in CWs. Additionally, as natural systems, the performance of CWs depends to some extent on seasonal changes and related enzyme activities. Hence, it is necessary to make variation rules of enzyme activity and the corresponding influence factors clear, from which we could also gain a better understanding on wetland function and mechanism of pollutant removal in CWs.

In China, the limited land resource is a non-negligible factor in CWs application [16]. Integrated vertical-flow constructed wetland (IVCW), with the advantages of land saving and predominant effectiveness, has been successfully applied in many cities and countryside areas in China since 1996 [14,17]. Although there are several papers reported the treatment performances of CWs on urban runoff, most of them were constructed with the configuration of horizontal subsurface flow (HSSF) or surface flow (SF) [18–20]. To date, studies on the treatment performance or substrate enzyme activity of a combination of the IVCW and other CW configurations (e.g. HSSF-CW, SF-CW) for urban runoff treatment have been rarely reported.

In this study, two parallel pilot-scale integrated constructed wetland (ICW) systems were constructed on the bank of Nanfeihe River, a seriously polluted urban river, for purifying urban runoff. The detail operation and pollution removal efficiencies were performed in another paper [21]; here, we focused on the spatial and temporal variation rules of substrate enzyme activity during a one-year-period operation and the correlations between enzyme activity and pollutant removal efficiency, water temperature, organic matter, and plant growth.

2. Materials and methods

2.1. Site description and design of the ICW

Two parallel pilot-scale ICW systems (each was composed of a IVCW and HSSF-CW in series) were constructed on the bank of Nanfeihe River, a tributary and the biggest pollution source of Chaohu Lake, in Heifei, China, which was described in detail by Wu et al. (31˚52´40.89´´N, 117˚13´22.89´´E; elevation, 13 m; called site 4 in their report) [21,22].

The ICW systems were constructed in early May 2011. Each was designed as a combination system of a down-flow chamber (DFC, 50 m^2 and planted with *Canna indica*), an up-flow chamber (UFC, 50 m^2 and planted with *Iris pseudacorus*), and a horizontal subsurface-flow chamber (HFC, 50 m^2 and planted with *Acorus calamus*) in series. Steel slag of 5–25 mm diameter and zeolite of 5–15 mm diameter were filled to a depth of 30 cm at the top of both UFC and DFC, respectively, and a 40-cm and 30-cm-thick layer of 10–30 mm diameter gravel at the bottom, respectively. The whole HFC was filled with gravel to a 60 cm depth. The average porosity of the ICW substrates was approximately 0.34. All the three species of plants were planted with an original density of 16 ind/m^2 and fully grown during the experiments until the winter (Fig. 1) [21].

2.2. Operation conditions

Adjoining the ICW systems, there was a sewage pumping station, which mainly received urban runoff from two open channels together draining a 750-ha catchment area. During the dry season, the urban runoff was mainly composed of domestic sewage from an unrenovated urban village, while dominated by stormwater runoff in the rainy season. The wastewater was pumped from the sedimentation tank in this

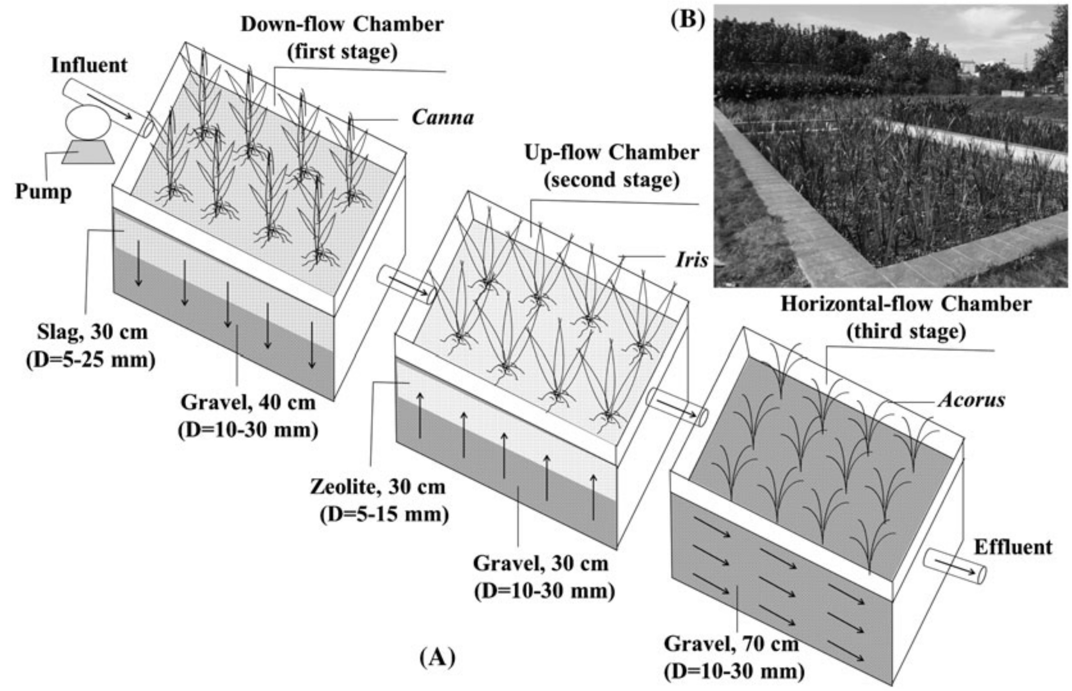

Fig. 1. Flow scheme (A) and photograph (B) of the ICW.

station and induced in each ICW unit per day to yield a hydraulic loading rate of 100 mm/d, and the theoretical hydraulic retention time was about 2.2 d. This study was carried out from January 2012 to January 2013.

2.3. Sampling and analysis

Water quality was monitored separately in these two parallel systems at monthly intervals from January 2012 to July 2012 and bimonthly from July 2012 to January 2013. Parameters of TP, TN, ammonium (NH_4^+-N), and chemical oxygen demand by dichromate method (COD_{Cr}) were analyzed according to the Standard Methods of Environment Monitoring in China [23]. Turbidity was analyzed using a turbidimeter (2100Q HACH, USA), and other physical and chemical characteristics including pH, dissolved oxygen (DO), and temperature were measured using a portable multimeter (YSI Proplus, USA).

Five representative rhizosphere samples were collected from five target plants in each flow-chamber (DFC, UFC and HFC) and homogenized well to make a composite sample once in every two months from mid-March 2012 to mid-January 2013 [9,24]. After all litter was removed, the field-moist samples were sieved (10 mm for steel slag and zeolite; 15 mm for gravel) and stored in a refrigerator at 4℃ for analysis of enzyme activities. Totally, three substrate enzyme activities were measured, namely (NR), urease (UR), and PP in the ICW system. NR activity was analyzed using KNO_3 as a substrate [25]. UR activity was measured using a buffered method [26]. PP activity was determined using *p*-nitrophenol phosphate as the substrate [27].

In order to explore the effect of plant growth stage on enzyme activities in this system, complete plants were collected once every two months during a vegetative life cycle from May to November 2013, with a square frame (30 cm × 30 cm) in three replicates at each sampling and biomass (by wet weight), height, total leaves number, and roots number were estimated for them.

2.4. Statistical analysis

Statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA; Version 13.0). The experiment data were presented as mean ± standard deviation. Comparison of the averages was analyzed using one-way ANOVA followed by LSD tests. Relationships between enzyme activities and other variables (i.e. plant biomass, height, leaf number, root number, and contaminant removal efficiencies) were tested with a Pearson correlation analysis.

3. Results

3.1. Removal efficiencies

Table 1 presented a concentrated overview of the physico-chemical characteristics of the influent and effluent and the removal rates of TP, TN, NH_4^+-N, and COD_{Cr} in the experimental system from January 2012 to January 2013, with 20 samples (14 samples for TN and NH_4^+-N).

The ICW system achieved an average removal efficiency of 64.8 ± 21.9% for TP. The release of TN and NH_4^+-N into the effluent was observed during March–May 2012 in this study, and the corresponding removal rates were 59.6 ± 31.4% and 52.7 ± 39.3%, respectively. As the probable reason of warm temperatures and litter decomposition, a sustaining decrease on the COD_{Cr} removal efficiencies was likewise noted during April–July 2012 and the average removal rate was 72.7 ± 14.0% for it. The average effluent concentrations of all these pollutants (except for NH_4^+-N) were below the permitted values of standard A, according to "Discharge standards of pollutants for municipal wastewater treatment plant (GB 18918-2002)" [28].

3.2. Plant status

All species were planted in mid-April 2011 and developed well before winter. The plants began to regrow from March 2012 and wither when the winter coming in late November. Biomass (by wet weight), height, root numbers, and leaf numbers showed a single-peak pattern for all species during the vegetative life cycle from March 2012 to January 2013 and achieved their maximum values generally during the period September to November (Fig. 2). Some differences on growth peak were observed among species. Both *I. pseudacorus* and *A. calamus* attained their maximum value of these parameters in September, while *C. indica* reached its peak in November.

3.3. Spatial and temporal variations of enzyme activities

Temporal and spatial variations of activities of NR, UR, and PP in the ICW systems were summarized in Fig. 3.

The enzyme activities were varied during the year. NR activities showed different trends over time among different chambers, and the maximum activities in DFC, UFC, and HFC were observed in July, January, and March, respectively. For UR and PP, the temporal patterns were similar among three chambers. The maximum activities of UR in DFC and UFC, were presented in September and November, respectively. Similarly, the maximum activities of PP were all concentrated in September and November in these chambers.

All these three enzyme activities were variable in different chambers and showed significantly higher activity in DFC ($p < 0.05$) in any sampling date, except for PP activity in July and January. PP activities of all sampling dates are significantly higher in UFC than those in HFC ($p < 0.05$), while for the activities of NR and UR, no evident regularity existed between the two chambers.

Table 1
Characteristics of the influent and effluent from ICW system for the period January 2012–2013

	Influent			Effluent					
Parameters[a]	Mean	Min	Max	Mean	Min	Max	% Removal	Class I-A[b]	n[c]
Temperature (℃)	16.0	5.3	25.3	14.8	4.1	25.5	–	–	20
Turbidity (NTU)	52.8	29.0	104.0	5.0	0.8	10.5	–	–	20
DO (mg/L)	2.2	0.4	4.8	0.8	0.2	3.3	–	–	20
pH	7.8	7.5	8.2	8.0	7.6	8.7	–	6–9	20
TP (mg/L)	1.9	0.6	4.9	0.4	0.1	1.0	64.8 ± 21.9	0.5	20
TN (mg/L)	27.9	9.0	76.4	10.8	1.3	29.8	59.6 ± 31.4	15	14
NH_4^+-N (mg/L)	21.7	7.6	47.3	8.5	0.9	22.6	52.7 ± 39.3	8	14
COD_{Cr} (mg/L)	168	60	400	33	50	12	72.7 ± 14.0	50	20

[a] The data from January to July 2012 were referred from Choi et al. [19] and data of TN and NH_4^+-N were exclusive of N-release period March–May 2012.

[b] Class I-A, one-class A permitted standard of GB 18918-2002 issued by EPA, China.

[c] n, sample number.

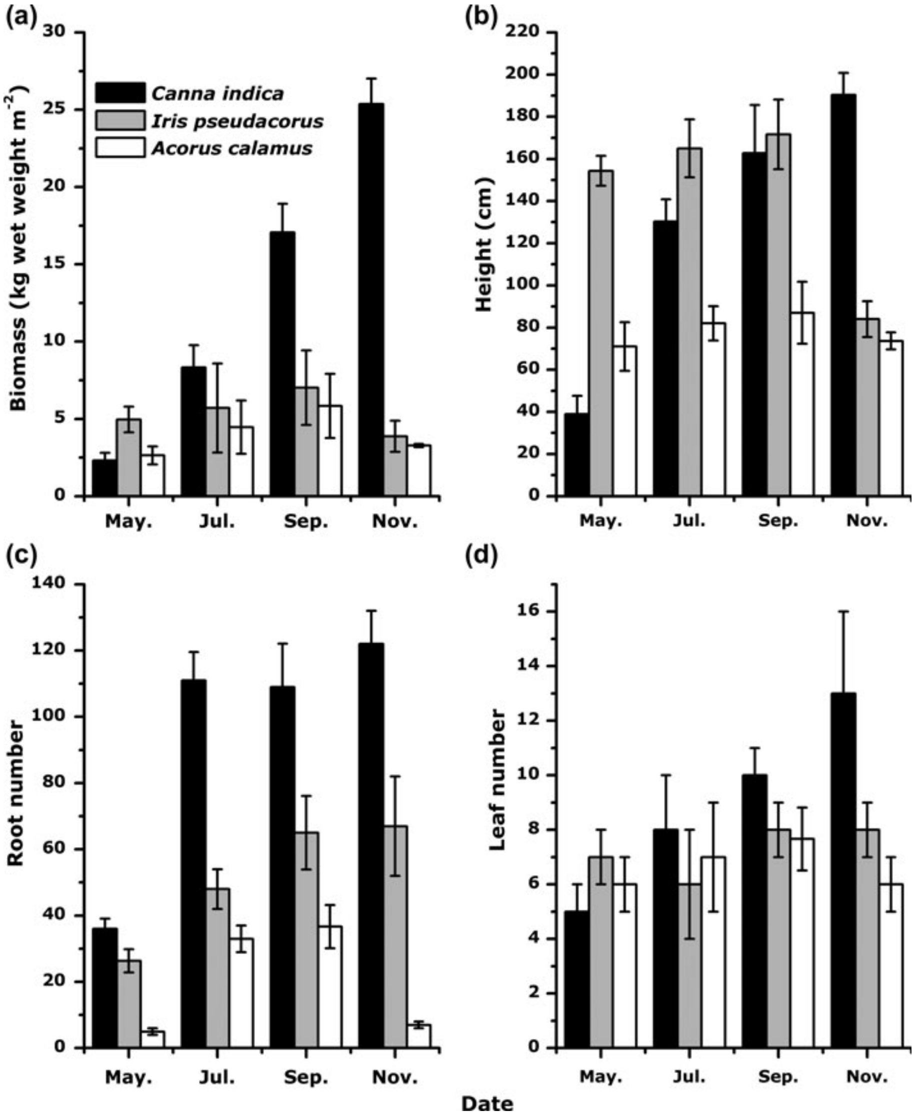

Fig. 2. Growth parameters of plants during a vegetative life cycle: (a) biomass (wet weight), (b) height, (c) root number, and (d) leaf number.

Regardless of NR or PP, the annual average activity was highest in DFC, followed by UFC and HFC. With regard to UR, the annual average activity in DFC was higher than those in UFC and HFC. The values of UR activities were higher in UFC than those in HFC at certain time points, but without significant difference.

3.4. Correlations of substrate enzyme activities and other variables

Pearson's correlation coefficients and *p*-values were calculated for all possible pairs of variables (enzyme activities and other parameters, such as pollutant removal rate, water temperature, substrate organic matter, biomass, height, leaf number, and root number of all species). There was a strong correlation between TP removal efficiency and the average PP activity of these three chambers (Table 2). Also, significantly positive correlation was detected between TN removal and the UR activity. Removal of COD was significantly related to the average PP activity but UR activity. With respect to plant species, the enzyme activities of both PP activity and UR activity were significantly correlated with all growth parameters of *C. indica* in DFC, except for UR activity and root number (Table 3). In UFC,

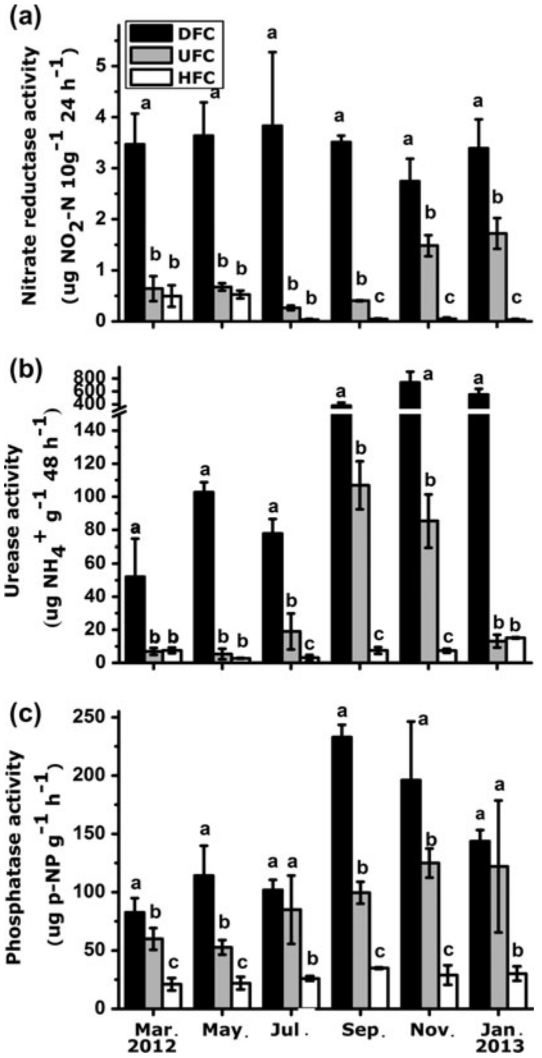

Fig. 3. Substrate enzyme activities in down-flow chamber (DFC), up-flow chamber (UFC), and horizontal subsurface-flow chamber (HFC) of the ICW system: (a) nitrate reductase, (b) urease, and (c) phosphatase.

significant correlations existed between root number of *I. pseudacorus*, and PP and UR activity. However, there were no significant correlations between all growth parameters of *A. calamus* and enzyme activities (PP, UR, and NR). It is notable that only UR activity in HFC was strongly negatively correlated with water temperature, and none of the enzyme activity presented significantly positive correlation with substrate organic matter in all three chambers.

Table 2
Correlations between substrate enzyme activities and contaminant removals in the ICW system

	TP (%)	TN (%)	COD (%)	NH_4^+-N (%)
AC-PP[a]	0.881*	–	0.878*	–
AC-UR	–	0.814*	0.634*	0.789
AC-NR	–	0.053	0.498	−0.110

[a] AC, average enzyme activity of all three chambers; PP, phosphatase; UR, urease; NR, nitrate reductase.

*Significant correlations at $p < 0.05$.

4. Discussion

In our study, the ICW system achieved an acceptable treatment effect for urban runoff, with an average removal efficiency of 64.8% for TP, 59.6% for TN, 52.7% for NH_4^+-N, and 72.7% for COD. In a 700-m^2 SF-CW, which was used for urban stormwater management, Birch et al. found the average TN and TP removal efficiency was only 22% and 12%, respectively [29]. Of course, there are also some studies got the similar results or higher removal efficiencies. For example, Johengen and LaRock designed a combined CW to treat urban stormwater runoff and the results showed the removal efficiencies for ammonia nitrogen and phosphate were 87% and 62%, respectively [20]. It was reported that a vertical-flow CW system presented considerable removal efficiencies for the on-site treatment of domestic wastewater: 94.4% for COD, 92.8% for NH_4^+-N, and 69.8% for TP [30].

As we all know, enzymes can catalyze the decomposition of macro-molecular pollutants into assimilable low-molecular moieties and some are related to the removal of nitrogen and phosphorus in CWs [8,10]. So substrate enzyme activity could be proposed to a vital factor for water quality amelioration in wetland systems. In this study, some strong relationships between various enzyme activities and pollutant removing were found, for example, the removal efficiencies of TP and COD presented significantly positive correlations with the average PP activity of the whole ICW, and the TN removing was related to the average UR activity. These were consistent with the results reported by Li et al. [31] that there were close relationships between UR activity and TN removal efficiency in a subsurface wastewater infiltration system. The positive correlation between PP activity and phosphorus removal was revealed in most of the wetlands [32], while inverse relationship between them was also found by Wu et al. [14].

In almost all sampling dates, we found the activities of NR and PP were the highest in DFC, and then in UFC and HFC, successively. The different contaminants loadings and various plant species possessing different rhizosphere microbes and root exudates

Table 3
Correlations between enzyme activities and other variables (water temperature, organic matter and plant growth parameters) in different ICW chambers

	Biomass	Height	Leaf number	Root number	Water-T	Organic-M
C. indica in DFC, I. pseudacorus in UFC and A. calamus in HFC						
DFC-PP [a]	0.739**	0.630*	0.747*	0.593*	0.084	−0.483
DFC-UR	0.920*	0.726*	0.726*	0.176	−0.245	−0.420
DFC-NR	0.304	−0.346	−0.346	0.268	−0.168	−0.144
UFC-PP	0.084	0.049	0.558	0.881*	−0.001	0.375
UFC-UR	0.161	0.049	0.408	0.755*	0.290	0.148
UFC-NR	−0.170	−0.144	−0.138	−0.293	−0.126	0.070
HFC-PP	0.490	0.502	0.513	0.372	0.077	−0.232
HFC-UR	0.119	0.270	0.073	0.189	−0.698*	0.395
HFC-NR	−0.294	−0.126	−0.297	−0.442	−0.154	−0.026

[a] DFC, UFC and HFC represent down-flow chamber, up-flow chamber and horizontal subsurface-flow chamber, respectively; PP, UR and NR represent phosphatase, urease and nitrate reductase, respectively.

*Significant correlations at $p < 0.05$.

**Significant correlations at $p < 0.01$.

might contribute to the result. Similar observations were noted by Cui et al. [33] and He et al. [34], who reported positive correlation between enzyme activity and contaminant concentration, although bioavailable nutrients can potentially decrease activity enzyme production by microbes according to “economic rules” [35].

Plants can influence enzyme activity directly by excreting exogenous enzymes and also affect microbial species composition by releasing exudates and oxygen into the rhizosphere that in turn affects enzyme activity indirectly [10,12]. It has been reported that the plant above-ground growth could affect the rates of root respiration and radial oxygen loss radial in the rhizosphere [36,37]. The oxygen availability may influence the activity of denitrification enzymes and removal efficiency of inorganic nitrogen in integrated vertical-flow CW [38]. It is revealed that the plant root biomass was positively related to urease, acid, and alkaline PP activities [38]. This result might be to some extent related to the effect of the higher root respiration rate on the oxygen content in the rhizosphere, since Cheng et al. concluded ammonia nitrogen removal was significantly related to the plant above-ground biomass and nitrogen removal highly correlated with the plant photosynthetic rate in micro-scale gravel-bed CWs [39]. In this study, some significant correlations were detected between plant parameters (biomass, root number, leaf number, and height) and enzyme activities (PP and UR) in DFC and UFC, and the maximum activities of NR, UR, and PP occurred during summer or autumn in DFC and UFC when plants were in the vigorous growth stage, which was consistent with a report of Kong et al., who found that many significant positive correlations between soil enzyme activity was positively related to root activity [32]. And it was also similar to one previous report that denitrifier enzyme activity significantly increased in the presence of plants, especially when they were growing during summer and autumn [40].

Substrates in CWs containing many different kinds of enzymes and microbial communities are also a living dynamic system, similar to soils [8]. Extra-cellular enzyme activity is sensitive to changes in substrate (micro-environmental) conditions and affected by many factors, including temperature, organic matter, microbial population, nutrient composition, and plant exudates [41]. No significant correlations were found between the activity of any enzyme except UR in HFC and water temperature in all chambers, though it could directly affect the plant growth rate and activity of microorganisms in CWs. It is well known that extracellular enzymes can mediate the degradation of organic matter and provide rapid evaluation for wetland system [42]. Organic matter in turn might also have had great influence on enzyme activity and associated microbial population. Different enzymes could be affected by complex carbon sources that provide a source of electrons for decomposition processes and increase the surface area available for microbial colonization, presenting some optimization for wetland efficiency [15]. In some CWs receiving nitrate-contaminated water with low concentration of organic carbon, it would be beneficial to enhance nitrate removal by increasing plant litter or available organic matter [43]. However, these do not confide with the results of our study, in which enzyme activity was not significantly correlated with substrate organic matter in any of the chambers and some negative-correlated trends occurred between them in DFC and HFC. Further specific researches are in great need.

5. Conclusions

This ICW could be used for urban stormwater management effectively for its acceptable removal efficiency of pollutants. Some strong relationships between enzyme activities and pollutant removal efficiencies were found, and therefore, enzyme activity of substrate could be a vital factor for water quality amelioration in CWs. From the spatial and temporal view, the maximum enzyme activities occurred in DFC and in the vigorous growth stage of plants, respectively. The different contaminants loadings and various plant species and its corresponding growth state might contribute to different temporal and spatial changes of substrate enzyme activity.

Acknowledgments

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