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Original Research

The Performance of Species Mixtures in Nitrogen and Phosphorus Removal at Different Hydraulic Retention Times

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Abstract

The effects of species compositions (two-species mixtures or monocultures) and hydraulic retention times (HRT; 1, 4, 8, 16, 24, 48 hours) on total nitrogen (TN) and total phosphorus (TP) removal from eutrophic water were studied in a constructed wetland. Two species mixtures showed higher efficiency to remove TN and TP than monocultures. Average removal efficiency of TN and TP was 49.6% and 34.0%, respectively. A longer HRT enhanced the removal efficiency of TN and TP, which suggested that species mixtures, HRT, and species mixtures × HRT interaction were useful for increasing the wastewater TN and TP removal.

Keywords: constructed wetland, eutrophic water, plant mixtures, pollutant removal, wastewater treatment

Introduction

Nitrogen (N) and phosphorus (P) were the two major nutrients that were important for the normal functioning of ecosystems, such as plant growth and survival. However, excess influent of N and P was the main cause of eutrophication [1], which caused water turbidity and often-unexpected biological changes, such as loss of biodiversity [2, 3]. Enhancing N and P removal from eutrophic water was a critical issue of wastewater treatment.

As a sustainable technique for nutrient removal from wastewater, ecological engineering has attracted extensive attention [1]. For example, constructed wetlands (CWs) have been widely used at present [4, 5] to remove excess nutrients and other pollutants from sewage in high efficiency [5, 6]. In CWs, N removal was performed by plant uptake and denitrification processes [7, 8], and P removal was performed by substrate (media) absorption and plant uptake [9]. Plants in CWs are functionally and morphologically different and may play both direct and indirect roles in nutrient removal [10, 11]. Plant species richness (number of plant species) can increase microbial and enzyme activities and N removal in CWs [12, 13]. Plant diversity may play an important role in the functions (plant biomass accumulation and nutrient removal) of wetlands [14-16]. However, studies using the number of species alone cannot describe community properties adequately [17]. Furthermore, differences in species compositions were more sensitive than diversity measures to detect the influence of management on the plant [18, 19]. The change of species compositions affects the function of the phytoplankton community in polluted aquatic ecosystems [20]. However, the relationship between plant species compositions and removal efficiency of TN and TP in CWs has not yet been sufficiently solved.

In CWs, some previous studies have built and screened out the suitable plant species [14, 21] or plant mixtures [13], and evaluated the removal efficiency of the CW at different

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hydraulic retention times (HRT) [22]. Many studies have suggested that the performance of CWs was generally a function of the HRT [22, 23]. So, for maximum nutrient removal, selection of proper HRT was required for the application of CW.

This study aims to investigate the effects of plant species composition and HRT on TN and TP removal functions in the CW. The results of this study can be used to suggest management choices to improve the removal efficiency of nutrients in CWs.

Table 1. Plant species and compositions used in the study.

Species compositions	No. of the plots
Ad, Ca, Cl, It, Ls, Td	18
Ca+Cl, Ca+Td, Ca+Ad, Ca+It, Ca+Ls, Cl+Td, Cl+Ad, Cl+It, Cl+Ls, Td+Ad, Td+It, Td+Ls, Ad+It, Ad+Ls, It+Ls	45

Ad – *Arundo donax* L., Ca – *Cyperus alternifolius* L., Cl – *Coix lacryma-jobi* L., It – *Iris tectorum* Maxim., Ls – *Lythrum salicaria* L., and Td – *Thalia dealbata*.

Materials and Methods

Site Description and Experimental Design

A subsurface vertical-flow CW (800 m^2) was built in 2009 at Dongyang City, Zhejiang Province, China. The structure of CW (40×20×1.1 m, length×width×depth) is outlined in Fig. 1. The CW was built with a 3-layer filter: one layer of coarse 1-2 mm sand (0.4 m) at the top followed by 6-12 mm gravel (0.2 m) in the middle and 50-120 mm gravel at the bottom (0.5 m). It was designed to receive a maximum treatment capacity of about 1,500 m^3·day^{-1}. During the experimental period (August to October, 2012), the sewage was supplied once every two days and the flow was controlled by a water-level activated pump. The CW was fed with pretreated real eutrophic water (average chemical oxygen demand (COD) = 9.6 mg·L^{-1}, biochemical oxygen demand in five days (BOD$_5$) = 21.2 mg·L^{-1}, total N = 60.1 mg·L^{-1}, and total phosphorus = 2.5 mg·L^{-1}) from Dongyang river.

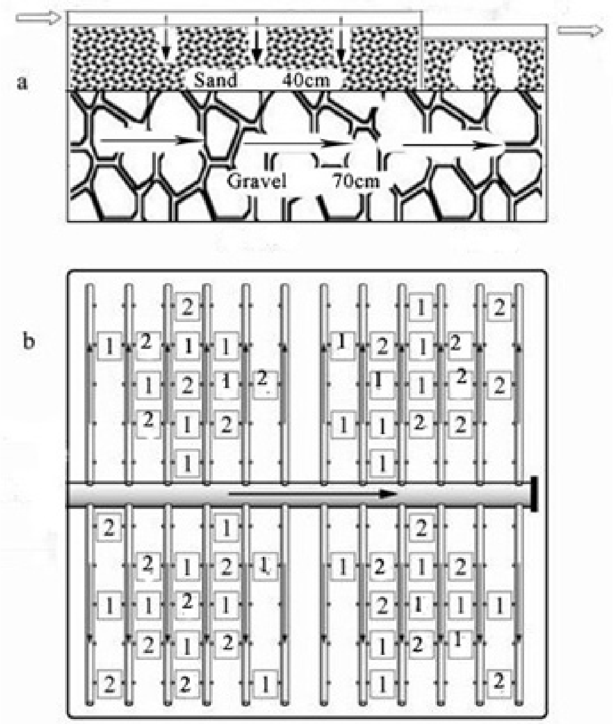

Fig. 1. The experiment was carried out in the wetland. Schematic drawing of the vertical structure of the CW was shown in Fig. 1a. The numbers in the empty rectangles indicated the number of species in the plots (b). At the surface of the CW, the small points on the pipe represented discharge holes (b).

Plots in the CW were 3×3 m, spaced 0.5 m apart. Polyvinyl chloride (PVC) pipes with discharging holes provided a consistent supply of water to each plot, ensuring that each plant had a similar growth environment in the down-flow chambers of the CW (Fig. 1). Water influent was 0-30 cm below the substrate surface. Water effluent was mixed 50 cm below the surface. There was no physical barrier to isolate the plots, so the samples were collected in the center of each plot at a depth of 30 cm to avoid the edge effect.

Plant species were *Cyperus alternifolius* L. (Ca), *Iris tectorum* Maxim. (It), *Arundo donax* L. (Ad), *Thalia dealbata* (Td), *Coix lacryma-jobi* L. (Cl), and *Lythrum salicaria* L. (Ls), which were commonly used in CWs of China. All planted species had similar length to ensure similar initial biomass and were transplanted eight seedlings per m^2 (Table 1). We planted all the species either in monoculture or with two-species combination in March 2010 (Table 1). Experimental species treatments were established by independent random draws (Fig. 1). Each treatment was triplicated with 63 plots in total (Table 1).

Sample Collection and Analysis

We made 189 PVC pipes with discharging holes (20 cm diameter × 30 cm deep) to collect substrate solutions. In the center of each plot, a water channel (2.5 m×0.3 m×0.2 m, length×width×depth) was dug in August 2012. The PVC pipes with discharging holes were inserted into the water channel at 50 cm. Eight vacuum pumps were used to collect the samples from the pipes. We collected the samples at the time of 1, 4, 8, 16, 24, and 48 hours after wastewater irrigation. Three subsamples of each plot were mixed to form a composite sample to estimate the effluent TN and TP concentrations. The samples were filtered with 0.45 μm ash-free filter papers prior to analysis. Total N was analyzed using the alkaline potassium persulfate digestion ultraviolet spectrophotometric method [24]. Total P concentrations were determined on acidified portions of each sample via persulfate digestion and the ascorbic acid method [24]. Removal efficiency (%) of TN and TP was calculated as ((influent-effluent)/influent)×100. Wastewater samples were collected on the 2 August, 2 September, and 2 October 2012.

Table 2. Mean and standard error (SE) values of TN and TP concentrations in influent and effluent from 8-2 to 10-2.

Date/month-day	Influent TN ($\mathrm{mg \cdot L^{-1}}$)	Effluent TN ($\mathrm{mg \cdot L^{-1}}$)	Influent TP ($\mathrm{mg \cdot L^{-1}}$)	Effluent TP ($\mathrm{mg \cdot L^{-1}}$)	Removal of TN (%)	Removal of TP (%)
8-2	59.8±10.2	29.1±11.3	2.48±0.82	1.59±0.98	51.3	35.8
9-2	61.2±11.6	31.9±9.6	2.57±0.43	1.81±0.91	47.8	29.6
10-2	59.3±12.3	29.8±13.1	2.45±0.95	1.55±0.92	49.7	36.7

Removal efficiency of TN and TP was calculated as ((influent-effluent)/influent)×100.

Harvesting of plants was performed in two parallel and evenly spaced 0.2 × 2 m strips in the center of each plot on 4 October 2012. Plant materials were divided into aboveground and belowground, and dried at 70ºC until constant weight to determine dry matter (DM) yield (referred to as aboveground DM, belowground DM and total DM – the sum of aboveground and belowground DM).

Statistical Analysis

Analysis of variance (ANOVA) was performed on the removal efficiency of TN and TP using SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA). The effects of the species compositions and HRT on the effluent TN and TP concentrations and removal efficiency were tested using ANOVA general linear models procedure. One-way ANOVA was used to evaluate the significance between different parameters according to species compositions and the HRT by the Tukey HSD tests. The level of significance of $P = 0.05$ was accepted. Significant P-values ($P < 0.05$) were in bold. The error estimates given in the text and error bars in figures are standard errors (SE) of means.

Results and Discussions

Both plant species mixtures and HRT were two important variables in influencing the removal efficiency of CWs [22, 23]. The concentration and removal efficiency of TN and TP for the experiment are shown in Table 2.

Effects of Species Mixtures on Removal Efficiency of TN and TP

Species compositions had a significant effect on TN concentrations in effluent and the removal efficiency of TN and TP (Fig. 2). Removal efficiency of TN and TP ranged from 47.8 to 51.3%, and 29.6 to 36.7%, respectively (Table 2). Effluent TN concentrations in the plots of two-species mixtures were lower than those in the monocultures (Fig. 2a). However, effluent TP concentrations in the plots of two-species mixtures and monocultures had no significant difference (Fig. 2b). Removal efficiency of TN and TP in the plots of two-species mixtures was higher than that in the monocultures (Fig. 2c, d), which may be due to the higher plant biomass in the mixture plants community [5, 13]. The greater

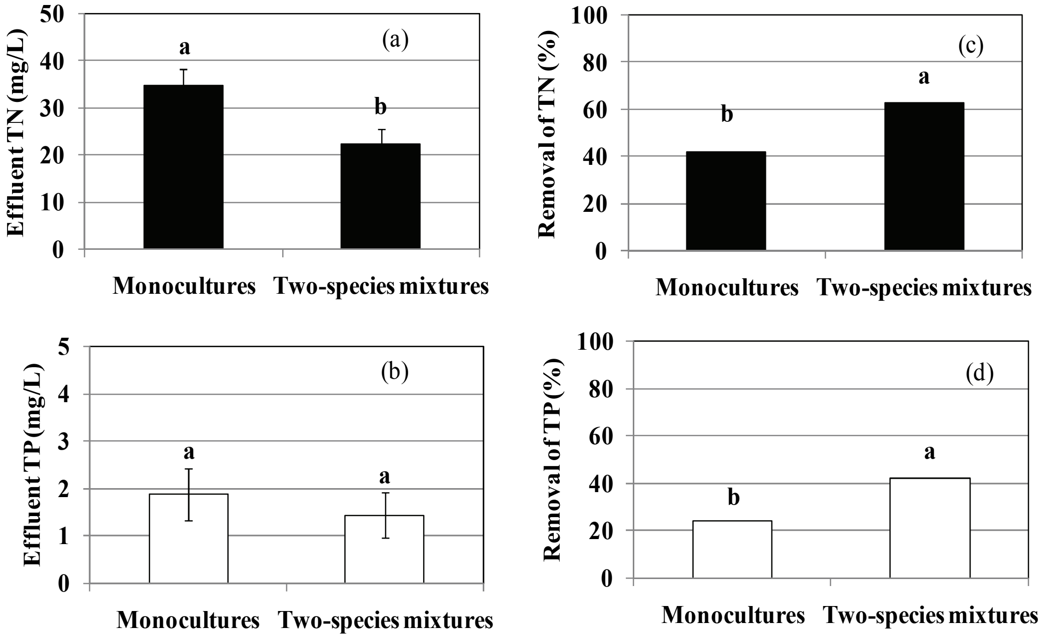

Fig. 2. Total N and TP concentrations in effluent (a, b) and removal efficiency of TN and TP (c, d) in the plots of monocultures and two-species mixtures. ANOVA tested for significant differences among treatment means. Values were means ± SE. Removal efficiency of TN and TP was calculated as ((influent-effluent)/influent)×100.

plant biomass, especially belowground biomass, would increase the plant N and P uptake or retention more efficiently through the crossed distribution of roots among different depths of substrate [14, 25, 26], leading to decreases in N and P concentrations in effluent [22, 23]. On the other hand, high biomass mixtures may increase oxygen diffusion and organic exudates in the rhizosphere [7, 16], which may increase N and P transformation by microbes [25, 27], thus led to a low TN and TP concentration in effluent of more diverse communities. As a result, the two-species mixtures produced the larger plant biomass than that in the monocultures (Fig. 3), but had lower TN and TP concentrations in effluent (Fig. 2a, b). It showed that plant mixtures could increase plant biomass accumulation and TN and TP removal in CWs.

Effects of Hydraulic Retention Time on Removal Efficiency of TN and TP

Hydraulic retention time (HRT) was one of main factors influencing the removal efficiency of the CW [22]. In this study, we want to know how different HRTs (1, 4, 8, 16, 24, 48 hours) affect the removal of TN and TP. Results showed that the HRT had a significant effect on TN and TP concentrations in effluent, and removal efficiency of TN and TP (Fig. 4), showing the importance of the HRT in TN and TP removal. Removal efficiency of TN and TP ranged from 8.3 to 62.1% and 6.1 to 43.2%, respectively (Fig. 4 c, d). With the HRT increased (1 to 48 hours), the TN and TP concentrations in effluent were decreased (Fig. 4 a, b), while the removal efficiency of TN and TP was increased (Fig. 4 c, d). The HRT could affect the duration of water within the CWs, flow depth and substrate porosity and plant growth through the nutrient supply in CWs [23], thus a longer HRT may enhance the removal of pollutants [23]. Likewise, the study showed that pollutant removals depended greatly on the HRT [23]. The longer contact of microorganisms with pollutants could improve the removal efficiency of TN and TP [23], which showed that the increase of HRT could increase TN and TP removal in CWs.

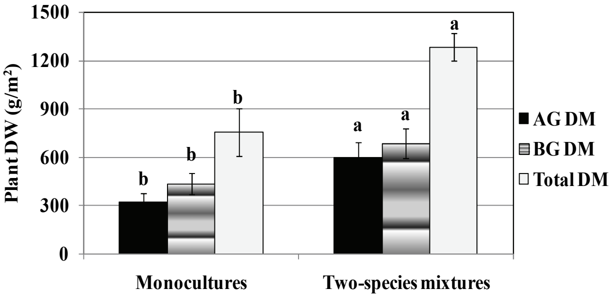

Fig. 3. Aboveground (AG) dry matter (DM), belowground (BG) DM, and total DM in the treatments of monocultures and two-species mixtures. ANOVA tested for significant differences among treatment means. Values were means ± SE.

Effects of Species Compositions and Hydraulic Retention Time on Removal Efficiency of TN and TP

Across all plots, we found that species compositions and HRT affect the removal efficiency of TN and TP (Table 3). The removal efficiency of TN and TP was improved by the species compositions, plus HRT and their interactions, showing that the interaction among the species compositions, and HRT was also important in influencing TN and TP removal. Some studies also found that NO_3-N removal attributed to the interaction of plant species richness and HRT [22, 23]. Our findings also confirmed that the highest removal efficiency of TP was in the CWs with two plant

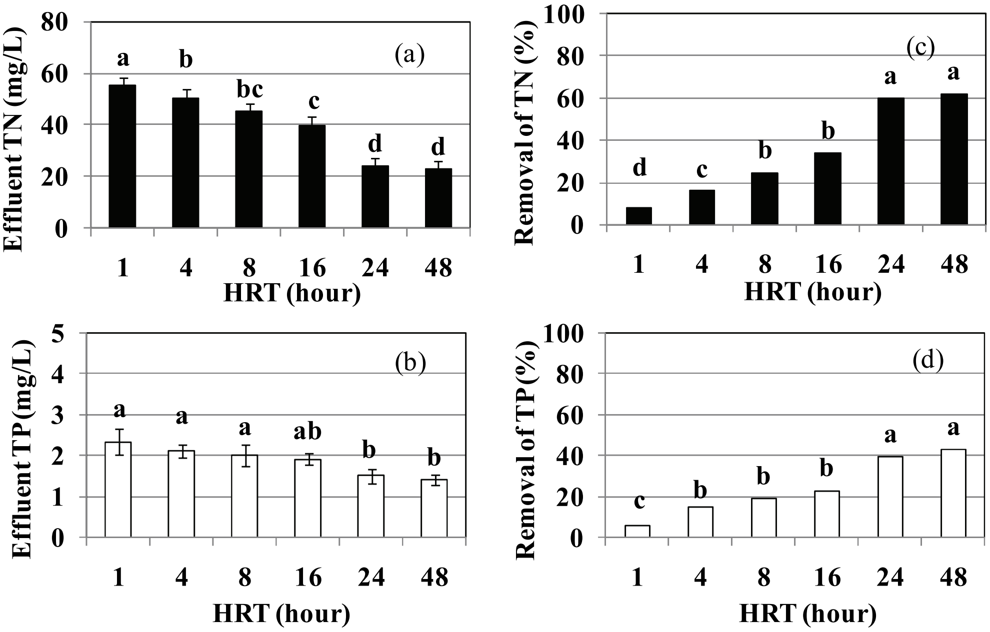

Fig. 4. Total N and TP concentrations of effluent (a, b), and removal efficiency of TN and TP (c, d) in different hydraulic retention times (HRT; 1, 4, 8, 16, 24, 48 hours). ANOVA tested for significant differences among treatment means. Values were means ± SE. Removal efficiency of TN and TP was calculated as ((influent-effluent)/influent) ×100.

Table 3. Effects of species compositions (SC), and hydraulic retention times (HRT), and aboveground (AG) DM, and belowground (BG) DM, and total DM on the removal efficiency of TN and TP. Results from ANOVA were included (F-test and P-values). Significant P-values ($P < 0.05$) were in bold. Removal efficiency of TN and TP was calculated as ((influent-effluent)/influent)×100.

Source of variation	Removal of TN (%)		Removal of TP (%)	
	F	*P*	*F*	*P*
Species composition (SC)	5.67	**0.03**	4.11	**0.04**
HRT	7.21	**0.01**	5.05	**0.03**
SC×HRT	4.63	**0.04**	4.08	**0.05**
AG DM	2.87	0.09	2.59	0.09
BG DM	4.12	**0.03**	5.04	**0.03**
Total DM	1.6	0.62	3.99	0.06

species at the longest HRT [23]. Plant uptake was regarded as an important removal pattern of TP. Likewise, this study also found that the belowground biomass had a significant effect on removal efficiency of TN and TP (Table 3). Prior papers also emphasized the importance of belowground biomass in nutrient dynamics of wetland systems [5, 26]. High diverse plant mixtures could have higher belowground biomass and may increase N and P combined uptake by plants and microbes [25, 27]. A longer HRT may cause a longer contact of wastewater with the plant roots and microbes in the CWs and improve the removal efficiency of pollutants [23, 28]. It showed that species mixtures × HRT interactions could increase TN and TP removal in CWs.

Conclusions

The study of a subsurface vertical-flow CW showed that average removal efficiency of TN and TP was 49.6% and 34.0%, respectively. The plant mixtures were more useful for improving wastewater TN and TP treatment efficiency than species in monoculture. A longer HRT enhanced the removal efficiency of TN and TP. Therefore, the results showed that diverse plant mixtures, longer HRT, and species mixtures × HRT interaction were useful for increasing wastewater N and P treatment efficiency in CWs.

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