Assessment of the Effects of Ornamental Wetland Polyculture Combinations on Urban Sewage Treatment

Min Wang
Sichuan Agricultural University
Yujue Zhou (monicawang456@163.com)
Sichuan agricultural university
Lin Xiang
Sichuan Agricultural University
Xiaoyang Ke
Sichuan Agricultural University
Hui Zhang
Sichuan Agricultural University
Li Guo
Sichuan Agricultural University
Yuanzhi Pan
Sichuan Agricultural University
Jun Ma
Sichuan Agricultural University
Zhuo Huang
Sichuan Agricultural University
Xi Li
Sichuan Agricultural University

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Abstract

Previous studies have shown that wetland plants can treat wastewater in a cost-effective and sustainable way, however, the studies on the performance of ornamental wetland plant diversity in treating urban sewage were scarce. Therefore, this study was conducted to assess and select wetland polyculture combination that was effective in urban sewage treatment in subtropical areas. We formed five combinations out of six ornamental wetland plant species including *Thalia dealbata*, *Cyperus alternifolius*, *Iris pseudacorus*, *Lythrum sastlicaria*, *Nymphaea tetragona*, and *Zantedeschia aethiopica*. The growth state and removal effects of each plant combination were systematically measured and assessed. The results indicated all the combinations exhibited remarkable total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), and chemical oxygen demand chromium (COD_{cr}) removal rate of 70.75%-77.67%, 63.86%-73.71%, 69.73%-76.85%, and 57.28%-75.69%, respectively. Additionally, pH was reduced to 7.54-8.00 in the sewage. The purification effect reached the best during 30-36th day. The comprehensive assessment showed the mixture of *Thalia dealbata + Cyperus alternifolius*, closely followed by *Thalia dealbata + Cyperus alternifolius+ Lythrum sastlicaria*, was highly effective at extracting various pollutants, and both of them could be used as favorable combinations to convert eutrophication and purify municipal wastewater. Linear regression showed that TP, TP, NH_4^+-N, and $COD_{cr.}$ were significantly related to plant biomass, indicating that plant biomass essential indicator for screening purification plants. Our study highlighted the importance of plant diversity in biological wastewater treatment, however the competition between plants was suggested to take into consideration in future studies.

Introduction

Urban water systems fulfill a variety of purposes, such as providing water resources, performing ecological functions, decorating the environment, and supporting city life (Y. Zhang et al., 2017). However, in recent years, many cities in China experienced water pollution due to a large amount of wastewater discharged into the urban rivers (Jiang et al., 2019; Wang et al., 2019; W. Zhang et al., 2017), in addition to the effects of dry and wet atmospheric deposits (Kuang et al., 2016; Ye et al., 2018) and the pollution of the surface runoff (Shajib et al., 2019; Teng et al., 2019). Excess nitrogen can result in eutrophication, and then ammonium toxicity for many plant species (Han et al., 2019). The excessive level of nitrogen and phosphorus addition into water system deteriorates the urban landscape, and puts human health, and the ecosystem under pressure (Anderson et al., 2002; Grattan et al., 2016; Xia et al., 2019).

Wastewater treatment in wetland systems is the result of physical, chemical, and biological interactions in the soil and water environment with the application of wetland plants (Skrzypiecbcef et al., 2017). To treat sewage is mainly to remove or reduce the excessive amounts of pollutants (Hegazy et al., 2016), such as total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), chemical oxygen demand chromium (COD_{cr}), and pH in the water body. Carbon, nitrogen, and microorganism activity are major indicators that contribute to the reduction and retention of pollutants (Parde et al., 2020; Sun et al., 2019). Thus, these indicators are essential in assessing water quality.

Conventionally, the municipal wastewater is treated biologically with the activated sludge process (ASP) (Daverey et al., 2019; Gorsalitz, 2012). Though the ASP is highly efficient in removing the chemical oxygen demand from municipal wastewater, it consumes a huge amount of energy and cost in the disposal of a large volume of waste sludge (Daverey et al., 2019; McCarty et al., 2011; Resende et al., 2019). Unlike conventional biological reactors, wetland systems do not produce secondary sludge (Sun et al., 2019). With the advantages of energy neutral and sustainability, the ecological purification and restoration of sewage, constructed wetlands (CWs) have been increasingly recognized as an effective method for sewage treatments (Senzia et al., 2003; Yeh et al., 2015), thanks to its high efficiency, low cost, easy operation, and supreme landscape effect (Coveney et al., 2002; Ojoawoa et al., 2015).

The vegetation in CWs plays an important role in wastewater treatment (Sandoval et al., 2019). However, there exist numerous species of wetland plants. The effect of plant purification is likely related to their ecological habits, absorption traits, nutrient accumulation, and distribution patterns (Zhang et al., 2009). Thus, the wetland plants may differ in their capacities and preferences to assimilate pollutants from sewage. Numerous studies have focused on the design and performance of CWs (H. Wu et al., 2015), however, effective choosing of suitable wetland plants and ensuring the aesthetic effects simultaneously remains a challenge. More importantly, recent works reported on the purification effect of monocultures (Fraser et al., 2004; Hernández et al., 2018; Li et al., 2015), which implies that the choosing of monoculture and polyculture significantly affects purification capacity (Calheiros et al., 2015; Leiva et al., 2018). Ornamental wetland plant appears as a viable option in urban CWs construction, due to its aesthetic and commercial value (Sandoval-Herazo et al., 2018; Zamora et al., 2019). However, the study of ornamental wetland plant polyculture combinations in wastewater treatment remains limited. It is essential to select appropriate ornamental plant species and configure feasible combinations to enhance the urban waterfront landscape and achieve better purification effects simultaneously.

The plant traits differ among different climatic zones. In this study, based on the survey of literature and wetland plants in Chengdu, Sichuan province, China, we found that six wetland plant species were widely used both locally and in the subtropical areas, among which, the combination of *Thalia dealbata* and *Cyperus alternifolius* were one of the most commonly used combinations in constructed wetlands (Zhang et al., 2020). Consequently, considering the aesthetic sense of space, variety, and color, in this study, the following species were chosen: emergent macrophytes, including *Thalia dealbata, Cyperus alternifolius, Iris pseudacorus*, *Zantedeschia aethiopica, Lythrum salicaria*; floating-leaved macrophyte, including *Nymphaea tetragona* (Sandoval et al., 2019; H. Wu et al., 2015). We used the combination of *Thalia dealbata* and *Cyperus alternifolius* as the base combination, and five combinations were configured to evaluate and compare the growth status and pollutant removal effects under greenhouse static water condition. We hypnotized that 1) all the combinations were effective in wastewater treatment; 2) more diversified plant species would incur better purification effects. The purpose of this study is to evaluate the effects of plant diversity in wastewater treatment and select the suitable plant combination with superior purification effect and landscape effect to provide scientific reference for municipal wastewater treatment and wetland constructions in subtropical areas.

Materials And Methods

Plant combination

Seedlings of the above-chosen wetland plants were purchased from the flower market in Wenjiang district, Chengdu, Sichuan province, China. The following five combinations were configured: *Thalia dealbata* + *Cyperus alternifolius* (T + C), *Thalia dealbata* + *Cyperus alternifolius* + *Lythrum salicaria* (T + C + L), *Thalia dealbata* + *Cyperus alternifolius* + *Iris pseudacorus* + *Nymphaea tetragona* (T + C + I + N), *Thalia dealbata* + *Cyperus alternifolius* + *Zantedeschia aethiopica* + *Nymphaea tetragona* (T + C + Z + N), *Thalia dealbata* + *Cyperus alternifolius* + *Iris pseudacorus* (T + C + I) (Fig. 1).

Test water

Test water was taken from the sewage outfall of the Yangliu River, Wenjiang District. The baseline indicators were determined as follows, TN: 42.16 mg/L,TP6.33 mg/LNH_4^+-N6.49 mg/L, COD_{cr}: 85.59 mg/L, pH value: 9.21.

Experiment design

From July 6th to August 25th, 2018, the experiment was conducted in the greenhouse on the roof of the 4th teaching building of Sichuan Agricultural University. A total of six treatments consisting of five hydrophyte combinations and one control group were set up in a laboratory-scale constructed wetland apparatus (Fig. 2). Static sewage without any plants was set as the control. Each plant combination comprised of nine culture containers and the control group comprised five. Each treatment except the control group was repeated three times. In total, there were 140 containers (5×9×3 + 5). The culture container used in the experiment was a blue polyethylene box (length, 64 cm; width, 32 cm; depth, 44 cm), with a volume of 90 L (Fig. 2). The container and the substrate were sterilized with 0.2% potassium permanganate for 12 hours and rinsed with de-ionized water. The bottom of the container was filled with sand to a depth of 15 cm and subsequently covered by a layer of small stones. Each container was added 30 L sewage. Prior to the experiment, healthy and uniformly sized seedlings were transplanted to the container after 14 days of adaptive cultivation in sewage. The initial biomass of each container was maintained at 45.21–46.78 g/L, and the plant cover area was kept less than 50%. Sewage was well-stirred before sampling. 250 ml sewage was taken every six days from 8:00 a.m. to 9:00 a.m.. Each time after sampling, the container was refilled by de-ionized water to stabilize the water level lowered by evaporation and transpiration. The experiment lasted for 48 days. When the plants were harvested at the end of the test, the biomass, root length, and height of each treatment were measured successively.

Data analysis

Water quality parameters were determined following the guidelines of *Monitoring and Analysis Methods of Water and Wastewater (GB 3838 − 2002)* (SEPA, 2002). TN was quantified by alkaline potassium persulfate digestion followed by UV spectrophotometry. TP was analyzed using the potassium persulfate molybdenum antimony spectrophotometry. NH_4^+-N was determined by Nessler's reagent spectrophotometry. COD_{cr} was measured by the potassium dichromate method, and pH was measured by the PHS-3c pH meter. The removal rate (R) of TN, TP, NH_4^+-N, and COD_{cr} was calculated as:

$R= (C_0- C_i)/ C_0 \times 100\%$ (1)

where C_i represents the concentration of TN, TP, NH_4^+-N, and COD_{cr} at the i*th* time; C_0 represents the initial concentration of TN, TP, NH_4^+-N, and COD_{cr}.

The fuzzy membership function was used to evaluate the overall purification capacity of the five combinations. The indexes of TN, TP, NH_4^+-N, and COD_{cr} were comprehensively compared. Since these four indexes were negatively correlated with the purification ability of the plant combination, the membership function was calculated as:

$R(X_i) = 1- (X_i -Xmin)/ (Xmax-Xmin)$ (2)

where $R (X_i)$ was the membership function value; X_i indicated the measured value of a certain indicator; *Xmin* represented the minimum value of this indicator; *Xmax* was the maximum value of this indicator. The membership function values of the indicators of the five combinations were calculated separately and then added and averaged.

Data of on the 6th d and 48th d was used to evaluate the relationships between the removal rate of total nitrogen, total phosphorous, NH¬4+-N, CODcr, pH by linear regression, because the data of plant biomass was measured on the 6th day and the last day (48th) to ensure enough samples used to purify sewage.

Statistics were conducted by SPSS19.0; Figures were drawn by Sigmaplot and Microsoft Excel.

Results

Growing state

Generally, the plants thrived and generated new shoots and tillers in the early stage (0-36thd) (Fig. S1, S1). Root lengths and plant heights increased distinctively (Fig. S1, S1). *Thalia dealbata, Cyperus alternifoliu, and Lythrum salicaria* The height of the *Thalia dealbata* increased the most from the initial 80.6 cm to 198.7 cm. *Cyperus alternifolius* produced a large number of fibrous roots, and the average root length increased the most from 20.1 cm to 45.6 cm. During 36-48th d, the growth slowed down and the flowers withered; some leaves of the *Thalia dealbata and Nymphaea tetragona* turned yellow, and even rotted in the water. After 48 days, the biomass of all the combinations noticeably went up with the rate of increase exceeding 45%, among which T+C+I+N, T+C+I, and T+C+L increased the most, and increments were 65.66, 62.32, and 59.12, g/L, respectively, significantly higher than that of T+C and T+C+Z+N ($P<0.05$) (Fig. 3).

Removal of total nitrogen

As shown in Fig. 4a and Table 1, the TN concentration of each combination declined evidently over time with the average removal rate of 70.75%-77.67%. Additionally, the treatment duration significantly affected the purification effect of TN (P<0.01). During 0-6 d, the concentration of TN was rapidly reduced with an average removal rate of 61.21%, and the removal rate of TN by T+C reached 73.06%, which was significantly higher than that of the control group (41.72%). During 12-30 d, the TN concentration decreased linearly, but the removal rate slowed down. Notably, on the 12th day, the TN concentration of all the combinations picked up, and average removal eased to 52.69%. During 30-48 d, the TN concentration proceeds to decrease slowly but fluctuated slightly. On the 30th day, the average removal rate of TN reached 94.38%, which indicated most of the nitrogen had been purified, and the combination of T+C+L exhibited the highest removal rate of 97.65%. The initial TN concentration was 42.16 mg/L, whereas the TN concentration dropped to 0.42-1.77 mg/L at the end of the experiment, distinctively lower than the control group (17.74 mg/L). The mixture of plants had an evident impact on the purification effect of TN (P<0.01). The removal ability of each combination differed significantly (P<0.05). The average removal rate was ranked in the following descending order: T+C+I>T+C+L>T+C+I+N>T+C>T+C+Z+N>CK, where the T+C+I showed the best purification effect of TN with an average removal rate of 77.67%. Its maximum TN removal rate of 99.01% appeared on the 48th day, significantly higher than that of the control group at the same time (57.93%).

Table 1: The removal rate of TN in the combinations of ornamental hydrophytes (*P*/%).

Treatment	*t/d*								Average Removal Rate
	6d	12d	18d	24d	30d	36d	42d	48d	
CK	41.72^{a}±0.54	28.72^{a}±0.61	38.58^{a}±0.96	45.70^{a}±1.05	43.23^{a}±0.71	57.26^{a}±0.31	61.21^{a}±0.50	57.93^{a}±1.00	41.60^{a}±2.09
T+C	73.06^{b}±0.61	55.46^{b}±0.81	74.81^{b}±0.93	82.86^{b}±0.30	92.75^{b}±0.46	97.59^{b}±0.06	97.53^{bd}±0.22	98.33^{bd}±0.06	71.59^{c}±3.79
T+C+L	53.86^{c}±0.68	44.09^{c}±1.13	65.46^{c}±0.78	90.64^{c}±0.48	97.65^{c}±0.11	97.60^{b}±0.33	97.05^{bc}±0.12	97.98^{bd}±0.11	74.71^{b}±3.50
T+C+I+N	57.14^{d}±0.85	54.14^{b}±0.53	77.85^{e}±0.45	89.95^{e}±0.25	90.71^{e}±0.30	94.86^{d}±0.53	96.05^{c}±0.41	95.81^{c}±0.33	72.94^{e}±3.53
T+C+Z+N	51.75^{c}±0.64	47.45^{d}±0.71	70.02^{d}±0.52	86.67^{d}±0.52	94.87^{d}±0.42	92.25^{c}±0.28	96.38^{c}±0.48	97.39^{b}±0.28	70.75^{d}±3.64
T+C+I	70.22^{e}±0.80	62.29^{e}±0.64	82.64^{f}±0.53	92.73^{c}±0.23	95.91^{d}±0.11	97.71^{b}±0.32	98.53^{d}±0.09	99.01^{d}±0.04	77.67^{f}±3.56

Different lowercase letters in the same column indicate significant differences between different combinations at the same time (P<0.05). T, *Thalia dealbata, Cyperus alternifolius;* I, *Iris pseudacorus*; Z, *Zantedeschia aethiopica*; L, *Lythrum salicaria*; N, *Nymphaea tetragona.*

Removal of ammonium nitrogen

As shown in Fig. 4b and Table 2, the overall trend of NH_4^+-N removal in each combination was similar to that of TN. The removal rate of NH_4^+-N reached 69.73%-76.85%. The treatment duration also considerably impacted the purification efficiency (P<0.01). During 0-6 d, the concentration of NH_4^+-N dropped fast with a removal rate exceeding 60%. During 12-30 d, the concentration of NH_4^+-N continued to fall, but the removal rate slowed down. Noticeably, on the 12th day, the concentration of NH_4^+-N picked up at different levels, and it returned to an initial 6.49 mg/L in the control group. During 30-48d, the concentration of NH_4^+-N fluctuated slightly. On the 30th, 36th, 42th, and 48th day, the average removal rate of NH_4^+-N was 92.93%, 94.71%, 94.12%, and 94.99% respectively. At the end of the experiment, the concentration of NH_4^+-N in each group reduced from 6.49 mg/L to lower than 0.45 mg/L, which was significantly different from the control group (2.56 mg/L). Plant mixtures also affected greatly the purification effect of NH_4^+-N (P<0.01), but not significant among combinations (P>0.05). The average removal rate was ranked in the following order: T+C+L>T+C +I+N>T+C+I>T+C+Z+N>T+C>CK, where the average removal rate of *Thalia dealbata*+*Cyperus alternifolius*+ *Lythrum salicaria* (T+C+L) was the highest (76.79%), in contrast to the control group (60.55%).

Table 2: The removal rate of NH_4^+-N in the combinations of ornamental hydrophytes (*P*/%).

Removal of total phosphorus

Treatment	*t/d*								Average Removal Rate
	6d	12d	18d	24d	30d	36d	42d	48d	
CK	34.31^{a}±2.41	0.04^{a}±3.06	60.40^{a}±1.93	61.33^{a}±0.36	54.36^{a}±4.59	57.39^{a}±1.62	59.79^{a}±2.09	60.55^{a}±1.84	41.97^{a}±2.80
T+C	62.32^{b}±2.36	45.27^{b}±2.10	50.00^{b}±1.76	80.72^{b}±0.83	92.16^{b}±0.60	93.26^{b}±0.27	96.57^{b}±0.23	96.88^{b}±0.13	69.73^{b}±3.57
T+C+L	82.54^{c}±1.22	65.60^{c}±1.54	82.54^{c}±1.18	90.38^{c}±0.83	92.20^{b}±1.21	95.03^{b}±0.03	90.02^{c}±0.5	92.80^{c}±0.33	76.79^{c}±3.35
T+C+I+N	72.97^{d}±2.34	60.64^{cd}±1.72	88.38^{d}±0.33	91.23^{c}±0.33	94.10^{b}±0.15	93.21^{b}±0.13	92.33^{bc}±3.32	93.56^{cd}±0.15	76.27^{c}±3.43
T+C+Z+N	68.69^{d}±1.58	64.24^{cd}±0.67	84.10^{ce}±0.43	84.42^{d}±0.15	95.16^{b}±0.11	94.42^{b}±0.32	95.66^{b}±0.15	95.94^{bd}±0.10	75.85^{c}±3.42
T+C+I	71.26^{d}±1.38	58.62^{d}±1.11	86.66^{de}±0.44	87.83^{e}±0.15	91.06^{b}±0.24	97.65^{c}±0.15	96.03^{b}±0.18	95.76^{bd}±0.11	76.10^{c}±3.48

Different lowercase letters in the same column indicate significant differences between different combinations at the same time (P<0.05). T, *Thalia dealbata, Cyperus alternifolius;* I, *Iris pseudacorus*; Z, *Zantedeschia aethiopica*; L, *Lythrum salicaria*; N, *Nymphaea tetragona.*

Fig. 4c indicated the TP concentration of each combination fell sharply during the experiment with a removal rate of 63.86%-73.71%, and the extension of treatment remarkably affected the TP removal effect (P<0.01). During 0 - 6 d, the concentration of TP rapidly decreased, and the removal rate of T+C+I+N reached as high as 81.07%, whereas the removal rate of T+C was only 25.24%. The difference between the combinations was significant. TP concentration continued to fall during 12 - 24 d but fluctuated during 30 - 48 d. The TP concentration of all the combinations turned up except for T+C on the 12th day. After 36 days, the average TP removal rate reached 95.40% (Table 3). At the end of the experiment, the concentration of TP dropped from 6.34 mg/L to 0.05-0.30 mg/L, significantly lower than that of the control group (2.20 mg/L). The assembling of combination greatly affected the purification effect of TP (P<0.01). the average TP removal rate of different groups was significantly different (P<0.05), and ranked in the following order: T+C+I+N>T+C+L>T+C+I>T+C+Z+N>T+C>CK, where the combination of T+C+I+N and T+C+L demonstrated the best purification effect with an average removal rate of 73.71% and 72.71% respectively, significantly different from the control group (25.32%) (P<0.05); the minimum TP concentration of 0.05 mg/L appeared in the T+C+L treatment on the 48th day, and the removal rate reached 99.21%, significantly different from the control group (65.30 %).

Table 3: The removal rate of TP in the combinations of ornamental hydrophytes (*P/*%).

Removal of chemical oxygen demand chromium

Treatment	*t/d*								Average Removal Rate
	6d	12d	18d	24d	30d	36d	42d	48d	
CK	35.65^{a}±1.23	10.57^{a}±1.28	28.71^{a}±0.81	47.63^{a}±0.79	45.58^{a}±0.74	63.88^{a}±0.42	61.36^{a}±0.33	65.30^{a}±1.56	39.85^{a}±2.60
T+C	25.24^{b}±1.37	33.28^{b}±0.18	56.47^{b}±0.33	82.33^{b}±0.57	90.22^{b}±0.33	96.53^{b}±0.09	94.79^{b}±0.24	95.90^{bd}±0.48	63.86^{b}±4.05
T+C+L	65.14^{c}±0.33	38.17^{c}±0.48	66.09^{c}±0.18	96.69^{c}±0.24	95.11^{c}±0.18	95.74^{bc}±0.40	98.26^{c}±0.18	99.21^{c}±0.18	72.71^{ce}±3.84
T+C+I+N	81.07^{d}±0.42	43.38^{d}±0.33	72.56^{d}±0.51	85.65^{d}±0.16	94.16^{c}±0.24	94.95^{c}±0.16	94.01^{bd}±0.24	97.63^{bc}±0.24	73.71^{c}±3.60
T+C+Z+N	60.57^{e}±0.33	43.38^{d}±0.33	63.56^{e}±0.24	87.22^{d}±0.27	92.27^{d}±0.33	93.85^{d}±0.31	93.38^{d}±0.33	95.27^{d}±0.42	68.78^{d}±3.55
T+C+I	71.29^{f}±0.24	33.75^{b}±0.27	61.83^{f}±0.24	92.13^{e}±0.77	95.11^{c}±0.27	95.90^{b}±0.09	97.32^{e}±0.18	98.26^{c}±0.18	71.73^{e}±3.86

Different lowercase letters in the same column indicate significant differences between different combinations at the same time (P<0.05). T, *Thalia dealbata, Cyperus alternifolius;* I, *Iris pseudacorus*; Z, *Zantedeschia aethiopica*; L, *Lythrum salicaria*; N, *Nymphaea tetragona.*

As shown in Fig. 4d and Table 4, the chemical oxygen demand chromium (COD_{cr}) in each combination decreased significantly overtime throughout the experiment. At the beginning of the experiment (0-6d), the COD_{cr} dropped rapidly with an average removal rate higher than 60%, remarkably different from the control group (27.16%). However, the COD_{cr} in each combination rebounded slightly on the12th day. In the following experiment (12-48d), it declined again but slowed down compared with the preceding period. At the end of the experiment, the COD_{cr} in the experimental group was reduced noticeably from an initial 85.59 mg/L to 9.65-21.66mg/L, contrastingly different from the control group (52.98 mg/L), which demonstrated advantages in the purification effect of COD_{cr} among the plant combinations. By Chinese law, the content of COD_{cr} of the recycled sewage for landscape use should be lower than 50mg/L in China. In this study, all the experimental treatments reached this standard. Differences of COD_{cr} removal rate were detected in combinations, it was ranked in the following descending order: T+C+L>T+C+I+N>T+C+I>T+C>T+C+Z+N>CK, among which the T+C+L combination performed the best, reaching a removal rate of 88.73%.

Table 4: The removal rate of chemical oxygen demand chromium (COD_{cr}) in the combinations of ornamental hydrophytes (*P/*%).

Treatment	*t/d*								Average Removal Rate
	6d	12d	18d	24d	30d	36d	42d	48d	
CK	27.16^{a}±0.73	14.69^{a}±0.89	18.65^{a}±0.69	28.69^{a}±0.84	28.78^{a}±0.59	36.70^{a}±0.68	35.16^{a}±0.68	38.10^{a}±1.38	28.49^{a}±0.81
T+C	60.36^{b}±1.99	34.21^{b}±1.02	41.57^{b}±1.11	63.66^{b}±0.91	59.47^{b}±0.65	70.62^{b}±0.63	76.91^{b}±0.65	78.43^{b}±0.47	60.65^{b}±0.93
T+C+L	71.92^{c}±1.48	46.30^{c}±0.52	60.64^{c}±0.39	69.23^{c}±0.43	77.05^{c}±0.42	85.23^{c}±0.42	87.07^{c}±0.47	88.73^{c}±0.41	73.27^{ce}±0.57
T+C+I+N	72.56^{c}±1.08	62.85^{d}±0.97	72.71^{d}±0.95	75.15^{d}±0.47	70.11^{d}±0.40	81.46^{d}±0.54	81.10^{d}±0.40	86.47^{d}±0.31	75.69^{c}±0.64
T+C+Z+N	70.46^{c}±0.75	33.47^{b}±1.05	45.15^{e}±0.53	53.00^{e}±0.71	54.42^{e}±0.22	61.37^{e}±0.59	65.70^{e}±0.46	74.69^{e}±0.37	57.28^{d}±0.59
T+C+I	66.11^{d}±0.98	37.68^{e}±0.58	44.69^{e}±0.40	59.64^{f}±0.35	65.72^{f}±0.56	66.96^{f}±0.32	80.09^{d}±0.37	81.73^{f}±0.36	62.83^{e}±0.49

Different lowercase letters in the same column indicate significant differences between different combinations at the same time (P<0.05). T, *Thalia dealbata, Cyperus alternifolius;* I, *Iris pseudacorus*; Z, *Zantedeschia aethiopica*; L, *Lythrum salicaria*; N, *Nymphaea tetragona.*

The correction of pH

Fig. 3e illustrated that the pH in the sewage of each plant group was significantly reduced during the experiment. During 0-24 d, the pH decreased steadily, but it fluctuated feebly during 24-48 d. The sewage demonstrated alkaline with an initial pH of 9.21. However, as wetland plants growing, the pH of each combination decreased over time. At the end of the experiment, the pH in the water of the experimental group was stable between7.54-8.00, while pH in the control group was always higher than 8.50, indicating the plant counteracted the pH of the sewage markedly.

The comprehensive evaluation of the removal effect

The membership function value method of fuzzy mathematics was employed to evaluate the purification capacity comprehensively. Based on the analysis mentioned above, pollutant removal was the most effective before 36d. Thus, each concentration index was taken on the 36th day. The results were listed in Table 5. The order for the five hydrophyte plant combinations in terms of sewage purification capacity was: T+C > T+C+L > T+C+I+N > T+C+I > T+C+Z+N, among which T+C and T+C+L exhibited the best extract effect, with final purification rates of TN and TP higher than 98%. T+C+I+N and T+C+I were classified to the combinations with strong purification ability with the highest biomass increment, while the purification ability of T+C+Z+N purification ability was moderate with the lowest biomass increment.

Table 5: Comprehensive score ranking of the five hydrophyte combinations.

Treatment	Membership function value					
	TN	NH_4^+-H	TP	COD_{cr}	Total Score	Ranking
CK	0	0	0	0	0	6
T+C	0.9970	0.8910	1.0000	0.7578	0.9114	1
T+C+L	0.9973	0.9349	0.9758	0.6915	0.8999	2
T+C+I+N	0.9295	0.8897	0.9516	0.5979	0.8422	3
T+C+Z+N	0.8650	0.9198	0.9179	0.0989	0.7004	5
T+C+I	1.0000	1.0000	0.9807	0.2069	0.7969	4

T, *Thalia dealbata, Cyperus alternifolius;* I, *Iris pseudacorus*; Z, *Zantedeschia aethiopica*; L, *Lythrum salicaria*; N, *Nymphaea tetragona.*

Relationships between total nitrogen, total phosphorous, NH4+-N, CODcr, pH, and biomass

Our results showed that total nitrogen, total phosphorous, NH4+-N, CODcr, were significantly positively related to plant biomass ($p<0.01$). Meanwhile, pH was significantly negatively related to plant biomass($p<0.01$).

Discussion

In order to evaluate the effects of wetland polyculture in sewage treatment and select suitable plant combinations for urban wetland construction and enhancing the waterfront landscape effect, we comprehensively determined and compared the pollutant removal effects of five polyculture combinations. Our results indicated that all the combinations exhibited distinctive pollutant removal effects, but significant differences were detected among them. Our findings are in some respects consistent with previous studies showing that plant mixture is generally highly effective at nutrient removal (Fraser et al., 2004; Wang et al., 2013) and that the possibility exists in mixed hydrophytes remediation system for processing wastewater (Polomski et al., 2008). Compared with previous researches, we are different from them that we considered the synergistic effects in the plant combinations and aesthetic value at the same time for the selection of the plant combinations. More importantly, we found that more diverse plants did not always performed better in wastewater purification. This research provides a solid theoretical basis for pollutant removal in the wetland system and recommends new ornamental plant combinations for urban wetland construction.

In this experiment, all the combinations grew well in the sewage with considerably increased but differed biomass (Fig. 3). The biomass of floating and emergent plant combination (T + C + I + N) increased the most, possibly because it could maximize the living space by growing laterally and upwardly on the water surface. However, when the emergent plant of the combination was replaced by *Zantedeschia aethiopica* (T + C + Z + N), the biomass increased the least, likely as a result of inter-species competition or exclusiveness among *Zantedeschia aethiopica* and other plants (Calheiros et al., 2015; Hong-xia et al., 2017). By analyzing the correlation between the increases in biomass and removal effect (Fig. 5), we found the removal rates of TN,TP, NH_4^+-N, CODcr, and pH were close related to the increment of plant biomass, implying the biomass increment was a vital mechanism for direct nutrient removal (Greenway et al., 2011), which could be used as an essential indicator for screening purification plants. This finding conforms with the study (Bachand et al., 2000; Wang et al., 2013) that the increased biomass enhanced the removal efficiency.

It is widely acknowledged that the purification of eutrophic water bodies is achieved by the synergized effects of vegetation uptake, substrate adsorption, microorganism accumulation and transformation, and other pathways (Vymazal, 2007; Wu et al., 2014; S. Wu et al., 2015). In our study, we found the treatment duration was also strongly linked to the removal efficiency. The purification effect was not ideal when the duration was too short, and secondary pollution would be incurred if it was too long. In this experiment, the removal rate of the five combinations exhibited a similar pattern of initial rapid reduction of pollutants followed by an eventually slight fluctuation. At the beginning (0–12 d), the fast growth of plants incurred intensive pollutant absorption through roots, coupled with substrate assimilation, the sewage was thus rapidly purified. However, the concentration of pollutants rebounded notably on the 12th day. The reasons were twofold, first, lots of pollutants enriched in the rhizosphere were decomposed by microorganisms and transferred again into the water, driving concentration to rise (Faulwetter et al., 2009); second, some substances released from the saturated substrate also increased the concentration (Johansson Westholm, 2006). However, the removal rate declined during 12–36 d, which might be attributed to descending plant growth, weakening pollutant absorption, and receding microbial degradation. All the combinations exhibited a remarkable removal rate of TN, TP, and NH_4^+-N over 90% from 30th to 36th day, which was distinctively higher than that of the control group, revealing the plants have already obviously reduced pollutants and improved water quality. At the final stage (36-48d), the concentration of pollutants fluctuated up and down. The TP in the control group was raised, which might be induced by the microorganism that died of scarce nutrients reversing the assimilated phosphorus to water (Polomski et al., 2008). In the experiment group, the raised TP might be ascribed to the phosphorus transferred into the water from the rotten flowers and leaves of the *Nymphaea tetragona* and *Thalia dealbata*. As a result, biomass harvesting time may affect the plant itself and the removal efficiency (Wang et al., 2015). In practical application, management should be strengthened to control the planting density and harvesting time to prevent the rotten plants from contaminating the water body.

Previous research indicates that the removal of organic matter is mostly driven by microorganisms and the uptake of plants (Korkusuz et al., 2005; Savin et al., 1998; Xu et al., 2016). In our study, all the experimental groups were efficient at reducing organic matter. By comparing the removal rates of COD_{cr}, the T + C + L manifested the best purification capacity. This is mainly attributed to the strong pollution resistance of the T + C + L under high pollutants concentration. Its fast-developing roots system encourages suitable habitats for microorganisms, additionally, plants add oxygen during the process of photosynthesis or by direct transport from the atmosphere through their stems and roots to the rhizosphere, which facilitates the absorption and degradation of COD_{cr} (Chen et al., 2016; Rehman et al., 2017). Overall, in the initial period (0-6d), when the plants were newly transplanted into sewage, a mass of organic matter was rapidly assimilated by the plants, so the COD_{cr} declined linearly. However, on the 12th day, the COD_{cr} turned up slightly, which might be explained by the fact that a large number of roots began to germinate, the intensive root respiration and microbial metabolism in the root zone released lots of organic substance which drove the COD_{cr} up. After 12 days, the plants entered into a rapid vegetative growth period. With the vigorous photosynthesis, the uptake and decomposition of the root system, the biosynthesis effect was stronger than the decomposition effect, resulting in the steadily decreased COD_{cr} in the final stage again.

The pH of the sewage samples was originally high due to many alkaline substances in the domestic sewage discharged into the river such as soap or laundry detergent. In our study, the pH was significantly corrected after the experiment. At the beginning of the experiment, the pH declined linearly, because the microbial activity was still weak, alkaline substances were mainly absorbed by the newly transplanted plants, which gave rise to relatively increasing H^+ concentration. However, the hydrolysis of nitrogenous compounds led to the release and ionization of ammonia, which consumed H^+ in the sewage, thus, the pH rebounded slightly on the 12th day. With the fast growth of the plants, the organic acid in the root exudates from the developed roots (Chen et al., 2016), and the nitrification of ammonia from nitrogenous compounds released H^+ into the water again, which reduced the pH steadily till the end. Our results echo the previous study on reducing the pH in the textile effluent by using the plants (Islam et al., 2013).

The comprehensive evaluation depicted the best extract efficiency of T + C, closely followed by T + C + L. The reason for this apparent superiority was that different plants could complement each other to enhance tolerance to abiotic stress or treatment efficiency of other toxins or nutrients (Fraser et al., 2004), thus ensured the stability of the purification system and the sustainability of the treatment effect. They grew rapidly with a well-developed root system to provide continuous aerobic-anaerobic-aerobic substratum for the attached microorganism (Meng et al., 2014; Wang et al., 2016). Moreover, root exudation of a wide variety of compounds (organic, amino, fatty acids.) provides better nutrient uptake for the rhizosphere microbiome in return (Rohrbacher et al., 2016) to accelerate growth and reproduction, which further improved the removal rate of pollutants. Both of them are very suitable for urban sewage purification and artificial wetland construction. Additionally, T + C + I + N and T + C + I demonstrated relatively high removal efficiencies which could be used as effective alternatives. The least effective combination was T + C + Z + N, likely owing to the mutual competitive and exclusive effect on the absorption of pollutants between the plant species, which inhibited the growth and biomass accumulation, hence the combined purification ability was decreased. Therefore, it is not recommended as a beneficial plant combination. We conducted a laboratory-scale experiment in a given time in this study. We suggest that long-term treatment of diversified hydrophyte species in real constructed wetland should be further studied.

Conclusions

(1) each hydrophyte combination grew robustly in the sewage with significantly increased biomass, which can be used as a major indicator to screen aquatic plants for the purification of water bodies; (2) five hydrophyte combinations exhibited generally high effectiveness at nitrogen, phosphorus, ammonium nitrogen, and organic matter removal in addition to pH correction in the sewage; (3) extension of treatment had a remarkable effect on the purification effect, which achieved the best during 30-36 days. In practical application, this study suggests choosing the appropriate plant combinations and the best harvesting time based on the local water pollution status to maximize the purification effects and landscape effects. (4) More diversified plant species is not always effective in wastewater treatment. *Thalia dealbata*+*Cyperus alternifolius* was most efficient at reducing various pollutants, closely followed by *Thalia dealbata* + *Cyperus alternifolius*+ *Lythrum sastlicaria*. Both of them were recommended for urban sewage purification as the most suitable plant combination of all in this experiment.

Declarations

Ethics approval and consent to participate

In research, decorum does not involve human beings and animals, it does not apply.

Consent to Publish

On behalf of Min Wang, Lin Xiang, Xiaoyang Ke, Hui Zhang, Li Guo, Yuanzhi Pan, Jun Ma, Zhuo Huang, Xi Li, I am submitting the article *Assessment of the effects of ornamental wetland polyculture combinations on the urban sewage treatment*. The authors declare that they agree with the submission and eventual publication of the Environmental Science and Pollution Research.

Authors Contributions

Yujue Zhou: Conceptualization, Supervision, Writing- Reviewing and Editing. Min Wang: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation, Visualization.Lin Xiang, Xiaoyang Ke, and Hui Zhang helped with figure drawing.Li Guo, Yuanzhi Pan, Jun Ma, Zhuo Huang, and Xi Li contributed to providing suggestions.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

Does not apply

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Figures

Figure 1

Five ornamental wetland plant combinations and concept image of their use in a constructed wetland.

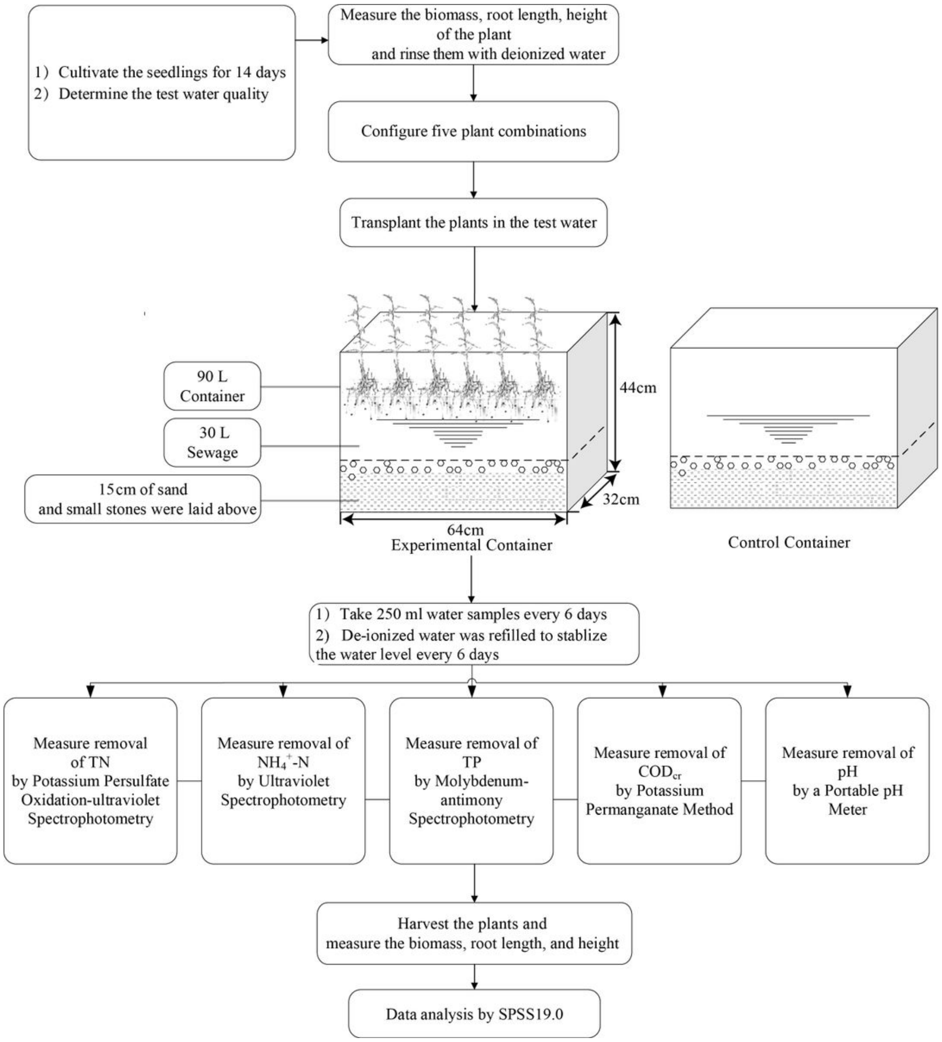

Figure 2

Schematic drawing of the laboratory-scale constructed wetland apparatus and the experiment process.

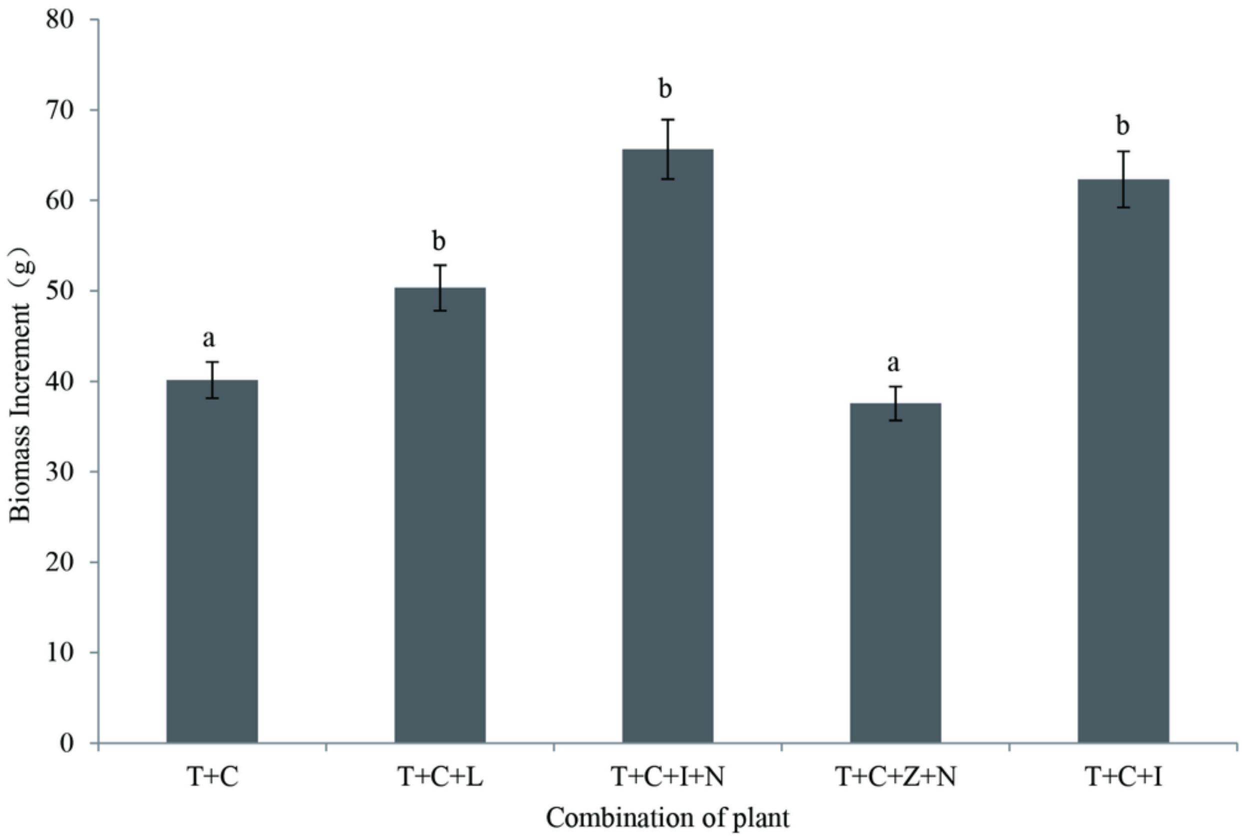

Figure 3

Variations in biomass increment of the five hydrophytes combinations. Note: Different lowercase letters indicate significant differences between groups (P<0.05). T, Thalia dealbata, Cyperus alternifolius; I, Iris pseudacorus; Z, Zantedeschia aethiopica; L, Lythrum salicaria; N, Nymphaea tetragona.

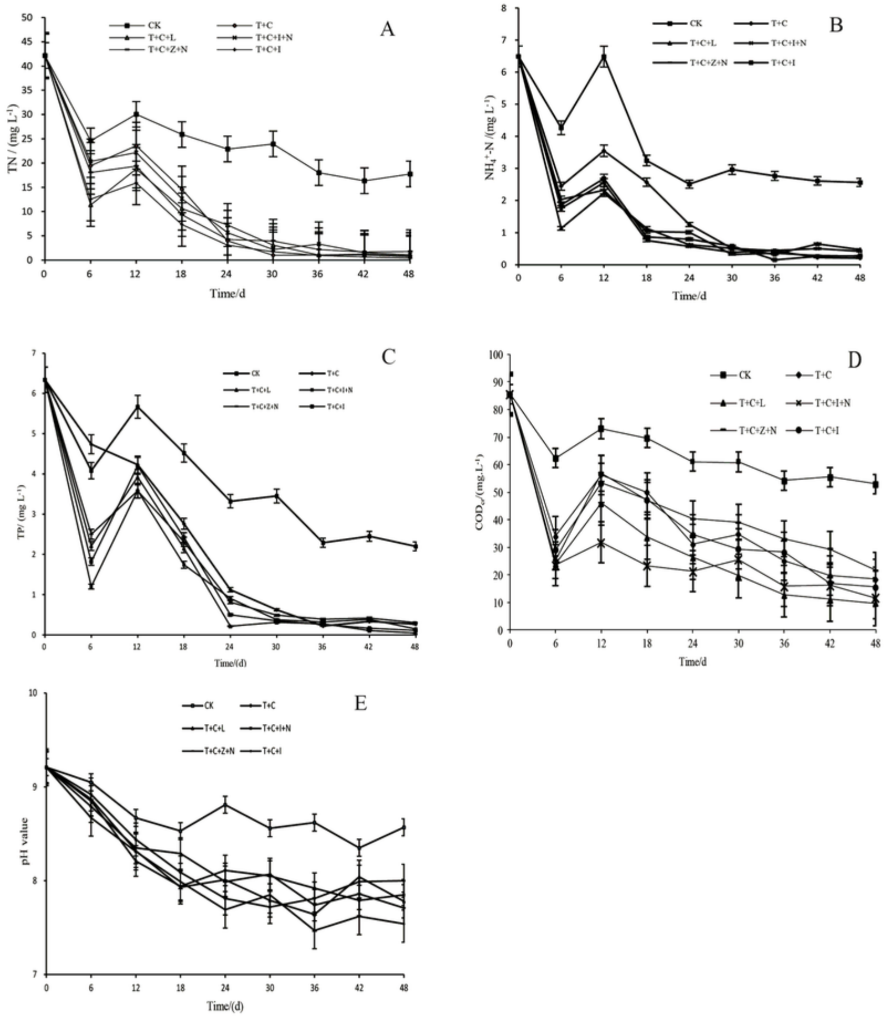

Figure 4

Dynamic changes in the concentration of pollutants in sewage. (a) Dynamic changes in total nitrogen concentration (TN). (b) Dynamic changes in NH¬4+-N concentration. (c) Dynamic changes in total phosphorus concentration (TP). (d) Dynamic changes in CODcr. (e) Dynamic changes in pH value (pH). T, Thalia dealbata, Cyperus alternifolius; I, Iris pseudacorus; Z, Zantedeschia aethiopica; L, Lythrum salicaria; N, Nymphaea tetragona.

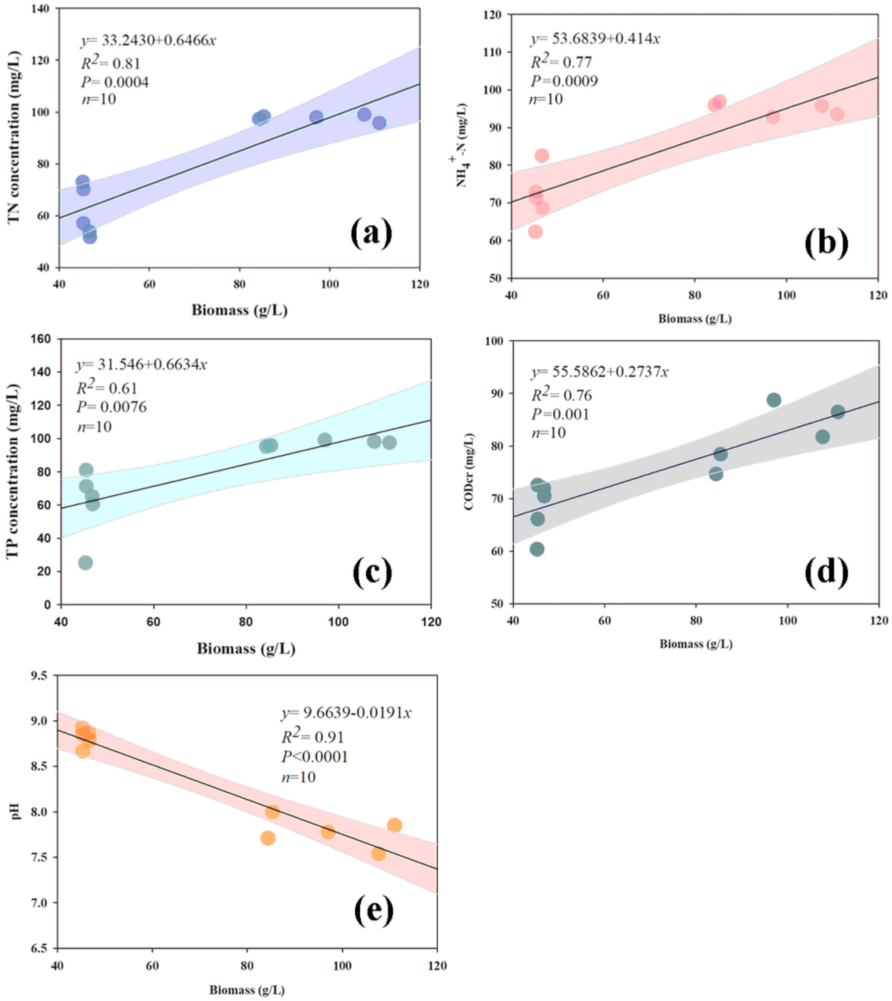

Figure 5

Relationships between the removal rate of total nitrogen, total phosphorous, NH¬4+-N, CODcr, pH, and plant biomass by linear regression. The shaded area represents the 95% confidence intervals of the linear regressions.

Supplementary Files

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