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Role of different plants on nitrogen and phosphorus removal at low temperature in lab-scale constructed wetlands

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Abstract. In this study, plant growth and nitrogen and phosphorus removal efficiency in lab-scale CWs by five plants (*H. vulgaris*, *N. peltatum*, *N. tetragona*, *N. pumilum*, *S. trifolia*) in winter in Sichuan basin was evaluated. *H. vulgaris* and *N. tetragona* would well adapt to the winter wetland environment, and the relative growth at the end of the experiment was 89.83% and 66.85%, respectively. In winter, *H. vulgaris* kept growing with accumulated stems and leaves, while growth of *N. tetragona* was mainly caused by the growth of roots and stems underwater. In addition, during the winter, removal efficiencies were 66.29%, 57.47%, 54.78%, 55.47%, 41.66% of TN and 62.40%, 69.75%, 69.97%, 65.65%, 76.55% of TP for each planted CWs respectively. The results indicated that the removal of nitrogen and phosphorus from CWs was mainly achieved by substrate, while a small portion was attributed by plant. However, plants like *H. vulgaris* and *N. tetragona*, in the CWs in winter can play the role of landscaping. Thus, *H. vulgaris* could be considered as a suitable and effective nutrient removal plant for treatment of nitrogen and phosphorus water in winter wetlands in Sichuan basin.

1 Introduction

Water eutrophication is the phenomenon that disturbs of the aquatic ecosystems equilibrium, caused by a large number of aquatic organisms proliferating abnormally due to excessive nutrients enter into water body. Among them, nitrogen and phosphorus are considered as one of the main inducing factors of eutrophication. As a new ecological restoration technology with low cost, good economic benefits and environmental friendliness, the constructed wetlands (CWs) are widely used in the remediation of water eutrophication [1]. Plants in the CWs can absorb nutrients in the water for their growth, thus nutrients in the water was removed [2-4].

More than 150 macrophytes are applied to CWs for water purification, but few species are widely used at present [5], including *Phragmites australis*, *Typha orientalis*, *Canna indica*, *Juncus effusus*, *Ceratophyllum demersum*, *Potamogeton distinctus*, *Myriophyllum verticillatum, Hydrilla verticillata* and so on. The removal effects of pollutants differed from plant species, which may be related to plant tolerance to pollutants, redox conditions in root zone, microbial activities and other factors [6,7]. Aquatic plants can not only absorbed nitrogen and phosphorus from water though leaves, stems and roots [8], but also affected the oxygen content in sediments by photosynthesis [9,10]. That would lead to differences in the absorption of nitrogen and phosphorus by plant species [11].

The seasonal alternation from summer to winter makes steep declines in environmental temperature, which would wilt plants and maybe weakened the oxygen transporting capacity of plants in CWs in winter, leading to deterioration of wastewater treatment in the CWs [12]. Therefore, it is particular important to ensure the nutrient removal capacity of CWs for plants at low temperature in winter. Seasonal plant arrangement, like *P. crispus* and *P. australi* series system, enhanced the performance of surface flow wetlands at low temperature [13]. In addition to plant configuration, it was also a useful way to improve CWs removal efficiency of pollutants in cold winter by planting cold-resistant plants or strong pollutants removal ability plants in winter. The removal rate of TN and TP in non-point agricultural wastewater can reach to over 70% after planting *O. javanica* in the cold winter in CWs [14]. To date, the growth and nutrients removal of suitable plant species in the winter in Sichuan Province CWs remains incipient.

Thus, the aim of this study was to evaluate the removal efficiencies of five leaf floats plants for nitrogen and phosphorus by lab-scale constructed wetlands, focusing on low temperature condition in Sichuan.

2 Materials and methods

2.1 Experiment setup

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The study was conducted at Yanting agricultural ecology station, CAS, in Mianyang City, Sichuan Province，which has a subtropical humid monsoon climate with an annual average temperature of 17.5°C and an annual rainfall of 826 mm. The average maximum temperature reached to 10~12°C, occurred at 2 or 3 pm. And the average minimum temperature was 2~4°C, occurred around sunrise. The lowest temperature in Sichuan basin occurred in January and the extreme low average temperature was -2~0°C.

The experiments were carried out in a lab-scale horizontal subsurface flow constructed wetland (referred as CW in the following), with diameter of 40 cm and height of 50 cm, packing 25 cm thickness for substrate. The influent water quality was artificially prepared to simulate rural domestic sewage, and the hydraulic retention time was 7 d.

Based on field investigation and literature review, CWs planted with five leaf floats plants were selected to monitoring variation of water quality in winter. The plants were *Hydrocotyle vulgaris, Nymphoides peltatum, Nymphaea tetragona, Nuphar pumilum, and Sagittaria trifolia L.*. Each plant was planted into three CWs system (with same plant density) and three unplanted CWs were also monitored as control. The experiment started from 15, November 2018 and ended in 23, March 2019, lasting 130 days.

2.2 Sample collection and analyses

The plants sample were collected before and after the experiment, and biomass of each plants were measured. Water sample (including both influent and effluent) were collected every two weeks from 15, November 2018 to 15, March 2019. AutoAnalyzer 3 (AA3, Bran+Lubbe, Norderstedt, Germany) were used to analyze water quality indexes of TP and TN. Meanwhile, water temperature, pH, and DO were recorded at each sampling time by portable instrument.

2.3 Data analysis

The relative growth of each plant was calculated as follows:

$$Relative\ growth\ \%=(B_t-B_0)\div B_0\times 100 \tag{1}$$

where, B_t is the biomass at the end of the experiment (g/m^2), and B_0 is the biomass in the beginning of the experiment (g/m^2).

The removal efficiency (R_e) of TN or TP for each sampling time was calculated as follows:

$$R_e=(C_{ii}-C_{ei})\div C_{ii}\times 100 \tag{2}$$

where, C_{ii} is the influent concentration of TN or TP at the *i-1* time water sampling (mg/L), and C_{ei} is the effluent concentration of TN or TP at the *i* time water sampling (mg/L). And the removal rate (R_t, mg/g/d) was calculated as:

$$R_t=\frac{(C_{ii}-C_{ei})\times V_i}{B_0\times t} \tag{3}$$

where, V_i is the volume of influent (L), and t is the duration time of the experiment (d).

Differences in biomass, removal efficiency, and removal rate of different plant species were analyzed by the LSD procedure and contrasts with 0.05 probability level using SPSS 19.0 software. Correlations between release amounts and initial characteristics were identified by Pearson correlation coefficient. Means and standard deviations (SD) were calculated using Microsoft Office Excel 2013. Figures were completed by Origin 8.1.

3 Results and discussion

3.1 Plant growth in the winter

Table 1 listed the planting density, biomass in the beginning and at the end, and relative growth of the five plants. There were significant differences in biomass among different plant species ($P<0.05$). At the end of the experiment, biomass of five plants in this study was much smaller than the emerged plants like *T. orientalis*, *P. australis* and *I. tectorum* (1.9, 18.2 and 3.3 kg/m^2) [15]. That may be because this study was lab-scale and performed in the winter. Also, environmental stresses, like high phosphorus concentration in this study, would inhibit plant growth and even damage wetland plants [1].

Table 1. Plant density and biomass of five plants.

Plant Species	Planting Density (/m^2)	B$_0$ (g/m^2)	B$_t$ (g/m^2)	Relative growth (%)
H. vulgaris	152	217.83±41.34b*	411.01±61.62b	89.83
N. peltatum	24	32.48±0.00d	18.03±1.86d	-44.49
N. tetragona	35	484.08±38.74a	805.44±40.07a	66.85
N. pumilum	24	276.11±23.89a	270.49±10.46c	-1.45
S. trifolia	24	153.11±0.00c	57.52±10.19d	-62.43

*means showed in each row followed by the same letter identifier are not significantly different (LSD, $P<0.05$)

Biomass of *H. vulgaris* and *N. tetragona* increased rapidly and the relative growth at the end of the experiment was 89.83% and 66.85%, respectively. During the experiment period, *H. vulgaris* performed exuberant vitality and mass of stems and leaves increased largely and continuously. However, the growth of tuberous plants, especially *N. tetragona*, was not satisfied in the early stage of the experiment. After gradually adapting to the environment in the later stage, they grew better. The biomass of *N. tetragona* increased mainly due to the simultaneous growth of tubers and leaves. No flowering phenomenon of *N. tetragona* was detected throughout the winter, indicating the underground roots of *N. tetragona* were wintered and developed [16], which was consisted with the study of Zhang et al. on perennial plants. While little changes was in biomass of *N. pumilum* before and after the experiment and biomass of *N. peltatum* and *S. trifolia* at the end of the experiment were less than that in the beginning. This suggested that *H. vulgaris* and *N. tetragona* were cold-tolerant plants, while *N. pumilum*, *N. peltatum* and *S. trifolia* were not suitable for cold winter in Sichuan.

3.2 Water environment for different planted CWs during the winter

Variations of water temperature of the CWs during the experiment were shown in Fig. 1. From November to March next year, the influent water temperature in CWs was about 6 to 16°C. However, the sampling time was at noon which made the monitored temperature was about 5°C higher than the average winter temperature in the CWs. During the experiment of CWs overwintering operation, water temperature after the treatment of CWs changed within 2°C compared with the influent water temperature measured at the same sampling time. There was no significant difference in water temperature between CWs with plants and the control CWs without plants and no remarkable difference between CWs planted with different plant species. That illustrated that the water temperature of CWs was invisibly regulated by the presence of plants.

Compared with the influent pH, the effluent pH of both planted and unplanted CWs significantly increased, as shown in Fig. 2. In the experiment, the influent pH fluctuated between 6.3 an 7.9, while the effluent pH can be reached about 9.0. The effluent pH increased since the influent water contained a large amount of nutrients. To some extent, the CWs systems would adjust the pH of water, maintaining the CWs in an alkaline environment. However, there was little influence made by plant species and the presence of plants on the effluent pH of the CWs. This kept in line with the research of Zhang et al., indicating that the substrate exhibited great pH buffering capacity, while pH changed by plant-induced was almost inconspicuous [3].

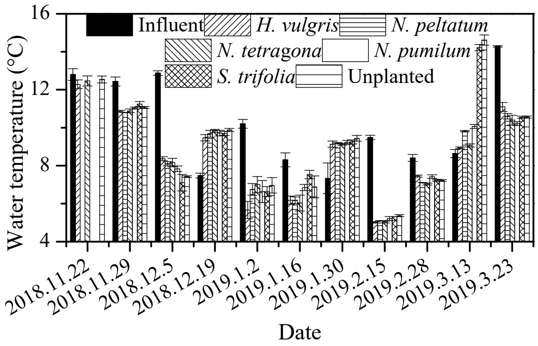

Fig. 1. Variation of water temperature in CWs during the experiment

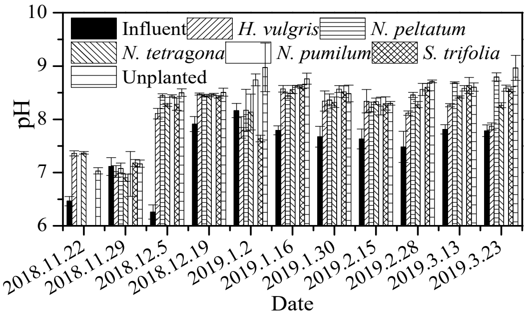

Fig. 2. Variation of pH of water in CWs during the experiment

DO is considered to be an important factor to estimate the water self-purification capacity, which directly affected the reduction of organic or inorganic contaminants [17]. Fig. 3 showed the DO contents in water of CWs during the experiment. The DO concentration of influent was range from 4.66 mg/L to 7.34 mg/L and 5.63 mg/L for average. Meanwhile, the average DO concentrations of *H. vulgaris, N. peltatum, N. tetragona, N. pumilum, S. trifolia* and unplanted CWs were 9.28 mg/L, 10.03 mg/L, 9.25 mg/L, 9.68 mg/L, 9.99 mg/L and 6.93 mg/L, respectively.

Although there was little difference of DO concentration between different plant species CWs, the DO in water of planted CWs was higher than that of unplanted CWs, which illustrated that the planting of overwintering plants in this study had a positive effect on oxygen condition of CWs. This was primarily correlated with plant photosynthesis and the release of oxygen from plant roots [3,12]. Contrary to the ornamental plants [18], plants in this study exerted great influence on the ability of oxygen uptake of CWs. This would promote the decomposition of plant organic carbon, be conductive to nitrification, and be unfavourable for denitrification [3], thus improved the removal performance of organic matter and nitrogen in CWs [19].

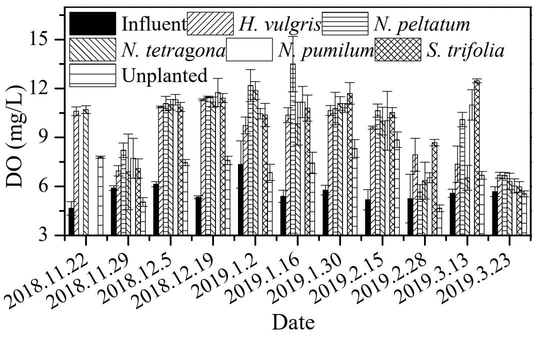

Fig. 3. Variation of DO concentration of water in CWs during the experiment

3.3 Removal of nitrogen and phosphorus from planted CWs in winter

Fig. 4 showed the variation of TN and TP concentration in the influent and effluent of the CWs during the experiment. The average TN and TP concentration in the influent was 4.26 mg/L and 14.05 mg/L. In the first month of the experiment, TN concentration in all CWs effluent fluctuated greatly and differed widely. There was a significant decline in TN concentration in planted CWs, especially in *H. vulgaris* and *N. tetragona* system, which may caused by plants uptake nitrogen for growth. When the temperature dropped to the lowest in January, the effluent TN concentration in all CWs almost unchanged and that in planted CWs was slightly higher than that in unplanted CWs. This phenomenon corresponded to the decrease of nitrate concentration at first and then increase. Ammonia in the CWs would be converted into nitrate and nitrite through nitrification firstly, and then the nitrate and nitrite might not be discharged from water due to the oxygen conditions were not beneficial to denitrification [2]. Thus, the TN concentration in water in planted CWs did not decrease but increase.

Variation of TP concentration in the effluent of CWs differed from that of TN. And effluent TP concentration was obviously lower than influent in all cases, indicating that the CWs conduct well performance of TP removal in winter. It was found that the effluent TP concentration in planted CWs was higher than that in unplanted CWs, inconsistent with previous studies that the presence of plants would increase the phosphorus removal capacity [4,18]. The possible reason was that plant communities with small biomass did not possess potential for phosphate retention [20].

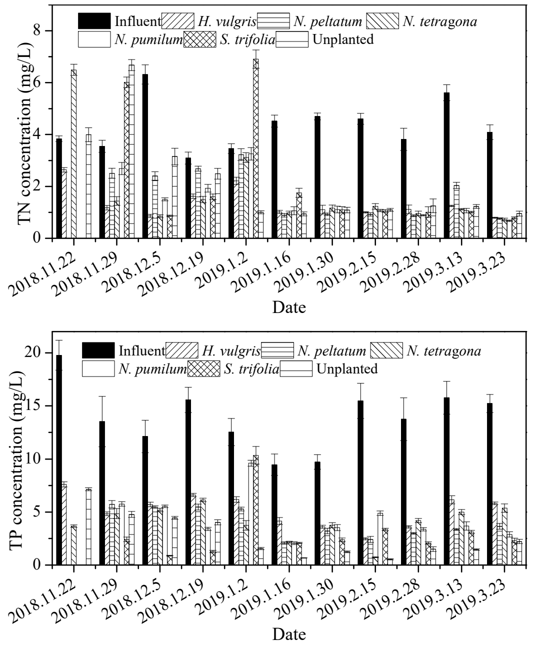

Fig. 4. Variation of TN and TP concentration of water in CWs during the experiment

As shown in Fig. 5, the TN removal efficiency in CWs planted with *H. vulgaris, N. peltatum, N. tetragona, N. pumilum, S. trifolia* and unplanted was ranging from 31.21% ~ 86.32%, 6.73% ~ 81.39%, -69.12% ~ -82.27%, -54.22% ~ 83.48%, -99.70% ~ 86.29%, and -88.29% ~ 79.10%, respectively. And the TP removal efficiency was ranging from 50.69% ~ 84.00%, 54.89% ~ 84.37%, 57.22% ~ 95.21%, 54.29% ~ 81.02%, 17.51% ~ 92.68%, and 63.28% ~ 96.45%, respectively.

The average TN and TP removal efficiency and removal rate for each plant of the CWs during the 130d duration time in the experiment was listed in Table 2. There was no difference in the TN removal efficiency between different plants, but some difference in TP removal efficiency. Except for *S. trifolia* CWs, the average TN removal efficiency of planted CWs was higher than that of unplanted CWs (45.67%), and the average TP removal efficiency of planted CWs was lower than that of unplanted CWs (71.35%), which indicated that the substrate sorption was the predominant way of TP removal in CWs [21]. The removal efficiency of TN in planted CWs was negatively correlated to that of TP, but the removal rate of TN was positively related to that of TP. Due to the small biomass of *S. trifolia*, the TN and TP removal rate of *S. trifolia* CWs (0.63 mg/g/d and 2.31 mg/g/d, respectively) was much higher than that of other plant in the experiment. And both TN and TP removal rate of *N. tetragona* CWs (0.04 mg/g/d and 0.18 mg/g/d, respectively) were the minimum among all planted CWs.

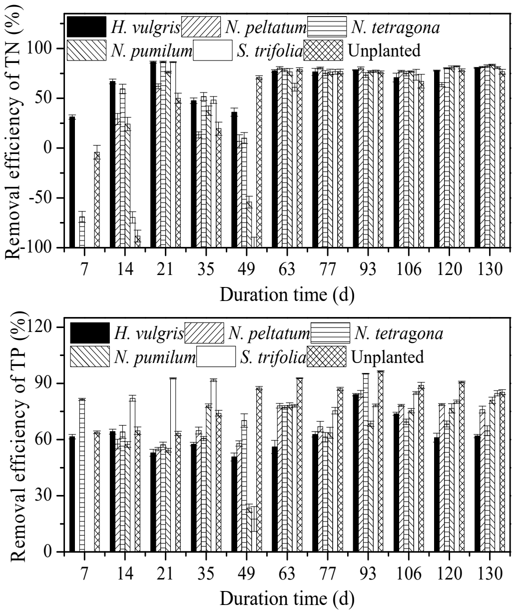

Fig. 5. Variation of TN and TP removal efficiency in CWs during the experiment

Table 2. TN and TP removal efficiency and rate of the CWs.

Plant species	TN		TP	
	Average removal efficiency (%)	Average removal rate (mg/g/d)	Average removal efficiency (%)	Average removal rate (mg/g/d)
H. vulgaris	66.29	0.11b*	62.40b	0.36bc
N. peltatum	57.42	0.63a	69.75ab	2.31a
N. tetragona	54.78	0.04b	69.97ab	0.18c
N. pumilum	55.47	0.06b	65.65ab	0.26bc
S. trifolia	41.66	0.09b	76.55a	0.55b
Unplanted	45.67	--	71.35a	--

*means showed in each row followed by the same letter identifier are not significantly different (LSD, $P<0.05$)

Plants as the mainly component of CWs, was an intermediate medium for nutrient transformation, would enhance the removal processes of nutrients [1,7]. The capacity of nutrient uptake by plants subjected to various factors was accounted for about 28.2% to 34.5% of nitrogen removed from CWs and 25.2% to 33.4% of phosphorus. While the substrate in CWs was considered for about 7.2% to 25.5% of nitrogen removed and 7.3% to 35.0% of phosphorus [1,2]. This common result was inconsistent with our study that although plants in this experiment can survive in winter, the nitrogen and phosphorus removal in CWs basically depended on substrate, especially for phosphorus [4,21]. At low nitrogen concentration of influent (about 4.26 mg/L), plants like *H. vulgaris* and *N. tetragona* grew in winter with little removal of TN, about 20% at most only accounting removal efficiency from plant [22]. It was found in our study that TN removal efficiency of planted CWs was affected by pH value in water, which in agreement with the results obtained by Zhang et al. [23]. The main reason was that pH in the water was closely related to the amount and activity of microbial communities (eg. nitrifying and denitrifying bacteria) [24]. Moreover, nitrification-denitrification was the essential way of nitrogen removal in CWs, accounting for 66.9%~80.5% of TN removal [23]. The TP removal efficiency of CWs in winter was independent of microbial environment, since the substrate adsorption and precipitation were the main approaches for phosphorus removal in CWs in winter [21]. With substrate CW system can completely remove phosphate from wastewater, while the removal efficiency for phosphate was only 89% or -14% without substrate [4].

4 Conclusions

Plant growth and removal of nitrogen and phosphorus from water in the surface flow lab-scale wetlands were investigated in winter in Sichuan basin with five plants, e.g. *H. vulgaris, N. peltatum, N. tetragona, N. pumilum,* and *S. trifolia*. Plant species significantly affected biomass. *H. vulgaris* and *N. tetragona* showed the higher relative growth at the end of the experiment (89.83% and 66.85%). Mean while, plant presence was an active factor that influenced DO value in CWs in winter. In addition，planted CWs performed well removal efficiency of TN and TP in winter, for CWs with *H. vulgaris, N. peltatum, N. tetragona, N. pumilum, S. trifolia* about 66.29%, 57.47%, 54.78%, 55.47%, 41.66% of TN and 62.40%, 69.75%, 69.97%, 65.65%, 76.55% of TP. However, a small part of TN and TP removed from water was attributed by plant. Through the comprehensive analysis of plant growth and nutrient removal efficiency, *H. vulgaris* could be a promising choice to plant in winter CWs in Sichuan basin. Further studies are required regarding the involvement of plant root morphology and microbial environment in winter.

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