Influence of vegetation type and temperature on the performance of constructed wetlands for nutrient removal

Hui Zhu, Qing-wei Zhou, Bai-xing Yan, Yin-xiu Liang, Xiang-fei Yu, Yoram Gerchman and Xian-wei Cheng

ABSTRACT

In this study, the influence of vegetation type and environmental temperature on performance of constructed wetlands (CWs) was investigated. Results of vegetation types indicated that the removal of most nutrients in polyculture was greater than those in monoculture and unplanted control. The greatest removal percentages of NH$_4$$^+$-N, total nitrogen (TN) and total phosphorus (TP) in polyculture were 98.7%, 98.5%, and 92.6%, respectively. In experiments of different temperatures, the removal percentages of NH$_4$$^+$-N, NO$_3$$^-$/NO$_2$$^-$/N, TN and TP in all CWs tended to decrease with the decline of temperature. Especially, a sharp decline in the removal percentages of NO$_3$$^-$/N (decreased by above 13.8%) and TN (decreased by above 7.9%) of all CWs was observed at low temperature (average temperature of 8.9 °C). Overall, the performance of CWs was obviously influenced by temperature, and the polyculture still showed best performance in the removal of nitrogen when the average temperature dropped to 19.8 °C. Additionally, the variations of urease activities in rhizosphere soil tended to decrease with the decreasing temperature. Overall, a substantial enhancement for nitrogen and TP removal in polyculture (Canna indica + Lythrum salicaria) was observed. In conclusion, CW cultivated with polyculture was a good strategy for enhancing nutrient removal when temperature was above 19.8 °C.

Key words | constructed wetlands, domestic sewage, temperature, urease activities, vegetation types

INTRODUCTION

The enrichment of surface water with nutrients from municipal wastewater can strongly affect water quality and lead to eutrophication of water bodies (Karczmarczyk et al. 2016). In addition, the consumption of water rich in nitrates can cause the disease known as blue baby syndrome (nitrate competition for oxygen in the blood), stomach problems in adults and even cancer (Magalhaes et al. 2016). Therefore, the removal of nitrogen and phosphorus from wastewater is one of the top priority concerns (Hong et al. 2016). The nutrient treatment has been a difficult issue in developing countries. Constructed wetlands (CWs), as an inexpensive, effective and reliable water treatment technology, have been widely used for nutrient removal. In CWs, the main removal mechanism for nutrient has been clearly identified in recent years (Van de Moortel et al. 2010). The efficient removal of nutrients can be achieved through a series of biotic and abiotic processes, especially those occurring around the rhizosphere of vegetation (Stottmeister et al. 2005). Hence, CWs with vegetation may have higher removal efficiency of nutrients than those without vegetation. Accordingly, to ensure the efficient removal of nutrients, vegetation species selection should be taken into consideration (Zheng et al. 2016). It has been reported that canna (Canna indica) and purple loosestrife (Lythrum salicaria) have been widely used to improve the quality of the water environment due to their high nutrient removal efficiency and aesthetic value (Uveges et al. 2002; Zhang et al. 2008). Therefore, Canna indica and Lythrum salicaria may serve as efficient plant species in CWs.

Whether polyculture has a significant improvement in the treatment efficiency of CWs is still a controversial
issue. Some studies indicated that polyculture outperformed monoculture, while others showed varied results. For example, Liang et al. (2011) has reported that system functioning benefited from higher plant diversity, and Coleman et al. (2001) also proved that to mix different plants can enhance the efficiency of CWs. However, Fraser et al. (2004) claimed that the polyculture had no significant improvement on the performance of CWs. Therefore, further studies are necessary to explore the differences between monoculture and polyculture, and to identify the driving mechanisms, thereby assessing whether polyculture wetland is a good strategy for enhancing nutrient treatment. The decreasing temperature would suppress the nutrient removal efficiency of CWs as proved by previous studies (Yan & Xu 2014), but how CWs with specific design (e.g., polyculture) would perform under decreasing temperature remains unclear. Therefore, relative studies are necessary to be conducted so as to clarify the feasibility of improving winter performance of CWs by optimizing the vegetation. Numerous studies showed that nutrient removal in CWs was mainly attributed to the metabolism of microbes and enzyme activities (Yang et al. 2016). However, the rate of biological decomposition may be limited by a variety of environmental variables, such as dissolved oxygen and temperature (Chang et al. 2015). Especially, temperature is considered as one of the dominant factors affecting the growth and activity of microbes. For example, the metabolism and activities of nitrifying and denitrifying bacteria can be significantly affected by temperature and tend to decrease when the temperature decreases to below 10 °C; nitrifying and denitrification could almost stop completely when the temperature is below 6 °C (Yan & Xu 2014). Therefore, understanding how the microbial activities vary with the temperature variations is critical for both exploring the mechanism of nutrient removal variation under a changing environment and creating effective CWs. However, available studies rarely focused on the change of microbial activities (e.g., urease activity) in CWs under reduced temperatures.

Due to the lack of knowledge as addressed above, four bench-scale CWs were established for nutrient removal in the present study. CWs were cultivated with polyculture (Canna indica + Lythrum salicaria), Canna indica monoculture, Lythrum salicaria monoculture and unplanted control, respectively. The specific objectives of this study were: (1) to examine the differences in the treatment performance between unplanted control, monoculture and polyculture; (2) to clarify the variations of removal efficiencies and rhizospheric microbial activity in CWs with the decreasing environmental temperature (average temperature from 25.5 to 8.9 °C). This study could provide useful information for enhancing the efficiency of CWs, as well as future design, application, and operation management of CWs in cold regions like Northeast China.

**MATERIAL AND METHODS**

**Experimental setup**

Four batch flow CWs with identical dimensions (described below) were established in the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China. This geographical region belongs to north temperate zone continental climate with four distinct seasons. The schematic diagram of CWs is given in Figure 1. They were rectangular tanks made of Plexiglas, with dimensions of 0.98 m long, 0.56 m wide and 0.5 m deep. Each CW included an inlet section (0.16 m long), operating section (0.66 m long), and outlet section (0.16 m long). Three sampling ports (a distance of 10 cm from bottom) were evenly distributed horizontally along the operating section to allow for the collection of effluent water samples. The inlet and outlet section were filled with gravel with a diameter of 24.8 ± 5.6 mm at a thickness of 40 cm. The gravel (a diameter of 15.0 ± 2.0 mm) as substrate was placed in the operating section with a thickness of 40 cm, and a 10-cm depth layer of typical black soil in Northeast China was placed on top of the substrate layer. Two plant species, Canna indica and Lythrum salicaria, were used in this study. Canna indica is not an original native species in Northeast China; however, it has been proved to be safe for local ecosystem and has been already used in many landscape gardening and ecological engineering operations in Northeast China (Liang et al. 2017). Lythrum salicaria is a commonly observed native plant species in Northeast China (Wang et al. 2006). The vegetation of the four CWs was as follows: W1 was cultivated without vegetation (unplanted control); W2 was cultivated with Canna indica monoculture; W3 was cultivated with Lythrum salicaria monoculture; W4 was cultivated with 50% of Canna indica and 50% of Lythrum salicaria (polyculture). All CWs with vegetation were cultivated at a density of 24 seedlings m⁻². Seedlings of Lythrum salicaria and Canna indica were initially collected from the nature wetlands in Sanjiang Plain and the Beihu Park in Changchun, China, respectively in 2016. Seedlings of both plants were grown in hydroponic...
solution until they were big enough (approximately 30 cm height) to be transplanted into CWs.

**Experimental operation**

The synthetic wastewater was prepared by dissolving NH$_4$Cl, KNO$_3$, KH$_2$PO$_4$, CaCl$_2$, and MgSO$_4$ in tap water. The CWs were operated intermittently and the hydraulic retention time (HRT) was 7 days for all experiments. Each wetland system was fed with 60 L synthetic wastewater on Day 1 of each operation cycle. The formal experiments were carried out after the vegetation had grown for 30 days within each respective CW for acclimation. Experiment 1 was designed to test the effect of vegetation types on CW efficiency. While, Experiment 2 was designed to highlight the correlations between CW performance and variations in temperature. The compositions of the synthetic wastewater employed in Experiments 1 and 2 are listed in Table 1. In Experiment 2, performance of CWs was tested under three temperature regimes, T1 (average of 25.5 °C), T2 (average of 19.8 °C), and T3 (average of 8.9 °C), as illustrated in Figure 2.

**Sample collection**

Samples of wastewater were collected daily during all the experiments. Three water samples were collected from each respective sampling port in each CW, and were mixed together to form a composite water sample.

<table>
<thead>
<tr>
<th>Nutrient concentrations (mg/L)</th>
<th>Experiment</th>
<th>NH$_4$-N</th>
<th>NO$_3$-N</th>
<th>TN</th>
<th>TP</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>27.5</td>
<td>30.1</td>
<td>68.0</td>
<td>6.7</td>
<td>6.67</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>27.5</td>
<td>30.3</td>
<td>68.9</td>
<td>8.5</td>
<td>6.78</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1** | Diagram of the experimental unit.

**Figure 2** | Variations in the daily environmental temperatures of the three temperature regimes tested in Experiment 2.
Rhizosphere soil samples were collected from each CW at each temperature regime during Experiment 2 to investigate the influence of temperature on microbial activity. Five rhizosphere soil samples were collected from the four corners and the center of each CW, and were then mixed together to form a composite sample. After removing the roots and macrofauna, field-moist samples were sieved (<2 mm), and immediately placed in separate plastic Ziploc bags. Samples were stored at 4 °C for further analyzing of urease activity.

Analytical methods

The parameters, such as NH$_4^+$-N, NO$_3^-$-N, total nitrogen (TN) and total phosphorus (TP), were measured using an automatic chemical analyzer (Mode Smartchem 200, Italy) after corresponding standard pretreatment and reagent addition according to standard methods (Ju et al. 2014). A recent study had indicated that enzymes were responsive to the intensity and direction of biological activities in CWs (Huang et al. 2012). Urease activity was proved to be related to the nitrogen removal in CWs (Huang et al. 2012). Hence, urease activity was selected as an indicator to investigate the microbial activity in CWs under different temperature regimes. Urease activity was measured by incubating 5 g of soil with 10 mL of 10% urea solution and 20 mL of citrate buffer (pH at 6.7) for 24 h at 37 °C. The formation of ammonium was determined spectrophotometrically at 578 nm by using an automatic chemical analyzer (TU-1901, China) and the activity was expressed as mg NH$_4^+$-N g$^{-1}$ soil, as described by Zhou et al. (2017). SPSS 19.0 (IBM Corporation, 2012, USA) statistical package for Windows was used for data analysis. Linear regressions between the urease activity and nitrogen removal percentages were carried out with Pearson bivariate correlations. All figures were prepared using the Origin 9.0 statistical packages.

RESULT

Effect of vegetation types on efficiency of CWs

As illustrated in Figure 3, the performance of CWs with different vegetation types against HRT was investigated. In general, the removal percentages of NH$_4^+$-N increased with the extension of HRT, and the greatest removal percentages

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![Figure 3](https://iwaponline.com/wst/article-pdf/77/3/829/213037/wst077030829.pdf)

Figure 3 | Removal efficiencies of CWs cultivated with different plant species; HRT represents hydraulic retention time; W1: unplanted control, W2: monoculture (Canna indica), W3: monoculture (Lythrum salicaria), W4: polyculture (Canna indica + Lythrum salicaria).
were observed on Day 7 in all CWs. On Day 7, the removal percentages of NH$_4^+$-N in W1, W2, W3 and W4 were 88.4%, 92.8%, 95.62% and 98.7%, respectively. Meanwhile, the polyculture showed better removal efficiency as compared to monoculture or unplanted control, and it took only 5 days to reach approximately 98.7% of the NH$_4^+$-N removal percentage. Analogously, the polyculture also showed better performance for TN removal as compared to W1, W2 and W3. The removal percentage of TN in W4 was 98.5%, which was 10.4, 3.3 and 2.3 percentage points greater than those in W1 (88.1%), W2 (95.2%) and W3 (96.2%), respectively. Overall, polyculture showed the best performance in the removal of NH$_4^+$-N and TN. Comparing the removal of NO$_3^-$-N in four CWs (Figure 3(b)), CWs cultivated with vegetation showed higher removal efficiency (took only 1 day to completely remove NO$_3^-$-N in the water) and there was not much difference between W2 (99.8%), W3 (99.8%) and W4 (100.0%). In contrast, the removal percentages of NO$_3^-$-N in W1 (92.5%) was lower than the CWs with vegetation. It could be concluded that CWs cultivated with vegetation obviously enhanced the removal of NO$_3^-$-N. In the reduction of TP, the removal percentages in W1 (77.0%), W2 (79.7%) and W3 (91.9%) were 15.7, 13.0 and 0.8 percentage points lower than that in W4 (92.7%), respectively. Polyculture substantially improved the reduction of TP in CWs. The above results indicated that the NH$_4^+$-N, TN and TP removal were enhanced by the polyculture (Canna indica and Lythrum salicaria) as compared to the monoculture culture and unplanted control, demonstrating that the polyculture can be used in CWs for improving the treatment of nutrient.

**Effect of temperature on CWs**

**Effect of temperature on removal efficiency of CWs**

The removal percentages of NH$_4^+$-N, NO$_3^-$-N, TN and TP in CWs under different temperatures are shown in Figure 4. When the temperature decreased from T1 to T3, the removal percentages of NH$_4^+$-N in W1, W2, W3, and W4 decreased from 98.7% to 88.3%, from 98.2% to 90.9%, from 99.6% to 89.1%, and from 99.6% to 92.7%, respectively. Higher and stable removal percentages of NO$_3^-$-N in W1, W2, W3, and W4, which were around 92.4%, 99.6%, 99.6%, and 99.9%, respectively, were achieved at T1 and T2. At T3, however, the removal percentages of NO$_3^-$-N in W1, W2, W3, and W4 decreased to only 59.7%, 79.2%, 87.5%, and 87.1%, respectively. The removal percentages of TN in W1, W2, W3, and W4 decreased from 88.8% to 75.3%, from 97.8% to 85.5%, from 97.8% to 88.4%, and from 83.3% to 79.2%.
98.6% to 86.9%, respectively, through T1 to T3. The removal percentages of TP in W1, W2, and W3 decreased from 97.3% to 76.5%, from 98.5% to 83.5%, and from 98.7% to 90.8%, respectively, through T1 to T3. However, the removal percentages of TP in W4 were comparatively stable (from 95.4% to 92.9%, through T1 to T3). Overall, the performance of CWs was obviously influenced by temperature, and the polyculture still exhibited best performance in the removal of nitrogen at relatively higher temperatures (e.g., T1 and T2). Additionally, polyculture also showed good performance for the treatment of TP even when the temperature dropped to T3. It could be concluded that the polyculture may be ideally suited for enhancing the reduction of TP, especially at low temperature of about 8.9 °C.

**Effect of temperature on microbial activity**

The variation of urease activity under different temperature regimes is illustrated in Table 2. When the temperature decreased from T1 to T3, the urease activities in W1, W2, W3 and W4 decreased from 0.27 to 0.11 mg NH₄⁺-N g⁻¹ soil, from 0.40 to 0.28 mg NH₄⁺-N g⁻¹ soil, from 0.51 to 0.33 mg NH₄⁺-N g⁻¹ soil, and from 0.73 to 0.36 mg NH₄⁺-N g⁻¹ soil, respectively. These results suggested that variations in temperature had a substantial influence on urease activity in CWs. Meanwhile, there was significantly positive correlation between urease activity and the reduction of TN in W2 ($R^2 = 0.995$, $P = 0.03243$). Similarly, significantly positive correlation between urease activity and the reduction of NO₃⁻-N was observed in W5 ($R^2 = 0.997$, $P = 0.02235$) (Figure 4(b) and 4(c), and Table 2). Therefore, it could be concluded that the urease activity was significantly related to the reduction of nitrogen in the monoculture of *Canna indica* or *Lythrum salicaria*. We might speculate that the reduction of nitrogen in polyculture was not only related to the activity of microorganism but also related to other impact factors, such as the activity of vegetation.

**DISCUSSION**

Vegetation is an important component of CWs and could contribute to the purification of nutrients, such as nitrogen and phosphorus (Ge et al. 2016). The contribution of vegetation to nitrogen removal in CWs varies with different designs and operation conditions of CWs, because the nitrogen removal process in CWs can be affected by various factors, e.g., the hydraulic condition of CWs, the type of substrate, the wetland plant species, the growth of plants, and the influent load (Allen et al. 2013; Nivala et al. 2015; Zhu et al. 2014; Liang et al. 2017). Although the contribution to nitrogen removal by various pathways remains unclear, plant uptake could usually contribute to at least 10% of the nitrogen removal in CWs based upon available literatures. For example, Chan et al. (2008) proved that plant uptake could account for 10–15% of nutrient (N and P) removal in CWs. In this study, a substantial enhancement of NH₄⁺-N, NO₃-N, TN and TP removal in CWs with vegetation was observed in comparison with unplanted control, and plant uptake accounted for approximately 10–15% of the nutrient removal, by comparing the nutrient removal percentages in CWs with vegetation and unplanted control (Figure 3). These findings were in agreement with the results found in previous studies (Chan et al. 2008). In addition to direct uptake, plant tissue also provided more surfaces for bacteria growth. Bastviken et al. (2005) reported that bacteria were much more abundant when grown attached to surfaces than when suspended in water. Besides providing necessary surfaces for bacteria growth, the plant root not only provided oxygen for complete nitrification, but also released organic carbon as a carbon and energy source for heterotrophic bacteria, such as the denitrifying bacteria (Bastviken et al. 2005; Akratos & Tsihrintzis 2007). Therefore, CWs cultivated with vegetation showed higher removal efficiency of NO₃-N.

It has been hypothesized that combining different plant species in CWs could improve the treatment efficiency of CWs by means of functional complementarity (Fraser et al. 2004; Qiu et al. 2011; Rodriguez & Brisson 2016). Liang et al. (2011) proposed that the pollutant removal efficiencies of polyculture might be better than in monoculture CWs because of temporal and spatial compensations in seasonal plant activity, root affinity for microorganism colonization and ability to take up nutrients. Our results

**Table 2** Variations of urease activity in rhizosphere soil under different temperatures

<table>
<thead>
<tr>
<th>Urease activity (mg NH₄⁺-N g⁻¹ soil)</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.27</td>
<td>0.40</td>
<td>0.51</td>
<td>0.73</td>
</tr>
<tr>
<td>T2</td>
<td>0.18</td>
<td>0.37</td>
<td>0.48</td>
<td>0.54</td>
</tr>
<tr>
<td>T3</td>
<td>0.11</td>
<td>0.28</td>
<td>0.33</td>
<td>0.36</td>
</tr>
</tbody>
</table>

also showed a substantial enhancement in the removal of nutrient in polyculture wetlands as compared to individual culture of *Canna indica* or *Lythrum salicaria* (Figures 3 and 4). The higher nutrient removal efficiency observed in the polyculture compared with the monoculture wetlands might be attributed to the functional complementarity between *Canna indica* and *Lythrum salicaria* over the course of the experiment. It was observed that the growth period of *Lythrum salicaria* began and finished earlier than *Canna indica*. Also, the height of *Canna indica* and *Lythrum salicaria* was also complementary for the better use of the sunlight (Figure 5). Therefore, it could be concluded that functional complementarity between *Canna indica* and *Lythrum salicaria* existed and promoted the nutrient removal efficiency in CWs. In addition, the stronger rhizosphere in polyculture can also stimulate microorganism activity (Kumari & Tripathi 2014; Peng et al. 2014). The urease activities in the polyculture (W4) tended to be greater than those in the monoculture wetlands as observed in this current study (Figure 5). Therefore, polyculture showed better performance as compared to monoculture and unplanted control. It is noteworthy that the pathways of nutrient (especially nitrogen) removal in CWs are complicated. In addition to plant uptake and nitrification, there are other pathways like sediment storage and volatilization occurring under specific conditions (Wu et al. 2013). The pH of influent water in this study was below 7 (Table 1); therefore, the volatilization might be very limited. Since the substrate and structure of CWs were all the same, the better performance of CW with polyculture could be attributed only to the functional complementarity between *Canna indica* and *Lythrum salicaria* in terms of both plant uptake and a better microorganism activity in rhizosphere, as discussed above. Previous studies have revealed that the removal percentages of NH\(_4\)-N, TN, and TP in winter (1-5 °C) were lowered by 12-40%, 12-27% and 6-34%, respectively, compared to those observed in summer (Ouellet-Plamondon et al. 2006; Song et al. 2006; Taylor et al. 2011). However, the influence of temperature on efficiency of CWs might vary with different design of CWs. Therefore, it is very important to quantify the influence of temperature on the performance of different CWs, thereby evaluating the capacity of CWs with specific design (e.g., polyculture) for resisting low temperature. As shown in Figure 4 and Tables 1 and 2, a positive relation was observed between the performance of CWs, urease activity and temperature, indicating that low temperatures had an obviously adverse impact on the performance of CWs and urease activity. This is consistent with the findings of a previous study (Ouellet-Plamondon et al. 2006). It was also reported that the metabolism and activity of denitrifying bacteria tended to decrease, when temperature fell below 10 °C, and the process of denitrification was almost stopped when temperature dropped to below 6 °C (Yan & Xu 2014). It was no wonder that the removal percentages of NO\(_3\)-N and TN were relatively stable during most of the operation time (average temperature above 19.8 °C), while a rapid decrease of NO\(_3\)-N and TN removal happened at T3 (average temperature of 8.9 °C) in this current study. For the reduction of TP, rapid decline of removal percentage in unplanted control was observed at T2 and T3 (Figure 4(d)), which may be caused by the reduced activity of phosphate-accumulating organisms (PAOs) as reported by Yan & Xu (2014). However,
the removal percentage of TP in CWs with polyculture was relatively stable and only lowered by 2.4 percentage points at T2 and T3 (Figure 4(d)); this might be due to the functional complementarity and the root exudates released from root, which stimulated the activity of PAOs (Headley et al. 2013). Although the dominant pathway for phosphorus removal in CWs might be sediment storage and/or substrate adsorption (Liang et al. 2017), the contribution of plant uptake and microbial action could not be ignored. Therefore, the polyculture still showed relatively high removal percentages of TP at a relatively low temperature. All in all, more specific research regarding the reduction of nutrient at low temperature is recommended in future studies for further revealing the mechanisms of plant influence.

CONCLUSION

Whether polyculture is more beneficial than monoculture for nutrient removal in CWs has been a controversial subject. In this current study, a comprehensive investigation was conducted to evaluate the effectiveness of CWs with different vegetation including both polyculture and monoculture under declining environmental temperatures. A substantial enhancement of nitrogen and TP removal in polyculture was observed as compared to unplanted control or monoculture of Canna indica or Lythrum salicaria. Both the decontamination of nutrient and the microbial activity in CWs were distinctly influenced by the decline of temperature. Compared with monoculture and unplanted control, the polyculture still showed better nutrient removal efficiency when the average environmental temperature dropped to 19.8 °C. All above results demonstrated that polyculture was an efficient way to enhance the purification of nutrients under most temperature regimes. It could be concluded that CWs with polyculture would be a good strategy for nutrient treatment in rural regions of Northeast China.

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