Correlation among soil microorganisms, soil enzyme activities, and removal rates of pollutants in three constructed wetlands purifying micro-polluted river water

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ABSTRACT

Three pilot horizontal subsurface flow constructed wetlands (CWs) were constructed to purify micro-polluted waters in Chongqing, P.R. China. The quantity of soil microorganisms (bacteria, fungi, and actinomycetes) and enzyme activities (dehydrogenase, catalase, and urease) were monitored, and they together formed the soil biological indicators in the experimental CWs. All three CWs had acceptable removal abilities for pollutants and were adaptable to water quality fluctuations. The removal rates of pollutants, number of soil microbes, and activities of soil enzymes in planted wetland were higher than those in unplanted wetland. There were significant correlations between the removal of total nitrogen (TN-N) \((p < 0.01)\) and temperature, as well as the number of soil microbes \((p < 0.01)\) and activities of soil enzymes \((p < 0.05)\). The 1 canonical variables can be used to represent the most information of the soil biological indicators in the three CWs. There were good linear relationships between the canonical variables of microbes and enzymes, and positive correlations could be observed in the planted CWs. The soil biological indicators in the planted CWs can be divided into two the same classes by the cluster analysis method, whereas there were two kinds of classification in the unplanted CW including two or three classes. Hydrophytes planted in the CWs can help make the relationships among the soil biological indicators closer. Soil biological activity can be improved more greatly by planting Arundo donax in summer and planting Acorus calamus in winter. There were negative correlations between the removal of ammonium \((\text{NH}_4^+ - N)\) and soil biological indicators in all the three CWs, as well as the permanganate index \((\text{COD})\) and total phosphorus \((\text{TP-P})\), but the soil biological indicators did not have obvious relationships with the removal of phosphorus or carbon in all CWs \((p > 0.1)\). The relationship between the removal of TN-N and soil biological indicators was very significantly positive in the planted CWs \((p < 0.05)\), and positive in the unplanted CW. Soil enzyme activity had a more significant relationship with the removal rate of TN-N than soil microorganism.

1. Introduction

Wetlands are defined as a half-way world between terrestrial and aquatic ecosystems, and exhibit some of the characteristics of each (Smith, 1980). The proven water purification capability of wetlands has encouraged engineers and scientists to construct artificial wetland systems to take advantage of this ability (Scholz and Lee, 2005). Constructed wetlands (CWs) are engineered wastewater treatment systems that encompass a plurality of treatment modules, including biological, chemical, and physical processes, which are all akin to processes occurring in natural treatment wetlands (Babatunde et al., 2008).

Currently, a wide range of wastewaters from domestic (Bahgat et al., 1999; Decamp and Warren, 2001; Nurk et al., 2005), industrial (Calheiros et al., 2007; Tao et al., 2007), and agricultural (Nguyen, 2000; Tanner et al., 2002) origins, and even landfill leachate (Kozub and Liehr, 1999; Sundberg et al., 2007) can be treated by CWs, which is an efficient ecological system with low maintenance requirements and construction costs (Inamori et al., 2007). In recent years, CWs have been introduced as a cost-efficient alternative to conventional technologies for micro-polluted surface and groundwater (Wu et al., 2011; Lin et al., 2008; Braeckeveld et al., 2008). The purification performance of CWs is mostly attributed to the combined functions of microbes and filtering materials, which may be complemented by plants (Truu et al., 2009). Some recent studies have reported that enzymes in wetland systems play very important roles in matter transformation (Shackle et al., 2000; Reboreda and Caçador, 2008; Zhou et al., 2005). Enzyme activity is one way of describing the general condition of soil microbial populations.
Enzymes are responsive to the intensity and direction of biological activities in CWs (Liang et al., 2003). The mineralization of organic matter is mainly carried out by microbes both under aerobic and anaerobic conditions. Nitrogen removal in CWs has mostly been assumed to result from the combination of nitrification and denitrification (Sundberg et al., 2007). Microbes may also play important roles in phosphorous removal (Oehl et al., 2004). There is increasing evidence that microbial properties (e.g., microorganism, enzymes, and basal respiration) can be used as potential indicators of CW management.

We selected a series of CWs that purify micro-polluted waters in southwestern China to study the relationships among microorganisms, enzymatic activities, and removal rate of pollutants. Our aims were to (1) determine the changes in soil biological properties and their relationship with nutrients during micro-polluted water purification in CWs; and (2) investigate the reason and mechanism of pollutant degradation in CWs from soil biological aspects.

2. Materials and methods

2.1. Description of CWs

Three pilot-scale subsurface horizontal flow CWs were constructed in 2008 to treat a micro-polluted river (30°42′16″N, 108°19′33″E) with a warm-temperature monsoonal climate. The climate was characterized by an annual precipitation of 1243 mm and an average temperature of 17.7 °C. All three experimental wetlands (WL1, WL2, and WL3) were built with brick and plastered with concrete 3 m long, 1.0 m wide, 1.0 m deep, and 2% base sloped. Each unit had an inlet, treatment zone, and outlet. The treatment zone was filled orderly with three successive layers of the following substrates: a 20 cm bottom layer of 40–60 cm-diameter washed river gravel, a 30-cm layer of 20–40 cm-diameter washed brick, a 30-cm layer of 10–20 cm-diameter washed river gravel, and a 10 cm layer of local soil at the top. The inlet and treatment zone were separated with perforation tracery to avoid short flow. The treatment zone and outlet were also separated to distribute effluent uniformity. The water levels in the beds were controlled at the bed surface during operation by a vertical standpipe at the effluent end (Fig. 1).

Arundo donax and Acorus calamus were planted in WL1 and WL2 in July 2008, respectively. WL3 was unplanted. The plant densities of the two planted CWs were approximately 12 plants/m². Both native hydrophyte species were transplanted from the bottomland beside the treated river. They were selected because they are very common in the area and also showed efficiency in the accumulation of nutrients and contaminants in CWs.

2.2. Sampling and analysis

The experimental study was performed from July 2010 to January 2011 adopting a continuous operation mode. The hydraulic load of the system during the period was controlled at 1.5 m³/m² day. Soil and water samples were collected once and three times every month during a 7-month operating period. The experiments were conducted in triplicate.

In each CW, three 0.9 m × 1.0 m plots were randomly established. In each plot, four soil sampling profiles were set up. Soil sampling horizons were taken from the left, right, and middle of each plot. Soil sampling profiles were set up with depths of 0.1 m. A total of 36 samples were collected (3 plots × 4 soil sampling profiles × 3 horizons) for each CW. To facilitate analysis, the soil samplings of each CW were completely mixed in the field. Samples were taken to the laboratory and stored at 4 °C. Visible plant and root residues were removed. Fresh soil was divided into two subsamples; one was air-dried for chemical analyses and the other was stored with filter samples at 4 °C for microbiological analyses.

The numbers of bacteria, fungi, and actinomycetes were determined by the serial dilution plate count method (Li et al., 2008). Subsequent dilutions were prepared by manually shaking the suspension for 20 min to resuspend the soil and filter. Then, 1 ml of dilution was transferred using a sterile pipette to 9 ml of sterile disinfected water in a test tube. This suspension was manually shaken for 10 s, and subsequent serial dilutions were similarly prepared with 10⁻⁴–10⁻⁶ for bacteria, 10⁻¹–10⁻³ for fungi, and 10⁻³–10⁻⁵ for actinomycetes, respectively. About 0.2 ml of suspension from each serial dilution was spread over an isolation selective agar medium (beef extract peptone, martin agar, and improved Gao I medium). The bacteria, fungi, and actinomycetes were incubated at 37 °C for 1 day, 28 °C for 3–4 days, and 28 °C for 5 days, respectively.

Soil enzyme activities were assayed in triplicate air-dried pooled samples as described by Li et al. (2008). Dehydrogenase activity (DA) was determined by the reduction of triphenyltetrazolium chloride (TTC) to triphenylformazone (TPF). It was measured by the colorimetric method and expressed in EUdea, where one unit is the amount of enzyme that produces 1 μg TPF/g soil in 6 h at 30 °C. Catalase activity (CA) was measured by the titrimetric method using potassium permanganate and expressed in Vdeq, where one unit is the amount of enzyme that uses 1 ml of 0.002 mol/l potassium permanganate (KMnO₄)/g soil. Urease activity (UA) was measured by the colorimetric method using toluene and 10% urea. UA was expressed in EUura, where one unit is the amount of enzyme that produces 1 mg NH₄⁺/g soil in 3 h at 38 °C.

All water samples were analyzed according to the procedures described in the Standard Method for Examination of Water and Wastewater (S.M., 2002). The physicochemical water parameters, i.e., water temperature, dissolved oxygen (DO), and pH were determined using instruments.

2.3. Data analysis

All experimental data were calculated using Microsoft Excel 2007 and analyzed with PASW Statistics 18.0, including canonical correlation, principal component, and hierarchical cluster analyses. Pearson’s test was used to test the significance level (p < 0.05 and p < 0.01) of the relationship among temperature, number of microbes (bacteria, fungi, and actinomycetes), soil enzyme activities, and removal rates of pollutants in different CWs. Significant differences among the soil characteristics in the three CWs were determined by one-way ANOVA, including soil microorganisms and soil enzyme activities. This process was followed by a Tukey–HSD test when appropriate.

3. Results and discussion

3.1. Purification efficiencies of pollutants in the experimental CWs

The water qualities of the influent and effluent in the experimental CWs are shown in Tables 1a and 1b, respectively. Given that the water quality of the influent greatly changed, the concentrations and removal rates pollutants in the CWs showed certain fluctuations. The average removal rates of NH₄⁺-N in WL1, WL2, and WL3 were 64.3%, 63.7%, and 53.3%, those of TN-N were 48.3%, 28.6%, and 26.2%, those of TP-P were 48.5%, 42.2%, and 38.9%, and those of COD were 23.1%, 23.4%, and 20.1%, respectively. Therefore, the system had acceptable removal ability of pollutants and was adaptable to water quality fluctuations.
Due to low concentrations of organic matter in river water, the number of heterotrophic aerobic bacteria was relatively small compared with high organic matter, indicating that nitrifiers and other autotrophic aerobic bacteria can easily obtain more DO (Wu et al., 2008). When the influent DO was 5.0 mg/L, nitrification was enhanced (Ruiz et al., 2003) and more NH$_4^+$-N was bio-oxidized to nitrate. On the other hand, when denitrification was influenced (Ovez et al., 2006), the removal of TN-N was limited. Low-concentration phosphorus limited the removal of TP-P, and the conditions in natural river water were not suitable for the microorganisms to remove phosphorus because of the high DO. Hence, the removal of TP-P was not entirely caused by the microorganisms in the experimental CWs. Due to the low concentration of COD in raw water, the amount of available organic carbon to microorganisms was small, and the purification of organic matter was adopted by aerobic bacteria with poor nutrition, algae, protozoa, and micro-metazoans (Boeije et al., 2000), where heterotrophic bacteria played an important role in the removal of COD. Thus, the removal of COD in all three CWs was low and there was no significant difference among the three CWs ($p > 0.05$).

Pollutant purification efficiencies can be improved by planting hydrophytes (especially for NH$_4^+$-N removal). The results of the $t$-test for the removal of NH$_4^+$-N were WL1–WL2 ($p > 0.05$), WL1–WL3 ($p < 0.05$), and WL2–WL3 ($p < 0.05$). These results indicated that there were significant differences between the planted CWs and unplanted CW, but not between the two planted CWs. Given the small grain diameter of the filler in the CWs, the interception of media was improved to help remove particulate pollutants. With the absorption of the filler and hydrophyte, the dissolved pollutants decreased in the system. In both planted CWs, the removal rates of the other pollutants were almost the same, except for TN-N. Both A. donax and A. calamus had strong and well-developed roots that provided good conditions for nitrifier and denitrifier, where nitrification was enhanced, thus resulting in reduced NH$_4^+$-N. Compared with A. calamus, A. donax had well-developed parts in the ground, which helped promote the absorption of nitrogen and phosphorus. To the removal of TN-N, there must be very significant differences between WL1 and WL2 ($p < 0.01$). Therefore, the removal rate of TN-N was higher in WL1, but the absorption ability of hydrophyte to TP-P was limited because of the low influent concentration. The results of the $t$-test for the removal of TP-P showed no significant difference among the three CWs ($p > 0.05$). In all three pilot-scale CWs, there was no significant correlation between the removal of pollutants and temperature, except for TN-N. However, there was a significant correlation between the removal of TN-N and temperature in WL1 ($r = 0.857$, $p < 0.05$), but not between WL2 ($r = 0.571$, $p > 0.05$) and WL3 ($r = 0.286$, $p > 0.05$).

### 3.2. Number of soil microbes

Soil microbes are the most important components of CWs. They play key roles in material cycles and purification in wetlands. C, N, and P are nutrient materials for soil microbes, and are also necessary nutrient elements for hydrophyte growth in CW. Soil microbes use pollutants in water by participating in various reactions (e.g., mineralization and assimilation, oxidation and deoxidization, and other processes). Hence, the species and number of microbes are significant in the removal of nitrogen and phosphorus pollutants. Fig. 2a–d, respectively showed that the numbers of soil bacteria, fungi, actinomycyes, and total microbes in both planted CWs were

**Table 1a**

<table>
<thead>
<tr>
<th>Influent–effluent</th>
<th>$T$ (°C)</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>NH$_4^+$-N (mg/L)</th>
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<tbody>
<tr>
<td>Influent</td>
<td>6.75 ± 0.35–29.15 ± 1.72</td>
<td>7.61 ± 0.10–7.73 ± 0.10</td>
<td>4.55 ± 0.66–7.07 ± 1.79</td>
<td>0.38 ± 0.10–1.35 ± 0.11</td>
</tr>
<tr>
<td>WL1</td>
<td>6.75 ± 0.21–28.8 ± 1.56</td>
<td>7.31 ± 0.20–7.42 ± 0.04</td>
<td>1.12 ± 0.24–2.29 ± 0.68</td>
<td>0.18 ± 0.01–0.42 ± 0.22</td>
</tr>
<tr>
<td>WL2</td>
<td>6.60 ± 0.00–28.94 ± 1.91</td>
<td>7.39 ± 0.08–7.50 ± 0.16</td>
<td>1.35 ± 0.16–4.33 ± 1.51</td>
<td>0.10 ± 0.09–0.42 ± 0.22</td>
</tr>
<tr>
<td>WL3</td>
<td>6.65 ± 0.07–29.01 ± 1.06</td>
<td>7.55 ± 0.13–7.64 ± 0.09</td>
<td>2.68 ± 0.47–5.82 ± 1.46</td>
<td>0.14 ± 0.12–0.45 ± 0.18</td>
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**Table 1b**

<table>
<thead>
<tr>
<th>Influent–effluent</th>
<th>TN-N (mg/L)</th>
<th>TP-P (mg/L)</th>
<th>COD (mg/L)</th>
</tr>
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<tbody>
<tr>
<td>Influent</td>
<td>0.94 ± 0.27–3.75 ± 0.87</td>
<td>0.050 ± 0.001–0.099 ± 0.011</td>
<td>4.84 ± 0.37–6.82 ± 0.03</td>
</tr>
<tr>
<td>WL1</td>
<td>0.21 ± 0.13–2.27 ± 0.81</td>
<td>0.027 ± 0.001–0.063 ± 0.016</td>
<td>3.54 ± 0.11–5.52 ± 0.05</td>
</tr>
<tr>
<td>WL2</td>
<td>0.46 ± 0.19–3.02 ± 0.48</td>
<td>0.029 ± 0.001–0.056 ± 0.023</td>
<td>3.55 ± 0.10–5.42 ± 0.01</td>
</tr>
<tr>
<td>WL3</td>
<td>0.51 ± 0.48–2.76 ± 0.17</td>
<td>0.033 ± 0.007–0.055 ± 0.008</td>
<td>3.85 ± 0.05–5.34 ± 0.01</td>
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**Fig. 1.** Schematic diagram of a subsurface horizontal flow constructed wetland.
higher than those in WL3. In soil microbe biota, the number of bacteria was the highest, i.e., more than 84%, 89%, and 89% of the soil total microbes in WL1, WL2, and WL3, respectively. Actinomycetes followed and the least were fungi. The numbers of soil microbes in the experiment were lower than those reported in wastewater treatment (Sleytr et al., 2007; Truu et al., 2009). The low concentration of pollutants was the main reason for the low number of soil microbes.

The number of soil bacteria was significantly different among all CWs (Fig. 2a). The highest number of soil bacteria was in WL2, which was significantly higher than in the other CWs. In WL1 and WL3, the numbers of soil bacteria decreased with decreased temperature and reached the lowest quantity in winter, which was significantly lower than summer and autumn, i.e., only 71% and 62% of the numbers of soil bacteria in summer. In WL3, the quantities of soil bacteria in summer were almost similar to those in winter. There were significant positive correlations between soil bacteria and temperature in WL1 ($r = 0.958$, $p < 0.01$) and WL3 ($r = 0.969$, $p < 0.01$), but not in WL2 ($r = 0.698$, $p > 0.05$).

Fungi play an important role in organic matter decomposition. The number of soil fungi was the highest in WL2 (Fig. 2b) and was significantly higher than in the other CWs. We conclude that the main reasons were that the iris rhizomes of A. calamus were developed, and the well-developed underground parts in WL2 inherited large quantities of plant debris. The microclimate in WL2 planted with A. calamus also suited the growth of soil microbes. The number of fungi in the planted CWs was higher than in the CW without hydrophyte, and the number of soil fungi in WL3 did not dramatically change with the seasons, unlike in WL1 and WL2. These results indicated that the number of soil fungi was influenced by the planting of hydrophyte in the CWs. Fig. 2b also shows that the number of soil fungi decreased with decreased temperature and reached the lowest quantity in winter. There were significant positive correlations between the number of soil fungi and temperature in WL1 ($r = 0.979$, $p < 0.01$), WL2 ($r = 0.981$, $p < 0.01$), and WL3 ($r = 0.936$, $p < 0.01$).

Actinomycetes are widely distributed. They are a major group that maintain the dynamic balance of biological communities in CWs. Actinomycetes also play important roles in organic matter and nitrogen decomposition. However, the numbers of soil actinomycetes in the planted CWs were higher than in the unplanted CW, and the number of soil actinomycetes in WL1 was the highest among the three experimental CWs, different from bacteria and fungi (Fig. 2c). According to the t-test results, there were very significant differences between WL1 and WL2 ($p < 0.01$), between WL1 and WL3 ($p < 0.01$), as well as between WL2 and WL3 ($p < 0.01$). Similar to bacteria and fungi, the number of actinomycetes decreased with decreased temperature and reached the lowest quantity in winter. There were significant positive correlations between the number of soil fungi and temperature in WL1 ($r = 0.943$, $p < 0.01$), WL2 ($r = 0.910$, $p < 0.01$), and WL3 ($r = 0.891$, $p < 0.01$). The trend of the number of total microbes was similar to that of the number of bacteria due to the domination of soil microbes in all CWs (Fig. 2d).

3.3. Soil enzymes activities

Soil enzyme activity has been proposed to be an important determinant factor of soil quality. As the first enzyme to catalyze the dehydrogenation of organic matter in electron transport systems, dehydrogenase plays a key function in organic matter degradation (Chaperon and Sauvé, 2007). DA is a useful indicator of overall microbial activity of soils (Tam, 1998; Megharaj et al., 1999). Hydrogen peroxide widely exists in the plant’s body and soil, and has toxic action to biology and soil. Fungus, bacterium and plant root in the soil can secrete catalase to relieve poisoning effect of hydrogen peroxide (Wan et al., 2008). There were
relationships between CA and microbe activities, and CA reflects the strength of the process of soil microbiology (Li et al., 2008). Urease plays a key role in hydrolysis of urea-like substrates into NH₄⁻-N and CO₂. NH₄⁺ is the direct origin of nitrogen, and this enzyme activity characterizes nitrogen pollutants (Liang et al., 2003).

DA, CA and UA in planted CWs were higher than unplanted CW (Fig. 3), and enzymes activity decreased with the decrease of temperature. The selected soil enzymes activities were extremely significantly different between WL2 and WL3 (p < 0.01), and between WL1 and WL2 (p < 0.05). There were very significant differences between WL1 and WL3 to DA (p < 0.01) and UA (p < 0.01), except CA (p > 0.05).

DA in WL1 was higher than that in WL2, while there was not a significant difference between them (p < 0.01) (Fig. 3a). DA in unplanted CW was the lowest in all CWs, which could be promoted by planting hydrophyte. A large amount of pollutants from river water was used for the fast growth of the hydrophyte. Organic compounds containing carbon and nitrogen were degraded to small molecule organic compounds and ammonia by dehydrogenation of dehydrogenase in CWs. With temperature decreasing from 28–29°C to 6–7°C, DA rapidly declined and reached the lowest in winter. Like microbes, the correlation between DA and temperature reached a significant level in WL1 (r = 0.821, p < 0.05), WL2 (r = 0.821, p < 0.05) and WL3 (r = 0.893, p < 0.01).

The average values of CA in planted CWs were similar to that in unplanted CW (Fig. 3b). It suggested that hydrophyte in CWs made little effect to CA. There were relationships among CA, soil respiration intensity and microbe (Li et al., 2008). Although the number of microorganisms in three CWs was different, order of magnitudes was same. On the other hand, hydrogen peroxide produced from hydrophyte planted in CWs treating micro-polluted water was proved to be slightly (Xu et al., 2010, 2011). Therefore, CA in all experimental CWs was almost same. Taking into account the impact of temperature on CA, correlation between CA and temperature was analyzed and the results showed that the correlation was very significant in all CWs (r > 0.9, p < 0.01).

Urease can promote hydrolyzation of protein from plant organisms, and ammonia produced from hydrolyzation is a direct nutrient source of plant growth. Thus, UA can indicate the condition of nitrogen pollutants. UA in planted CW was higher than unplanted CW (Fig. 3c), because UA could be promoted by the well-developed roots, stems and leaves of A. donax and A. calamus. Hydrophyte planted in CWs could strengthen the hydrolysis of organic N compound by stimulating the secretion of urease. Urease was also affected by temperature, and the correlation between them was very significant in WL2 (r = 0.893, p < 0.01), and significant in WL1 and WL3 (r = 0.821, p < 0.05).

3.4. Soil biological characteristics of the CWs

CW soil can provide not only a large number of different species of microorganisms, protozoa, and metazoa, but also good conditions for the survival of hydrophytes. The soil biological characteristics of CWs may include activity, distribution, as well as influence on microbes, plant roots, and soil enzymes. In this manuscript, the relationships between the microbial quantities and enzyme activities in the CW soils were discussed and analyzed by statistical methods.

According to the canonical correlation analysis of the number of microbes and enzyme activities in the three CWs, the I canonical correlation coefficient was the highest among the three in each CW (0.994, 0.992, and 0.998). There were significant correlations between microbes and enzymes in CWs as revealed by Wilk-Lambda and Chi-square tests (Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Wilk-Lambda and Chi-square tests of canonical correlation coefficients.</th>
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<tr>
<td>CWs</td>
<td>Correlation coefficients</td>
</tr>
<tr>
<td>WL1</td>
<td>0.994</td>
</tr>
<tr>
<td>WL2</td>
<td>0.992</td>
</tr>
<tr>
<td>WL3</td>
<td>0.998</td>
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</table>

*Correlation was significant at the 0.05 level.

Three pairs of canonical variables were identified, i.e., U1 and V1 to WL1, U2 and V2 to WL2, as well as U3 and V3 to WL3, where \( U \) was a canonical variable of microbe, \( V \) was a canonical variable of enzyme activity, \( X_1 \) was bacteria, \( X_2 \) was fungi, \( X_3 \) was actinomycetes, \( Y_1 \) was dehydrogenase, \( Y_2 \) was catalase, and \( Y_3 \) was urease. The formulas of the canonical variables are shown in Table 3.

According to the canonical correlation coefficients shown in Table 3, there were positive correlations between \( U \) and bacteria in WL2 and WL3, and a negative correlation in WL1. These correlations were opposite to those for fungi. The correlations between \( U \) and actinomycetes were negative in all CWs, where the correlation coefficient was the highest. There were negative correlations between \( V \) and dehydrogenase in WL2 and WL3, and a positive correlation in WL1. These correlations were opposite to those for catalase. The correlations between \( V \) and urease were negative in all CWs, where the correlation coefficient was the highest in WL1 and WL2. In WL3, the correlation coefficient of dehydrogenase was the highest. Canonical redundancy rate analysis showed that the canonical variables can explain more than 65% variability of the number of microorganisms and enzyme activities in the three CWs. These results indicated that the I canonical variable can be used to represent the most information of the soil biological indicators.

The relationship between the canonical variables of the microbe and enzyme (\( U \) and \( V \)), was suggested from experimental data on the number of microorganisms and soil enzyme activity derived from the formulas of \( U \) and \( V \) in the different CWs (Fig. 4).

There were good linear relationships between \( U \) and \( V \), and the relationship was positively correlated in the planted CWs, where the canonical variables of the soil enzymes decreased with decreased canonical variables of microbes. Hence, the relationship between microorganisms and soil enzyme activity was improved by planting hydrophytes in the CWs. When \( V \) changed from –5 to –30, there was little change to \( U \) in WL3 (10–15), different from those in the planted CWs. There was another difference between the planted CW and unplanted CW. The canonical variable of the microbes was negative in the planted CW, whereas the canonical variable of the microbes was positive in the unplanted CW. In both planted CWs, the variation range of \( U \) in WL1 was larger than that in WL2, but the variation range of \( V \) was bigger in WL2 than in WL1. The number of microbes can be improved by planting A. donax, whereas soil activity can be stimulated by planting A. calamus.

Hierarchical cluster analysis with squared Euclidean distance was used to indicate the two soil biological characteristics, and the tree diagrams of the three CWs are shown in Fig. 5.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Formulas of canonical variable and canonical redundancy rate analyses.</th>
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<tbody>
<tr>
<td>CWs</td>
<td>Formulas of canonical variable</td>
</tr>
<tr>
<td>WL1</td>
<td>( U_1 = 10^4 \times (0.394 X_1 – 0.382 X_2 – 0.993 X_3) )</td>
</tr>
<tr>
<td></td>
<td>( V_1 = 0.405 Y_1 – 0.460 Y_2 – 0.290 Y_3 )</td>
</tr>
<tr>
<td>WL2</td>
<td>( U_2 = 10^4 \times (0.010 X_1 – 0.300 X_2 – 0.067 Y_3) )</td>
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<tr>
<td></td>
<td>( V_2 = 0.407 Y_1 + 0.334 Y_2 – 0.927 Y_3 )</td>
</tr>
<tr>
<td>WL3</td>
<td>( U_3 = 10^4 \times (1.275 X_1 – 0.322 X_2 – 1.886 X_3) )</td>
</tr>
<tr>
<td></td>
<td>( V_3 = 0.721 Y_1 + 0.523 Y_2 + 0.415 Y_3 )</td>
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</tbody>
</table>
Although the tree diagram of WL1 differed from that of WL2, the result of the cluster analysis was similar according to the cluster analysis divided into two classes (Fig. 5a and b). Actinomycetes, fungi, and CA were formed one class; and bacteria, DA, and UA formed another. Compared with WL1 and WL2, the cluster analysis of WL3 had two classifications (Fig. 5c). First, actinomycetes and fungi formed one class; bacteria, DA, and CA formed a second class; and UA was the last class. Another classification was UA being on a class of its own, and the remaining soil biological indicators comprised another class.

The differences between the planted and unplanted CWs indicated that hydrophytes planted in CWs can help make the relationship among soil biological indicators similar and closer. In the planted CWs, CA was affected by the number of actinomycetes and fungi, and UA and DA were affected by the number of bacteria. In the CW without hydrophyte, DA and CA were affected by the number of bacteria, and the effect of actinomycetes and fungi on soil enzyme activity were insignificant. In all three CWs, there were relationships between bacteria and DA. DA is a kind of soil oxidoreductase widely distributed in soil microenvironments. Given that dehydrogenase is an important enzyme in bacterial steroid degradation (Ye et al., 2010), the number of bacteria directly affected the activity of dehydrogenase. Urease is present in most bacteria, fungi and plants. Therefore, bacteria and UA had good correlations in the planted CWs. In WL3, the numbers of bacteria and UA were not in the same class, implying that hydrophytes can promote the relationship between bacteria and UA. Although fungi can secrete urease, the relationship between fungi and UA was not close due to the small number of fungi in the CWs.

To scrutinize soil biological activity in CWs, principal component analysis was performed to integrate two variables, namely, biomass and soil enzyme activity, into a comprehensive index. According to the results of factor analysis, there was only one principal variable to refer to the microbe and enzyme in each CW, and the cumulative was above 85%. The total variance and component matrix are shown in Tables 4 and 5.

According to the component matrix, all soil biological indicators had big roles in the principal variables, except bacteria in WL2 and UA in WL3. All six variables can be replaced with a principal variable in the experimental CWs. Three principal component synthesis models were developed from the components of six soil biological indicators.

### Table 4

<table>
<thead>
<tr>
<th>CW</th>
<th>Total</th>
<th>% variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WL1</td>
<td>5.690</td>
<td>94.831</td>
<td>94.831</td>
</tr>
<tr>
<td>WL2</td>
<td>5.227</td>
<td>87.112</td>
<td>87.112</td>
</tr>
<tr>
<td>WL3</td>
<td>5.331</td>
<td>88.851</td>
<td>88.851</td>
</tr>
</tbody>
</table>
indicators and the cumulative of the principal variable, as follows:

\[ P_1 = 0.404X_1 + 2.442X_2 + 0.407X_3 + 0.297Y_1 + 0.428Y_2 + 2.293Y_3, \text{ in WL1} \]

\[ P_2 = 0.349X_1 + 2.828X_2 + 0.346X_3 + 2.684Y_1 + 0.340Y_2 + 2.884Y_3, \text{ in WL2} \]

\[ P_3 = 0.423X_1 + 2.172X_2 + 0.445X_3 + 2.208Y_1 + 0.425Y_2 + 2.050Y_3, \text{ in WL3} \]

The values of the principal variable obtained from the above formulas were derived from the experimental data on the number of microorganisms and soil enzyme activities, which changed with temperature. The results are shown in Fig. 6.

There were extremely significant positive correlations between the principal variables obtained from soil biological indicators and temperature, and the correlation coefficients were 0.962 in WL1 (p < 0.01), 0.876 in WL2 (p < 0.01), and 0.957 in WL3 (p < 0.01). The values of the principal variables in the planted CWs were higher than those in the unplanted CW. Before October 2010, WL1 had higher soil biological activity than WL2. Thereafter, the soil biological characteristics were higher in WL2 than in WL1. Therefore, soil biological activity can be greatly improved in CWs planted with A. donax in summer and with A. calamus in winter. The gap between WL1 and WL3 also became smaller with decreased temperature, whereas the gap between WL2 and WL3 became bigger.

3.5. Relationship among soil microbes, enzyme activity, and removal of pollutants in CWs

To investigate the relationship among soil microbes, enzymes, and removal of pollutants in the CWs purifying micro-polluted river, the correlations between pollutants and soil biological indicators were analyzed by Pearson’s test, and the results are shown in Table 6.
Table 6
Correlation between the removal rates of pollutants and soil biological indicators (n = 21).

<table>
<thead>
<tr>
<th>Pollutants</th>
<th>CWs</th>
<th>T</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
<th>Total microbe</th>
<th>DA</th>
<th>CA</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4(^+)-N</td>
<td>WL1</td>
<td>-0.569</td>
<td>-0.559</td>
<td>-0.625</td>
<td>-0.742</td>
<td>-0.642</td>
<td>-0.831</td>
<td>-0.704</td>
<td>-0.797</td>
</tr>
<tr>
<td>N</td>
<td>WL2</td>
<td>-0.741</td>
<td>-0.466</td>
<td>-0.805</td>
<td>-0.860</td>
<td>-0.759</td>
<td>-0.829</td>
<td>-0.712</td>
<td>-0.806</td>
</tr>
<tr>
<td>%</td>
<td>WL3</td>
<td>-0.503</td>
<td>-0.559</td>
<td>-0.474</td>
<td>-0.700</td>
<td>-0.612</td>
<td>-0.748</td>
<td>-0.677</td>
<td>-0.741</td>
</tr>
<tr>
<td>TN-</td>
<td>WL1</td>
<td>0.831*</td>
<td>0.811*</td>
<td>0.842*</td>
<td>0.762*</td>
<td>0.798*</td>
<td>0.785*</td>
<td>0.826*</td>
<td>0.796</td>
</tr>
<tr>
<td>N</td>
<td>WL2</td>
<td>0.813*</td>
<td>0.826*</td>
<td>0.837*</td>
<td>0.919*</td>
<td>0.874*</td>
<td>0.783*</td>
<td>0.880**</td>
<td>0.888**</td>
</tr>
<tr>
<td>%</td>
<td>WL3</td>
<td>0.324</td>
<td>0.399</td>
<td>0.428</td>
<td>0.241</td>
<td>0.354</td>
<td>0.262</td>
<td>0.338</td>
<td>0.062</td>
</tr>
<tr>
<td>TP-</td>
<td>WL1</td>
<td>-0.651</td>
<td>-0.601</td>
<td>-0.556</td>
<td>-0.498</td>
<td>-0.564</td>
<td>-0.138</td>
<td>-0.559</td>
<td>-0.333</td>
</tr>
<tr>
<td>P</td>
<td>WL2</td>
<td>-0.236</td>
<td>-0.196</td>
<td>-0.143</td>
<td>-0.085</td>
<td>-0.127</td>
<td>0.105</td>
<td>0.072</td>
<td>-0.112</td>
</tr>
<tr>
<td>%</td>
<td>WL3</td>
<td>-0.114</td>
<td>-0.153</td>
<td>-0.195</td>
<td>-0.255</td>
<td>-0.188</td>
<td>-0.268</td>
<td>-0.192</td>
<td>-0.465</td>
</tr>
<tr>
<td>COD</td>
<td>WL1</td>
<td>-0.537</td>
<td>-0.689*</td>
<td>0.455</td>
<td>-0.398</td>
<td>-0.517</td>
<td>-0.444</td>
<td>-0.473</td>
<td>-0.420</td>
</tr>
<tr>
<td>%</td>
<td>WL2</td>
<td>0.090</td>
<td>-0.062</td>
<td>-0.032</td>
<td>-0.154</td>
<td>-0.127</td>
<td>0.171</td>
<td>0.216</td>
<td>-0.198</td>
</tr>
<tr>
<td></td>
<td>WL3</td>
<td>-0.029</td>
<td>-0.216</td>
<td>0.090</td>
<td>-0.451</td>
<td>-0.295</td>
<td>-0.388</td>
<td>-0.041</td>
<td>-0.388</td>
</tr>
</tbody>
</table>

* Correlation was significant at the 0.05 level.
** Correlation was significant at the 0.01 level.

There were negative correlations among the removal of NH4\(^+\)-N and soil biological indicators in the three experimental CWs, as well as between COD and TP-P. The relationships between the removal of TN-N and soil biological indicators were significantly positive in the planted CWs (p < 0.05) and positive in the unplanted CW. The average correlation coefficients among TP-P or COD and the soil biological indicators were less than 0.50, which suggested that the soil biological indicators did not have close relationships with phosphorus or carbon removal in all CWs. The number of soil biological indicators correlated with the removal of nitrogen, was larger in the CW with than without hydrophyte, especially for the removal of TN-N. The relationship between the removal of TN-N and soil biological activity was improved by planting hydrophytes in the CW. In the two planted CWs, the relationships between TN-N and the soil biological indicators were almost the same. As regards NH4\(^+\)-N, soil enzyme activity had a more significant relationship with the removal rate than with the soil microorganisms. CA had almost the same roles in the removal of NH4\(^+\)-N in the three CWs, whereas parts of DA and UA used to remove NH4\(^+\)-N were almost similar in the planted CWs, which were higher than those in the unplanted CWs. Plants can also create a correlation between microbes and the removal of NH4\(^+\)-N by improving the conditions of the microenvironment in the CWs for the microbes and simulating the activities of enzymes. The indicators of soil biology related to NH4\(^+\)-N were higher in WL2 than in WL1 because the roots of A. calamus were more developed than those of A. donax.

Urease was significantly and positively correlated with the removal of TN-N, and negatively correlated with that of NH4\(^+\)-N. Nitrogen pollutants was consumed by urease as the N source, with NH4\(^+\)-N and other nitrogen-containing gas as the final products; thus, urease can indicate the nitrogen pollution and removal level in CWs. Dehydrogenase was also significantly and positively correlated with the removal of TN-N and negatively correlated with the removal of NH4\(^+\)-N. Dehydrogenase and catalase had similar functions with Urease in CWs. Compared with CA, DA and UA can better indicate the nitrogen pollution level and size of soil biological activity. All soil numbers of bacteria, fungi and actinomycete were significantly correlated with the removal of TN-N. The main reason was that the nitrogen pollutants in the CWs were decomposed and transformed by enzymes and microbes (Erler et al., 2011). Hence, the number of microbes and enzyme activities can be used as indicators to evaluate nitrogen removal.

According to the results of the principle correlation analysis, P can indicate the number of soil microbes and soil enzyme activity in each CW. Thus, three biological variables and three soil enzyme variables can be unified into a principle variable, which can more effectively suggest correlations among microbes, enzymes, and pollutant removal rates. The results of the correlation analysis are shown in Table 7.

The results in Table 7 are similar to those in Table 6. The negative correlation of NH4\(^+\)-N or TP-P with P was not significant (p > 0.05), whereas the positive correlation of TN-N with P was significant (p < 0.05) in the planted CWs. The negative correlation between COD and P in WL1 was significant (p < 0.05), different from those in WL2 and WL3. These results suggested that the relationship between the removal rate of COD and the principal variable obtained from soil biological indicators was improved by planting A. donax, different from the results in Table 6.

4. Conclusion

(1) The three CWs had acceptable abilities for pollutant removal, and were adaptable to water quality fluctuations when treating micro-polluted river water. There were significant differences among the numbers of microbes in each experimental CW, and the trend was bacteria > actinomycetes > fungi. The pollutant purification efficiencies, numbers of microbes, and soil enzyme activities can be improved by planting hydrophytes.

(2) Canonical redundancy rate analysis showed that there were linear positive relationships between the canonical variable of microbes and enzymes in the planted CWs. The number of microbes can be improved by planting A. donax, and soil enzyme activity can be stimulated by planting A. calamus. Cluster analysis revealed that the soil biological indicators in the planted CWs can be divided into two the same classes, different from those in the unplanted CW. Principal component analysis showed that all six soil biological indicators can be combined with a principal variable in the each CW (cumulative > 85%). There were extremely significant positive correlations between the principal variable and temperature (p < 0.01).

(3) The relationships between the removal of TN-N and soil biological indicators were significantly positive in the planted CWs (p < 0.05) and positive in the unplanted CW. Soil biological indicators did not have close relationship with phosphorus or carbon removal in all CWs (p > 0.1). As regards NH4\(^+\)-N, soil

Table 7
Relationship between principal variables and pollutant removal.

<table>
<thead>
<tr>
<th>Principle variable</th>
<th>NH4(^+)-N</th>
<th>TN-N</th>
<th>TP-P</th>
<th>COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (WL1)</td>
<td>-0.643</td>
<td>0.798*</td>
<td>-0.563</td>
<td>-0.874</td>
</tr>
<tr>
<td>P2 (WL2)</td>
<td>-0.518</td>
<td>0.759*</td>
<td>-0.128</td>
<td>-0.127</td>
</tr>
<tr>
<td>P2 (WL3)</td>
<td>-0.613</td>
<td>0.353</td>
<td>-0.436</td>
<td>-0.296</td>
</tr>
</tbody>
</table>

* Correlation was significant at the 0.05 level.
enzyme activities had more significant relationships with the removal rate than the soil microorganisms.

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References


