Plant growth and nutrient uptake in treatment wetlands for water with low pollutant concentration
Gilles Vincent, Kankan Shang, Guowei Zhang, Florent Chazarenc and Jacques Brisson

ABSTRACT
The objective of this study was to determine how macrophytes commonly used in treatment wetlands (TWs) respond to water with low pollutant concentration. We measured pollutant removal efficiency and compared growth and nutrient uptake of five macrophytes in demonstration scale units (volume >40 m³) irrigated by water with pollutant concentrations representative of average urban stormwater quality. All species showed a strong productivity gradient along the beds, starting with high biomass – high density near the inlet, then decreasing progressively with distance. Cyperus was by far the most productive species. Phragmites and Thalia had higher biomass in the first few metres of the beds than Typha and Arundo. In terms of pollutant removal, decreasing plant growth may be interpreted as indicative of high efficiency when caused by nutrient depletion. Differences in aboveground biomass between species did not translate into measurable differences in removal efficiency at the outlet. Although Phragmites australis is the species most commonly used in TWs, under the low nutrient load, Cyperus had twice its biomass, and higher N and P uptake. These results highlight the importance of considering wastewater characteristics when selecting macrophyte species for TWs.

Key words | diluted wastewater, macrophytes, Phragmites, treatment wetlands, Typha

INTRODUCTION
Eutrophication due to discharge of nutrients such as phosphorus and nitrogen is a major cause of water resource degradation in many freshwater ecosystems around the world. In countries with a shortage of freshwater resources, there is increasing demand to treat pond or river water characterized by low to medium concentrations of contaminants in a way that produces a high-quality output. Treatment wetlands (TWs, or constructed wetlands, CWs) have become a popular alternative to conventional methods due to low operational and maintenance costs, high pollutant removal efficiency and beneficial environmental outcomes. While TWs are commonly used to treat municipal and other highly charged wastewaters, they can also ameliorate water with a low to medium pollutant load, such as stormwater runoff (Mungasavalli & Viraraghavan 2006), greywater (Li et al. 2009), recreational wastewater (Vincent 1992, 1994), and several types of low-nutrient industrial wastewater (Wu et al. 2015). They are also widely used for tertiary treatment of pre-treated domestic wastewater. TWs thus represent an interesting alternative for removing excess nutrients from pond or river waters.

Surface flow TWs are the favored option for stormwater wetlands as well as tertiary TWs designed to polish minimally polluted effluents (Kadlec & Wallace 2009). Horizontal flow subsurface TWs are used under certain specific conditions, such as in northern climates with harsh winters where freezing may impair wastewater flow or damage pipes (Mæhlum et al. 1995; Wallace et al. 2001). Subsurface flow TWs may also be preferred under subtropical or tropical climates, where open water may contribute to mosquito breeding and thus pose a health risk (Kivaisi 2001). Subsurface flow TWs may be more efficient at removing nutrients, because the media contribute to phosphorus adsorption or serve as a substrate for microbial development. Lowest possible output concentrations (background concentrations) are higher in free water TWs compared to
horizontal flow subsurface TWs because the gravel matrix isolates the water from some of the background generating processes such as wildlife activity or the presence of phytoplankton (Kadlec 2009).

Macrophytes growing in subsurface flow TWs stimulate microbial activity, stabilize the surface, and prevent vertical flow TW systems from clogging. They may also contribute to pollutant removal through uptake, although their role is often considered unimportant in highly concentrated polluted water compared to other processes (Brix 1994; Brisson & Chazarenc 2009; Vymazal 2011). The relative role of plant uptake in nutrient removal may be more important in minimally polluted water, where high-quality outflow is required. The most commonly used plants in TWs are large macrophytes with fast growth, such as Phragmites australis, Typha spp. and Scirpus spp. (Vymazal 2011). The high nutrient load of domestic wastewater maximizes their high density and productivity, thereby fostering optimal conditions for treating domestic wastewater. Their relative efficiency may differ in minimally polluted water, especially as available nutrients may become limiting for fast-growing species.

The aim of this study was to evaluate how macrophytes commonly used in TWs respond to polluted water with low nutrient concentration. We were particularly interested in evaluating the possible gradient in plant development resulting from decreasing nutrients along the TW, a process that cannot be observed in mesocosms and thus necessitates large-scale experimental units. We measured pollutant removal efficiency and compared growth and nutrient uptake for five macrophytes in demonstration scale units (total vol >40 m³), in a subtropical climate. The removal efficiency of the systems was also evaluated by comparing planted with unplanted units.

**METHODS**

**Site and experimental set-up**

The study was carried out on a pilot horizontal flow subsurface TW system in Chenshan Botanical Garden (31°04′39″ N, 121°11′12″E) in Shanghai, one of the most urbanized cities in China, located on the eastern edge of the Yangtze River Delta plain. The pilot system was constructed with 12 separated parallel units, 13 m long by 4.5 m wide, providing a total filter bed surface of 58.5 m² (Figure 1). Each bed was filled with 60 cm of granitic river gravel (Ø = 10–20 mm). Clumps of five common macrophytes were planted (two replicates) in March 2016: Phragmites australis (Cav.) Trin. ex Steud., Typha orientalis C. Presl., Cyperus alternifolius L., Thalia dealbata Fraser ex Roscoe, and Arundo donax L. (hereafter referred to as Phragmites, Typha, Cyperus, Thalia and Arundo). The clumps were collected from wetlands around Chenshan Botanical Garden, and planted in the TW at a density of 2 clumps/m². Starting at the end of March 2016, all units were fed with water from an artificial lake in Chenshan Botanical Garden (Table 1). Pollution concentration was slightly below our target average value for our model typical polluted river in China.

![Image](https://iwaponline.com/wst/article-pdf/77/4/1072/493856/wst077041072.pdf)
Physico-chemical analysis

Wastewater treatment performance was monitored every two weeks over the 14-week sampling period, between May 12 and November 21, 2016. Inflow and outflow grab samples were collected simultaneously, filtered and processed on the same day. The following parameters were also measured in the laboratory, immediately after sampling: dissolved oxygen and temperature (Hach HQ30d, USA), pH (Hach HQ411d, USA), turbidity (Hach 2100Q, USA), and electrical conductivity (Leici Company, China). Samples of COD and biological oxygen demand (BOD$_3$) were kept refrigerated and analyzed the day after sampling, and samples for TN and TP were kept frozen until processed for analysis. The following parameters were measured according to APHA (2005): COD, BOD$_5$, NH$_3$-N, TN, and TP. Evapo-transpiration was estimated by evaluating the volume of water that refilled the basin after one or two days had elapsed since feeding, during periods without rain.

Plant species analysis

At the end of the growing season, we measured the allocation biomass by harvesting the aboveground plant portion in 1 m$^2$ adjacent plots crossing the central part of the beds from the inlet to the outlet, for a total of 11 plots. We weighed the fresh biomass, then oven-dried it to a constant weight at 70°C, and weighed the dry biomass. For P and N measurements, we divided each bed into three regions of roughly equal growth: maximal growth (in the first few metres), medium growth, and low growth (usually the second half of the beds). The exact size of these regions varied according to species. We collected plant samples from the middle of each of these regions. Total nitrogen and total phosphorus were analyzed using an automatic intermittent analyzer (AMS/Westco Smart-Chem 200, Italy) after digestion with H$_2$SO$_4$ in glass tubes.

RESULTS

Total aboveground dry plant biomass varied from 29.1 kg/bed (Typha) to 80.2 kg/bed (Cyperus) (Figure 2). Cyperus produced far more biomass than Arundo, Phragmites and Thalia. Cyperus also accumulated the most nitrogen and phosphorus in its aboveground biomass (342.8 gN and 25.6 gP) (Table 2). Although aboveground biomass of Thalia was nearly equal to that of Arundo and Phragmites (43.2 kg compared to 40.0 kg and 42.9 kg respectively), it accumulated a much greater quantity of nutrients in its aboveground tissue (278.5 gN and 11.8 gP, compared to 132.6 gN and 4.22 gP for Arundo, and 158.4 gN and 4.5 gP for Phragmites (Table 2, Figure 2). Plant biomass was not equally distributed in the beds, all species showing a strong decreasing gradient of plant growth from inlet to outlet (Figure 2). Thalia, Cyperus and Phragmites produced more than 50% of their total cumulative biomass in the first 2 m of the beds, after which growth decreased sharply. The decline in growth was a little more gradual for Typha and Arundo (Figure 2). The ratio between belowground and aboveground plant biomass increased from inlet to outlet for all species (Figure 3). At the outlet, this ratio was

Table 1 | Characteristics of the water at the inflow, before and from August 1st, 2016

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before August 1st</th>
<th>From August 1st</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>12.5 ± 1.5</td>
<td>14.1 ± 1.4</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>5.3 ± 1.4</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>7.45 ± 1.56</td>
<td>9.84 ± 1.64</td>
</tr>
<tr>
<td>NH$_3$ (mg/L)</td>
<td>2.05 ± 0.98</td>
<td>6.86 ± 1.10</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>0.22 ± 0.08</td>
<td>1.85 ± 0.13</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>9.4 ± 3.3</td>
<td>7.9 ± 0.6</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>4.3 ± 1.3</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>8.3 ± 0.4</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>EC (μS cm)</td>
<td>500 ± 21</td>
<td>525 ± 15</td>
</tr>
</tbody>
</table>

(Suzhou River; chemical oxygen demand (COD) 20 mg/L, NH$_4$-N: 7.0 mg/L, total nitrogen (TN) 10 mg/L, total phosphorus (TP): 0.4–0.8 mg/L (Zhang et al. 2009)). To get closer to these values, from August 1st of the same year, the pollutant load was increased slightly through addition of a 15:15:15 nutrient solution (percentage per weight, of nitrogen-phosphorous-potassium: N-P-K containing micro-elements (Table 1). The pilot-scale system was fed for approximately 12–15 hours per day, five days a week, at the same hydraulic rate of 4 m$^3$/d (or 5.6 cm/d). Flow rate thus varied but the theoretical hydraulic retention time (HRT) averaged 5 days. The water was first pumped into a 4 m$^3$ container, then fed to the units by gravity through a 5 m long perforated pipe located on the surface and perpendicular, at the inlet of the TW units, in order to ensure homogeneous distribution of wastewater (Figure 1). During the experiment, water level was maintained under 2 cm below the surface of the units. Data on rainfall and temperature were collected from a meteorological station located at Chenshan Botanical Garden, close to the pilot-scale system.

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above 1 (more than half of the total plant biomass was belowground) except for Phragmites (Figure 3). Nitrogen and phosphorus concentrations in plant tissue also varied along the beds, but no clear pattern common to all species emerged. The strongest gradient was revealed in Phragmites, which showed a sharp decrease in nutrient tissue concentration from inlet to outlet (Table 2).

Unplanted beds were less efficient in pollutant removal than planted beds for all pollutants measured except COD (Figure 4). Indeed, because the comparison is based on concentration at the outlet, the difference in removal between planted and unplanted is even greater if we account for the greater water loss through evapotranspiration in planted beds (Figures 4): a water balance analysis showed that this underestimation was in the range of 5% (data not shown). From mid-summer, nutrient output in planted beds was always below 4 mg/L for TN and 0.4 mg/L for TP. There were no clear differences between plant species in terms of pollutant removal when comparing concentrations at the outlets, although in Thalia and Cyperus beds, TP concentration was particularly low, ranging from 0.04 to 0.17 mg/L over the season (Figure 4).

DISCUSSION

There is a growing need to develop water treatment approaches with low nutrient outputs, considering that even modest concentrations of phosphorus and nitrogen may result in eutrophic conditions. Camargo & Alonso (2006) estimated that total nitrogen concentration lower than 0.5–1.0 mg/L might be necessary to prevent eutrophication of aquatic ecosystems by inorganic nitrogen pollution (excluding those ecosystems with naturally high N levels). Yin et al. (1992), in an enclosure experiment conducted in a lake in China, determined that a concentration of 0.019 mg/L of phosphorus was sufficient to stimulate algal growth. In TWs, healthy plants and high growth are often considered necessary for high pollutant removal (Brix 1994). Yet, there is an apparent contradiction between requiring a very low nutrient output and the nutrient concentration necessary for plant growth in the TW. If nutrient levels are sufficient to maintain an optimal and homogeneous plant cover across the whole surface of the TW, it means that nutrient content in the outflow would

![Figure 2](https://iwaponline.com/wst/article-pdf/77/4/1072/493856/wst077041072.pdf)

**Figure 2** Aboveground dry biomass and cumulative proportion of aboveground dry biomass in relation to distance from the inlet.

### Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Phosphorus</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aboveground concentration (mg/g)</td>
<td>Aboveground concentration (mg/g)</td>
</tr>
<tr>
<td></td>
<td>Inlet</td>
<td>Middle</td>
</tr>
<tr>
<td>Thalia</td>
<td>0.17 ± 0.14</td>
<td>0.16 ± 0.11</td>
</tr>
<tr>
<td>Arundo</td>
<td>0.11 ± 0.06</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>Cyperus</td>
<td>0.39 ± 0.21</td>
<td>0.23 ± 0.10</td>
</tr>
<tr>
<td>Typha</td>
<td>0.20 ± 0.28</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Phragmites</td>
<td>0.23 ± 0.17</td>
<td>0.10 ± 0.03</td>
</tr>
</tbody>
</table>
still be at the minimum level sufficient to sustain high plant growth, and could thus be detrimental to the receiving water body. In our experiment, the decrease in plant growth along the units is most likely due to nutrient deficiency. The increasing ratio of belowground/aboveground biomass with distance from the inlet may not be signs of TW malfunction. On the contrary, they may be indicative of highly efficient nutrient removal in the wetland. Plant species selection is important for maximizing pollutant removal, and species that may be highly efficient in a CW for treating highly charged water such as domestic wastewater may not be the optimal species under a low-nutrient regime. In our experiment, *Cyperus* was by far the most productive species and accumulated the most nutrients. In a TW experiment conducted in Italy with wastewater with a very high nutrient content, *Arundo donax* produced more than 50% more aboveground biomass than *Cyperus alternifolia* (Tuttolomondo et al. 2015), while in our experiment, *Cyperus* had twice the aboveground biomass of Arundo. *Phragmites*, which is known to be highly efficient in CWs treating domestic wastewater, performed rather poorly under the low pollutant conditions of our experiment. It must also be noted that aboveground biomass was only partly correlated to nutrient uptake: *Thalia* showed a much higher level of nutrient uptake despite showing similar total biomass compared to *Arundo* and *Phragmites*.

The fact that our results do not take into account nutrients stored in belowground biomass may partially explain this lack of correlation. Thus, if nutrient removal through plant harvesting is the goal, our results confirm that comparing plant productivity alone may not be sufficient to determine plant species efficiency.

In addition to plant nutrient uptake, other factors may be responsible for a low nutrient concentration in the outlet of a TW. First, a large wetland in relation to a low water flow may allow a decrease in concentration along the flow path, down to the background pollutant concentrations (Kadlec 2009). In our case, plants showed full typical growth near the inlet, but their performance decreased strongly 2.4 m further along the TW. At 11 meters long, our experimental wetlands were oversized for the feeding regime we applied, at least for the first year of operation. Second, the substrate has maximum phosphorus sorption capacity in the first year of operation. This capacity will decrease over time, so that background pollutant concentration will be attained further along the TW. Running the system for additional years would be necessary in order to fully test the current parameters and survey progression of plant growth along the beds.

Our results show that *Cyperus* was the best-performing species among those tested for treating water with low concentrations of contaminants combined with a simultaneous need for a high-quality output, under subtropical climatic conditions. However, to further increase pollutant removal, selecting a single plant species best adapted to this situation may not be the best solution to the sharply decreasing gradient in phosphorus and nitrogen concentrations along a TW. Establishing more than one species along the gradient and selecting multiple plant species with progressively better relative nutrient uptake might be an optimal design. Also, as we move toward the outlet of a TW, plant species colonized with mycorrhizal fungi may increase total nutrient removal efficiency in a TW (Chagnon & Brisson 2017). By expanding the volume of soil explored by the root system, the mycorrhizal symbiosis may provide growth-limiting nutrients and alleviate plant stress. This may, in turn, improve water quality at the outlet by further decreasing nutrient output.

Studying a decreasing gradient in pollutant concentration and its effect on plant growth presents a methodological challenge because it cannot be reproduced in small experimental units. In our case, we had the exceptional opportunity to use large-scale experimental TWs, with replicates, to study this gradient. This process is not amenable to experiments using single mesocosms, by far
the preferred experimental unit size allowing replications. An alternative, however, would be to use mesocosms connected in series, with each unit progressively receiving less nutrients. This would allow further testing of different species combinations. Balancing plant growth requirements in TWs for treating minimally polluted water with the need for a low nutrient output will require further research, as the current state of knowledge, based mostly on data collected from CWs treating domestic wastewater, may not apply.

**ACKNOWLEDGEMENTS**

This study was financially supported by a grant from the Shanghai Administration Bureau of Landscape and City Appearance (Grant No. G152426, G162415). The authors would like to thank Karen Grislis for comments on a previous version of the manuscript, and Kou Shumeng and Sebastian Rodriguez for their technical assistance. We are grateful to the Shanghai Chenshan Botanical Garden for the implementation of the study site.
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