Effects of interspecific competition on the growth of macrophytes and nutrient

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2	removal in constructed wetlands: a comparative assessment of free water surface
3	and horizontal subsurface flow systems
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15	
16	Abstract
17	The outcome of competition between adjoining interspecific colonies of <i>Phragmites</i> and

*Typha* in two large field pilot-scale free water surface (FWS) and subsurface flow (SSF)
CWs is evaluated. According to findings, the effect of interspecific competition was
notable for *P. australis*, whereby it showed the highest growth performance in both

21	FWS and SSF wetland. In a mixed-culture, <i>P. australis</i> demonstrates superiority in
22	terms of competitive interactions for space between plants. Furthermore, the
23	interspecific competition among planted species seemed to cause different ecological
24	responses of plant species in the two CWs. For example, while relatively high density
25	and shoot height determined the high aboveground dry weight of P. australis in the
26	FWS wetland, this association was not evident in the SSF. Additionally, while plants
27	nutrients uptake accounts for a higher proportion of the nitrogen removal in FWS, that
28	in the SSF accounts for a higher proportion of the phosphorous removal.
29	
30	Keywords: Interspecific competition; plants growth; nutrients uptake;
31	constructed wetland.
32	
33	1. Introduction
34	Constructed wetlands (CWs), as a cost-effective and eco-friendly wastewater treatment
35	technology, have been widely applied for the treatment of various types of wastewater,
36	as well as polluted river and lake waters due to their easy operation and maintenance
37	(Rai et al., 2013; Svensson et al., 2015; Vymazal, 2013c; Wu et al., 2015). CWs are
38	typically classified into free water surface flow (FWS) and subsurface flow (SSF)
39	systems. The efficient removal of water pollutants is achieved through a number of
40	biotic and abiotic processes, especially around the rhizosphere of macrophytes, as the
41	wastewater flows through the CWs substrate materials (Stottmeister et al., 2003).

42	The macrophytes growing in CWs perform several direct and indirect roles in relation to
43	the treatment process such as uptake and assimilation of nutrients and heavy metals,
44	provision of substrates for the growth of attached bacteria, the release of oxygen and
45	exudates, surface insulation, hydraulic condition regulation and wind velocity reduction
46	(Vymazal, 2013b). In fact, CWs with plants have been proven to be more efficient
47	compared with unplanted CWs (Boog et al., 2014). Nonetheless, recent findings
48	indicate that bacteria richness and the performance of CWs varied greatly in relation to
49	different plant species (Toscano et al., 2015).
50	
51	Therefore, to ensure efficient performance of CWs, the macrophyte species to be
52	planted should be considered as an integral design component by careful selection. To
53	increase CWs performance, mixed planted systems have been considered to treat
54	different types of wastewater. The multi-species wetlands were considered less
55	susceptible to seasonal variation and more effective in pollutants removal than
56	single-species wetlands (Chang et al., 2014; Liang et al., 2011). However, these CWs
57	were mainly tested at laboratory-scale or short experimental periods. Moreover,
58	interspecific competition for resources among planted species, such as space, light, and
59	nutrients, is one of the important factors in determining wetland vegetation.
60	Nevertheless, due to the high variability in this interspecific competition among planted
61	species, the development and stability of different species in mixed planted wetlands
62	during long-term operation remain unclear (Agami and Reddy, 1990; Amon et al., 2007).

Besides, to date, no studies have directly compared the performance or evaluated the
competitive interaction between plant species of the mixed culture plants in FWS and
SSF wetlands under the same wastewater loading and environmental conditions at the
field scale.

67

The common reed (*Phragmites australis*) and cattail (*Typha* spp.) are the most often 68 used plant species in CWs (Vymazal, 2013a), because of their high flood-tolerant and 69 reproduction abilities. However, the application of these two plants in mixed cultures in 70 FWS and SSF wetlands is rare. *Phragmites* spp. and *Typha* spp. are both colonial 71 macrophytes that share several morphological traits, such as tall, unbranched shoots and 72 both rhizomes and roots as underground structures. They also share similar habitats and 73 74 a large range of site conditions, including resistance to saline conditions (Miklovic and Galatowitsch, 2005). Like Phragmites, Typha often forms dense stands due to their 75 strong vegetative propagation, and both *Typha* species and their hybrid display invasive 76 tendencies in disturbed wetlands (Shih and Finkelstein, 2008). Consequently, the 77 contact zone between *Phragmites* and *Typha* stands is probably characterized by intense 78 competition for space, the outcome of which is best revealed by the spatial dynamics at 79 that contact zone over time as one species progress to the detriment of the other. Thus, 80 81 adjoining colonies of *Phragmites* and *Typha* represent an ideal model for testing 82 hypotheses about competitive interactions between these clonal species and developing an understanding of interspecific plant competition in CWs. 83

84	In this study, local P. australis and T. latifolia were equally planted in monospecific
85	colonies in pilot-scale FWS and SSF wetlands to treat highly polluted river water over
86	two years. The pollutants treatment performance and the roles of plants were evaluated
87	in a side-by-side comparison of the FWS and SSF wetlands. The specific objectives
88	were to: (1) evaluate pollutant removal in pilot-scale FWS and SSF wetlands over two
89	full years of operation; (2) assess the effects of interspecific competition between P.
90	australis and T. orientalis in terms of growth characteristics, species range extension
91	and nutrients accumulation abilities under mixed culture conditions; and (3) analyze the
92	different interspecific competition characteristics of P. australis and T. orientalis in
93	FWS and SSF wetlands.
94	
95	2. Materials and methods
96	2.1 Description of the pilot wetlands
97	The pilot-scale FWS and SSF wetlands were constructed on the eastern bank of the
98	Zaohe River in Xi'an, northwestern China (34°22'54"N, 108°51'05"E). The area has a
99	sub-humid continental monsoon climate, which is cold and lacks rainfall during the
100	winter. The FWS CW was designed with a length of 45 m, width of 20 m and height of
101	0.6 m, and was filled with sand (0.06-10 mm, initial porosity about 30%) to a depth of
102	0.35 m. The water depth was controlled at 0.4 m. The SSF wetland was designed with a
103	length of 34 m, width of 20 m and height of 0.8 m, and was filled with gravel (1-70 mm,

105	m. Both wetlands were lined with high-density polyethylene to prevent the seepage of
106	polluted water to the underlying groundwater. The bottom slope of all the CWs was
107	0.5%. The chemical characteristics of the gravel and sand substrates are shown in Table
108	1. Water from the Zaohe River is pumped into an elevated feeding tank for
109	sedimentation and subsequent distribution to the CWs continuously. The inflow rate to
110	the FWS and SSF was 90 $m^3/d$ and 68 $m^3/d$ , respectively, both of which correspond to a
111	surface loading of 0.1 m/d. Local P. australis and T. orientalis with similar size obtained
112	from the field near the riverbank were selected and washed with tap water. They were
113	then planted in equal proportions in the CWs at a density of 9 shoots/m <sup>2</sup> and a height of
114	about 20 cm in September. Plants harvesting was carried out in November, for the two
115	successive years, when the plants began to wither. Each year is defined here as
116	November to October. The pilot wetlands were commissioned in November 2010.
117	

118 **2.2 Water sampling and analysis** 

During the two-year experimental period, water samples were collected weekly from the
influent and effluent of the two CWs. All of the water samples were transported to the
laboratory for chemical analyses within 24 h. The parameters measured include SS,
COD, BOD<sub>5</sub>, NH<sub>3</sub>-N, TN and TP. Standard methods (MEPC and WWMAA, 2002)
were followed for all the chemical analyses. Water temperature and dissolved oxygen
(DO) were measured on site by using a portable meter (HQ30d53LED<sup>TM</sup>, HACH,
USA). The removal efficiencies for each wetland were calculated from the difference in

126	concentration between the influent and effluent of the CWs. Significant differences
127	were determined at $\alpha$ =0.05 by paired samples t-tests and one-way analysis of variance
128	(ANOVA).
129	
130	2.2 Plant sampling and analysis
131	During the experimental period, the plant heights in the two CWs were measured
132	monthly in three randomly selected quadrats of 0.25 $m^2$ . The number, weight and
133	coverage of <i>P. australis</i> and <i>T. orientalis</i> in the two CWs were measured before the
134	harvesting in November. The selected harvested plants were separated into stems, leaves
135	and flowers and washed with distilled water to remove the adhering water and
136	sediments. Plant parts were then oven-dried at 80 °C to a constant weight, and their dry
137	biomass were determined. All dried plant samples were ground separately to pass
138	through a 0.25 mm mesh screen, digested and analyzed for total N and P according to
139	the routine analysis method for soil agro-chemistry (Bao, 2000). The average nutrients
140	concentration in the aboveground biomass was calculated as follows:
	$C_{total} = \frac{(DM_{leaves} \times C_{leaves}) + (DM_{stems} \times C_{stems}) + (DM_{flowers} \times C_{flowers})}{(DM_{leaves} + DM_{stems} + DM_{flowers})} $ (1)
141	where $DM = dry$ matter of a particular shoot part (g), $C = concentration of nutrients in$
142 143	the respective plant parts (% DM).
144	The amount of nutrients uptake by the aboveground biomass was calculated according
145	to the following equation:

$$N_{total} = (DM_{leaves} \times C_{leaves}) + (DM_{stems} \times C_{stems}) + (DM_{flowers} \times C_{flowers})$$
(2)

where DM values represent the total biomass of leaves, stems and flowers, and C 146 147 represents the average concentrations of N and P in these respective plant parts. N values represent the amount of nutrients uptake by the aboveground biomass of plants 148 149 The relative importance value (I.V.) (Hong et al., 2014) of each species was calculated 150 as a sum of the relative density and relative coverage in each CW, to represent a 151 plant-sociological result, after the interspecific competition among the planted species. 152 The competitive value (C.V.) of each species was used to compare their growth 153 responses to interspecific competition. The C.V. (Eq. 3) provides a means to determine 154 interactions among plant groups (Hong et al., 2014). 155  $C.V. = 100 (X_2 - X_1)/X_2$ (3)

where  $X_1$  is the dry weight of a particular species grown alone, and  $X_2$  is the dry weight of a particular species grown with neighbours.

158

#### 159 3. Results and discussion

#### 160 **3.1 Overall performance of the FWS and SSF wetlands**

161 Figure 1 shows the variation in the concentrations of COD, BOD<sub>5</sub>, TN, NH<sub>3</sub>-N, TP, and

- 162  $PO_4^{3-}$ -P in the river water, the average of which were 303.6±10.3, 98.3±4.6, 39.6±0.7,
- 163 29.1±0.8, 3.6±0.1, and 1.6±0.1 mg/L, respectively (Table 2). These concentrations
- indicated that the river water was highly polluted and was similar to that of domestic

165	wastewater. This high level of pollution was to be expected as currently the main
166	function of the Zaohe River is an urban drainage channel to receive effluents from
167	several domestic wastewater treatment plants, urban runoff, and untreated industrial
168	wastewater. Moreover, the treatment performance of two CW types is also shown in
169	Figure 1 and Table 2. While both wetland types achieved significantly high levels of
170	organic matter removal, the levels of nutrients removal were moderate. Nonetheless, the
171	COD removal in the SSF wetland was significantly higher than that in the FWS ( $p$ <0.05,
172	Fig. 1a), whereas the $BOD_5$ concentration of the effluents in the two wetlands were
173	similar and below 10mg/L (p>0.05, Fig. 1b and Table 2). Furthermore, the microbial
174	population in the SSF wetland $(10^6 \text{ cfu/ml})$ was nearly one order of magnitude higher
175	than that of the FWS wetland $(10^5 \text{ cfu/ml})$ during the experimental period. However, the
176	DO concentration in the SSF wetland (about 0.48 mg/L) was lower than that of FWS
177	wetland (about 1.53 mg/L). As the biodegradation of organics proceeds more rapidly
178	under aerobic conditions (Li et al., 2014), the FWS wetland was expected to show
179	higher BOD <sub>5</sub> removal. However, the organics removal in CWs has also been shown to
180	be accomplished by the physical processes of sedimentation, filtration, and interception
181	(Wang et al., 2015), which resulted in both wetlands achieving similar BOD <sub>5</sub> removal.
182	Nevertheless, the COD removal efficiency in the SSF wetland was higher.
183	
184	The TN and $NH_3$ -N concentrations in the FWS wetland effluent (18.5 and 12.9 mg/L)

<sup>185</sup> were significantly lower than that in the SSF (21.7 and 17.0 mg/L) (p<0.05, Fig.1 c-d

X

186	and Table 2). The removal of $NH_3$ -N in the FWS wetland was higher, because the DO
187	concentration was higher, which benefited nitrification (Li et al., 2014). Moreover, the
188	removal of TN in the FWS wetland was also higher than that of the SSF wetland. The
189	minimal concentrations of nitrified nitrogen in the effluent of the two wetlands indicated
190	that simultaneous nitrification and denitrification occurred in the wetlands. On the other
191	hand, the TP and $PO_4^{3-}$ -P concentrations in the FWS wetland (1.6 and 1.1 mg/L) was
192	significantly higher than that in the SSF (1.1 and 0.7 mg/L) (p<0.05, Table 2). The
193	effluent concentrations of P in the FWS wetland was observed to increase slight above
194	that of the influent during the spring season (Fig.1 e-f). This increase in effluent P
195	concentration could be attributed to the desorption of P by the substrate. Overall, the
196	higher P-sorption capacity of the gravel (0.8 mg/g) compared with sand (0.1mg/g) and
197	the flow conditions of the SSF wetland resulted in a higher phosphorus removal in the
198	SSF wetland. Nonetheless, plants uptake also plays important roles in nutrients removal
199	in CWs (Vymazal, 2013b). Additionally, the microbial population and communities
200	diversity around the rhizosphere and roots of plants are reported to be higher than the
201	other regions in the wetlands (Berendsen et al., 2012; Hallin et al., 2015). Based on this
202	consideration, the roles and evolution of the mixed-planted macrophytes in the two
203	wetlands were further examined.

204

#### 205 **3.2 Comparative analysis of plant growth**

206 During the experimental period, the average air temperature in the wetland area was

207	about 18.0 °C. The average temperatures in summer and winter were 28.9 °C and 2.2 °C,
208	respectively. Although the average water temperature in the two wetlands were similar,
209	the heights of plants in the FWS wetland were about 20 cm higher than that in SSF
210	wetland. The heights of T. orientalis were higher than P. australis in both wetland types.
211	0
212	Before the harvesting in autumn, the average plant density in the FWS wetland was 119
213	shoots/m <sup>2</sup> in the first year, which increased to 160 shoots/m <sup>2</sup> in the second year (Fig. 2a).
214	In the SSF wetland, shoot densities increased from 99 shoots/m <sup>2</sup> to 111 shoots/m <sup>2</sup>
215	(Fig.2a). The initial planted density in the two wetlands were 9 shoots/m <sup>2</sup> each.
216	Therefore, the observed increase in the plant density mainly occurred during the first
217	year when there was much more space available for the plants to grow into. However,
218	the slightly higher plant density in the second year resulted in little space available for
219	new plants to grow, which consequently increased interspecific competition among the
220	plants (Agami and Reddy, 1990). Furthermore, the density of plants in the FWS was
221	higher than that in the SSF wetland (Kadlec and Wallace, 2008). This difference further
222	increased in the second year (Fig. 2b), which indicated that the FWS wetland provided
223	much more suitable conditions for the macrophytes to grow. Although the heights of T.
224	orientalis were higher, the densities of P. australis in both wetlands were about three
225	times higher than that of T. orientalis. In addition, the P. australis/T. orientalis ratio
226	increased in the second year, especially in the SSF wetland (3.2-3.5). This increase
227	indicated that <i>P. australis</i> had better reproductive and adaptive capabilities than <i>T</i> .

228 *orientalis* under the mixed culture conditions, especially in the SSF wetland. However,

the opposite results were reported by Kercher and Zedler (2004).

230

231	As the plants were cut about 20–30 cm above the ground, the amount of plants biomass
232	at harvesting can be regarded as the net increase. The average biomass production of
233	plants in the FWS CW was 1.8 kg/m <sup>2</sup> in the first year, which increased to $3.4 \text{ kg/m}^2$ in
234	the second year. In the SSF CW biomass production increased from $1.1 \text{ kg/m}^2$ to $1.4$
235	kg/m <sup><math>2</math></sup> (Fig. 2b). As with the plant density, the plants biomass production in the FWS
236	was 1.6-2.4 times higher than that in the SSF wetland in the first and second year. This
237	difference indicated that the plants in the FWS CW were denser than those in the SSF
238	CW (Vymazal, 2013a; Leto et al., 2013). As indicated above, the plant density in the
239	two wetlands increased slightly in the second year. Nevertheless, compared with the
240	plant density, the biomass of plants in the two wetlands types increased significantly,
241	especially in the FWS CW (Fig. 2), demonstrating that the plants became heavier in the
242	second year (Leto et al., 2013).

243

Furthermore, the dry weights of *P. australis* in both wetlands were about 1-2 times higher than that of *T. orientalis* during the whole experimental period. The higher density of the *P. australis* resulted in the higher dry weight. The higher aboveground biomass of *P. australis* indicated a higher pollutants uptake capacity, as well as a higher belowground biomass and microbial population (Peng et al., 2014). Additionally, the

249	densities and dry weights of the plants in these two CWs were found to be higher than
250	those reported in the literature (Leto et al., 2013; Liang et al., 2011).
251	
252	3.3 Nutrients contents in plant tissues and plant uptake
253	The nutrients concentrations in the aboveground tissues of plants in the two wetlands
254	were similar, which both increased slightly in the second year of the experimental
255	period (Fig. 3a, b). In the second year, the nutrients content of <i>P. australis</i> and <i>T.</i>
256	orientalis tissues were 36.0 mg N/g and 3.3 mg P/g, and 31.6 mg N/g and 3.6 mg P/g,
257	respectively, in the FWS CW. In the SSF CW, nutrient contents were 34.1 mg N/g and
258	3.4 mg P/g ( <i>P. australis</i> ), and 29.8 mg N/g and 3.5 mg P/g ( <i>T. orientalis</i> ) (Fig.3a, b).
259	Overall, the nitrogen concentration in the T. orientalis was lower than that in P. australis
260	in the two CW types (Fig. 3a, b). The opposite was found for phosphorus. However, this
261	difference was not significant. Moreover, the nutrients concentrations in the
262	aboveground tissues of plants were within the range of values reported in other previous
263	studies (Kadlec and Wallace, 2008).
264	

As shown in Figure 3c, the nitrogen uptake by the aboveground parts of *P. australis* and *T. orientalis* in the FWS were 31.7 g N/m<sup>2</sup> and 20.8 g N/m<sup>2</sup>, respectively, in the first year, which increased to 80.0 g N/m<sup>2</sup> and 37.6 g N/m<sup>2</sup>, respectively in the second year. The phosphorus uptake rates increased from 3.0 g P/m<sup>2</sup> and 2.5 g P/m<sup>2</sup>, respectively, in the first year to 7.3 g P/m<sup>2</sup> and 4.2 g P/m<sup>2</sup>, respectively, in the second year (Fig. 3d). In

270	the SSF wetland, nitrogen uptake by the aboveground parts of <i>P. australis</i> and <i>T.</i>
271	<i>orientalis</i> increased from 20.4 g $N/m^2$ and 11.1 g $N/m^2$ , respectively in the first year, to
272	29.2 g N/m <sup>2</sup> and 14.2 g N/m <sup>2</sup> , respectively in the second year (Fig. 3c). Similarly, the
273	phosphorus uptake increased from 2.0 g P/m <sup>2</sup> and 1.4 g P/m <sup>2</sup> , respectively in the first
274	year, to 2.9 g P/m <sup>2</sup> and 1.7 g P/m <sup>2</sup> , respectively in the second year (Fig. 3d). As the
275	amount of nutrients uptake by plants were calculated by the nutrients content in the
276	plants tissues and the dry weight of the plants (Kadlec and Wallace, 2008), the
277	significant difference in the plants' dry weights resulted in the plants in the FWS CW
278	showing much higher nutrients uptake ability than those in the SSF CW, especially in
279	the second year. Moreover, the nutrients uptake by <i>P. australis</i> in both wetlands were
280	about 1-2 times higher than that of <i>T. orientalis</i> during the whole experimental period.
281	Therefore, in order to improve the nutrients uptake ability of plants and consequently,
282	the performance of the wetlands, the plant species with high adaptation and
283	reproduction ability should be selected. Additionally, despite the interspecific
284	competition among the plant species in these two CWs, the nutrients uptake by P.
285	australis and T. orientalis were found to fall within the range of values reported in other
286	previous studies (Leto et al., 2013; Liang et al., 2011).

#### 287

#### 288 **3.4 Significance of plant for nutrients removal**

- As plants play important roles in nutrients removal in CWs, it is important to
- 290 comprehend the different roles of plants planted in different CW types for nutrients

291	removal. Table 3 shows the nutrients mass balance components for each CW during the
292	experimental period. Overall, as the plant and other components of the two CWs were
293	much mature in the second year, the amount of nutrients removed was to increase in
294	both wetlands. The amount of nitrogen removed in the FWS wetland was higher than
295	that of the SSF wetland. In contrast, the SSF wetland showed higher phosphorous
296	removal than FWS wetland (Table 3). However, while the plants nutrients uptake
297	accounted for a greater proportion of the phosphorus removal in FWS wetland than the
298	nitrogen, the opposite was found in the SSF wetland (Table 3). Thus, the impact of plant
299	uptake, microorganisms degradation, and substrates adsorption were quite different for
300	the removal of nutrients in FWS and SSF wetlands (Kadlec and Wallace, 2008).
301	
202	
302	Although the amount of nutrients uptake by plants in the two wetlands both increased in
302	Although the amount of nutrients uptake by plants in the two wetlands both increased in the second year, the proportion attributable to plants for nutrients removal in the FWS
303	the second year, the proportion attributable to plants for nutrients removal in the FWS
303 304	the second year, the proportion attributable to plants for nutrients removal in the FWS and SSF wetlands were significantly different (Table 3). For the FWS wetland, the
303 304 305	the second year, the proportion attributable to plants for nutrients removal in the FWS and SSF wetlands were significantly different (Table 3). For the FWS wetland, the proportion attributable to plants for both TN and TP removal increased from 8.6% and
303 304 305 306	the second year, the proportion attributable to plants for nutrients removal in the FWS and SSF wetlands were significantly different (Table 3). For the FWS wetland, the proportion attributable to plants for both TN and TP removal increased from 8.6% and 9.9% in the first year, to 13.9% and 13.9% in the second year. This increase highlighted
303 304 305 306 307	the second year, the proportion attributable to plants for nutrients removal in the FWS and SSF wetlands were significantly different (Table 3). For the FWS wetland, the proportion attributable to plants for both TN and TP removal increased from 8.6% and 9.9% in the first year, to 13.9% and 13.9% in the second year. This increase highlighted the important roles that the plants in the FWS wetland played in nutrients removal.
303 304 305 306 307 308	the second year, the proportion attributable to plants for nutrients removal in the FWS and SSF wetlands were significantly different (Table 3). For the FWS wetland, the proportion attributable to plants for both TN and TP removal increased from 8.6% and 9.9% in the first year, to 13.9% and 13.9% in the second year. This increase highlighted the important roles that the plants in the FWS wetland played in nutrients removal. More particularly, as the biomass of plants increased, the microbial activities in the

312	attributable to plants for TP removal was increased from 4.6% to 4.8%. This possibly
313	occurred because as the reproduction of plants in SSF wetland enhanced the
314	nitrification-denitrification process around the rhizosphere of the plants, and the
315	P-sorption capacity of the gravel was decreased with the long-term operation
316	(Berendsen et al., 2012; Hallin et al., 2015; Kadlec and Wallace, 2008; Stottmeister et
317	al., 2003). Additionally, P. australis contributed much more to the TN and TP removal
318	than T. orientalis in both FWS and SSF CWs. Therefore, considering the important
319	direct and indirect roles of plants in nutrient removal in the FWS wetland, the selected
320	plant species should possess the ability to regenerate and grow vigorously. However, in
321	the SSF wetland, as the direct impacts of plants were minimal, the planted species
322	should possess the ability to improve the diversity and activity of the microorganisms.
323	
324	Moreover, due to the importance of plants in CWs especially in the FWS wetlands,
325	several studies have suggested that the overall nutrients removal would be higher if a
326	multiple or earlier harvesting scheme is adopted (Batty and Younger, 2004; Kadlec and
327	Wallace, 2008). However, multiple or earlier harvesting can be detrimental to the plants
328	because they would not have sufficient opportunity to withdraw nutrients and
329	nonstructural carbohydrates from the shoots to belowground plant parts (Van der Linden,
330	1980, 1986). Besides, frequent aboveground harvesting can slow growth and biomass
331	development. Therefore, the annual harvesting of the plants should be based on the
332	consideration of the economic, climatic and wetland operation conditions. According to

333	Zheng et al. (2015), long term nutrient removal by annual harvesting of plants stands in
334	autumn season can be considered as a good plant management approach in the
335	prevailing conditions of northwestern China. This is because harvesting at the end of the
336	growing season in autumn does not only promote the new plants' uptake of more
337	nutrients. It also increases the belowground biomass and stimulates the microbial
338	activities in the wetlands, both of which enhances CWs performance.
339	9
340	3.5 Plant-sociological results after interspecific competition
341	The importance values (I.V.) of 132.3 and 145.3 for <i>P. australis</i> in the FWS and SSF
342	wetlands, respectively, and 67.7 and 54.7 for T. orientalis, respectively, indicated that
343	after two growing seasons (2010–2012), P. australis was the predominant species in the
344	two wetlands. The competitive values (C.V.) of the plant species, which were in the
345	following order: P. australis in FWS (64.7), P. australis in SSF (64.3), T. orientalis in
346	SSF (35.7), and <i>T. orientalis</i> in FWS (35.3), indicate that <i>P. australis</i> grew better than <i>T.</i>
347	orientalis under interspecific competition in both FWS and SSF wetlands (Table 4).
348	G
349	From the plant-sociological results of I.V. and C.V., P. australis showed the overall
350	highest growth performance in the two CWs. This high growth performance of P.
351	australis coupled with its high aboveground dry weight value resulted in the species
352	expanding its coverage in both the FWS and SSF wetlands (Fig. 4). This finding is

353 particularly interesting for the FWS wetland because the continuously inundated

354	condition during the growing season would be expected to confer competitiveness on
355	Typha spp., rather than Phragmites spp., which prefer well-drained or intermittently
356	inundated conditions. According to Asaeda et al. (2005), the growth of <i>Typha</i> spp.
357	seedlings is usually facilitated in a continuously flooded condition, and decreases in a
358	well-drained condition. Kercher and Zedler (2004) also reported that the growth of
359	Typha spp. is not negatively influenced by an inundated condition, and could result in a
360	competitive advantage to Typha spp. over Phragmites spp
361	
362	Nonetheless, despite the relatively high growth performance in both wetlands,
363	interspecific competition of <i>P. australis</i> showed different ecological characteristics, in
364	terms of the correlation between growth parameters, such as shoot height, density, and
365	dry weight. For example, while relatively high plant density and shoot height
366	determined the high above ground dry weight of <i>P. australis</i> in the FWS wetland ( $p < p$
367	0.01), this association was not evident in the SSF wetland. This finding suggests that
368	competition likely leads to different ecological responses among plant species in
369	different wetland systems. Consequently, such different responses could affect the
370	competitive status and vegetation composition in constructed wetlands (i.e., competitive
371	effect of competitive ability) (Goldberg and Landa, 1991; Keddy et al., 1994). This
372	differential response is further demonstrated in Figure 4, whereby the encroachment of P.
373	australis into the T. orientalis stands was exhibited differently in the two wetland
374	systems. In the FWS wetland, the encroachment started in the inflow zone, which later

 $\leq$ 

375	spread to the outflow zone. In the SSF wetland, however, P. australis started to
376	encroach simultaneously at the inflow, outflow and plant border zones.
377	
378	Furthermore, the speed of encroachment was different in the FWS and SSF wetlands.
379	The expansion of <i>P. australis</i> into <i>T. orientalis</i> stands in the SSF wetland was much
380	more rapid than that in the FWS wetland (Fig. 4). This phenomenon could be explained
381	by the fact that <i>P. australis</i> has a much stronger growing capability, resource utilization
382	capacity, competitive potential and higher potential leaf photosynthesis capacity than T.
383	orientalis (Fu et al., 2011). These advantages were more evident in the SSF wetland,
384	where there were no conducive conditions for the growth of plants. Therefore, it
385	becomes difficult to maintain single plant colonies within the mixed culture of wetlands
386	due to the progressive dominance of the most aggressive species. Thus, wetland plants
387	should be carefully selected during the planning stage when mixed culture is to be used
388	in a particular CW.
389	
390	

#### 391 **4. Conclusions**

The effect of interspecific competition was notable for *P. australis*; it showed the highest growth performance in both FWS and SSF wetlands. In mixed-culture, *P. australis* demonstrates superiority in terms of competitive interactions for space between plants. Furthermore, the interspecific competition caused different ecological

396	responses	of plant	species	in the two	CWs.	Additionally,	while	plants	nutrients	uptake
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- 397 accounted for a higher proportion of the nitrogen removal in FWS, that in the SSF
- accounted for a higher proportion of the phosphorous removal. Special management
- effort is, thus, required to maintain habitat characteristics and the design macrophyte
- 400 diversity in mixed culture CWs.

401

#### 402 Acknowledgements

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406

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#### 533 List of figure captions

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- 535 Figure 1 Variations in (a) COD, (b) BOD<sub>5</sub>, (c) TN, (d) NH<sub>3</sub>-N, (e) TP, (f) PO<sub>4</sub><sup>3-</sup>-P
- concentrations in the pilot FWS and SSF wetlands during the operation period (n=77
- 537 for BOD<sub>5</sub> and  $PO_4^{3-}$ -P, n=62).

538

539 Figure 2 Average plant densities in FWS and SSF wetlands (a) and total mean

aboveground dry weights in FWS and SSF wetlands (b) at the end of the growing

season during the experimental period.

542

543 Figure 3 Average nutrients content (a, b) and nutrients uptake by the aboveground

biomass of *P. australis* and *T. orientalis* in the two wetland types (c, d) at the end of the

545 growing season during the experimental period.

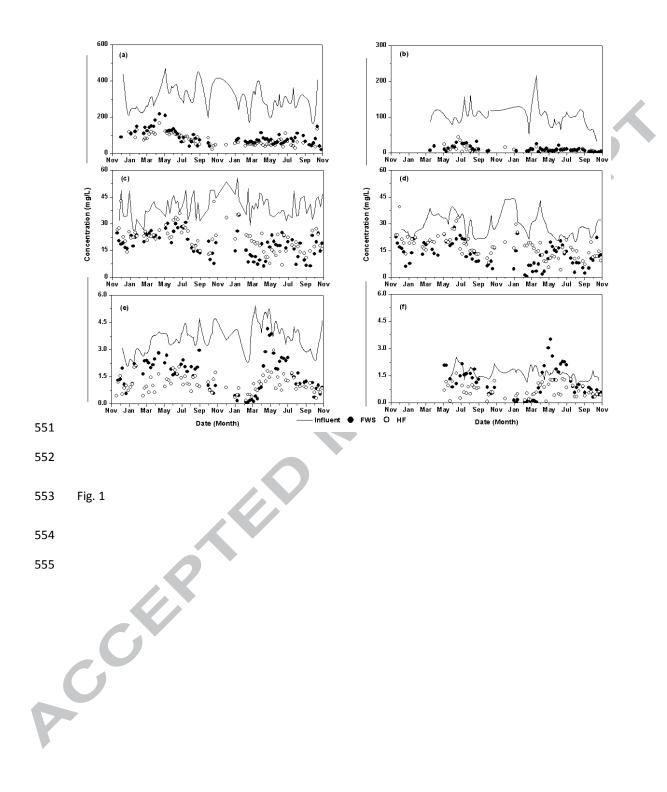
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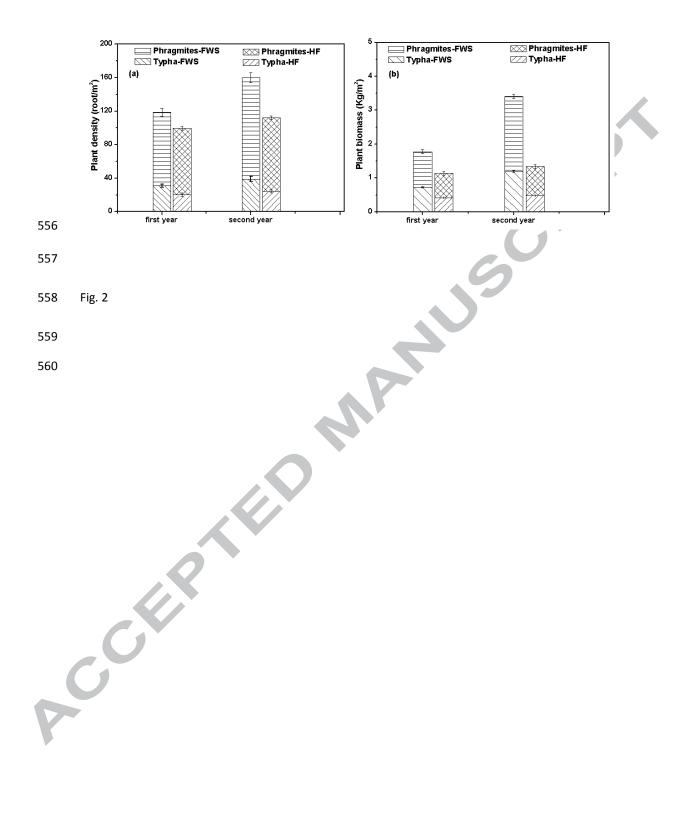
547 Figure 4 Changes in the growth patterns of *P. australis* and *T. orientalis* in FWS and

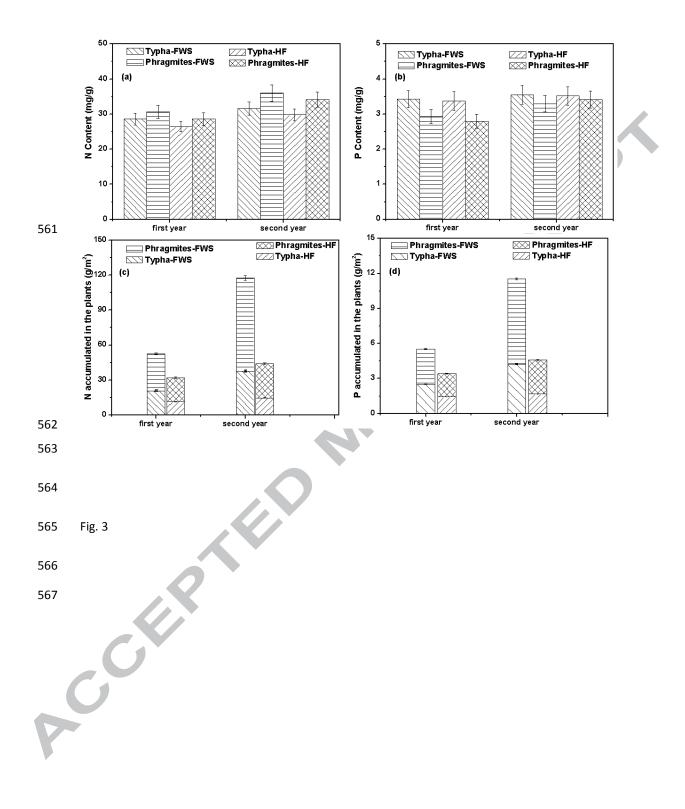
548 SSF CWs during the time of operation.

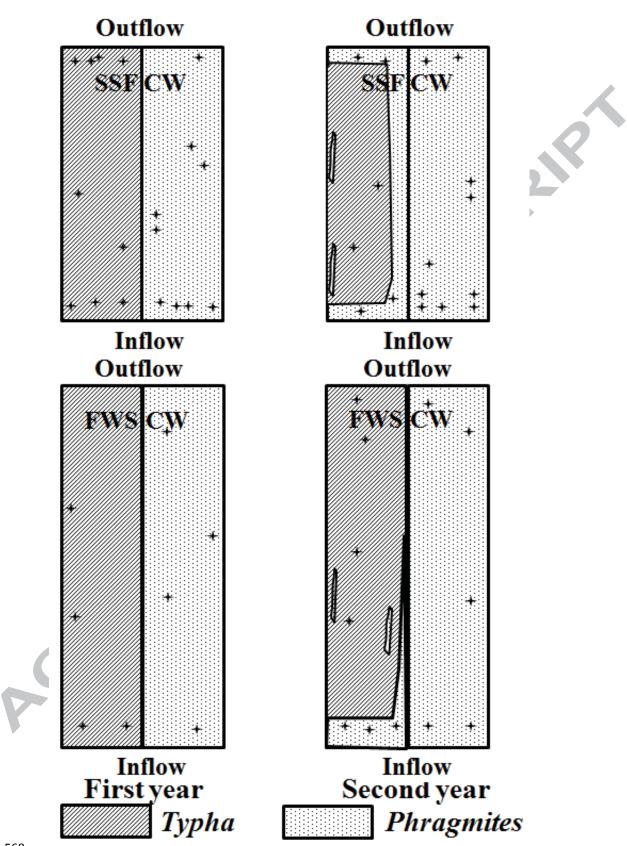
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gravel 8.76 51.61 1.11 1.01 8.79 27.58 4.05 0.73 - 5.1	0	Table 1 C	hemica	l pro	oper	ties	of the s	ubstrat	es						
C       N       P       O       Na       Mg       Al       Si       K       Ca       Ti       Fee         gravel       8.76       -       -       51.61       1.11       1.01       8.79       27.58       4.05       0.73       -       5.1         Sand       7.14       -       -       56.82       2.99       0.23       7.67       26.25       3.55       0.87       -       1.6         1       2       3       -       -       56.82       2.99       0.23       7.67       26.25       3.55       0.87       -       1.6         1       -       -       -       56.82       2.99       0.23       7.67       26.25       3.55       0.87       -       1.6         2       -		Carls at set of	лU				Chem	nical co	omposi	tion ma	ass perce	entage	(%)		
Sand 7.14 56.82 2.99 0.23 7.67 26.25 3.55 0.87 - 1.6		Substrate	рп	С	N	Р	0	Na	Mg	Al	Si	K	Ca	Ti	Fe
		gravel	8.76	-	-	-	51.61	1.11	1.01	8.79	27.58	4.05	0.73	2	5.12
		Sand	7.14	-	-	-	56.82	2.99	0.23	7.67	26.25	3.55	0.87	-	1.62
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#### **Table 1** Chemical properties of the substrates

Table 2 Pollutant concentrations and loadings at the influent and effluent of the two 575

TN           a         SD <sup>a</sup> 6         0.7           6         24	n	<b>SD</b> <sup>a</sup>	Mea n			<sup>3-</sup> -P SD <sup>a</sup>
6 0.7	n		n			SD
6 0.7	n		n			
	29.1	0.8	36			
8 24			5.0	0.1	1.6	0.1
	1005	28	124. 4	3.5	55.3	3.5
5 0.7		0.7	1.6	0.1	1.1	0.1
8 18	560	15	70.6	1.6	17.6	1.1
7 0.8	17.0	0.7	1.1	0.1	0.7	0.05
9 16	416	14	87.0	2.0	32.1	1.3
7	7 0.8	7 0.8 17.0	7 0.8 17.0 0.7	7 0.8 17.0 0.7 1.1	7 0.8 17.0 0.7 1.1 0.1	

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						0	0	
Period	Wetland	parameter	Influent (g/m <sup>2</sup> )	Effluent (g/m <sup>2</sup> )	P. autralis (g/m <sup>2</sup> )	T. orientalis (g/m <sup>2</sup> )	Total plant uptake (g/m <sup>2</sup> )	Plant uptake $(\%)$
	EW/C	NT	1335.1	723.5	31.7	20.8	52.5	8.6
Linet moon	2 M J	TP	116.2	60.4	3.0	2.5	5.5	9.9
rust year		NT	1335.1	831.2	20.4	11.1	31.5	6.2
	700	TP	116.2	42.2	2.0	1.4	3.4	4.6
		NT	1401.9	556.3	80.0	37.6	117.6	13.9
Concert Lance	2 M L	TP	132.5	49.6	7.3	4.2	11.5	13.9
second year	C C F	NT	1401.9	662.5	29.2	14.2	43.4	5.8
	100	TP	132.5	36.1	2.9	1.7	4.6	4.8
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Plant-sociological value	FWS	wetland	SSF	wetland
-	P. australis	T. orientalis	P. australis	T. orientalis
.V. <sup>1</sup>	132.3	67.7	145.3	54.7
C.V. <sup>2</sup>	64.7	35.3	64.3	35.7
Species importance valu	e			5
Species competitive valu	ue. Lower value	es indicate low c	ompetitiveness	under an
nterspecific competitive	condition.		$\mathbf{\mathcal{O}}$	
			·	
	$\mathcal{O}$			

595 • I	nterspecific competition of <i>Phragmites</i> and <i>Typha</i> was investigated in two large
596 C	CWs
597 • <i>H</i>	? australis showed higher growth performance in mixed cultured FWS and SSF
598 v	vetlands
599 • I	nterspecific competition caused different ecological responses of plant species
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PC	