

**Improving low-temperature performance of surface flow constructed wetlands  
using *Potamogeton crispus* L. plant**

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**Abstract:**

In this study, enhanced organics and nitrogen removal efficiency in SFCWs by  
different submerged plants for polluted river water treatment under cold temperature  
was evaluated. High average removal efficiencies of COD (92.45%),  $\text{NH}_4^+\text{-N}$   
(93.70%) and TN (55.62%) were achieved in experimental SFCWs with *P. crispus*  
compared with SFCWs with other plants. SFCWs with underground *P. australis* root  
also presented better performance than the unplanted systems, indicating its positive  
role of contamination removal in winter. The results of this study indicated SFCWs  
with hardy submerged plant *P. crispus* could be a more effective and sustainable  
strategy for removing organics and nitrogen in shallow nutrient enriched river water  
ecosystems under cold climate.

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**Keywords:** Cold climate; Surface flow constructed wetlands; Organics and nitrogen removal; Polluted river water

## 1. Introduction

Constructed wetlands have been a competitive option for point and non-point pollution source control, ecological restoration as well as water sources protection (Wu et al., 2015). Thereinto, surface flow constructed wetlands (SFCWs) become more and more attractive in treating numerous polluted rivers (mainly receiving the effluent from municipal sewage plant with COD of 50 mg/L and NH<sub>4</sub>-N of 5 mg/L) in China, in order to meet the Grade-III (COD 20 mg/L, NH<sub>4</sub>-N 1 mg/L) national surface water standards (GB3838-2002) (Wu et al., 2015; Fan et al., 2013). About 7800 ha SFCWs have been constructed in Shandong province and accordingly 400 million m<sup>3</sup> polluted river water were purified every year.

The seasonal alteration from summer to cold winter brings the biggest challenge for the sustainable operation of the extensive SFCWs. Under cold temperature, apart from microbial activities are inhibited, plant decay is one of the main reasons account for poor removal efficiency of pollutants (Wu et al., 2015). Most wetland plant species such as *P. australis*, *T. orientalis*, *lotus*, *Acorus calamus*, *Canna generalis*, *Juncus effuses* perform better in warmer climates and deteriorate during winter. (Yan and Xu, 2014). It was reported that 70 percent of *P. australis* leaves and 50 percent of *T. latifolia* leaves turned yellow in October and completely declined by the end of November in northern regions (Yan and Xu, 2014), thereby impacting microbial metabolism and growth with subsequent depressed contaminant removal (Pang et al.,

2015). Plants are important structural and biological component of CWs and play an essential role in wetland succession. They not only take up nitrogen and phosphorous directly as nutrients for growth and reproduction, but also act as intermedium for a variety of chemical and microbial processes by increasing the environmental diversity, carbon and oxygen supply in the rhizosphere which in turn promotes removal performance in CWs (Ong et al., 2010).

Plant selection is therefore proved to be extremely important for operation and maintenance of SFCWs located in regions with cold months. Cold resistant plant species especially submerged aquatic vegetation can be selected to supply microbial attachment and increase land covering in winter. Submerged plants also greatly help in lakes restoration due to effective nutrient uptake from the water through their shoots (Dierberga et al. 2002). *Potamogeton crispus* L. (*P. crispus*) is a typical submerged herbaceous perennial plant and the total biomass can be obtained by higher nitrogen and phosphorus contents of sediment (Wang et al. 2013). Little attention has been paid to determining the feasibility of *P. crispus* in SFCWs during cold winter. Its roles of supplying oxygen for microbial community and removing nitrogen and phosphorous in SFCWs remain unclear and need to be investigated.

The main objective of the present investigation was to evaluate the performance of the microcosm SFCWs by different submerged plants in treating polluted river water under cold winter. The contribution of *P. crispus* as well as underground part of *P. australis* on simultaneous transformation of organics and nitrogen were also focused. The results of this study might provide significant implications for

sustainable operation of SFCWs in low-temperatures regions during winter.

## 2. Materials and methods

### 2.1. Microcosm wetland system

The CWs were developed in Jinan, northern China (36°40'36"N, 117°03'42"E), with the sub-humid continental monsoon climate, which is characterized by an annual precipitation of 670.7 mm and average temperature of 14.7 °C.

Twelve microcosmic SFCWs were divided into four groups with different plants under a transparent rain shelter in November, 2013. Each system was 50 cm deep and 40 cm in diameter, with an outlet at the bottom. A 20 cm layer of washed sand (particle size <2 mm, mainly Si<sub>2</sub>O<sub>3</sub>, Al<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>) was used as the substrate. Water depth was kept 25cm above the sand surface to facilitate the growth of plants.

Three native submerged plants species were transferred from Nansi Lake, Jinan, and then cultivated with a 10% modified Hoagland's solution for one week before use. Fifteen shoots approximately 0.10~0.15 m high were adopted while one group unplanted as the control (A: control, B: *P. australis* roots, C: *Vallisneria spiralis*, D: *P. crispus*). After planting, the CWs were acclimated for about half months.

### 2.2. Experimental procedure

In order to minimize variability in the experiment, synthetic sewage treatment plant effluent (Class IA) was used in this study by mixing the following components in tap water: 44.53 mg/L sucrose, 23.57 mg/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.19 mg/L KH<sub>2</sub>PO<sub>4</sub>, 50.5 mg/L KNO<sub>3</sub>, 10 mg/L MgSO<sub>4</sub>, 10 mg/L FeSO<sub>4</sub>, and 10 mg/L CaCl<sub>2</sub>. Table 1 shows the composition and the average concentrations of pollutants in the feed.

88 The experiment cycle was 9 days because of low temperature. The influent was  
 89 supplied in batch mode into CWs within 15 min at about 8:00 am on the first day of  
 90 each cycle. Effluent was manually discharged through a valve at the bottom of each  
 91 system. Eleven cycles lasted from November 2013 to March 2014. An aquarium  
 92 heater was adopted to avoid freezing when temperature was below zero.

### 93 **2.3. Water sampling and analysis**

94 At 8:00 am every day during the cycle, water samples were collected using a syringe  
 95 tube at the middle water depth of all 12 units. The samples were analyzed  
 96 immediately for COD (closed reflux method), nitrogen (persulfate and  
 97 spectrophotometry method) and total phosphorus (TP, ascorbic acid reduction method)  
 98 (APHA, 2005). Dissolved oxygen (DO) and temperature were measured by a DO  
 99 Meter (HQ 30d 53LED™ HACH, USA) in situ.

### 100 **2.4. Statistics**

101 All statistical analyses were performed through the statistical program SPSS 18.0  
 102 (SPSS Inc., Chicago, USA). Two-sample t-tests were used to evaluate the significance  
 103 of differences between means. In all tests, differences and correlations were  
 104 considered statistically significant when  $P < 0.05$ .

## 105 **3. Results and discussion**

### 106 **3.1. DO profile during experiment cycle**

107 The water temperature during the studies was  $3.9 \pm 2.3$  °C. Fig. 1 shows the  
 108 cyclic DO distribution of the twelve CWs. Distinct oxygen consumption and  
 109 reoxygenation phase were clearly observed in all CWs. The difference among various

plants could also be well distinguished from the DO profile. The DO concentration in all CWs changed immediately once influent was pumped in and decreased sharply during the first three days. The highest oxygen consumption rate was observed in the wetlands D with *P. crispus* than those in others, mainly because the well growth condition during winter offered more attachment sites and photosynthetic oxygen for efficient biodegradation process of the contaminants (Eriksson and Weisner, 1997). The results were consistent with previous study of Wang et al., 2016, which reported that the plant roots remained active even when the aboveground was dormant, and also enhanced microbial abundance compared with the unplanted wetlands. The DO concentrations gradually increased after three-day degradation of pollutants. The *P. crispus* with lush leaves in winter exhibited remarkable higher reoxygenation ability than another submerged plant *V. natans*, reflecting high utilization potentiality in pollution removal during cold winter. In our study, positive role of underground *P. australis* root in wetlands reoxygenation was found compared with the control. Wang et al. (2015) observed that about  $22.95 \pm 17.57 \text{ mg/m}^3 \cdot \text{day}$   $\text{O}_2$  concentration was provided by *P. australis* root from radial oxygen loss under  $5.56 \pm 1.78 \text{ }^\circ\text{C}$  in winter.

### 3.2. Organic matter removal

The average influent concentrations of COD,  $\text{NH}_4^+\text{-N}$ , TN, and TP were 61.68, 4.87, 14.20 and 0.71 mg/L, respectively. Table 1 shows the performance of the twelve SFCWs during the experimental period. The *P. crispus* CWs performed best on organic matters degradation and nutrient removal. The removal of the contaminants during the typical cycle (9 days) was further analyzed.

Fig. 2 (a) shows the COD profiles in SFCWs with different plants. Contrary with other wetlands, the COD concentration decreased rapidly in *P. crispus* wetlands immediately after feeding and changed slightly after three days, which is corresponding to the DO profile. The average COD removal rate in wetlands D (92.45%) was markedly higher than those (67.32%, 72.60% and 80.36%) in wetlands A, B and C, respectively ( $P < 0.05$ ). This could also be reflected by the reaction coefficient based on the kinetic models. The estimated first-order removal rate constants ( $k$ ) for COD removal in wetlands D were  $0.68 \pm 0.05 \text{ d}^{-1}$ , while much lower  $k$  values of  $0.14 \pm 0.09$ ,  $0.20 \pm 0.06$  and  $0.36 \pm 0.08 \text{ d}^{-1}$  in A, B and C was found, respectively. Only 2 days are enough for wetlands D to meet the Grade-III national surface water standards whereas more than 8 days were required for other wetlands. Both the highest oxygen consumption rate and reoxygenation rate demonstrated the exuberant vitality of *P. crispus* at low temperature, which consequently facilitated microbial diversity in the wetlands (Yan and Xu, 2014). The degradation of aerobic heterotrophic bacteria as well as plant uptake all contributed to high removal efficiency of COD (Fan et al., 2010). The outstanding COD removal rate in *P. crispus* wetlands was far superior to other studies reported at similar temperature (Jing et al., 2001; Li et al., 2008), showing its great potential in wastewater treatment during winter.

### 3.3. Nitrogen removal

The conversion profile of various nitrogen forms is shown in Fig. 2 (b, c and d). The wetlands D with *P. crispus* exhibited surprisingly superior nitrification efficiency

154 compared with other planted and unplanted wetlands. The estimated first-order  
155 removal rate constants ( $k$ ) for  $\text{NH}_4\text{-N}$  removal in *P. crispus* wetlands were  $0.68 \pm 0.10$   
156  $\text{d}^{-1}$ , higher than those of  $0.28 \pm 0.06$ ,  $0.51 \pm 0.11$  and  $0.58 \pm 0.15 \text{ d}^{-1}$  in wetlands A, B  
157 and C, respectively. As shown in Table 1, *P. crispus* systems demonstrated much  
158 lower effluent concentration of  $\text{NH}_4\text{-N}$  ( $0.31 \pm 0.03 \text{ mg/L}$ ) than other wetlands  
159 ( $P < 0.05$ ), which satisfied the standards of Grade-III national surface water. The  
160 highest  $\text{NH}_4\text{-N}$  removal (93.70%) was even superior to many results obtained in  
161 summer with high temperature (Jing et al., 2001). Compared to *P. australis* and *V.*  
162 *natans* in present study, *P. crispus* with an average length of 0.45 m after rapid growth  
163 provided favorable surface area for the attached growth of microorganisms. With  $\text{O}_2$   
164 production during daylight, nitrification of the epiphytic microbial communities could  
165 be of great importance for  $\text{NH}_4\text{-N}$  conversion (Eriksson and Weisner, 1997).

166 Without vegetation, the removal of  $\text{NO}_3\text{-N}$  in the control was limited compared  
167 with planted systems, mainly because of lacking in attachment for microorganisms.  
168 The unaccounted part of the  $\text{NO}_3\text{-N}$  removal was presumably lost through  
169 denitrification. In Fig. 2(c), the removal rate constants ( $k$ ) of *P. crispus* systems ( $0.26$   
170  $\pm 0.05 \text{ d}^{-1}$ ) were not as competitive as  $\text{NH}_4\text{-N}$ , which could be attributed to two  
171 reasons. Firstly, high  $\text{NH}_4\text{-N}$  conversion efficiency to  $\text{NO}_3\text{-N}$  slowed removal rate of  
172  $\text{NO}_3\text{-N}$ . Secondly, the low influent COD/N ratio (4.34) was insufficient for further  
173 removal of  $\text{NO}_3\text{-N}$  via denitrification. However, none excessive accumulation of  
174  $\text{NO}_3\text{-N}$  was observed in *P. crispus* systems. Denitrification during the night might  
175 made important contribution when  $\text{O}_2$  saturation in the wetlands was much lower than

176 daylight.

177 As a result of promotion in nitrification and denitrification, the removal rate  
 178 constants ( $k$ ) for TN removal in *P. crispus* wetlands was  $0.39 \pm 0.04 \text{ d}^{-1}$ , still higher  
 179 than other wetlands. Vymazal (2007) observed TN removal varied between 40% and  
 180 55% with removed load ranging between 250 and 630  $\text{g N m}^{-2} \text{ yr}^{-1}$  depending on CWs  
 181 type and influent loading. In present study, about 55.62% of influent TN was removed  
 182 from the *P. crispus* wetlands D, showed no significant difference compared with  
 183 43.47%, 47.48% and 50.31% in wetlands A, B and C ( $P > 0.05$ ), respectively. Relative  
 184 studies on sustainably operation of SFCWs in cold temperature were hard to be found  
 185 (Yan and Xu, 2014). Considering positive results obtained under the temperature of  
 186  $3.9 \pm 2.3 \text{ }^{\circ}\text{C}$ , *P. crispus* in SFCWs could be a promising choice to intensify nitrogen  
 187 removal performance for the polluted river water treatment in winter.

#### 188 **4. Conclusion**

189 The application of submerged plant *P. crispus* significantly enhanced the  
 190 performance of SFCWs during the treatment of synthetic polluted river water under  
 191 cold temperatures. Average removal efficiencies of COD,  $\text{NH}_4^+\text{-N}$  and TN were  
 192 92.45%, 93.70% and 55.62%, respectively. The estimated first-order removal rate  
 193 constants ( $k$ ) also reflected the superior pollutants transformation rates in *P. crispus*  
 194 systems. Adoption of *P. crispus* in SFCWs could be a more effective and sustainable  
 195 strategy for organics and nitrogen removal from shallow nutrient enriched river water  
 196 ecosystems. Further studies on the microbial mechanisms should be carried out to  
 197 support the initial findings in this study.

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249 **Figure Captions:**

250 **Fig. 1.** Typical profile of DO in the SFCWs with different plants under low  
251 temperature.

252 **Fig. 2.** Dynamic removals of COD and nitrogen in the SFCWs with different plants  
253 during the typical operating period.

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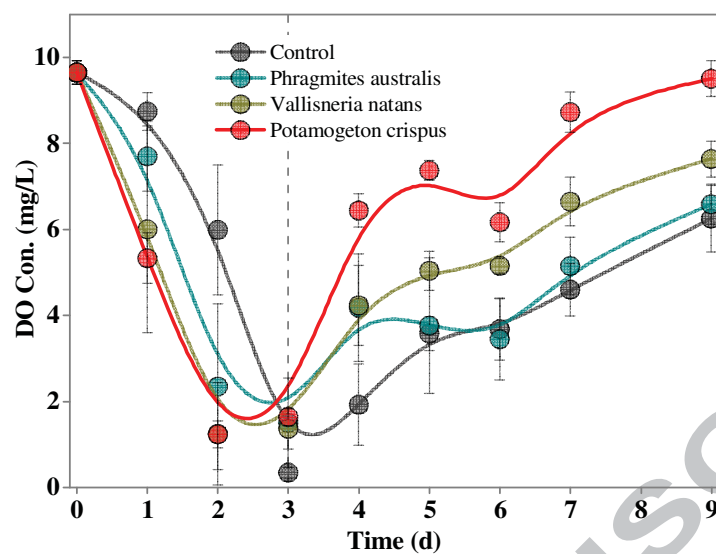
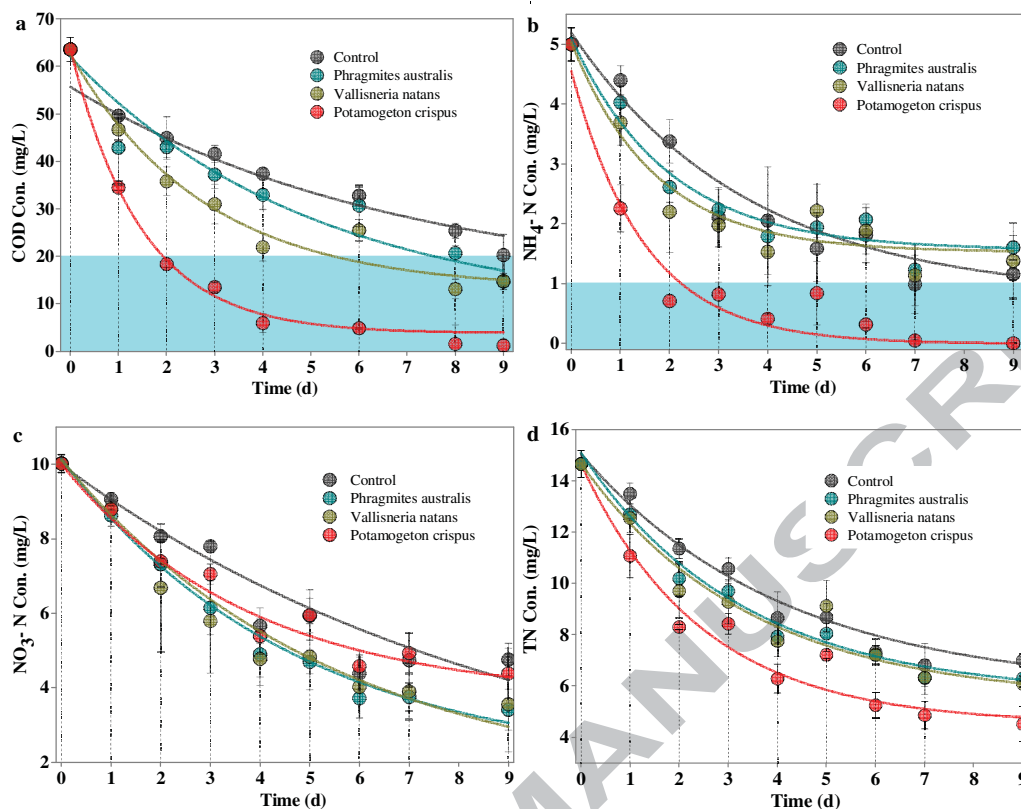


Fig. 1. Typical DO profile in the SFCWs with different plants under low temperature.

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Fig. 2. Dynamic removals of COD and nitrogen in the SFCWs with different plants during the typical operating cycle.

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**Table 1** Treatment performance of the SFCWs during the experimental period (mean  $\pm$  SD, n = 11).

Parameter	Wetland	Effluent (mg/L)	Removal (%)	First order kinetics (K-C* model)	R <sup>2</sup>	k (d <sup>-1</sup> )
COD	A	20.16 $\pm$ 4.86	67.32 $\pm$ 7.8	y=12.70+43.05*exp(-0.14*x	0.9	0.14 $\pm$ 0.0
			8	)	6	9
	B	16.90 $\pm$ 3.19	72.60 $\pm$ 5.1	y=7.88+54.19*exp(-0.20*x)	0.9	0.20 $\pm$ 0.0
			8		7	6
	C	12.11 $\pm$ 2.45	80.36 $\pm$ 3.9	y=13.08+49.57*exp(-0.36*x	0.9	0.36 $\pm$ 0.0
			7	)	6	8
	D	4.66 $\pm$ 0.51	92.45 $\pm$ 0.8	y=3.83+59.96*exp(-0.68*x)	0.9	0.68 $\pm$ 0.0
			2		9	5
NH <sub>4</sub> <sup>+</sup> -N	A	1.35 $\pm$ 0.15	72.30 $\pm$ 3.1	y=0.77+4.42*exp(-0.28*x)	0.9	0.28 $\pm$ 0.0
			4		8	6
	B	1.28 $\pm$ 0.07	73.61 $\pm$ 1.3	y=1.56+3.54*exp(-0.51*x)	0.9	0.51 $\pm$ 0.1
			9		5	1
	C	1.49 $\pm$ 0.13	69.29 $\pm$ 2.6	y=1.53+3.50*exp(-0.58*x)	0.9	0.58 $\pm$ 0.1
			7		5	5
	D	0.31 $\pm$ 0.03	93.70 $\pm$ 0.6	y=-0.0054+4.56*exp(-0.68*x)	0.8	0.68 $\pm$ 0.1
			3		8	0
TN	A	8.03 $\pm$ 1.01	43.47 $\pm$ 7.0	y=5.83+9.22*exp(-0.24*x)	0.9	0.24 $\pm$ 0.0
			5		8	5
	B	7.46 $\pm$ 0.64	47.48 $\pm$ 4.5	y=5.63+9.46*exp(-0.30*x)	0.9	0.30 $\pm$ 0.0
			3		9	4
	C	7.06 $\pm$ 0.47	50.31 $\pm$ 3.3	y=5.43+9.24*exp(-0.29*x)	0.9	0.29 $\pm$ 0.0
			3		8	5
	D	6.30 $\pm$ 0.29	55.62 $\pm$ 2.0	y=4.52+10.14*exp(-0.39*x)	0.9	0.39 $\pm$ 0.0
			9		6	4
TP	A	0.15 $\pm$ 0.04	79.28 $\pm$ 5.2	—	—	—
			9			

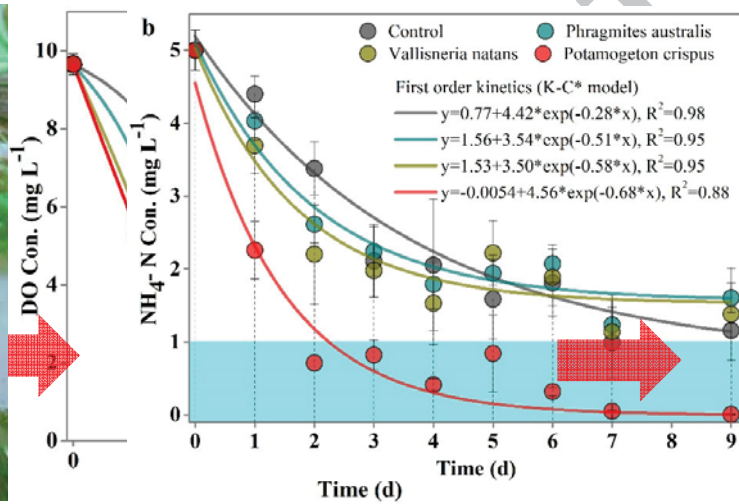
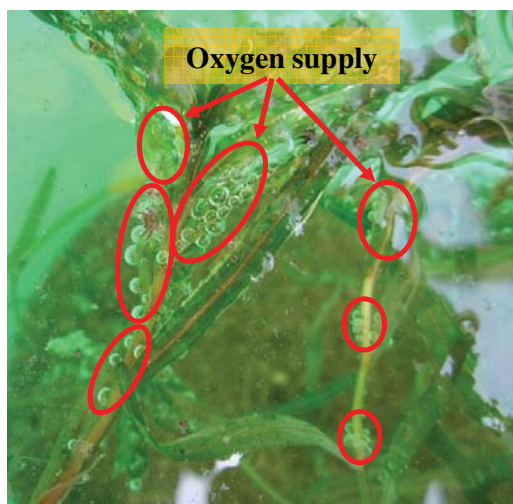
B	0.15±0.02	78.53±3.1	—	—	—
		0			
C	0.21±0.05	70.49±6.7	—	—	—
		1			
D	0.049±0.00	92.97±0.6	—	—	—
	5	6			

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276 **Research Highlights**

277 1) High oxygen supply efficiency is observed in SFCWs with *P. crispus* in cold  
278 winter.

279 2) High average removal rates of COD (92.45%) and  $\text{NH}_4^+\text{-N}$  (93.70%) were  
280 obtained.

281 3) The underground part of *P. australis* enhanced wetlands performance in winter.

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