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Enhancement of surface flow constructed wetlands performance at low temperature through seasonal plant collocation

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Abstract:

In the present study, a novel seasonal plant collocation system (SPCS), specificially the *Potamogeton crispus* and *Phragmites australis* series system, was investigated to enhance the performance of surface flow constructed wetlands (SFCWs) at low temperature. Results of a year-round experiment showed that SPCS conquered the adverse effect of low temperature and achieved sustainable nutrients removal. In addition, during winter, removal efficiencies of NH_4-N, TP, COD, and TN in SPCS were 18.1%, 17.6%, 10.1% and 5.2% higher than that in the control, respectively. *P. crispus* and *P. australis* complemented each other in terms of plant growth and plant uptake during the experiment period. Furthermore, it emerged that *P. crispus* could increase the quantity of ammonia oxidizing bacteria by 10.2%, due to

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its high oxygen enrichment ability. It is suggested that seasonal plant collocation has a promising future in SFCWs of areas being affected by climate change, e.g. northern China.

Keywords: Constructed wetlands; Nutrients removal; Low temperature; Seasonal plant collocation.

1. Introduction

Constructed wetlands (CWs) are engineered systems that enable natural treatment processes to occur within a more controlled environment. They have been widely used for wastewater treatment because of their ability to remove nutrients, organic matter, pathogenic bacteria and other pollutants (Wu et al., 2016). Compared with conventional purification systems, CWs are economical, easy to operate and maintain (Li et al., 2014). Removal of contaminants in CWs is highly dependent on the combined effects of filtration, sedimentation, plant absorption and complex microbial processes. These include the biodegradation of organics, nitrification–denitrification of nitrogen, uptake of nitrogen and phosphorus and adsorption of phosphorus, etc. (Ávila et al., 2015; Wu et al., 2016).

Unlike other traditional wastewater treatment processes, CWs are man-made nature systems, which are subject to changes in seasonal temperature (Rai et al., 2015). Previous studies have shown that treatment performance of CWs clearly declined obviously at low temperature (Bojcevska & Tonderski, 2007; Wang & Li, 2015). Wang et al. (2012) reported that removal efficiency of ammonia nitrogen (NH_4-N), total nitrogen (TN) and total phosphorus (TP) declined by 15%, 45% and 16%,

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respectively, in cold winter compared to that in warm seasons in a long-term study of a two-stage surface flow constructed wetland (SFCW). Common thermophile plants (e.g., *P. australis* and *Arundo donax*) in CWs are in a state of senescence at low temperature, which leads to weak metabolism (Van de Moortel et al., 2010). As a result, both plant nutrient uptake rates and the microbial quantities around plant roots declined. Moreover, microbial activities can also be depressed when at low temperature. Various studies on wastewater treatment reported that both nitrite and nitrate bacteria were strongly inhibited below 10 °C (Kim et al., 2006; Randall & Buth, 1984), which led to poor efficiency in nitrogen removal. Thus, in the regions like northern China, strategies should be studied and implemented to enhance treatment efficiency of CWs at low temperature. Doing so will make it possible to overcome the fluctuations in performance that are caused by climate change.

To date, strategies for improving CWs treatment efficiency at low temperature have mainly concentrated on optimizing hydraulic loading, psychrotrophic bacteria selection, and insulation. Zhang et al. (2006) reported that removal efficiencies of NH_4-N and COD of a wetland when treating polluted river water in winter increased from 14% to 39% and 20% to 31%, respectively, when hydraulic loading rate decreased by 50%. However, all the above methods have some disadvantages. Low hydraulic loading could improve wastewater treatment efficiency in winter, but at the cost of reduced treatment capability. Psychrotrophic bacteria have poor competitive ability in wetland, and insulation measurements can be costly. It is therefore critical

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and urgent to develop an efficient and economical method to improve CWs' performance at low temperature.

Plants are essential structural components of CWs, and play an important role in wastewater treatment because of their uptake, storage, and release processes. Koottatep and Polprasert (1997) reported that plant uptake contributed 43% of the TN removal in a study that explored the role of plant uptake in nitrogen removal in wetlands. Greenway and Woolley (2000) also reported that plant uptake accounted for 44%-65% of TP removal in a study on plant biomass and nutrient removal in a wetland. Wetland plants show significant differences in physiological properties. Many thermophilous plants (e.g. *P. australis* and *Acorus calamus*) decline in winter while many cold-resistant plants (e.g. *P. crispus* and *Ceratophyllum demersum*) are still growing. A submerged and emergent plants collocation system, which consisted of *P. australis* and *P. distinctus,* was proposed by Weisner et al. (1994) to study the characteristics of organic carbon release in the system. They found that the removal of nitrogen improved theoretically. Liang et al. (2011) compared monoculture and mixed wetlands, and observed that mixed wetlands had significant advantages in terms of treatment performance, adaptability for seasonal variations, and abundance of microbial populations compared to monoculture wetlands. However, no study has to date reported using seasonal submerged and emergent plants collocation to enhance the treatment performance of SFCWs at low temperature.

Therefore, the aim of this study was to evaluate the efficiencies of seasonal plant collocation system (SPCS) for intensifying pollutants removal, focusing on low

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temperature condition. In addition, the mechanisms of nutrients removal in the SPCS were studied as well.

2. Materials and Methods

2.1. Experiment setup

The experiment was conducted under a transparent rain shelter in Baihua Park in Jinan, northern China (36°40′ 36″N, 117°03′42″E). The climate is characterized by annual precipitation of 670.7 mm, and the highest temperature is above 35 °C in summer while the lowest temperature can reach below 0 °C in winter. The experiment period lasted from March 2, 2015 to January 31, 2016. In February 2016, which is generally known as the freeze-up period, the experiment was stopped.

P.crispus, one of the common submerged plant species found in north China, was used for this study. Ye and Guo (2011) constructed a series of water purification wetlands with winter vegetation, and *P.crispus* indicated a continuously purification effect in winter. The SPCS comprised *P. crispus* and *P. australis* in a series, while normal plant composition system (NPCS), acting as the control was planted using only the *P. australis* species. Each system was composed of two units in a series, and each unit was constructed by plexiglass with the following dimensions: 100cm height, 90cm length and 60cm width (Fig. 1a). Dimensional gradation substrate was used in the treated units: a 10cm bottom layer of gravel (1–2 mm in diameter) and a 30cm top layer of washed river sands (1-2mm in diameter). In SPCS, the first treated unit (FTU) was planted with *P.crispus* and the second treated unit (STU) was planted with *P. australis*, while both treated units were planted with *P. australis* in NPCS (Fig. 1b). *P.*

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crispus and *P. australis* were planted at a density of 120 rhizomes per square meter and 45 rhizomes per square meter, respectively. All plants were transferred from Xiaomei River wetland, Liaocheng, China. After planting, the systems were fed with low concentration of wastewater and stabilized for two weeks before the experiment began. *P. crispus* did not survive the summer, so the wilted *P. crispus* was removed in September 9, 2015, and was reseed in September 15, 2015. The systems' influent was met for the water quality: 50mg/L COD, 5mg/L NH_4-N, 20mg/L TN and 1mg/L TP. All systems were fed continuously with a hydraulic retention time (HRT) of 5d.

2.2. Plant physiology

Plant heights of the *P. crispus* and *P. australis* were monitored every month during the experiment period. In the middle of April, July, October and January, mature leaves of the plants were collected to measure chlorophyll content according to the method reported by Wellburn (1994). Results of the four months represented for the chlorophyll content in spring, summer, autumn and winter, respectively. *P. crispus*'s absorbing capacity for NH_4-N and NO_3-N were tested using hydroponic *P.crispus,* which were cultivated in basins accompanying the purification systems. The experiment parameters and ambient conditions were kept consistent with the purification systems except the static inflow form. On the fifteenth day of each month, 5 plants of *P. crispus* in the basin were taken to the laboratory and tested for absorption rates for NH_4-N and NO_3-N. *P. crispus* was put into a beaker filled with artificial wastewater, and the NH_4-N and NO_3-N concentrations were 5 and 15mg/L, respectively. A series of solution samples were taken at 5min intervals for 20min to

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estimate NH_4-N and NO_3-N absorption rates, which could be calculated by linear regression analyses of the concentration versus time (Romero et al., 1999).

2.3. Water sampling and analysis

In each system, water samples were collected from the influent and effluent of the treated units every 5 days. After sampling, all samples were then taken to the laboratory immediately and analyzed for NH_4-N, TP, COD and TN following the standard methods (APHA, 2005). Dissolved oxygen (DO) and water temperature were measured at the mid-water depth in situ using a DO meter (HQ 30d 53LED™ HACH USA).

2.4. Microbial analysis

At the end of each season, microbial samples in every unit were collected by collecting sand samples from the top layer (5 to 10cm) in five spots in every unit (Wang et al., 2015). MOBIO PowerSand™ DNA Isolation Kits were used to extract the DNA of the mixed substrate of the five sand samples. To characterize the amount of ammonia oxidation bacteria (AOB), the *amoA* genes were detected by quantitative PCR (Q-PCR) using Roche LC-480 (USA). Subunit A of ammonia monooxygenase (*amoA*), which encoding ammonia monooxygenase, is widely used as specific functional genes to indicate AOB in microbiology analysis (Pratscher et al., 2011). The reaction mixture was 20 μL, which was composed by: 10 μL of SYBR® Premix Ex Taq™, 7.2 μL of nuclease-free water, 0.4 μL of each of the forward and reverse primers, and 2 μL of template DNA. PCR programs were as follows: initial

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denaturation for 60 s at 94 °C, followed by 35 cycles of 94 °C for 60 s, 54 °C for 60 s, and 72 °C for 3 min.

2.5. Statistical analysis

Statistical analysis was undertaken utilizing the statistical program SPSS 22.0 (SPSS Inc., Chicago, USA). Significance of differences between means of the results of the experiment group and control group in each season were evaluated through two-sample t-tests. Significant difference was considered to be $P < 0.05$.

3. Results and discussion

3.1. Complementarity of seasonal plants

The temperature of air and water during the experiment period was presented in Fig. 2a, which revealed the four distinct seasons. Throughout the experiment period, *P. crispus* and *P. australis* grew well without any obvious symptoms emerging of toxicity or nutrient deficiency. The growth curves of the two plants are shown in Fig. 2b. The mature plant height was 80 and 179cm for *P. crispus* and *P. australis*, respectively, and this is in the range reported by Chen et al. (2011) in their study. The chlorophyll content of the plants is shown in Fig. 2c, and the variation trend during the four seasons was in accordance with the plant growth rate. Fig. 2d presents the results of the *P. crispus*'s absorption rates for NH_4-N and NO_3-N. *P. crispus* maintained high absorption rates for nitrogen in spring and winter, but these declined to a minimal value in summer (Fig. 2d).

P. crispus and *P. australis* both had highest growth rates during spring (0.47cm/d and 0.77cm/d, respectively). Significant differences in growth rates of the two plants

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were observed in the subsequent three seasons. *P. australis* was still growing in summer, when *P. crispus* had gradually stopped growing. During autumn and winter, *P. crispus* began to germinate and grow, while *P. australis* was in the dormant stage (Fig. 2b). Compared to the deficiency of the plant growth in NPCS in autumn and winter, the plant grew in a stable fashion during the long-term experiment in SPCS, and showed growth complementarity in different seasons. In terms of plant growth, SPCS was more stable than NPCS in the long-term study.

Chlorophyll content reflected plant growth conditions from another perspective. Both plants had relatively high chlorophyll content in spring (3.81mg/g FW for *P. crispus* and 3.46 mg/g FW for *P. australis*), indicating strong photosynthesis that enabled rapid plant growth during that season (Fig. 2c). This explained the highest plant growth rate observed in spring (Fig. 2b). A variation in chlorophyll content was similar with plant growth in the three seasons as well. For *P. crispus*, chlorophyll content fell to 0.59 mg/g FW in summer, while from autumn to winter, it increased from 2.11 to 3.33 mg/g FW. This was corresponded with the plant growth rate of *P. crispus*. In summer, *P. crispus'* plant growth rate dropped to the lowest level, and the increasing trend was also obtained from autumn to winter (Fig. 2b). Chlorophyll content of *P. australis* showed a sustained downward trend from summer to winter (Fig. 2c), and the plant growth rate was also in falling state from summer to winter (Fig. 2b).

P. crispus showed significant higher absorption rates for NH_4-N towards NO_3-N (Fig. 2d), which was due to different absorption mechanisms for the different forms of

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nitrogen. In the plant uptake process of nitrogen, NO_3-N uptake is more sensitive to low energy conditions than NH_4-N. The uptake of NH_4-N relied on passive diffusion with no energy requirement, whereas NO_3-N uptake was based on the energy requirement process of active absorption (Mengel & Viro, 1978). Even if the absorption rate differed between the two types of nitrogen, *P. crispus* showed the same seasonal variation characteristic in the absorption rate for both nitrogen forms, and in accordance with the *P. crispus*'s growth condition. In spring, when *P. crispus* had reached its highest growth rate, plant uptake also achieved its strongest state to meet the demands of growth, so the highest absorption rates for NH_4-N and NO_3-N were observed in this season. In autumn and winter, *P. crispus* continued to grow, during which time *P. crispus* could sustain the absorption of nitrogen. Especially in winter, the absorption rates for NH_4-N and NO_3-N were 1.59 and 0.62 $mgNmin^{-1}$ gDW^{-1}, just lower than that in spring. *P. crispus* showed least absorption for NH_4-N and NO_3-N as it began to decline and subsequently plant uptake was weakest. Conversely, plant uptake of *P. australis* was significantly high in spring and summer, but virtually ceased in winter. Therefore, in terms of plant uptake for nutrients, SPCS showed complementarity in the long-term study as well.

3.2. Effect of SPCS on wastewater treatment performance

Effluent concentrations of NH_4-N, TP, COD and TN during the long-term experiment are shown in Fig. 3. During the experiment, the treatment performance remained stable in SPCS and a significant improvement was observed in winter compared to the NPCS.

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In the year-round experiment, NH_4-N removal performance of SPCS had a clear advantage compared with NPCS. In SPCS, the best treatment efficiency regarding NH_4-N was 76.9% which was observed in spring, and this declined to 65.9% in summer. In the followed autumn and winter, treatment efficiency was higher than in summer even when the temperature fell (Fig. 3a). Compared to NPCS, the efficiencies in treating NH_4-N in SPCS were improved by 6.2%, 14.6%, and 18.1% in spring, autumn and winter, respectively ($P<0.05$), and no significant difference was observed in summer between the two systems. It is generally accepted that DO and plant uptake are two key factors which influence NH_4-N removal (Fan et al., 2016). Oxidation via nitrification is the key process in NH_4-N removal by microbial activities, and DO above 1.5 mg/L is essential for nitrification to occur (Wu et al., 2011). Sui et al. (2016) reported that under high DO conditions (above 3.75 mg/L), NH_4-N removal efficiency was improved by 35% relative to DO conditions below 1.50mg/L. To study the different outcomes caused by the DO factor on NH_4-N removal in the two systems, 24h DO monitoring began at 6:00am in the two systems and the results are shown in Fig. 4. In the two systems, DO reached maximum values at about 5:00pm, and reached minimum values at 6:00am. Photosynthesis and respiration are two key factors that influence DO concentration in the plant dominant pond. During the daytime, photosynthesis was stronger than respiration and DO was in an enrichment state. At night, photosynthesis almost stopped, respiration became the dominant factor, and DO was consumed at night, so the minimum values appeared at 6:00am. A significantly larger DO concentration was observed in SPCS compared to NPCS (Fig.

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4). Since *P. crispus* grew below the water's surface, oxygen produced by photosynthesis released into the water directly, which increased DO enrichment in SPCS. According to the discussion in section 3.1, photosynthesis is strong with relatively high levels of chlorophyll during spring, autumn, and winter. DO concentration was high in the three seasons and was in accordance with the NH_4-N treatment performance.

Plant uptake is another important way in the NH_4-N removal in CWs. Gottschall et al. (2007) reported that plant uptake accounted for 21% of NH_4-N removal during the growing season in a CW treating agricultural wastewater. In NPCS, plant uptake of NH_4-N almost completely ceased in winter because the *P. australis* was in the resting stage, and this was consistent with the treatment performance in NPCS (Fig. 3a). In SPCS, *P. crispus* retained impressive absorption ability for NH_4-N, and furthermore the enrichment of DO, resulted in the stable treatment efficiency of NH_4-N during winter. Even if *P. crispus* gradually declined in summer in SPCS, no significant difference in NH_4-N removal was observed with NPCS, when the NPCS functioned well in summer ($P>0.05$). Numerous studies have shown that the *P. australis* system performed better in summer than other seasons (Abou-Elela et al., 2013; Wu et al., 2013), which could make up for the adverse effects caused by decline of *P. crispus* in SPCS. Table 1 summarizes the contribution ratios in nutrient removal of the FTU, from which the plant complementary effect could be seen obviously in SPCS. The FTU's highest contribution ratio for removing NH_4-N from SPCS was

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82.9%. This was observed in winter while the lowest was 60.7% observed in summer, indicating that the two plant types both alternatively played a major purification role.

The highest TP treatment efficiency was observed in the first 60 days during spring in both systems, i.e. 86.9% and 86.8% for SPCS and NPCS, respectively (Fig. 3b). In summer, treatment efficiency of TP declined by 26.3% and 23.5% in SPCS and NPCS, respectively. No significant difference was observed between the two systems in spring and summer ($P>0.05$). In autumn and winter, TP treatment performance increased again in SPCS and no significant variation was observed in NPCS. The treatment efficiencies significantly were improved by 8.4% and 17.6% in SPCS compared to NPCS. TP removal mechanisms in CWs mainly include accumulation by substrates and plant uptake. In spring, *P. crispus* and *P. australis* had considerable uptake ability since both plants were still growing, and the substrates' adsorption capacity had reached the maximum level (Ciupa, 1996). Kadlec (2005) reviewed TP treatment performance in emergent plants dominated SFCWs observing that except for the enhanced removal in the spring growth period, seasonal effects on TP removal were minimal. This was consistent with the performance of TP removal in NPCS. In TP removal processes, temperature had little influence on sorption reactions. In this circumstance, TP removal performance changed little when the substrates' adsorption capacity and plant uptake were stable in NPCS during autumn and winter. In SPCS, *P. crispus* began to germinate, and this process restored the ability to adsorb phosphorus in autumn. Concerning the rapid growth stage of *P. crispus* in winter, more phosphorus was absorbed by *P. crispus* to meet the needs of

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growth. Meanwhile, radial oxygen loss and root respiration of *P. crispus* improved the substrates' porosity, which enhanced their adsorption in autumn and winter in SPCS (Gillis & Miller, 2000). The two factors gave the reasons for the better TP removal performance in SPCS during autumn and winter. The FTU's lowest contribution ratio was 64.2% observed in summer, and it increased by 7.6% and 17.2% in autumn and winter, respectively. Variation in the contribution ratio of FTU in SPCS for TP removal was similar with NH_4–N removal (Table 1), and commensurate with plant growth. *P. crispus* played a major role in TP removal in autumn and winter, making up for the adverse effects caused by the declining *P. australis.*

In the long-term experiment period, COD removal efficiency ranged from 32.9% (observed in summer) to 40.7% (observed in winter) in SPCS, while the removal efficiency ranged from 35.6% (observed in winter) to 41.3% (observed in summer) in NPCS. Significant differences in COD removal were found in summer and winter between the two systems ($P<0.05$). As can be seen in Fig. 3c, COD effluent concentration was generally higher in SPCS than that in NPCS during summer, especially in the last thirty days. In winter, COD treatment performance was poorer than that in summer in NPCS, while SPCS was significantly better than NPCS. Jing et al. (2001) reported that COD removal efficiency ranged from 13%-51% in a SFCWs for polluted river treatment. In winter, COD removal efficiency was generally below 30%, which was similar to how well NPCS performed. In comparison, SPCS showed a great advantage in winter with COD removal efficiency improving by 10.1% compared to NPCS. In winter, since the oxygen supply from the growing *P. crispus*

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root zone can enhance microorganisms' removal of organic material in the sediment or on the surfaces of stems and roots, COD removal did improve. The contribution ratio of the FTU in COD removal in SPCS was highest in winter (77.7%), demonstrating that the *P. crispus* dominated system played critical purification process. *P. crispus* was declined in summer, when the stems and leaves fell to the bottom of the water. Organic matter was released from the decaying plant tissue, leading to rising larger loading of COD in SPCS. This in turn resulted in significantly lower treatment efficiency in SPCS compared to NPCS in summer. FTU's contribution ratio was observed to be the lowest (57.6%) in summer, indicating the declined treatment capacity for COD of *P. crispus* simultaneously. It is very important to note that the *P. crispus* needs to be harvested in time to avoid secondary pollution when using the SPCS.

The mean effluent TN concentrations in the purification system throughout the study period are displayed in Fig. 3d. TN removal efficiencies were 43.2%, 44.3%, 46.3% and 44.7% for spring, summer, autumn, and winter in SPCS, respectively, which were consistence with the results reported by Vymazal (2007). In NPCS, no significant difference in TN removal performance was found between both systems except during winter ($P>0.05$). TN treatment efficiency slightly increased by 5.2% during the operating period in winter in SPCS compared to NPCS ($P<0.05$). In SFCWs, the complete nitrogen removal was relied on nitrification-denitrification and plant uptake. Nitrogen removal via denitrification is an anaerobic and heterotrophic microbial process, and it can be restricted by various factors such as insufficient

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organic carbon source and excess oxygen. Ding et. al (2012) reported that optimal COD/N ratio was 9.0 for TN removal in a study of nitrogen removal through microorganism process in CWs. However, the COD/N ratio was 2.5 for the two systems, which was significantly lower than the optimum value of 9.0. Therefore, in both systems COD/N was the limiting factor in microbial activities for TN removal. In this case, plant uptake was the deciding factor for TN removal in the two systems. SPCS showed capacity to engage in sustainable absorption for nitrogen in winter, and compared to the defect of plant uptake in NPCS, SPCS proved to be superior in TN removal performance in winter.

3.3. Microorganism quantities in SPCS

It is believed that oxidation of ammonia to nitrite is a critical step in nitrification, and the process is carried out by obligate aerobes AOB (Sims et al., 2012). Fig. 5 presents the differences in AOB quantities among the wetlands in sands based on the *amoA* gene in the two systems. In the FTU in SPCS, the highest *amoA* gene copy numbers was 1.4×10^8 copies/g soil observed in spring, which was 1.2, 1.5 and 1.6 times that of the number in summer, autumn, and winter. In the STU in SPCS, there were significantly fewer *amoA* gene numbers observed compared to FTU ($P<0.05$), and the highest number in STU was 4.6×10^7 copies/g soil observed in summer, which was 2.7, 1.2 and 4.9 times that of the number in spring, autumn, and winter. Total AOB quantities in SPCS improved by 10.2% during the experiment compared to the NPCS. Results further indicated that treatment performance of NH_4-N corresponded with the AOB abundance distribution in SPCS. *P. crispus* showed

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significant DO enrichment ability, and NH_4-N loading was higher in FTU in SPCS, which both created favorable conditions for AOB growth. However, many studies have shown that AOB was depressed at low temperature (Urakawa et al., 2008; Vazquez-Padin et al., 2011). In this study, even when at low temperature, AOB quantities were significantly higher in SPCS compared to NPCS. Wang et al. (2012) used a specific type of moss to enhance nitrogen removal under cold temperatures, and concluded that sustaining oxygen transfer to growing moss roots and cold-adapted AOB community explained the stable NH_4-N removal. In this study, *P. crispus* grew well in winter, the high DO concentration level and oxygen transfer to roots may have promoted the cold-adapted AOB community form, and this helped maintain stable AOB quantities in winter. Ratios of AOB quantities of FTU to STU in SPCS were 8.7, 2.5, 6.7, and 5.6 for spring, summer, autumn and winter, respectively, which was also similar with variation trend of contribution ratios of FTU of NH_4-N removal efficiency in SPCS. Throughout the long-term experiment period, stable NH_4-N removal performance partly benefitted from the improved AOB quantities enhanced by plant collocation

4. Conclusions

Seasonal plant collocation can effectively enhance pollutant removal efficiency in SFCWs at low temperature. In winter, removal efficiency of NH_4-N, TP, COD, and TN improved by 18.1%, 17.6%, 10.1% and 5.2% in SPCS, respectively. Long-term monitoring of plant growth indicated that seasonal plants showed complementary in plant uptake, ensuring the stable treatment performance in SPCS. Q-PCR results

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demonstrated that SPCS facilitated the growth of AOB, and the quantity of AOB improved by 10.2% during the experiment period. Finally, a seasonal plant collocation strategy may assist in the popularity and application of SFCWs in climate change areas in the future.

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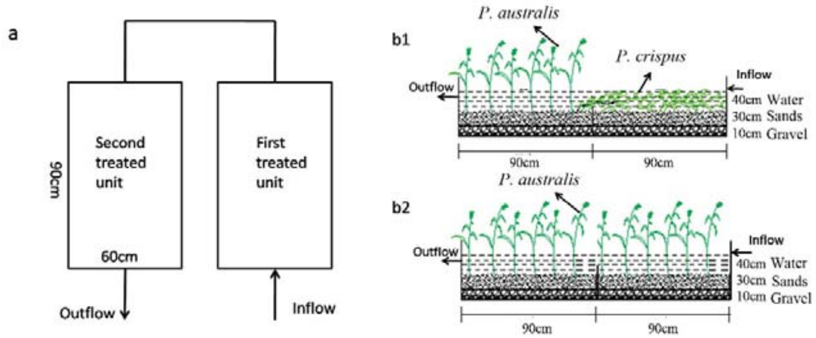

Fig. 1: The experiment setup plan (a), and the profiles of SPCS (b1) and NPCS (b2).

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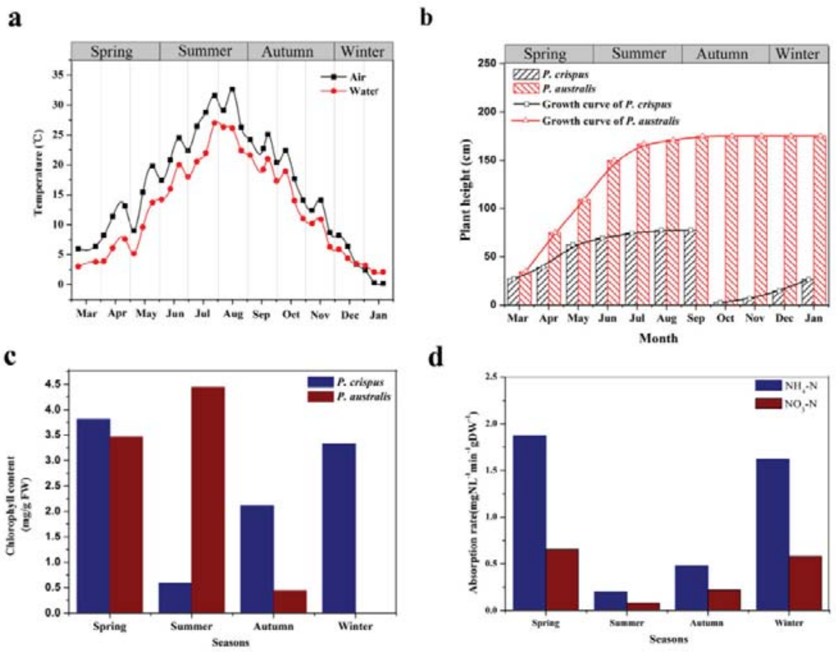

Fig. 2 Air, water temperature (a), growth curves of plants (b), chlorophyll contents (c) and *P. crispus*' absorption rates for NH_4-N and NO_3-N (d) during the experiment period.

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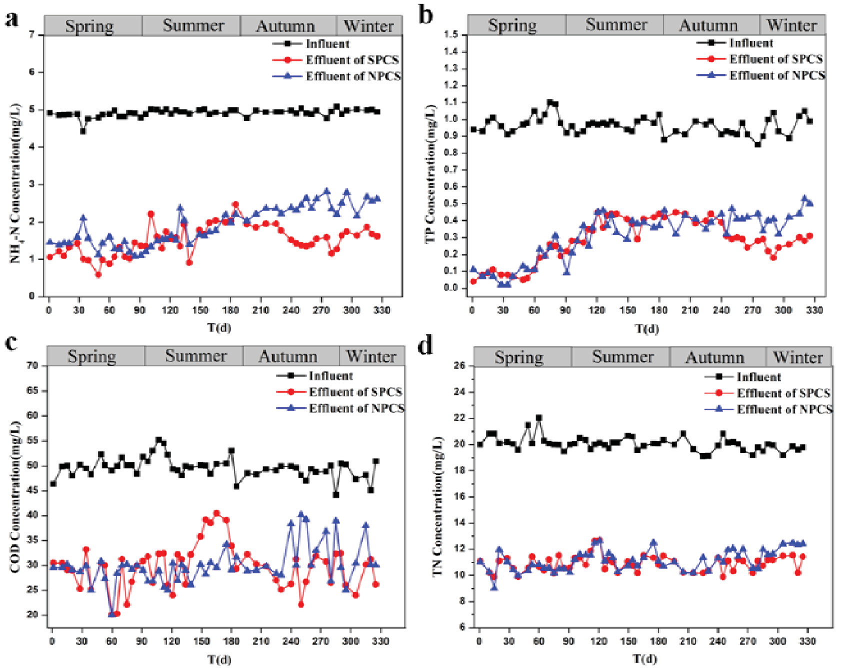

Fig. 3 Removal of pollutants in the two systems during the experiment period, a (NH_4-N), b (TP), c (COD) and d (TN).

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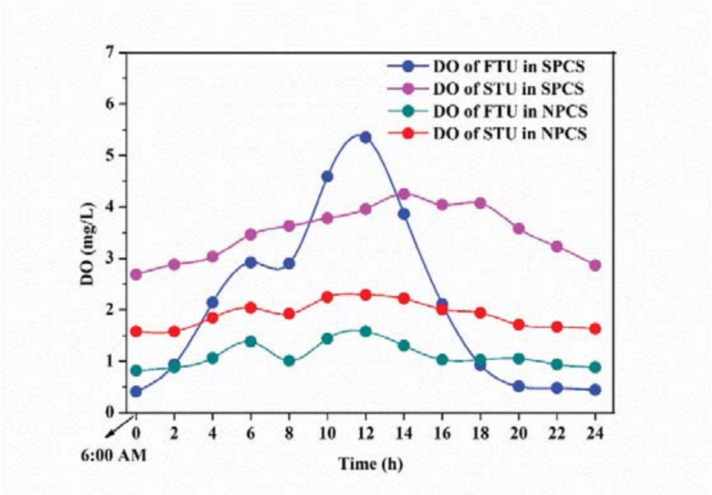

Fig. 4 DO 24h variation in the two systems.

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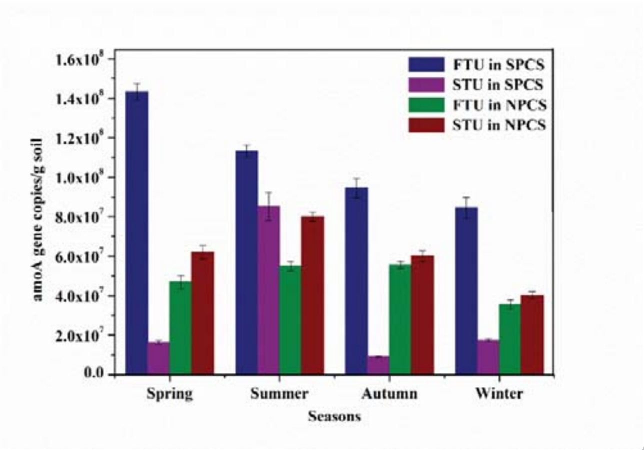

Fig. 5 AOB quantities in different seasons in the two systems.

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Table 1

Contribution ratios in nutrients removal of the FTU in the two systems.

Parameters	System	Contribution ratios (%)			
		Spring	Summer	Autumn	Winter
NH_4-N	SPCS	80.90	60.70	68.10	82.90
	NPCS	80.70	65.80	66.80	67.20
TP	SPCS	79.50	64.20	71.80	81.40
	NPCS	82.00	73.70	69.30	68.20
COD	SPCS	76.50	57.60	62.50	77.70
	NPCS	71.50	64.50	60.20	59.30
TN	SPCS	77.50	72.80	72.00	83.60
	NPCS	80.00	78.20	77.50	74.60

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Highlights

- Seasonal plant collocation wetland system performed well at low temperature.
- Plant growth and plant uptake were sustainable during the year-round experiment.
- DO and ammonia oxidizing bacteria were enriched benefitting from plant collocation.

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