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Effect of Different Plant Monocultures on Nitrogen Removal Performance in Wetland Microcosms

Rujun Wang[1], Ying Quan[2], ShaoKui Zheng[2], Xueyu Zhang[3*]

[1] China Academy of Transportation Sciences, Beijing, 100029, China

[2] MOE Key Laboratory of Water and Sediment Sciences/State Key Lab of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing, 100875, China

[3] Beijing Municipal Ecological Environment Appraisal and Complaint Center, Beijing, 100161, China

* Corresponding author's email: zhangxueyu@mail.bnu.edu.cn

ABSTRACT

This study investigated the nitrogen removal performance in wetland microcosms individually planted with different plant monocultures, including emergent, free-floating and submerged plants during ammonia removals, or large- and small-leaf free-floating plants during nitrate removal. For ammonia-dominated wastewater, both emergent (common reed) and free-floating (water hyacinth) plants in wetland microcosms achieved higher total nitrogen removals than a submerged plant (eelgrass) that significantly improved the microbial nitrifying performance. For nitrate-dominated wastewater, efficient nitrate removals in wetland microcosms planted with free-floating plant were achieved by both a full cover of water surface and the concentration of organic oxygen-consuming substances, which resulted in low dissolved oxygen levels and boosted microbial denitrification in wetland microcosms. FWS-CW developers and managers should thus pay close attention to the selection of wetland plant types and optimize their design to achieve optimum nitrogen removal performance.

Keywords: ammonia, constructed wetland, free-water surface, nitrate, optimization mechanism.

INTRODUCTION

Because of their better performance, constructed wetlands (CWs) have gradually become regarded as a promising solution for treating wastewater, especially for nitrogen and phosphorus removal (Guo et al., 2017; Jain et al., 2020; Zuo et al., 2020). Applied in the treatment plan for oil-contaminated, CWs showed a significant degrading effect on COD, heavy oil and recalcitrant organic compounds, as well (Xuegong et al., 2005; Vander Meulen et al., 2022). Nitrogen in CWs is transformed via plant uptake and microbial nitrogen removal, and the latter is thought to be the most complete and probably most important nitrogen removal mechanism in CWs (Bachand & Horne, 2000). The complete microbial nitrogen removal process is achieved by alternate aerobic nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) and anoxic denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) that require almost the opposite dissolved oxygen (DO) conditions (Garcia-Lledo et al., 2011). By contrast, wetland plants supply water oxygenation, surfaces for bacterial attachment, and organic carbon (Veraart et al., 2011).

CWs can be categorized into free-water surface CWs (FWS-CWs), and subsurface flow CWs (SSF-CWs) based on water flow regime (Gao et al., 2019). Like natural marshes, FWS-CWs can harbor more wetland plant types, including free-floating, emergent, and submerged plants (Vymazal, 2007), than SSF-CWs where only emergent plants perform these functions. Different wetland plant types occupy different habitats and display a variety of shapes. Emergent plants have roots in the sediment, stems in the water, and leaves and flowers above the water surface. Free-floating plants do not root in the sediment but float on the surface of the water, sometimes forming extensive green mats on the water surface.

Submerged plants are rooted with stems and leaves entirely underwater, and may form a low-growing "meadow" near the sediment. The various wetland plant types supported in FWS-CWs makes them a nature-based sustainable resource management technology that provides both pollutant removal and ecological services (Chen, 2011). It seems that FWS-CWs should be designed, constructed, and operated on the basis of the effect of wetland plant types on pollutant removal when its ecological services are fully considered.

Many studies demonstrated that different plant types strongly influenced the nitrate nitrogen removal in CWs (Zheng et al., 2020; Ruan et al., 2021), and the highest denitrification efficiencies were frequently reported in FWS-CWs planted with free-floating plants (Veraart et al., 2011; Li et al., 2020). It was suspected that different plant types exerted different influences on oxygen conditions in FWS-CWs, and that free-floating plants can provide a barrier ('close mat') to atmospheric oxygen transfer to promote microbial denitrification (Kadlec, 2008; Veraart et al., 2011). However, it is not clear if the different oxygenation performance of various plant types can influence the microbial nitrification performance in FWS-CWs, since aerobic nitrification process is thought to be the rate-limiting step in the microbial nitrogen removal process (Shammas, 1986). Also, no experimental data are available to verify the hypothesis on efficient nitrate removals in free-floating plant-based FWS-CWs. It seems that FWS-CWs still lack some critical elements needed to optimize their treatment function and system sustainability.

This study investigated the nitrogen removal performance in wetland microcosms individually planted with different plant monocultures. Firstly, we established three wetland microcosms, individually planted with monocultures of typical emergent, free-floating, or submerged plants, to compare their abilities to remove pollutants from ammonia-dominated wastewater with that of an unplanted microcosm as a control. In subsequent microcosm experiments, we investigated the effect of different free-floating plants (large- or small-leaf free-floating plants with an abiotic cover as control) and organic oxygen-consuming substance concentration (represented by initial chemical oxygen demand (COD_{in}) levels) on nitrate removal performance in FWS-CWs planted with free-floating plants.

As a rhizomatous perennial wetland emergent plant, *P. australis* typically forms homogenous belts (monocultures) in various wetlands (Wirsel, 2004; Shukla et al., 2021). Among the free-floating plants, *E. crassipes* and duckweeds have been extensively studied for their nutrient-removal potential in FWS-CWs because of their fast growth rate and large uptake of nutrients and contaminants (Sooknah & Wilkie, 2004; Jampeetong et al., 2012; Kalengo et al., 2021). As a dominant submerged plant species in some lakes (Li et al., 2010), *V. spiralis* is a perennial rooted plant with a high adaptive capability and a wide geographical range (Wang & Yu, 2007; Malschi et al., 2018); it can expand its distribution through stolons, forming monospecific beds in both stagnant and lotic freshwater environments (Racchetti et al., 2010). In this study, these wetland plants were chosen as model plant species because they are the most widely distributed wetland plants worldwide. The level of plant coverage is often considered to be a key factor controlling nitrogen removal in wetlands (Garcia-Lledo et al., 2011). To avoid the effect of different levels of coverage on pollutant removal performance, *V. spiralis, E. crassipes,* and *L. aequinoctialis* were all established at 100% coverage.

MATERIALS AND METHODS

Effect of wetland plant type on the treatment of ammonia-dominated wastewater

A 10-cm layer of local lake sediment was placed into the bottom of three experimental wetland microcosms (0.72×0.52×0.45 m), which were then planted with monocultures of common reed (*Phragmites australis*), water hyacinth (*Eichhornia crassipes*), or eelgrass (*Vallisneria spiralis*) collected from a local pond or brook. Another microcosm was left non-vegetated to serve as a control. The planting density for *P. australis* was 101 clumps/m^2, whereas 100% coverage was created for *V. spiralis* and *E. crassipes*. To improve microbial concentrations in the wetland microcosms, a microbial enrichment culture was independently cultured under aerobic conditions in 1000 L river water (pH 7–8) by the addition of 2 kg local soil, 80 g peptone, and 1.3 g KH_2PO_4 for seven days. Initially, the wetland plants were pre-grown in 168 L river water for two weeks. Subsequently, the water in each microcosm was replaced by an equal volume of enrichment culture. The DO and pH were then measured in situ using a HQ30d DO meter with an LDO101 optimal DO probe (Hach, Loveland, CO, USA) and a PHS-3C pH meter (Kexiao Instruments, Zibo, China), respectively, and daily water samples were taken for the measurement of pollutant concentrations. Since wetland plants can release organic carbon following senescence (Veraart et al., 2011), dead leaves were removed every day to avoid their potential effect (organic carbon of plant sources) on microbial nitrogen removal process.

Effect of wetland plant type (water coverage) on the treatment of nitrate-dominated wastewater

In this study, the simulated nitrate-dominated agricultural runoff was prepared by the addition of glucose, KNO_3, and KH_2PO_4 in river water to investigate the nitrate reduction mechanism in FWS-CWs fully covered by water hyacinth or duckweed. Three wetland microcosms were constructed with 100% coverage of monocultures of *E. crassipesor* duckweed (*Lemna aequinoctialis*). An additional microcosm was covered with 4-cm-thick foam sheets as a control. To ensure that there were sufficient denitrifying bacteria in the wetland microcosms, a microbial enrichment culture was prepared in 24 L river water (pH 7–8) by the addition of 2 kg local soil, 2.3 g glucose, 1.7 g KNO_3, and 0.2 g KH_2PO_4 for three days. The plants were pre-grown in 151 L river water for two weeks. Subsequently, 1 L microbial enrichment culture, 15 g dissolving glucose, 11 g KNO_3, and 1 g KH_2PO_4 were added to each microcosm containing 151 L river water. In situ DO and pH measurements were then conducted, and water samples were taken daily for the measurement of pollutant concentrations.

Effects of organic oxygen-consuming substances on nitrate removal performance in the wetland microcosms

Three *L. aequinoctialis* microcosms (100% coverage) were created by dissolving 6.8 g KNO_3 and 0.8 g KH_2PO_4 in 94 L river water. Approximately 0, 4.7, and 15.1 g glucose were added to the three individual wetland microcosms to investigate the potential effect of the concentrations of organic oxygen-consuming substances on nitrate removal performance in the wetland microcosms planted with *L. aequinoctialis*. All other experimental methods were as described above for the previous treatments.

Sample analyses

Water samples were pre-filtered (0.45-µm polyether sulfone membrane) prior to the determination of COD, total nitrogen (TN), nitrite-nitrate nitrogen (NO_x-N), and NH_3–N. COD was determined using a spectrophotometer (Hanna DR/3000, Hanna Co., Italy), and TN, NO_x-N, and NH_3-N were analyzed using flow injection analysis on a Skalar San++ Automated Ion Analyzer (Skalar Co., The Netherlands). All pollutant concentrations were corrected based on practical evapotranspiration to avoid an overestimation of concentrations (Weisner & Thiere, 2010).

RESULTS AND DISCUSSION

Effect of wetland plant type on treatment of ammonia-dominated wastewater

In recent decades, there is a growing interest in the use of CWs to purify a variety of wastewaters, most of which focus on municipal wastewater (Zheng et al. 2010), a kind of ammonia-dominated wastewater. The simulated ammonia-dominated wastewater presented here (35–50 mg COD L^{-1}, 6.9–7.4 mg TN L^{-1}, 4.6–5.9 mg NH_3-N L^{-1}, and 0.2–0.4 mg NO_x-N L^{-1}) approximated to the low-strength municipal sewage reported in our previous study (73 mg COD L^{-1}, 6.9 mg TN L^{-1}, 5.39 mg NH_3-N L^{-1}, and 0.3 mg NO_x-N L^{-1}) (Zheng et al., 2010). The ammonia-dominated wastewater was supplied to the three wetland microcosms planted respectively with monocultures of *P. australis*, *E. crassipes*, and *V. spiralis*, and their pollutant removal performances were compared with an unplanted control.

Figure 1 shows the time courses of COD, TN, NH_3-N, and NO_x-N levels in four wetland microcosms during a 14-day experimental period. COD removals of 74–75% were achieved in the *P. australis* and *E. crassipes* microcosms by the end of the experiment, but COD removals were only 36% and 46% in the *V. spiralis* microcosm and control, respectively. Similarly, TN removals of 87% and 86%, respectively, were achieved in the *P. australis* and *E. crassipes* microcosms, 75% and 70% in the *V. spiralis* microcosms and the control. NH_3-N was the predominant form of inorganic nitrogen (approximately 67–83%) at the onset of the experiment and was reduced to almost zero in all treatments by the end of the experiment, with removals of 99% and 91% in the planted microcosms and the control, respectively. It is well known that aquatic plants in CWs facilitated nutrient removal mainly through plant uptake, fixation of inorganic and organic particulates, and oxygen release from the plant roots to create an oxidized rhizosphere (Zheng et al., 2010). It seems that, in most cases, the pollutants in planted microcosms were removed more efficiently than those in unplanted microcosms, except for the COD in the *V. spiralis* microcosm. Furthermore, the *P. australis* and *E. crassipes* microcosms removed pollutants more efficiently than the *V. spiralis* microcosm. In a previous study, free-floating plants (water hyacinth, pennywort, and water lettuce) also achieved NH_3-N and soluble COD removals of 99% and 68%, respectively, for ammonia-rich anaerobically digested flushed dairy manure wastewater (Sooknah & Wilkie, 2004). Kalengo et al. (2021) investigated the NH_3-N removal efficiency of *Eichhornia crassipes* for aquacultural effluents treatment, datas show that summer and autumn have high NH_3-N removal efficiencies with 98%.

Alongside the reduction in NH_3-N concentrations, a significant increase (four times) in NO_x-N concentrations was observed in the *V. spiralis* microcosm. Previous investigations have demonstrated the importance of DO concentrations for nitrification, and low nitrification efficiency is often attributed to a lack of available DO (Wang & Yu 2007). Oxygen is released from emergent (Bezbaruah & Zhang, 2005) and free-floating (Sooknah & Wilkie, 2004) plant roots or submerged plant leaves (Toet et al., 2005) through photosynthesis and is a main oxygen supply in FWS-CWs (along with atmospheric diffusion). However, it seems that only the submerged plants can increase oxygen concentrations in the water column, which may create the aerobic environment required for the growth of nitrifying bacteria in FWS-CWs. However, because of their low COD and TN removal efficiencies, submerged plants should be used as the terminal wetland plant type in FWS-CWs to further purify wastewater prior to discharge.

Generally, evapotranspiration from vegetation is dominated by transpiration (Kadlec, 2006), whereas evaporation is dominant for barren surfaces (Kadlec, 2008). Evapotranspiration has been suggested to be higher in wetlands with dense emergent plant coverage than in unplanted sites (Batty et al., 2006). Kirzhner et al. (2008) found that the evapotranspiration rates of emergent (*Scirpus maritimus* and *Eleocharis palustris*) and free-floating (*E. crassipes* and *Pistia stratiotes*) plants were 10–18 and 2–4 mm/d, respectively. In the present study, the average evapotranspiration rates of emergent, free-floating, submerged plants, and the control were 11, 6, 2, and 7 mm/d, respectively (data not shown), in agreement with previous findings. The emergent plant microcosm consistently had

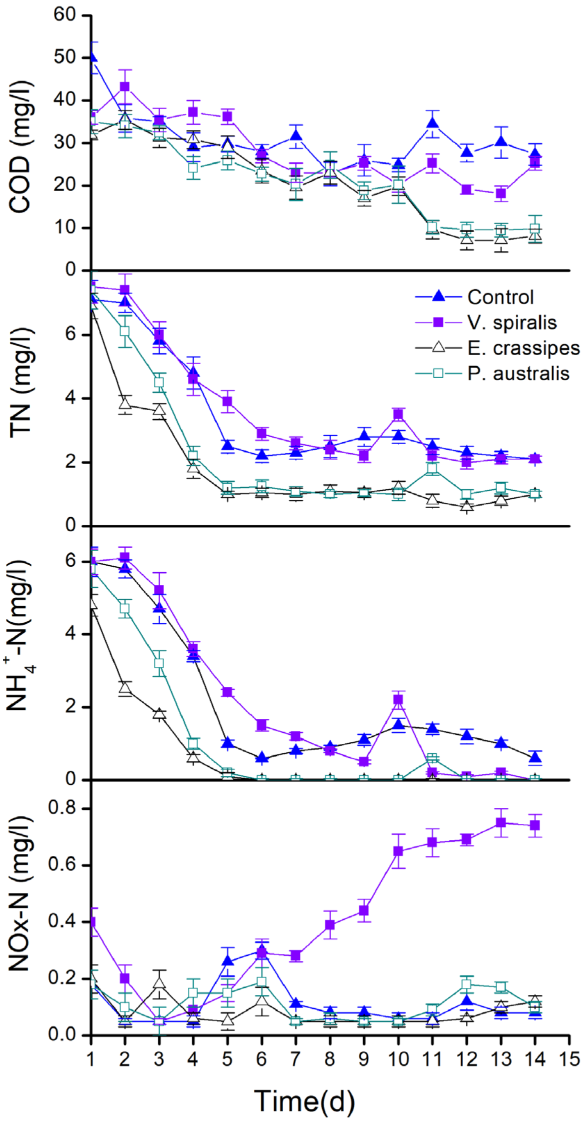

Figure 1. Time courses of COD, TN, NH_3-N and NO_x-N levels in four wetland microcosms during 14 days.Results are presented in mean ± SD which is represented as error bars (n = 3)

higher evapotranspiration than the other three microcosms. Therefore, although the emergent plants exhibited good pollutant removal performances, similar to free-floating plants, their high evapotranspiration rate may limit their applicability in arid or semi-arid regions. Furthermore, free-floating plants have the following advantages over other wetland plants: (1) higher productivity than several free-floating plants; (2) higher nutritive value of floating plants relative to many emergent species; and (3) ease of stocking and harvesting (Sooknah & Wilkie, 2004). Therefore, free-floating plants should be considered as a candidate species for purifying ammonia-dominated wastewater in FWS-CWs. In fact, many species of free-floating plants, such as *E. crassipes*, water lettuce(*Pistia stratiotes*), duckweeds, and watermoss (*Salvinia spp.*), have been used in FWS-CWs (Sooknah & Wilkie, 2004; Jampeetong et al., 2012).

Effect of wetland plant type (water coverage) on the treatment of nitrate-dominated wastewater

Nitrate removal from agricultural runoffs, e.g., plant nursery and aquaculture wastewater in which nitrate frequently makes up the majority of the nitrogen, is always very important in view of the adverse impacts of nitrate on humans and the environment (Yang et al., 2008). In previous studies, free-floating plants achieved NO_x-N removals of 86% (Lin et al., 2002) or higher. In this study, we investigated the effect of full coverage of a water surface by large-leaf water hyacinth (*Eichhornia crassipes*) or small-leaf duckweed (*Lemna aequinoctialis*) on nitrate removal performance, in comparison to an abiotic coverage (foam sheets) as a control (Fig. 2). The simulated nitrate-dominated wastewater presented here (79–82 mg COD L^{-1}, 12–14 mg TN L^{-1}, 1–2 mg NH_3-N L^{-1}, and 10–11 mg NO_x-N L^{-1}) approximated to those agricultural runoffs reported in previous study (10.1 mg TN L^{-1} (74% as NO_x-N L^{-1}) (Huett et al., 2005).

Figure 2 shows the time courses of COD, TN, NH_3-N, NO_x-N, DO, and pH levels in three wetland microcosms during an 11-day experimental period. At the end of the experiment, COD removals of 58–63% were achieved in the three microcosms. Approximately 99% NO_x-N removal and 92% TN removal were achieved in the *L. aequinoctialis* microcosm and the control, while only 63% NO_x-N removal and 53% TN removal were achieved in the *E. crassipes* microcosm.

The DO levels decreased sharply to < 1 mg L^{-1} since the third day of treatment in the *L. aequinoctialis* microcosm and the control, whereas most measurements were > 1.5 mg L^{-1} in the *E. crassipes* microcosm. Correspondingly, after

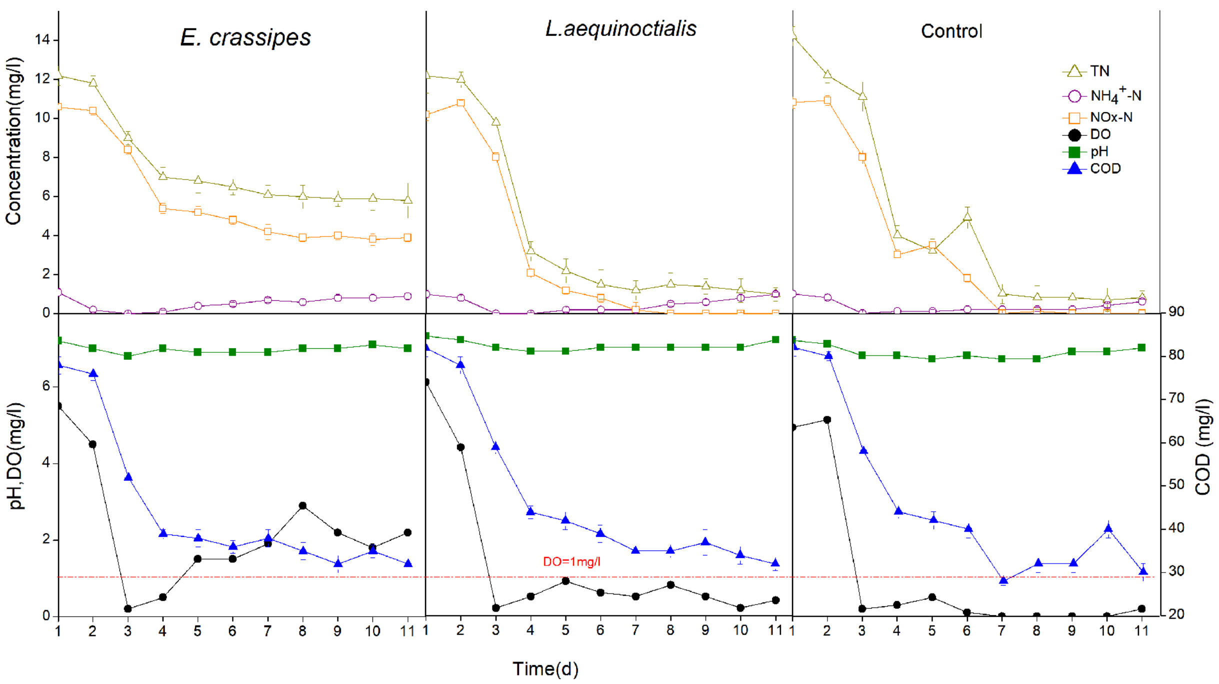

Figure 2. Time courses of COD, TN, NH_3-N and NO_x-N, DO and pH levels in three wetland microcosms during 11 days. Results are presented in mean ± SD which is represented as error bars (n = 3)

three days of treatment, further nitrate removal was observed in the *L. aequinoctialis* microcosm and the control, but there was no further nitrate removal in the *E. crassipes* microcosm. These results suggest that the DO level critically affected the nitrate reduction performance in the three microcosms, and lower DO levels improved the transformation of NO_x-N into N_2 via a microbial denitrification process. In this study, the pH values in the three microcosms were maintained between 6.9 and 7.3, which falls inside the optimal pH range (pH 6–8) for high denitrification activity (Jansson et al., 1994).

Although the large-leaf *E. crassipes* was more productive than small-leaf duckweed (Sooknah & Wilkie, 2004)., our results suggested that the duckweed microcosm had a higher NO_x-N removal rate than the *E. crassipes* microcosm. It appears that *E. crassipes* cannot completely cover the water surface like *L. aequinoctialis* and the foam sheet, possibly due to its larger leaves and spatial structure, which resulted in atomospheric diffusion to some extent. The abiotic coverage (foam sheet) without an oxygen release capability also achieved NOx-N and TN removals as high as those recorded for *L. aequinoctialis*, demonstrating a mechanism of nitrate reduction in free-floating plant-based FWS-CWs, i.e., free-floating plants provide a barrier to atmospheric oxygen transfer into the FWS-CWs by fully covering the water surface. The resulting low DO levels in FWS-CWs further boosted denitrification and prevented nitrate-nitrogen levels from increasing through the nitrification of ammonia (Nahlik & Mitsch, 2006). This also explains how a floating-raft hydroponic system can achieve a NO_x-N removal of ~97% for nitrate-rich agricultural runoff (Yang et al., 2008).

Effects of organic oxygen-consuming substances on nitrate removal performance in the wetland microcosms

In addition to surface coverage, the concentration of organic oxygen-consuming substances potentially influences the DO concentrations in FWS-CWs. The effect of the concentrations of organic oxygen-consuming substances on nitrate removal performance in the wetland microcosms planted with *L. aequinoctialis* was investigated at COD_{in} levels of 85, 58, and 28 mg L^{-1}, respectively. With an increase in COD_{in} level, COD removal was significantly increased from 7% to 61%, NO_x-N removal increased from 21% to 99%, and TN removal increased from 20% to 89% (Fig. 3). Three and nine days were required to achieve 0 mg DO L^{-1} in the microcosms at COD_{in} levels of 82 and 58 mg L^{-1}, respectively, while the DO level was maintained at > 2 mg L^{-1} at a COD_{in} level of 28 mg L^{-1} throughout the experimental period (Fig. 4).

There appears to be a positive correlation between the NOx-N removals and the CODin level (and thus DO concentration) in these microcosms. Higher CODin levels will deplete oxygen concentrations in the FWS-CWs, creating DO conditions that are favorable for microbial denitrification, and donate the electrons for denitrification process. In contrast, carbon limitation may hamper the nitrate removal potential of free-floating plant-based FWS-CWs. Therefore, merely the formation of a closed mat of free-floating plants is not enough to achieve a better nitrate reduction performance. The presence of sufficient organic oxygen-consuming substances is also necessary to optimize the nitrate reduction performance of FWS-CWs.

CONCLUSIONS

This study demonstrated that different wetland plant types strongly influence pollutant removal in ammonia-dominated wastewater. Free-floating plants were the best candidates for purification of ammonia-dominated wastewater when both pollutant removal performance and evapotranspiration were considered simultaneously. On the other hand, optimum nitrate reduction in free-floating plant-based FWS-CWs will occur only when there are enough organic oxygen-consuming substances present, as well as a fully covered water surface. FWS-CW developers and managers should thus pay close attention to the selection of wetland plant types and optimize their design to achieve optimum nitrogen removal performance.

Acknowledgments

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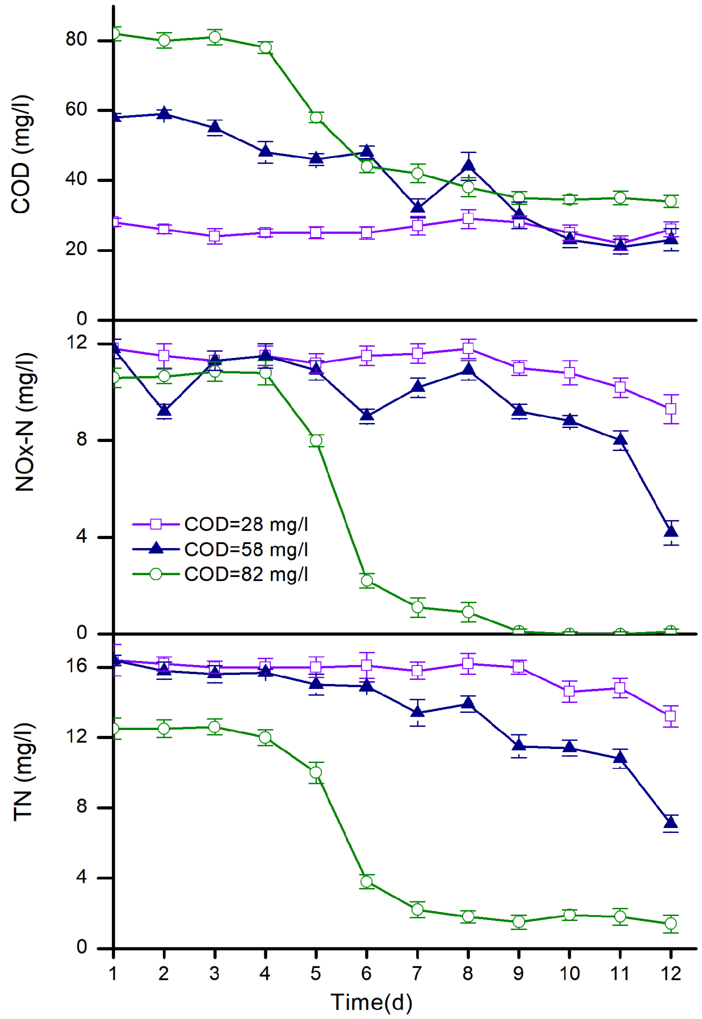

Figure 3. Pollutant removal performance of three duckweed (*L. aequinoctialis*) microcosms containing different initial COD levels during 12 days. Results are presented in mean ± SD which is represented as error bars (n = 3)

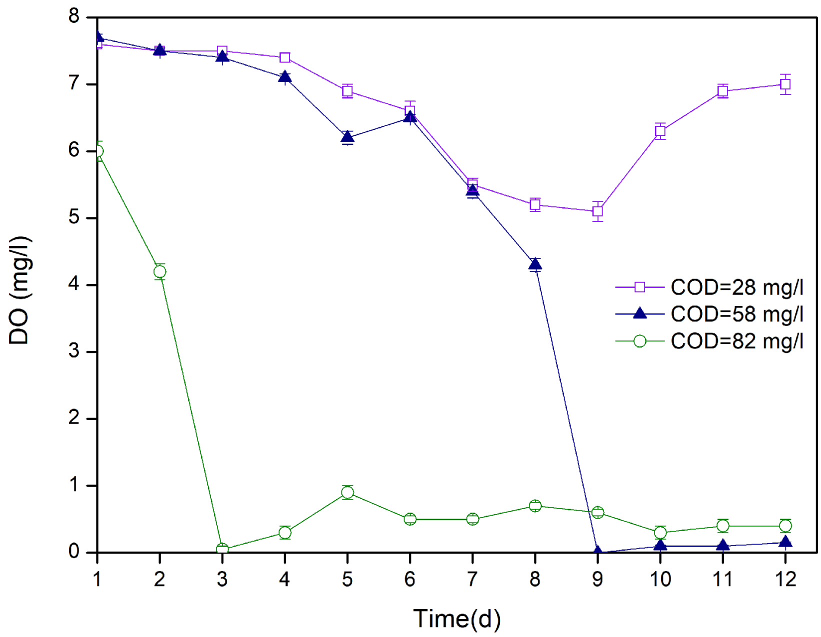

Figure 4. Time courses of DO levels in three *L. aequinoctialis* microcosms with different initial COD levels during 12 days. Results are presented in mean ± SD which is represented as error bars (n = 3)

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