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Treating performance of a commercial-scale constructed wetland system for aquaculture effluents from intensive inland *Micropterus salmoides* farm

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In intensive inland fish farming, discharge of untreated effluents adversely affects adjacent water bodies and causes water pollution. Thus, it is highly necessary to treat the effluents from inland fish farm. In this study, we built a commercial-scale integrated constructed wetland (CW) system with vertical subsurface flow, and monitored the purifying effect. During fish farming and discharge of effluents periods, the water samples were collected to detect the total nitrogen (TN), total phosphorus (TP), ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), nitrite nitrogen ($\text{NO}_2^-\text{-N}$) and chemical oxygen demand (COD_{Mn}). Results showed that the system was stable and significantly improved water quality from fish pond. During the fish farming period, the removal efficiency for TN, TP, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and COD was 24.93–43.72%, 61.92–72.18%, 56.29–68.63%, 56.66–64.81%, 56.42–64.19% and 28.37–42.79%, respectively. Similarly, these parameters were also markedly decreased by the integrated CW system during sewage discharge period, and the average total removal rate for TN, TP, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and COD was 50.24%, 64.48%, 61.36%, 62.65%, 56.16% and 37.32%, respectively. It was worth noting that three key parameters for effluents detection TN, TP and COD values were below the threshold values of water quality of Class II in freshwater sewage discharge standard of China (SCT9101-2007). In conclusion, this study evidently demonstrated that application of CW system was an environmental sustainable sewage treatment strategy in intensive inland fish farming.

KEYWORDS

constructed wetland, removal efficiency, total nitrogen, aquaculture effluents, recirculation aquaculture system

Introduction

Aquaculture products are key protein source in the global food production, which are increasing constantly in recent decades (FAO, 2020). They are mainly from marine water culture, inland freshwater culture and capture fisheries. In China, inland freshwater culture is the most important farming model, with a total area of 5,116.32 thousands ha and production of 30.14 million tons in 2019 (Yu et al., 2020). In the intensive inland freshwater farming, high-quality freshwater inflow was required. In turn, discharge of effluents can contaminate adjacent water bodies, which causes many environmental issues.

Like livestock farming and husbandry, large amounts of waste in aquaculture are generated, such as uneaten feed, feces, and dissolved material, which may be released into surface water and cause water pollution (Sindilariu et al., 2009). The pollution problem has aroused widespread concern as it exerts an adverse effect to aquatic ecological integrity (Turcios and Papenbrock, 2014). Excessive input nitrogen (N) and phosphorus (P) in aquatic ecosystems can promote the growth of algae and other phytoplankton, and subsequently cause dissolved oxygen (DO) depletion, water quality deterioration, and even death of aquatic animals (Bhateria and Jain, 2016; Wang et al., 2021). In China, the discharge standard of aquaculture effluents has been issued to prevent the harmful environmental effect (SCT9101-2007) (Ministry of Agriculture and Rural Affairs, 2007). However, existing methods for the treatment of aquaculture effluents, such as bio-filter and physical filter, require high investment and increase the cost of farming, which is beyond the reach of most of small producers. Therefore, it is highly necessary to seek effective method with a relatively low cost to address sustainability issues of inland fish farming.

Constructed wetland (CW) is one of the most promising methods for dealing with aquaculture effluents as it possesses some advantages, such as low cost, ecological benefit and ease of operation and maintenance (Tepe, 2018; Hang Pham et al., 2021). When integrated with special matrix and aquatic plants, CW can effectively remove many types of contaminants from wastewater, including organic matter, N, P, suspended solids, heavy metals and pathogenic microorganism through the coordinated action of biological, physical and chemical processes (Almuktar et al., 2018; Wang et al., 2021). Meanwhile, substrate microorganisms (e.g. nitrifying bacteria and denitrifying bacteria) play a pivotal role in conversion of nutrients, especially N, in wastewater (Liang et al., 2003; Li et al., 2018).

In aquaculture, CW has been applied to treat the discharged effluents from farms (Schulz et al., 2003; Sindilariu et al., 2007), or as a water filter unit to connect with recirculating aquaculture system (Zhang et al., 2010; Shi et al., 2011). According to surface area, the CW is classified into lab-scale CW ($< 10 \text{ m}^2$) (Saeed and

Sun, 2013; Jesus et al., 2017), pilot-scale CW ($10\text{-}100 \text{ m}^2$) (Papaevangelou et al., 2016; Uggetti et al., 2016) and commercial-scale CW ($> 100 \text{ m}^2$) (Tilley et al., 2002; Shi et al., 2011) in research or farming practice regarding aquaculture. Early study reported that a hybrid free water surface-horizontal subsurface flow CW (surface area of 5 m^2) could remove 95% of total inorganic N and 32-71% of P in effluents from milkfish (*Chanos chanos*) pond (Lin et al., 2002). Horizontal subsurface flow CW (28 m^2) effectively reduced the total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) concentrations in effluents from the flow-through trout fish farm (Chazarenc et al., 2007). Vertical flow CW (80 m^2) for treatment of aquaculture ponds water proved to be very effective and the reduction amounted to 61.5% of ammonia nitrogen ($\text{NH}_4^+\text{-N}$), 68% of nitrate nitrogen ($\text{NO}_3^-\text{-N}$) and 20% of P (Li et al., 2007). Shi et al. (2011) built a combination CW (221.0 m^2) to purify brackish effluent from commercial recirculating and super-intensive shrimp farming systems, and it exhibited good removal of TN (67%), TAN (71%) and TSS (66%). It is apparent that the most of these studies were conducted at lab-scale and pilot-scale systems. However, there was relatively little published research regarding practical application of a commercial-scale CW in aquaculture of China, and thus, its treatment performance needs to be further evaluated.

Based on the issues addressed above, we built a commercial-scale integrated CW system ($2,400 \text{ m}^2$) with vertical subsurface flow, which connected with fish ponds with a total surface area of $19,200 \text{ m}^2$ to form a recirculating pond system. The objective of this study was to evaluate the performance of the CW system to manage the pond water quality with no water exchange during the farming period. Further, we examine whether the system improved the quality of effluents to comply with the discharge standard at the end of farming.

Materials and methods

Aquaculture system and constructed wetland design

The experiment was conducted in Wujiang modern agriculture industrial park (Suzhou, China), where we built an integrated CW system with a vertical subsurface flow. The CW system was connected with 7 fish ponds with a total surface area of $19,200 \text{ m}^2$ to form a recirculating pond system (Figure S1).

The CW system (total surface area $2,400 \text{ m}^2$) was consisted of sedimentation pond (250 m^2), aeration pond (250 m^2), constructed wetland ($1,800 \text{ m}^2$) and stabilize water pond (100 m^2) (Figure S1). The wetland was filled with three layers of media. The bottom was filled with 30 cm of cobblestone

(diameter 8–10 cm), the middle layer was filled with 25 cm of peledith (diameter 4–6 cm) and the top was filled 25 cm of peledith (diameter 2–4 cm). Three types of macrophytes *Thalia dealbata*, *Iris germanica* and *Canna indica* were selected to plant into the wetland due to stronger uptake capacity of N, P and metal. The planning density was one plant/m². The mean hydraulic loading rate (HLR) was 0.6 m³/m² d and hydraulic retention time (HRT) was 0.83 d. The water in CW-fish pond recirculating system was not renewed during the experiment. The system ran from early Jun to late November 2020.

Fish culture

The largemouth bass (*Micropterus salmoides*) were provided by Suzhou Tongli Modern Agriculture Development Co. LTD (Suzhou, China). The stocking density was 30,000 fish/hm² with an average initial weight of 20 ± 2 g/fish. The fish were fed on a commercial pellet diet containing 47% crude protein, 5% crude lipids, 18.0% crude ash and 3.0% crude fiber (Changzhou Haida Biological Feed Co., Ltd, Changzhou, China). During the farming period, the fish were fed 3–4 times daily and feeding amount was approximately 2–4% of the fish weight. The management of fish farming was operated according to the method reported by Sun (Sun, 2020). The use of fish was approved by the Freshwater Fisheries Research Centre (FFRC) of the Chinese Academy of Fishery Sciences, Wuxi, China.

Water sampling and parameters detection

During the fish farming period, water samples were collected from the fish pond, sedimentation pond, aeration pond (inlet of wetland) and outlet of wetland once per 24–25 days from July 10 to October 20, 2020. During the sewage discharge period, water samples were collected from the fish pond, sedimentation pond, aeration pond (inlet of wetland) and outlet of wetland once per 9 days from November 1 to November 21, 2020. In each sampling site, we randomly collected the water samples *via* the five-point sampling method (Jiang et al., 2017).

The temperature and DO were detected using a YSI-DO 200 (YSI Inc., Ohio, USA), and the pH was measured using PHS-3CT acidometer (Shanghai Huyeming Scientific Instrument Co., LTD, China). The water quality parameters including COD_{Mn}, TN, NO₃⁻-N, NO₂⁻-N, NH₄⁺-N and TP were measured using standard methods (Wei, 2002). The COD content was measured using potassium permanganate as an oxidant. The TN concentration was detected according to alkaline potassium persulfate digestion method. The TP concentration was determined by ammonium molybdate spectrophotometric method. The NO₃⁻-N concentration was tested with UV spectrophotometry method. The NO₂⁻-N was examined using

N-(1-naphthalene) –ethylenediamine as a chromogenic agent. The NH₄⁺-N concentration was analyzed by nessler's reagent spectrophotometry method. Removal efficiency (%) of the CW for each water quality parameter was calculated using following equation.

$$\text{Removal efficiency (\%)} = (C_0 - C_1)/C_0 \times 100$$

Where C₀ and C₁ is the concentration of the target parameter in outflow and inflow of wetland, respectively.

Statistical analysis

Data are analyzed using SPSS 24.0 and values are expressed as the mean ± standard error of the mean (SEM). Normal distribution was assessed by Kolmogorov–Smirnov test, and homogeneity of variance was examined by Levene teste. Difference among different groups were analyzed by One-way analysis of variance (ANOVA) with LSD *post hoc* test. *P* value less than 0.05 were considered to be statistically significant.

Results

Running parameters of the integrated CW system

During the experiment period, there was a similar change of temperature in fish pond, sedimentation pond, aeration pond, and CW outlet, ranging from 20.7°C to 32.2°C. Average pH value was 7.31 ± 0.15, 7.29 ± 0.19, 7.17 ± 0.34 and 7.09 ± 0.28 in fish pond, sedimentation pond, aeration pond and CW outlet, respectively. The average DO value was 5.82 ± 0.51 mg/L, 5.07 ± 0.37 mg/L, 6.38 ± 0.58 mg/L and 2.07 ± 0.29 mg/L in fish pond, sedimentation pond, aeration pond and CW outlet, respectively. The DO reduction showed an oxygen depletion in the CW, which may be related to the aerobic respiration of heterotrophic microorganisms (Hang Pham et al., 2021). During the experiment period, the integrated CW system operated stably.

Changes of water quality parameters during fish farming period

During the fish farming period, the TN concentration successively decreased when the water flowed through sedimentation pond, aeration pond and CW, and significant difference was observed between CW inlet and CW outlet (*p* < 0.05; Figure 1A). The average TN concentration was 8.65 mg/L, 8.25 mg/L, 7.82 mg/L and 5.33 mg/L in fish pond, sedimentation pond, aeration pond (CW inlet) and CW outlet, respectively. Total TN removal rate of the integrated CW system ranged from 24.93 to 43.73% during the monitoring period (Figure 1B).

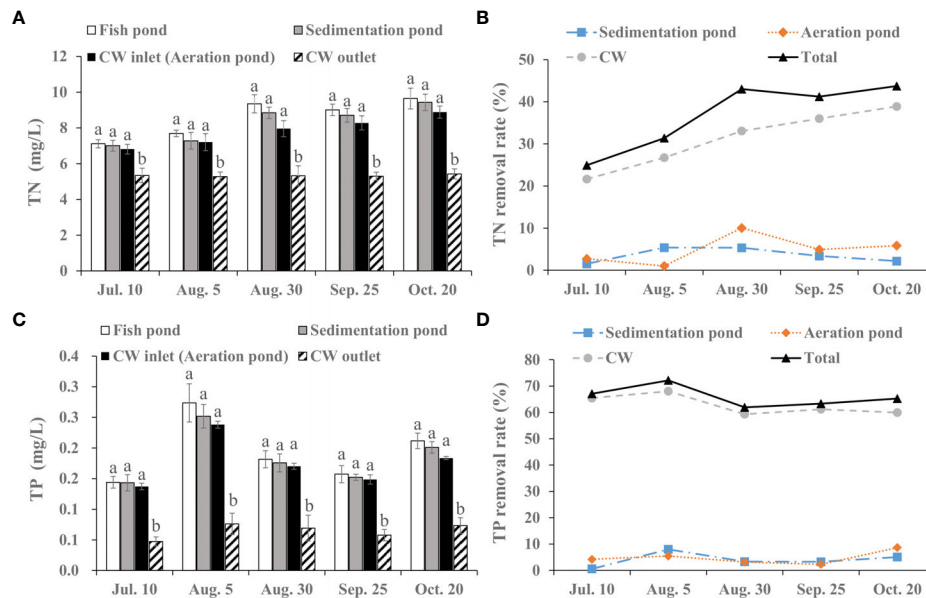


FIGURE 1

Removal efficiency of integrated CW system on TN and TP during the fish farming period. (A) TN value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (B) TN removal rate of the sedimentation pond, aeration pond and CW. (C) TP value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (D) TP removal rate of the sedimentation pond, aeration pond and CW. The value is expressed as mean \pm SE (n = 5). Different small letters in bar graph indicate significant difference ($p < 0.05$) among fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet.

The integrated CW system significantly removed TP concentration in effluents from fish pond, and TP value at the CW inlet was clearly higher than that at the CW outlet ($p < 0.05$; Figure 1C). Meanwhile, there was a stable total removal rate on TP ranging from 61.92 to 72.19%. Average removal rate was 4.02%, 4.76% and 62.79% in sedimentation pond, aeration pond and CW, respectively (Figure 1D).

As shown in Figure 2A, average $\text{NH}_4^+\text{-N}$ concentration in water generally decreased from fish pond to CW outlet, and the decrease exhibited significant difference between the CW inlet and CW outlet during fish farming period ($p < 0.05$). Average reduction rate of $\text{NH}_4^+\text{-N}$ was 2.75%, 2.82% and 60.81% in the sedimentation pond, aeration pond and CW, respectively (Figure 2B).

The variation of $\text{NO}_2^-\text{-N}$ level followed a similar trend to $\text{NH}_4^+\text{-N}$, decreasing from 0.04 mg/L (mean) at the fish pond to 0.016 mg/L (mean) at the CW outlet when the water flowed through sedimentation pond, aeration pond and CW (Figure 2C). Specifically, $\text{NO}_2^-\text{-N}$ level was obviously reduced at the CW outlet compared with the CW inlet during the July 10–October 20 ($p < 0.05$), and average total reduction rate of $\text{NO}_2^-\text{-N}$ was 60.54% (Figure 2D).

The integrated CW system showed a high removal effect for $\text{NO}_3^-\text{-N}$, with an average total removal rate of 59.66% (Figure 2F). In addition, average $\text{NO}_3^-\text{-N}$ concentration was 0.135 mg/L, 0.127 mg/L, 0.117 mg/L and 0.055 mg/L in fish pond, sedimentation pond, aeration pond (CW inlet) and CW

outlet, respectively, and there was a significant difference between the CW inlet and CW outlet ($p < 0.05$; Figure 2E).

The COD_{Mn} is frequently used to evaluate COD content in aquaculture effluents. Concentration of COD_{Mn} displayed a nonlinear decrease in different sampling time when the water flowed through sedimentation pond, aeration pond and CW (Figure 2G). Notably, COD_{Mn} value at CW inlet was higher than that at CW outlet ($p < 0.05$). Meanwhile, this system showed a stable removal efficiency for COD_{Mn} with 30.51–42.79% removal rate (Figure 2H).

Changes of water quality parameters during the effluents discharge period

During the effluents discharge period, average TN concentration decreased from 9.89 mg/L to 4.92 mg/L when the water from fish pond flowed through the CW system (Figure 3A). Compared with CW inlet, TN value was clearly reduced at CW outlet ($p < 0.05$), with an average reduction rate of 43.17% (Figure 3B).

Similarly, The TP concentration was effectively reduced by the CW system. The TP concentration was prominently lowered from 0.25 mg/L at fish pond to 0.09 mg/L at CW outlet, with a relatively stable average removal rate of 64.48% ($p < 0.05$; Figure 3C). The removal rate was 2.18–8.49% in sedimentation

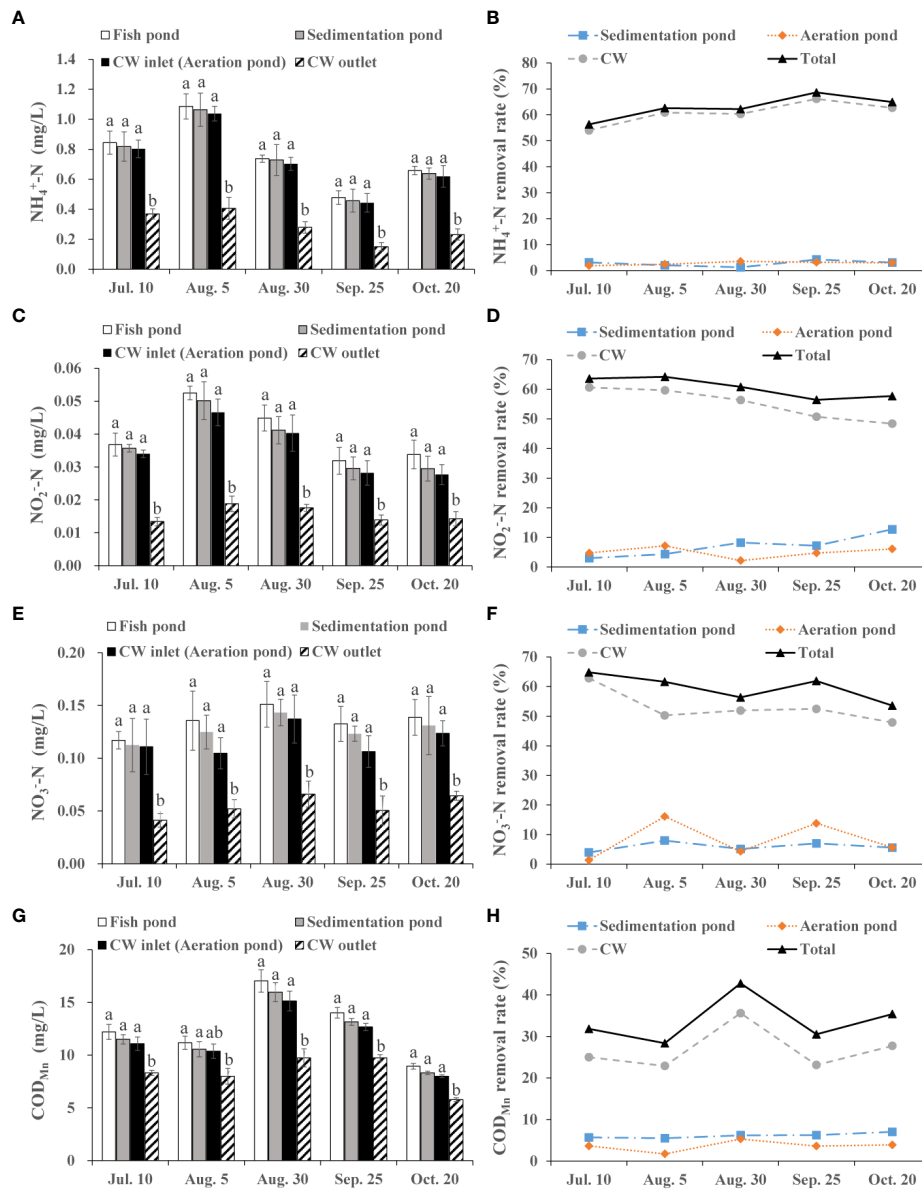


FIGURE 2

Removal efficiency of integrated CW system on NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and COD_{Mn} during the fish farming period. (A) NH₄⁺-N value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (B) NH₄⁺-N removal rate of the sedimentation pond, aeration pond and CW. (C) NO₂⁻-N value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (D) NO₂⁻-N removal rate of the sedimentation pond, aeration pond and CW. (E) NO₃⁻-N value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (F) NO₃⁻-N removal rate of the sedimentation pond, aeration pond and CW. (G) COD_{Mn} value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (H) COD_{Mn} removal rate of the sedimentation pond, aeration pond and CW. The value is expressed as mean ± SE (n = 5). Different small letters in bar graph indicate significant difference (p < 0.05) among fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet.

pond, 7.16–10.10% in aeration pond and 55.37–62.89% in CW (Figure 3D).

As shown in Figure 4A, The NH₄⁺-N concentration showed a decreasing tendency in water from fish pond to CW outlet, and there was a significant difference between the CW inlet and CW outlet (p < 0.05). The average removal rate was 5.73%, 9.81% and

54.57% in the sedimentation pond, aeration pond and CW, respectively (Figure 4B).

The NO₂⁻-N concentration was successively decreased in fish pond, sedimentation pond, aeration pond (CW inlet) and CW outlet, and the decrease showed significant difference between the CW inlet and CW outlet (p < 0.05; Figure 4C). The integrated

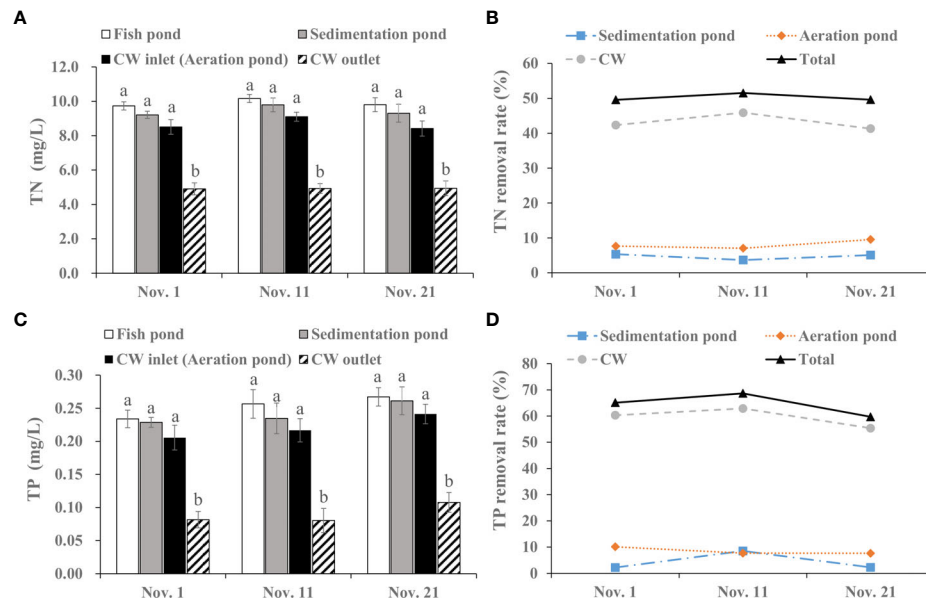


FIGURE 3

Removal efficiency of integrated CW system on TN and TP during the effluents discharge period. (A) TN value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (B) TN removal rate of the sedimentation pond, aeration pond and CW. (C) TP value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (D) TP removal rate of the sedimentation pond, aeration pond and CW. The value is expressed as mean \pm SE (n = 5). Different small letters in bar graph indicate significant difference ($p < 0.05$) among the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet.

CW system has a stable removal rate ranging 54.27% to 58.55% during the effluents discharge period (Figure 4D).

Variation of the NO_3^- -N level followed a similar trend to NO_2^- -N. The average NO_3^- -N level was 0.126 mg/L, 0.115 mg/L, 0.107 mg/L and 0.047 mg/L in the fish pond, sedimentation pond, aeration pond (CW inlet) and CW outlet, respectively (Figure 4E). The integrated CW system strongly reduced NO_2^- -N level in the aquaculture effluents with a total average removal rate 62.65% (Figure 4F).

During the effluents discharge period, the average COD_{Mn} concentration was 11.03 mg/L, 10.30 mg/L, 9.95 mg/L and 6.84 mg/L in the fish pond, sedimentation pond, aeration pond and CW outlet, respectively (Figure 4G), and the value at CW outlet was apparently lower than that at other sampling sites ($p < 0.05$). Meanwhile, the integrated CW system has a distinct impact on COD_{Mn} reduction, and removal rate ranged from 32.33% to 43.04% (Figure 4H).

Discussion

In aquaculture effluents, inorganic N is a key pollutant, primarily generated from residual feeds and feces (Piedrahita, 2003). Overloading N can cause eutrophication, algal blooms and water anoxia, which may further lead to abnormal changes in physiology and behavior of aquatic animals (Banerjee et al.,

2021). Thus TN level is a common parameter used to assess water quality in aquaculture. A CW (9.5 m²) was used to treat effluents from a recirculating aquaculture system farmed *Oreochromis niloticus*, and there was a 95.5% removal rate for TN (Behrends et al., 1999). Similarly, Lin et al. (2002) built a hybrid CW (5 m²) to treat effluents of *Chanos chanos* farming, and TN concentration was significantly removed by the CW system. Zhang et al. (2010) used a farm-scale CW (320 m²) to improve water quality in *Ictalurus punctatus* farming, and the TN removal rate was 48%. In addition, TN removal rate was related to types of CW systems, such as, 12.4% in floating-bed CW, 64.7% in horizontal subsurface flow CW, and 23.0% in surface flow CW (Bai et al., 2020). In the present study, the TN removal rate was 36.85% during the fish farming period and 50.24% during the effluents discharge period, indicating the integrated CW system has a high removal rate for inorganic N. In addition, the average TN value was 4.92 mg/L at CW outlet during the effluents discharge period, which met the water quality of Class II in the freshwater sewage discharge standard of China (SCT9101-2007).

In aquaculture practice, NH_4^+ -N, NO_2^- -N and NO_3^- -N are ubiquitous pollutants, and have received the most attention due to high toxicity on aquatic animals (Molayemraftar et al., 2022). High levels of NH_4^+ -N and NO_2^- -N have been reported to inhibit growth performance, induce tissue damage or even death in aquatic animals (Ip and Chew, 2010; Kroupova et al., 2018).

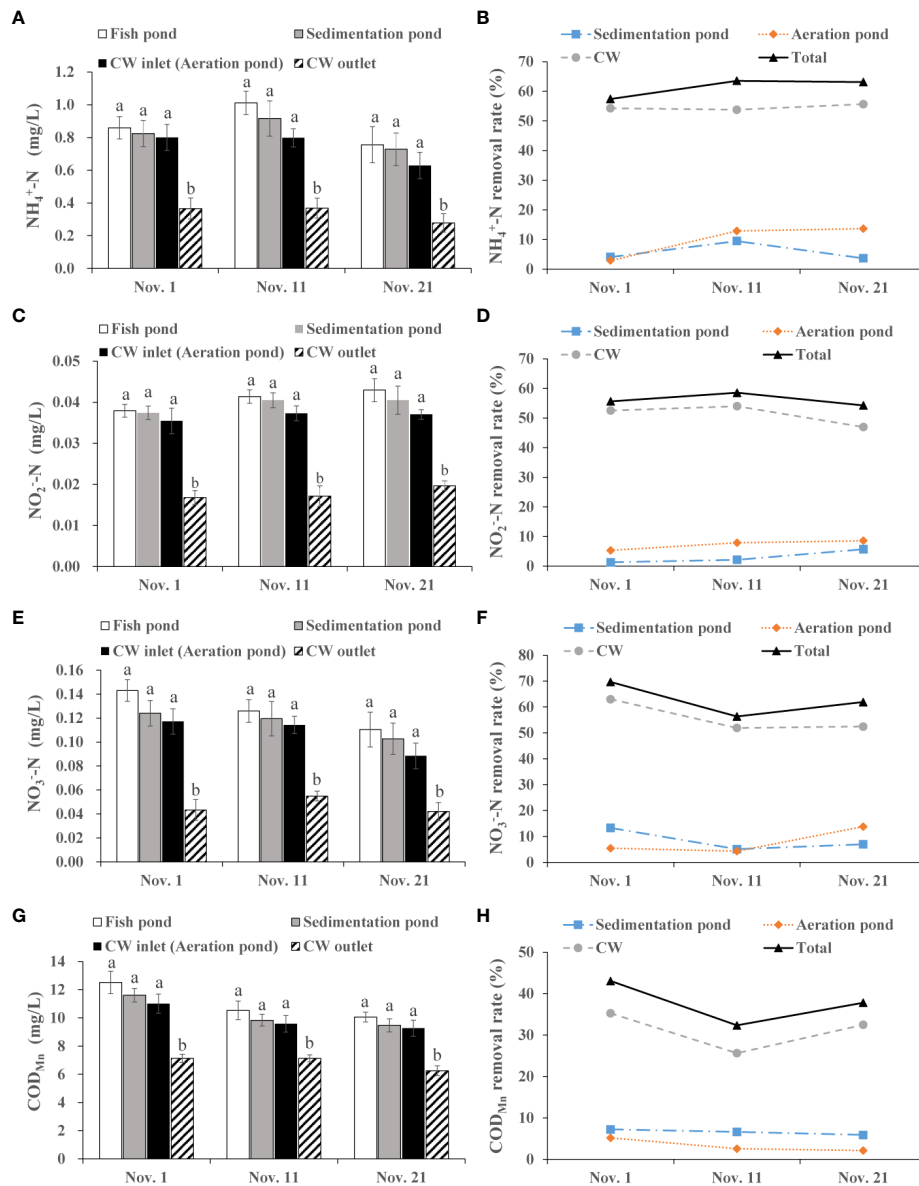


FIGURE 4

Removal efficiency of integrated CW system on $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ and COD_{Mn} during the effluents discharge period. (A) $\text{NH}_4^+\text{-N}$ value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (B) $\text{NH}_4^+\text{-N}$ removal rate of the sedimentation pond, aeration pond and CW. (C) $\text{NO}_2^-\text{-N}$ value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (D) $\text{NO}_2^-\text{-N}$ removal rate of the sedimentation pond, aeration pond and CW. (E) $\text{NO}_3^-\text{-N}$ value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet. (F) $\text{NO}_3^-\text{-N}$ removal rate of the sedimentation pond, aeration pond and CW. (G) COD_{Mn} value in the fish pond, sedimentation pond, CW inlet (aeration pond) and CW out. (H) COD_{Mn} removal rate of the sedimentation pond, aeration pond and CW. The value is expressed as mean \pm SE ($n = 5$). Different small letters in bar graph indicate significant difference ($p < 0.05$) among fish pond, sedimentation pond, CW inlet (aeration pond) and CW outlet.

Chronic $\text{NO}_3^-\text{-N}$ exposure reduces growth, increases susceptibility to hypoxia, disrupts endocrine system and affects the health status in fish (Monsees et al., 2017; Kellock et al., 2018; Isaza et al., 2021). It has been reported that CW was an efficient and cost-effective management practice to maintain reasonable concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ (Lin et al., 2010).

Mahmood et al. (2016) structured a CW-commercial ponds of *Macrobrachium rosenbergii* system, and found that the 43.8% $\text{NO}_3^-\text{-N}$, 25.7% $\text{NH}_4^+\text{-N}$ and 14.3% $\text{NO}_2^-\text{-N}$ were removed. In a lab-scale closed recirculation aquaculture system, horizontal subsurface flow CW removed 99% $\text{NO}_2^-\text{-N}$ and 82–99% $\text{NO}_3^-\text{-N}$, but had lower removal rate for $\text{NH}_4^+\text{-N}$ (10%) (Hang Pham

et al., 2021). In this work, the The CW system exhibited a high and stable removal rate for $\text{NH}_4^+\text{-N}$ (62.93%), $\text{NO}_3^-\text{-N}$ (59.66%) and $\text{NO}_2^-\text{-N}$ (60.54%), and improved substantially water quality from the fish pond during the fish farming period. It is worth noting that the average $\text{NH}_4^+\text{-N}$ concentration at CW outlet was below 0.5 mg/L during the effluents discharge period, meeting environmental quality standard of Class II for surface water in China (GB3838-2002). The data also indicated that the integrated CW system had a high removal efficiency for inorganic N in aquaculture effluents, which reduced aquaculture-included environmental contamination.

In CW system, N removal is a complex process, which may be related to sedimentation, uptake of aquatic plant, adsorption of media and biodegradation (Liu et al., 2019; Lu et al., 2020). N as an essential nutrient can be absorbed by aquatic plants, thus some aquatic plants are planted in CW to remove N. CW planted *Thalia dealbata* and *Pontederia cordata* displayed 66.44% and 68.56% TN reduction rate and 54.09% and 77.39% $\text{NH}_4^+\text{-N}$ reduction rate (Liu, 2011). In a CW planted *Canna indica*, the removal rate for $\text{NH}_4^+\text{-N}$ was 45.3–84.5% (Zhu et al., 2004). In this study, the *Thalia dealbata*, *Iris germanica* and *Canna indica* were planted into the CW to clean the effluents from fish pond, which reflected that the inorganic N removal was related to uptake of the aquatic plants. However, some researchers suggested that CW removed N primarily through ammonification, nitrification and denitrification, but not aquatic plants uptake (Uusheimo et al., 2018; Liu et al., 2019; Lu et al., 2020). In addition, the substrates of CW, such as pebbles and peledith, have been reported to have strong adsorption on N (Wang et al., 2018). Previous study suggested that, in commercial-scale CW system, TN removal rate was often around 40%, of which limiting factors were organic carbon concentration and low DO level (Xie et al., 2018; Lu et al., 2020). In this study, we speculated the major limiting factor for N removal was the DO level (2.07 ± 0.29 mg/L in CW outlet), because heterotrophic microorganisms played an important role in N removal process (Friedland, 2004).

Apart from N, P level is another important parameter evaluating water quality. P can promote algal growth and has a significant impact on microcystins production by *Microcystis aeruginosa* in aquatic environment (Dai et al., 2016). High concentration of P deteriorated not only water quality (Smith et al., 1999), but caused microcystins accumulation though enhancing *Microcystis* biomass (Wang et al., 2010). CW has the potential to treat P in aquaculture effluents. For example, CW as a recirculation filter in large-scale shrimp aquaculture was effective in removal of TP (65%) (Tilley et al., 2002); a laboratory-scale CW markedly removed the TP (average reduction rate 31%) from *Litopenaeus vannamei* farming water (Hang Pham et al., 2021). In line with previous studies, our data showed that the integrated CW system effectively removed TP with an average removal rate of 65.95% during the fish farming period. Interestingly, our data also displayed that the average TP

concentration was 0.09 mg/L at CW outlet, which lower than the threshold value (0.5 mg/L) in the freshwater effluents discharge standard Class II of China (SCT9101-2007). P removal in CW is a manifold process including physical, chemical and biological forces (Sindilariu et al., 2007). It also depends on the ecological situations, type of CW and planted macrophytes (Kumar and Dutta, 2019). P as an essential mineral can be absorbed by macrophytes in CW system. Various macrophytes possesses different uptake capacity of P, such as 48.1% for *Canna indica* and 76% for *Thalia dealbata* (BU et al., 2010; Yang et al., 2021). In addition, the P removal was related to adherence capability of a range of filter media in CW (Vohla et al., 2011; Wu et al., 2015).

Like TN and TP, COD concentration is a key monitoring parameter during aquaculture effluents discharge period. It is also used to evaluate organic pollutant in aquatic environment. Numerous studies suggested that CW had a strongly positive impact on the removal of organic matter with 59.7–89.0% COD reduction rate (Tuszyńska and Obarska-Pempkowiak, 2008). In the current study, the COD concentration was also clearly reduced by CW system, with 28.37–42.79% reduction rate. Meanwhile, the average COD concentration was 6.84 mg/L, below the threshold value (15 mg/L) in freshwater effluents discharge standard of China (SCT9101-2007). In CW, the COD can be removed by sedimentation and filtration suspended solids (Hang Pham et al., 2021). However, our data showed that the COD removal rate of sedimentation was 5.88–7.33% which was much lower than that of CW, reflecting the COD may be removed mainly via filtration of CW media. On the other hand, biodegradation by aerobic and anaerobic microorganisms was also an important removal mechanism of organic matter (Kumar and Dutta, 2019). In the aerobic biodegradation process, oxygen transfer may be a limiting factor for COD removal. Thus, the low COD reduction in this study may be related to DO level (2.07 ± 0.29 mg/L at CW outlet).

Conclusion

In the study, we built a commercial-scale vertical subsurface flow CW connected with fish ponds, which run stably during the fish farming and effluents discharge periods. During the fish farming period, the integrated CW system had high and stable removal efficiency for TN of 24.93–43.72%, TP of 61.92–72.18%, $\text{NH}_4^+\text{-N}$ of 56.29–68.63%, $\text{NO}_3^-\text{-N}$ of 56.66–64.81% and $\text{NO}_2^-\text{-N}$ of 56.42–64.19%. During the effluents discharge period, average value of TN, TP and COD, three key parameters for effluents detection, was 4.92 mg/L, 0.09 mg/L and 6.84 mg/L, respectively, which met the water quality of Class II in freshwater effluents discharge standard of China (SCT9101-2007). Finally, this study evidently demonstrated that application of CW was an environmental sustainable sewage treatment strategy in intensive fish culture system.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Author contributions

BL: Conceptualization, writing—original draft preparation, software; RJ: methodology, investigation validation, formal analysis, resources, YH: data curation, writing—review and editing; JZ: visualization, supervision, project administration, funding acquisition. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.1000703/full#supplementary-material>

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