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Intensified nutrients removal in constructed wetlands by integrated *Tubifex tubifex* and mussels: Performance and mechanisms

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ABSTRACT

The synergy of *Tubifex tubifex* (*T. tubifex*) and mussels on SFCWs (named SFCW-MT) performance was well studied in laboratory throughout a year. The SFCW-MT were steady operated with high TN and TP treatment, with the removal efficiencies of 37.85 ± 5.22% and 39.26 ± 5.20% even in winter. The mussels had excellent NH_4-N removal efficiency, and avoid the shortage of NH_4-N removal with *T. tubifex* in winter. Simultaneously, the SFCW-MT improved the NO_3-N treatment by 51% than that in control group. The plant growth was improved in SFCW-MT, which reflected in the improvement of total chlorophyll contents and plant heights. The N and P absorbed by wetland plants and adsorbed by substrate were both increased with mussels. Microbial analysis results revealed that, the mussels could keep the abundance of nitrifiers despite the negative effect of *T. tubifex*. On that basis, the improved proportions of denitrifiers (*Firmicutes*) have a significantly recognized role in NO_3-N transformation in SFCW-MT. The gut and membrane sections of mussels, as well as *T. tubifex*, also has proportions of denitrifiers and part of nitrifiers, and thus changed the microbial community in substrate. This evidence indicated that the co-existence of *T. tubifex* and mussels have potential application for simultaneous removal of NH_4-N and NO_3-N in CWs.

1. Introduction

Constructed wetlands (CWs) are used worldwide for removing and transforming pollutants from wastewater through the effects of plants absorption, substrate adsorption, and microbial metabolism (Kumari and Tripathi, 2015). However, insufficient nutrients removal at low temperature limited the long-term run of CWs. When temperature decreases, the denitrification rates were found to decline sharply (Li et al., 2016). To ensure a reliable nitrogen (N) removal capacity throughout the whole year in northern China, the denitrification rate at the lowest ambient temperature (the secondary effluent temperature of 6–8 °C) would be a serious problem.

As an ecological engineering, the CWs have sustainable environment for aquatic animals. A growing interest has been given to the pollution control and transfer by bioaccumulation through aquatic trophic chains (Ding et al., 2015; Kang et al., 2017). Many aquatic animals, such as *Tubifex tubifex* (*T. tubifex*), fish, oysters, and mussels, are considered for bio-remediates of pollutants (Gifford et al., 2007). In our previous study, we have found that *T. tubifex* is a typical meiofauna that has the ability to reduce nutrients such as N and phosphorus (P), especially nitrate (NO_3-N) (Kang et al., 2016). The *T. tubifex* enhanced the microbial decomposition rates through the enhancement of material exchange (Kristensen, 2000). But some limitations existed during the depuration of *T. tubifex* in aquatic environment. In winter, the removal efficiency of ammonium (NH_4-N) by *T. tubifex* was little declined due to the transformation and dissimilated nitrate reduced to ammonia (DNRA) process extraction (Kang et al., 2016).

It has been reported that some macrofauna has water purification capacity, such as loach, escargots, and oyster (Jones et al., 2015; Li et al., 2015). Freshwater mussels is a typical macrofauna and filter

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feeder, which was also considered for algal blooms control, aquaculture wastewaters treatment, and eutrophication reduction. Recent studies demonstrated that, mussels has important role in improving water quality as biofilters. It was shown that, the uptake of N and P by mussels arrived 100.9 mg/g dry weight (DW) tissue and 9.3 mg/g DW tissue. The zebra mussel could also remove 90% of organic carbon in wastewater, and a single mussel was capable of filtering 40 L water in one day (McLaughlan and Aldridge, 2013). The mussels can improve water quality substantially, and also perform an important restorative function in enhancing habitat, and increasing invertebrate abundance and/or diversity like an "ecosystem engineers" (Ismail et al., 2015). Recent studies have demonstrated that the mussels could oxygenate sediments through bioturbation, enhance bio-deposition of sediments and faeces through filter feeding, and provide a habitat environment for other taxa and conspecifics (Sousa et al., 2011). But the fate of trace contaminants transformation in CWs by mussels is not well studied. Even though meiofauna are orders of magnitude much more abundant than macrofauna in most aquatic environments, the macrofauna still has higher water purification volume than that with meiofauna. Biological interactions between macrofauna and the microfauna, and their combined action in regulating nutrients transformation are important in structuring stable ecosystem in aquatic sediments (Nascimento et al., 2012). Meanwhile, the treatment efficiency of NH_4-N by *T. tubifex* decreased in winter, while the NH_4-N removal was high with mussels. Both of the mussels and *T. tubifex* could enhance NO_3-N removal efficiency in aquatic environment. Given the potentially supplementary effect of mussels and *T. tubifex*, suggested applications of these two benthic fauna was proposed to enhance the long-term run of CWs.

To further enhance the performance of CWs, this study details the potential synergy effect of mussels and *T. tubifex* in CWs under laboratory conditions, and provides valuable data for the application of different faunal groups in CWs. Specific objectives were: 1) to evaluated the CWs performance in a whole year (including water treatment, greenhouse gas emission) influenced by two benthic fauna, especially in winter; 2) to study the biological interaction between two benthic fauna, plants and substrate in ecosystem function; 3) microbial study to elucidate the mechanisms of pollutant removal with mussels and *T. tubifex* in CWs.

2. Materials and methods

2.1. Configuration and operation of the experimental units

The surface-flow CWs (SFCWs) used in this study were established at Shandong University, Shandong, Northern China (36°40'36"N, 117°03'42"E), which performed a warm-temperature monsoonal climate and cold winter. The whole year exposure experiment were lasted from June 2016 to May 2017. The air temperature were 29.7 ± 0.57 °C, 6.49 ± 0.91 °C, 2.16 ± 0.50 °C, and 17.0 ± 1.25 °C in summer, spring, autumn and winter, respectively. In 2016, the plants were harvested at November.

The five identical outdoor microcosm units (length of 60 cm, width in 40 cm, and height of 60 cm were non-transparent. A schematic of the unit setup is shown in Fig. 1. The SFCWs were filled from bottom to top with gravel (2–3 cm in diameter, 5 cm depth), and washed river sands (1–2 mm in diameter, 20 cm depth). The inlet was constant level outflow with a flow rate of 8.33 mL/min using a peristaltic pump, and was set up at the top of the side of each unit.

The treated effluent of each unit was stable discharged from the outlet at the water-sediment interface to keep the water surface elevation at 15 cm. The influent wastewater was Class I (B) level according to the Wastewater Discharge Standard (EPAP, 2002). The composition of synthetic wastewater were as follows (mg per liter): sucrose 51.33, $(NH_4)_2SO_4$ 37.60, KH_2PO_4 10.33, KNO_3 76.60, $CaCl_2$ 10.00, $MgSO_4$ 10.00. The hydraulic retention time (HRT) of each unit was 3 days, corresponding to the hydroaulic loading rate (HLR) of 5.0 cm/day. The

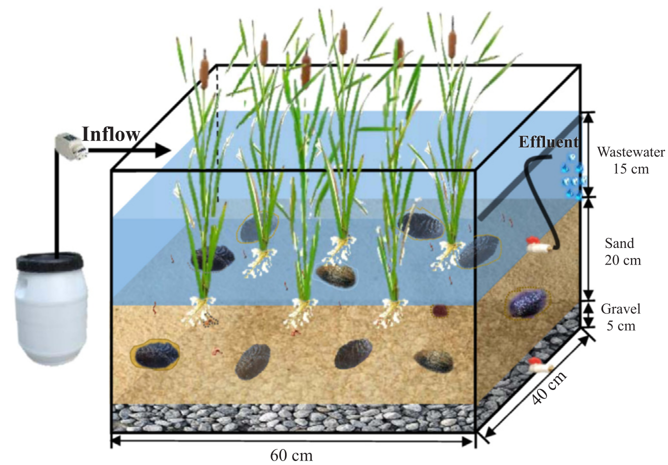

Fig. 1. Schematic diagram of the experimental units.

SFCWs were planted with *Typha orientalis* (*T. orientalis*, 12 stems per unit). Planting took place at the Arbor Day at March 12, 2016, and replanted at the Arbor Day in 2017 after harvested in November 2016. Three different types of SFCWs were studied (SFCW-C, SFCW-M, and SFCW-MT), the SFCW-M and SFCW-MT has two replicates per type, the SFCW-C only had one unit. The SFCW-M contained freshwater mussels, SFCW-MT contained both of freshwater mussels and *T. tubifex*, and SFCW-C was studied as the control group without benthic fauna.

2.2. Benthic fauna preparation

The freshwater mussels used in experiments were *Lamprotula leai*, collected from Henan, China. The length of *Lamprotula leai* used in experiments varied with the average length of 5.5 ± 0.8 cm and the average weight of 80.76 ± 5.19 g. After rinsed and scrubbed of shells, mussels were placed in air-saturated freshwater at 15 °C and fed with *Chlorella* vulgaris with small size (Svenningsen et al., 2012). Before added to experimental use, the mussels were depurated in synthetic freshwater for 72 h. Each experimental unit contained 15 individuals, according to the optimum condition reported by Izumi et al. (Izumi et al., 2012). The mussels were dispersed and uniformly distributed along the surface substrate. Then, the mussels transferred to the deep substrate by themselves. The preparation and density of *T. tubifex* was according to the previous research (Kang et al., 2016). The *T. tubifex* (15–20 mm in length and 0.5 mm in diameter) was washed and added into the CW units, with the density of 12,800–13,000 individuals/m^2.

2.3. Sampling and analysis

2.3.1. Plant physiology

The height and chlorophyll contents of the *T. orientalis* were measured before plant sampled. Chlorophyll concentration was determined as described by Chen and Pei (2016). The above-ground plant biomass was carried out one time during the whole experimental period: seasonal sampling in autumn (November 2016). After collection, the plant samples were dried at 35 °C for 72 h to a constant weight. The TN and TP concentration were measured by Xinpu Environmental Technology Company (Shanxi, China).

2.3.2. Water and sediment qualities monitoring

Water samples were taken from the influent and effluent of SFCWs every 3 days. The NH_4-N, NO_3-N, NO_2-N, TP, and organic matter (COD and TOC) were immediately analyzed in laboratory. All analyses were performed according to the standard methods (APHA, 2005). The sand as substrate was gathered with spatulas. Substrate samples were collected in five randomized cores (0–10 cm) and thoroughly mixed into