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Short communication

# Nutrient removal, biomass accumulation and nitrogen-transformation functional gene response to different nitrogen forms in enhanced floating treatment wetlands

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ABSTRACT

Enhanced floating treatment wetlands (EFTWs) were established to treat water with different concentrations of ammonia and nitrate, the ratios of which were set at 0:3, 1:2, 2:1 and 3:0, respectively. The results showed that the average total nitrogen (TN) removals were 80.7%, 79.2%, 47.0% and 10.1% in those four treatments, respectively. EFTWs had much better removal ability of $NO_3^-$-N (82.8–98.1%) than that of $NH_4^+$-N (25.1–59.4%). *Canna indica* L. in the water containing both $NH_4^+$-N and $NO_3^-$-N experienced higher relative growth rate and absorption capacity of N (7.7 g/m$^2$) and P (1.5 g/m$^2$) than those with the sole N source. The relative abundance of *narG* and *nirK* on the plant roots and biofilm carriers in the six nitrogen functional genes accounted for 71.1–85.6% and 58.5–90.3%, respectively, leading to high denitrification in EFTWs. Therefore, N forms in water could affect biomass accumulation and attached microorganisms, and thus affect the water purification efficiencies of EFTWs.

## 1. Introduction

Floating treatment wetland (FTW) has become widely accepted as an ecological engineering device in terms of water quality improvement over the past two decades (Headley and Tanner, 2012). Its main application lies in the treatment of eutrophic river and lake water, storm water, sewage, water supply reservoirs and aquaculture pond water (Borne et al., 2014; Yeh et al., 2015). However, there is still some limitation of FTW in practical application, including the instability of purification efficacy and limited biomass and growth rate of plants utilized (Cao and Zhang, 2014). Hence, enhanced floating treatment wetland (EFTW) has been developed as a novel water remediation technology. Biofilm carriers such as rice straw, plastic filling materials, AquaMats, combination filler and elastic packing were added into the traditional FTW (Zhao et al., 2012; Cao and Zhang, 2014; Wu et al., 2016; Zhang et al., 2016). The biofilm carriers support a large attaching surface area for the microorganisms to improve the purification efficiency of FTW, especially for the reductions of nitrogen and chemical oxygen demand (COD) (Wu et al., 2016).

The nutrient removal efficiencies of EFTWs in treating river water, simulated eutrophic water and urban lake water were enhanced by adding biofilm carriers in the previous study (Zhang et al., 2016). Nitrogen forms in those different kinds of water were distinctive. The responses of plant growth and microorganisms might also be quite different, leading to different purification efficiencies of EFTWs. To date, the overall performances of EFTWs in the water with different N forms and related mechanisms have been poorly documented. Therefore, the objective of this study was (1) to compare the nutrient removal capacities of EFTWs in the water with different N forms, (2) to investigate the plant growth and N functional gene responses to N forms, and (3) to estimate the fate of nitrogen in EFTWs.

## 2. Materials and methods

### 2.1. Experimental set up

A mesocosm experiment was carried out in twelve (4 sets × 3 replicates) polyethylene tanks (0.8 m length × 0.6 m width × 0.6 m depth). Each tank was filled with 240 l synthetic wastewater and covered with a piece of Styrofoam floating mat (0.6 m length × 0.4 m width × 0.04 m thickness). *Canna indica* L. (*C. indica*) as a native plant is commonly used in ecological restoration. Five shoots were planted on each floating mat with the density of 20 plants/m$^2$. The average weight and height were 49.0 g and 64.2 cm for each shoot, respectively. The

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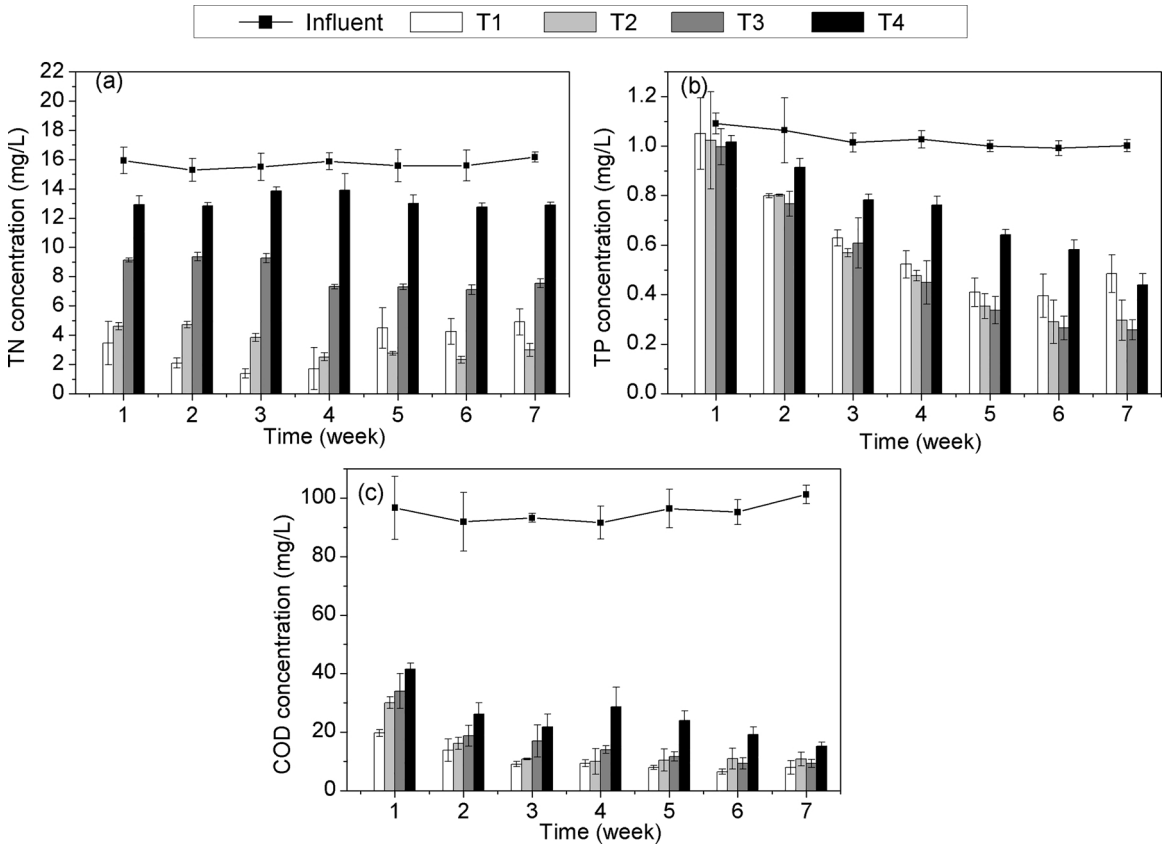


**Fig 1.** The influent and effluent concentrations of TN, TP, and COD in the EFTWs.

AquaMats selected as the biofilm carrier were described in our previous study (Zhang et al., 2016). One (1) $m^2$ AquaMat was divided into 6 pieces (50 cm × 33 cm), which were evenly fixed on the foam board in each tank using nylon rope. The experimental water was simulated with tap water and chemical reagents (glucose, $KH_2PO_4$, $NH_4Cl$ and $KNO_3$) with analytical reagent grade, and changed once a week. The setting characteristic of the tested water for COD, TN and total phosphorus (TP) was 90.0 mg/L, 15.0 mg/L and 1.0 mg/L in the four treatments. The ratio of ammonia ($NH_4^+$-N) and nitrate ($NO_3^-$-N) were 0:3, 1:2, 2:1 and 3:0 in the four EFTWs, respectively.

### 2.2. Water sampling and measurement

Water samples were collected from the middle point of water depth before and after each replacement, and analyzed immediately. pH and dissolved oxygen (DO) were determined in situ using a probe system (Thermo Orion 5 star, USA). COD was determined using HACH DR2800 and DRB200 (HACH, USA), while $NH_4^+$-N, $NO_3^-$-N, TN and TP were analyzed according to standard methods (State Environmental Protection Administration of China, 2002).

### 2.3. Plant sampling and analysis

At the beginning and end of the experiment, four shoots were randomly selected and oven dried at 75 °C for 48 h. N and P concentrations of the root, stem and leaf were analyzed, according to Zhu et al. (2011). At the end of the experiment, a piece of fresh leaf was cut, and used to measure chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid concentrations, following the methods by Peng et al. (2013).

### 2.4. Quantitative real-time polymerase chain reaction (qPCR)

At the end of the experiment, two pieces of biofilm carrier (2 cm × 3 cm) and 2 g plant roots were collected from each tank for DNA extraction. The samples were shaken in a 150-ml erlenmeyer flask with 100 ml deionized water and 10 glass beads at 25 °C and 140 rpm/min for 1 h, and then treated by ultrasonic for 30 min. Water DNA kits D5525-01 (Omega, USA) was used to extract and purify the total genomic DNA from samples. Extracted DNA was stored at −20 °C until use.

The16S rRNA fragments of the functional genes include anammox, ammonia monooxygenase (*amoA*), membrane-bound nitrate reductase (*narG*), copper-containing nitrite reductase (*nirK*), cd1-containing nitrite reductase (*nirS*), and nitrous oxide reductase (*nosZ*). All primers were chosen according to Zhi and Ji (2014), synthesized by Majorbio Bio-pharm Technology Company (Shanghai, China), and diluted to 10 mmol/L. qPCR was performed by a Real-time PCR Detection System (ABI7500, Applied Biosystems, USA), using the final 25 μl reaction mixtures containing: 12.5 μl SybrGreen PCR master mix (Applied Biosystems, USA), 2 μl template DNA, 1 μl forward and reverse primers, and 9.5 μl sterile water. qPCR was performed following a three-step thermal cycling procedure, and the protocol and parameters for each target gene were chosen according to Zhi and Ji (2014). The amplification size were 120, 257, 100, 515, 425, 473 bp for the standards of *amoA*, anammox, *narG*, *nirK*, *nirS* and *nosZ*, respectively. They were diluted to yield a series of 10-fold concentrations and then used for standard curves. The $R^2$ value exceeded 0.99 and efficiency was between 81 and 101% for each standard curve.

### 2.5. Statistical analysis

One-way ANOVA was used to evaluate the difference of water characteristics, plant growth and functional gene abundance among the four EFTWs at the 0.05 level. Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).