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Effect of *Eichhornia crassipes* on production of N_2 by denitrification in eutrophic water

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ABSTRACT

The floating aquatic macrophyte may stimulate the gaseous nitrogen production via denitrificaiton in eutrophic water. In order to justify this hypothesis, an improved floating-chamber method was deployed to directly measure the productions of N_2 and N_2O from the eutrophic water with or without growth of *Eichhornia crassipes*. The abundance of denitrifiers attached to *E. crassipes* root surface and in the water was measured. The interactions between *E. crassipes* and sediment on mediating N_2 production in eutrophic water were also analyzed. Within the experimental duration of 12 days, the values of N-15 at.% excess of N_2-N were significantly ($p < 0.05$) higher with the growth of *E. crassipes* than that without. Regardless of added sediment or not, the total removal amounts of N_2 and N_2O from the water were significantly ($p < 0.05$) higher in the planted treatments (4.9–6.7 g N m^{-3} without added sediment; 9.8–12.4 g N m^{-3} with added sediment) than non-planted treatments (2.9–3.4 g N m^{-3} without added sediment; 3.6–6.0 g N m^{-3} with added sediment). The total removal rates of N_2 and N_2O from the water were significantly ($p < 0.05$) higher in the planted treatments (63–71% without added sediment; 161–179% with added sediment) than non-planted treatments (41–44% without added sediment; 79–81% with added sediment). Regardless of plant presence or absence, the total removal amounts and rates of N_2 and N_2O from the water were significantly ($p < 0.05$) higher in the treatments with added sediment than without. The positive interactions ($p < 0.01$) between *E. crassipes* and sediment on promoting N_2 production from the water were observed. The presence of *E. crassipes* significantly promoted the abundances of *nosZ* ($p < 0.05$), *nirK* ($p < 0.01$) and *nirS* gene ($p < 0.01$) in the water. The results indicated that cultivation of *E. crassipes* could have a stimulating effect on the gaseous production of N_2 by denitrification in the eutrophic water.

1. Introduction

Rapid economic and social developments have been accelerating the processes of eutrophication in countries around the world (Albay et al., 2003). Eutrophic water is enriched with nutrients that stimulate excessive growth of phytoplankton (Jørgensen and Richardson, 1996). Eutrophication not only degrades aquatic ecosystem function, but also impacts the availability of scarce water resources (Khan and Ansari, 2005). In consequence, in situ bioremediation to remove excess nutrients from eutrophic lakes and rivers at low cost is becoming attractive (Babourina and Rengel, 2011).

Eichhornia crassipes (Mart.) Solms, a floating macrophyte, is a tropical species belonging to the pickerelweed family (Pontederiaceae). In many cases, this floating macrophyte is thought to be invasive weeds, posing a risk to aquatic ecosystems (Williamns et al., 2005). However, its high capability to assimilate nutrients and reduce suspended detritus is receiving attention for the treatment of eutrophic water body (Ayaweera and Kasturiarachchi, 2004).

The success of large-scale cultivation of *E. crassipes* depends on the confined growth of *E. crassipes*, safe harvest and disposal of its biomass. In China, large-scale confined growth of *E. crassipes* has been used to reduce nutrients levels in Lakes Taihu and Dianchi (Liu et al., 2010; Wang et al., 2012a). After nitrogen and phosphate assimilation, *E. crassipes* was mechanically harvested and processed to produce organic fertilizer, silage, and biogas (Jayaweera et al., 2007; Bai et al., 2011; Wang et al., 2011). This

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process removed a large amount of nutrients from the water (Zheng et al., 2008). As *E. crassipes* assimilates not only nutrients but heavy metals, the method to dispose of its biomass has been widely discussed in treating wastewater with dense concentrations of heavy metals (Chowdhury and Mulligan, 2011). For two large lakes of Taihu and Dianchi in china, the concentrations of metals in water phase generally below the "National Recommended Water Quality Criteria" from USEPA (Tao et al., 2012). The concentrations of metals in the organic fertilizer and silage made from the biomass of harvested *E. crassipes* were monitored (Bai et al., 2009, 2011). They can meet the national recommended standard for organic–inorganic compound fertilizer (GB18877-2009) and hygienical standard for feeds (GB13078-2006), respectively.

An ecological engineering project of large-scale cultivation of *E. crassipes* with coverage area of ~4.3 km^2 was carried out in the open ultra-eutrophic Lake Caohai, China, in 2011. Assimilation by *E. crassipes* removed 52% of the total N influent (936,000 kg). The N mass balance suggested that about 36% of total removed N was via the processes of denitrification in 2011 (Wang et al., 2013). A further study of using N-15 tracing method combined with mass balance estimation revealed that ~17.7% of the added $^{15}NO_3^-$ could be lost via denitrification. This proportion was enhanced to ~25.0% by cultivation of *E. crassipes* when compared with non-planted treatments (Gao et al., 2012). However, there was still lack of direct evidence concerning N_2 production via denitrification during treatment of eutrophic water using *E. crassipes* so far.

Denitrification is of special important in the management of aquatic ecosystems, resulting in permanent removal of anthropogenic N from ecosystems. In the process of denitrification, intermediate gaseous product of N_2O is a critical greenhouse gases whereas the N_2 final product is highly inert and thus has no adverse environmental consequences. In most circumstances, N_2 was the main gaseous product by denitrification in aquatic ecosystems (McCutchan et al., 2003a; McCutchan and Lewis, 2008). However, it is difficult to detect changes in N_2 in the environment due to high background of N_2 in the air. In this study, an improved floating-chamber method was adapted to directly measure production of N_2 and N_2O from eutrophic water in the presence of *E. crassipes*.

Sediment is a storage place for nutrients and contaminants (Bootsma and Hecky, 1999), where strong exchanges of nutrients and denitrification processes occur at sediment-water interface (Soto-Jimenez et al., 2003). The interactions between *E. crassipes* and sediment on mediating the sediment-water N exchanges and denitrification processes were rarely studied. Although the fibrous roots of *E. crassipes* do not grow into sediment, it can alter the balance of N-exchange at sediment-water interface (Wang et al., 2012b), and may also indirectly impact the denitrification processes at surface layer of sediment or sediment-water interface in the shallow water bodies. Zhang (2009) reported that the releasing of nutrients from sediment was slower than the nutrients being significantly removed by *E. crassipes*.

In this work, we investigated the influence of *E. crassipes* on denitrification through directly measuring the gaseous production of N_2 and N_2O from eutrophic water with or without sediment. We tested the following hypotheses to determine whether the presence of *E. crassipes* would stimulate the denitrification process. Firstly, *E. crassipes* roots would provide the necessary heterogeneous micro-habitats and substrates for denitrification (e.g. organic carbon, oxygen, etc.). Therefore, we predicted that abundance of denitrifiers attached to *E. crassipes* root surface and in the planted water would be higher compared to the non-planted water. Secondly, emission of N_2 from eutrophic water would be higher with *E. crassipes* than without, and the presence of *E. crassipes* had a positive effect on the gaseous nitrogen production in eutrophic water with or without sediment. Thirdly, the interactions between *E. crassipes* and sediment would not have a negative effect on mediating N_2 production in eutrophic water. The results would significantly contribute to clarify the role of floating macrophytes on mediating denitrification process. To what extent would N_2 and N_2O production contribute to nitrogen removal from eutrophic water in the presence of plants would also be quantified in the present study.

2. Materials and methods

2.1. Preparation of floating macrophytes

E. crassipes was collected from the No. 2 Pond at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. The pond received and stored domestic wastewater and rainwater. The concentration of total nitrogen (TN) in this pond ranges from 2.0 to 5.8 mg L^{-1}, total phosphorous (TP) ranges from 0.21 to 0.34, and pH ranges from 7.5 to 7.7 during the year in 2011. Full-size individuals of *E. crassipes* grown under natural light and having a length of approximately 15 cm above the root were collected from the pond in July 2011 for the experiment. Each replicate received ~1.0 kg (fresh weight) of *E. crassipes*.

2.2. Preparation of eutrophic water

Eutrophic water was pumped directly from the eutrophic pond into 24 cement containers (0.80 m length × 0.60 m width × 0.70 m height). For increased NO_3-N treatment, 5 mg L^{-1} of N-15 labeled KNO_3 was added to the water, allowed to equilibrate for one day before experiment. The initial concentrations of nitrogen in each container were measured afterwards (NH_4-N 6.0–7.2 mg L^{-1}, NO_3^-N 0.81–1.59 mg L^{-1}, NO_2^-N 0.15–0.33 mg L^{-1}, TN 6.3–9.2 mg L^{-1}).

In the treatments with application of sediment, each cement container had ~0.10 m depth of sediment (~130 kg dried sediment/container). Sediment was collected from Lake Taihu, naturally dried under shelter and ground to pass a 2 mm mesh sieve. Nitrogen concentrations (dry weight basis) in the sediment were NH_4-N 32.4 mg kg^{-1}, NO_3^-N 0.41 mg kg^{-1}, TN 13.5 g kg^{-1}.

2.3. Experimental design

The experiment was conducted at the field experimental station of JAAS from 1 July to 12 July, 2011. The prepared eutrophic water was reserved in the cement containers without water exchange during the experiment. The experimental design was multivariate with time effects and three between-subject variables (N-15 labeled nitrate, macrophyte and sediment), combined with one overall control (pure water with sodium hypochlorite) to check the diffusion of air-derived N_2. Each treatment had three replicates. The detailed information for treatments was shown in Table 1.

An improved floating chamber method was used to study the production of N_2 and N_2O from eutrophic water. The sketch diagram of the chamber for in situ collecting N_2O and N_2 released from water with or without presence of *E. crassipes* was shown in Fig. 1. The floating chambers were made from Plexiglass with 0.45 m length × 0.35 m width × 0.45 m height. In order to minimize N_2 background in the chamber headspace, each Plexiglass chamber was completely submerged into the water before starting the experiment. The inlet and outlet on the top of the chamber were all open at this time, which made the exclusion of air much easy. Special cares should be taken not to trap any air bubbles at the start. Then, the inlet and outlet were closed and a gas mixture (78% He, 21% O_2, 1% CO_2) was forced into the chamber. This made the chamber floating on the water, leaving a headspace of 0.45 by 0.35 by