Pol. J. Environ. Stud. Vol. 32, No. 3 (2023), 2601-2609
DOI: *10.15244/pjoes/160175* ONLINE PUBLICATION DATE: 2023-03-08

Original Research

Enhanced Nutrients Removal from Eutrophic Water by Aerobic Denitrifiers Assisting Floating Plants

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Received: 21 September 2022
Accepted: 1 February 2023

Abstract

Accelerated eutrophication is one of the most serious problems confronting freshwater systems worldwide. More effective methods to reduce nutrients from eutrophic water with high nitrogen but low carbon source are now urgently needed. In this study, the methods of floating plant remediation and aerobic denitrification were combined for seeking an effective strategy. Four aerobic denitrifying bacteria were adopted. Each strain was co-cultured with *Eichhornia crassipes,* and then the effects of different combinations on the removal of nitrogen and phosphorus were compared. Meanwhile, their impacts on the growth and physiological characteristics of plants were also measured. The results showed that the association of aerobic denitrifying bacteria with *E. crassipes* was effective in enhancing the simultaneous removal of nitrogen and phosphorus. *E. crassipes* helped bacteria to eliminate the accumulation of nitrites under the condition of limited carbon sources. Cooperation between *Rhizobium rosettiformans* and *E. crassipes* exhibited the best remediation performance. The four aerobic denitrifying bacteria increased the plant growth and phosphorus content, and had different effects on the plant root activity, leaf chlorophyll, leaf soluble sugar and leaf soluble protein concentration. Although the overall nutrients removal efficiency was greatly improved by the synergic association, the percentage contribution of plant uptake remained constant.

Keywords: aerobic denitrifying bacteria, aerobic denitrification, floating plant remediation, eutrophication, nitrogen, phosphorus

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Introduction

As industrial and agricultural activities increase, a large number of nutrients are released into the environment, making freshwater eutrophication a concern on a global scale. Nitrogen and phosphorous are identified as the primary nutrients that must be controlled to alleviate the severe eutrophication situation and the threat to the ecological health of aquatic systems [1]. Compared to the physical and chemical techniques, the bioremediation methods, including the use of specific microorganisms, aquatic plants, or aquatic animals, are more preferred for their cost-effective and sustainable characteristics [2].

The heterotrophic-aerobic denitrifying bacteria have recently attracted much attention because they provide a new pathway for biological removal of nitrogen [3-4]. They are of higher removal efficiency and more convenience with simultaneous nitrification and denitrification, compared to the traditional mode (aerobic autotrophic nitrification and anaerobic heterotrophic denitrification) [5]. Many researches have focused on the isolation of more efficient aerobic denitrifying bacteria and the elucidation of their detailed characteristics [6-7]. However, eutrophication is frequently caused by the wastewater with a low C/N ratio [8]. Organic carbon sources are essential for aerobic denitrifiers and can affect nutrient removal throughout the denitrification process [9-10]. The addition of external carbon sources will raise the cost of bioremediation. Floating aquatic plants are also preferable in the treatment of eutrophic water, because of their high growth rate for nutrient assimilation and low lignin content for use as bioenergy feedstock [11]. But the phytoremediation efficacy still needs to be enhanced [12]. In addition to screening plants with high removal rates [13], a combination with other methods is also required [14]. Organic matters derived from aquatic plants have been shown to serve as carbon sources and increase the abundance of denitrifying bacteria consortia [15]. Therefore, there exists a carbon basis for the cooperation between specific aerobic denitrifying bacteria and floating aquatic plants. But root organic exudates of aquatic plants are complex, including amino acids, organic acids, sugars, polysaccharides, and etc [16]. Different carbon sources can significantly affect denitrification performance [17]. And interestingly, the fraction of root exudates from some plant like *Lemna minor* was found to have an inhibitory effect on the nitrogen-removal efficiency of aerobic denitrifying bacteria [18]. Moreover, there is evidence that some bacteria are unique and compatible only in the rhizosphere of some plant species [16]. All of these bring uncertainty about the joint effect of specific aerobic denitrifying bacteria and floating aquatic plants to remove nutrients. Many researchers have focused on the combination of specific bacteria and plants in the remediation of heavy metals and organic pollutants, and highlighted the role of specific bacteria in the health-keeping and growth-promoting of terrestrial plants [19-20]. But how the aerobic denitrifying bacteria affect aquatic floating plants is still unknown.

Taking into account the aspects mentioned above, four aerobic denitrifying bacteria belonging to different classes were adopted as research objects in this work. *E. crassipes* was used as the cooperative floating plant due to its massive root system and strong nutrient removal ability. Then, the effects of the combination of different aerobic denitrifying bacteria and *E crassipes* on the removal of nitrogen and phosphorus from eutrophic water were examined. Meanwhile, the impacts of different aerobic denitrifying bacteria on the growth and physiological characteristics of *E. crassipes* were also measured. We hope it is helpful to find the collaborating potential of aerobic denitrifying bacteria and floating aquatic plants, and seek an effective strategy to further enhance the removal of nutrients from eutrophic water.

Experimental

Strain and Culturing Conditions

Microbacterium oxydans XJ, *Pseudomonas stutzeri* ZJ4, *Bacillus velezensis* B2 and *Rhizobium rosettiformans* 3-20 were isolated from water bloom samples in the aquaculture ponds of the Taihu Lake Basin. Our previous experiments indicated that they were all aerobic denitrifiers and could use nitrate nitrogen or ammonium nitrogen as the only nitrogen source. These strains were aerobically grown in batch cultures using Luria-Bertani (LB) medium at 25ºC, respectively. All media were sterilized at 121ºC for 20 min before use.

E. crassipes was collected from a concrete breeding pool at Guli Horticultural Farms (Suqian, China) and pre-incubated in 1/10 modified Hoagland's medium [21] for 30 days. The selected individuals were cleaned of debris, washed with tap water, immersed in 0.01% potassium permanganate for 15min and then washed with sterile deionized water. These pretreated plants were then used for coexistence experiments. The artificial climate room was pre-disinfected with ultraviolet irradiation for 12 h and maintained at 30°C with a constant relative humidity of 75% and illuminated by cool-white fluorescent lamps (2800 Lux) under a light/dark cycle of 16/8 h.

Experimental Design

In each treatment, 12L of 1/10 aseptic modified Hoagland's medium were prepared in cuboid-shaped plastic containers. These containers were pre-disinfected with 1.5mg L^{-1} chlorine dioxide and then washed with sterile deionized water. Ten treatments included: (1) only 1/10 modified Hoagland's medium (CK); (2) *M. oxydans* XJ inoculation alone (Mic);

(3) *P. stutzeri* ZJ4 inoculation alone (Pse); (4) *B. velezensis* B2 inoculation alone (Bac); (5) *R. rosettiformans* 3-20 inoculation alone (Rhi); (6) *E. crassipes* alone (P); (7) coexistence of *E. crassipes* and *M. oxydans* XJ (P-Mic); (8) coexistence of *E. crassipes* and *P. stutzeri* ZJ4 (P-Pse); (9) coexistence of *E. crassipes* and *B. velezensis* B2 (P-Bac); (10) coexistence of *E. crassipes* and *R. rosettiformans* 3-20 (P-Rhi). The inoculum of each bacteria was 0.01% (v/v). The initial concentrations of *M. oxydans* XJ, *P. stutzeri* ZJ4, *B. velezensis* B2 and *R. rosettiformans* 3-20 in the containers were 1.02×10^5 cells mL^{-1}, 1.10×10^5 cells mL^{-1}, 1.00×10^5 cells mL^{-1} and 1.11×10^5 cells mL^{-1}, respectively. The biomass of *E. crassipes* in each treatment was about 190 g. All treatments were aerated intermittently (30 min of aeration and 30 min of non-aeration) at an airflow rate of 80 L h^{-1} and supplemented with sterilized glucose solutions at a final concentration of 10mg L^{-1} every day to maintain the low-carbon environments. All pipes for aeration were also pre-disinfected with 1.5 mg L^{-1} chlorine dioxide. During the experiment period of 14 days, water samples were collected for analysis at 2-day interval. After the 14-day experiment, the plants were harvested for analysis. All experiments were conducted with three independent replicates under non-aseptic environment. The artificial climate room conditions were maintained as described above.

Analysis of Water Samples

Water samples were filtered through a 0.45 μm Millipore membrane and analyzed according to the methods described by [22]. Nitrate-N (NO_3^--N) concentration was analyzed using the UV spectrophotometric screening method. Ammonium-N (NH_4^+-N) concentration was determined using Nessler's reagent spectrophotometry method. Nitrite-N (NO_2^--N) concentration was determined using N-(1-naphthyl)-1, 2-diaminoethane dihydrochloride method. Dissolved total nitrogen (DTN) was measured using alkaline potassium persulfate digestion-UV spectrophotometric method. Dissolved total phosphorous (DTP) was measured using alkaline potassium persulfate digestion-ammonium molybdate spectrophotometric method. DTN or DTP removal rate was calculated using equation as follow:

$$R\ (\%) = (C_0 - C_t) \times 100 / C_0 \quad (1)$$

Where R is the removal rate; C_0 is the initial concentration of nutrient; and C_t is the final concentration of nutrient after 14 days cultivation.

Analysis of Plant Samples

The harvested plants were washed with deionized water and dried with absorbent paper. Their fresh weights (FW) were recorded as initially. The biomass production rate was calculated using equation as follow:

$$PR\ (\%) = (F_t - F_0) \times 100 / F_0 \quad (2)$$

Where PR is the biomass production rate; F_0 is the initial fresh weights; and F_t is the final fresh weights after 14 days cultivation.

Half of the plants in each treatment were used for the determination of physiological characteristics, and the others were used for the determination of total Nitrogen (TN) and total Phosphorous (TP) content. A part of the plants was heated at 105ºC for 15 min to deactivate enzymes and maintained at 65ºC for 48 h to achieve constant weight. Then the dry plant samples were ground into powder for analysis of TN and TP content. According to [23], TN contents of plants were measured with the H_2SO_4-H_2O_2 digestion-Kjeldahl method, and TP contents of plants were measured with the H_2SO_4-H_2O_2 digestion-ammonium molybdate spectrophotometric method. Fresh plant samples were used for the determination of physiological indexes. The content of total chlorophyll pigments in leaves was determined spectrophotometrically after extraction with 80% acetone [24]. The soluble sugar content in leaves was determined by sulfuric acid-anthrone method using a commercial assay kit (Suzhou Grace Biotechnology Co., Ltd, China). The soluble protein content in leaves was measured by Bradford method [25]. Root activity was measured by triphenyltetrazolium chloride (TTC) method using a commercial assay kit (Suzhou Grace Biotechnology Co., Ltd, China).

Statistical Analysis

Data were presented as mean standard deviation (SD). Differences between controls and treatments were determined by one-way ANOVA. Post hoc test was used to perform statistical multiple comparisons. LSD adjustment was adopted when the assumption of equal variances was met, and Games-Howell adjustment was adopted when the assumption of equal variances was not met. Student's t-test was used to assess the correlation between groups of parameters. Statistical significances were conformed at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM SPSS Inc., Chicago, IL, USA).

Results

Effect of Bacteria and Floating Plants on the Nutrients Removal

The main form of nitrogen in Hoagland's medium was NO_3^--N. As shown in Fig. 1a), all four denitrifying bacteria were effective in removing NO_3^--N from eutrophic water even under a low-carbon source condition. After 14 days, there was no significant

difference in the removal efficiency of NO_3^--N among the four bacteria. Compared to the single bacteria treatments, the reduction of NO_3^--N was dramatically promoted in all four plants-bacteria treatments. The removal capacity of NO_3^--N by P-Rhi treatments was significantly higher than that of the other three plants-bacteria treatments. The 14-day removal capacity of NO_3^--N by P-Rhi treatments was on average 2.98 times that of P treatments and 3.62 times that of Rhi treatments.

Compared to the controls, all four denitrifying bacteria could accelerate the reduction of NH_4^+-N in eutrophic water in 2 days. But the overall reduction of NH_4^+-N in 14 days showed a periodic fluctuation. Especially on the 6th day, the release of NH_4^+-N reached the highest point in all single bacteria treatments. The combination of plants and denitrifying bacteria reduced the amount of NH_4^+-N which was released back into water again. There was no significant difference in NH_4^+-N removal efficiency among all treatments after 14 days (Fig. 1b).

Unlike the reduction of NO_3^--N and NH_4^+-N, all four denitrifying bacteria caused NO_2^--N accumulation under a low-carbon source condition. Compared with the initial value, their concentrations of NO_2^--N in water all increased first, then decreased, and then maintained at a high level. The accumulation of NO_2^--N caused by *B. velezensis* was significantly higher than that of the other three bacteria after 6 days. The maximal accumulation of NO_2^--N in all four plants-bacteria treatments were significantly lower than that of the single bacteria treatments. After 10 days, the NO_2^--N concentrations in all four plants-bacteria treatments were all close to zero (Fig. 1c).

As shown in Fig. 1d), the reduction of DTP by the four denitrifying bacteria showed a fluctuating upward trend. The fluctuation period of DTP reduction was similar to that of NH_4^+-N. After 14 days, there was no significant difference in the removal capacity of DTP among the four bacteria. In comparison with the single bacteria treatments, the reduction of DTP was sharply improved in all four plants-bacteria treatments after 2 days. The 14-day removal capacity of DTP by P-Rhi treatments was on average 2.40 times that of P treatments and 2.68 times that of Rhi treatment.

The 14-day removal rates of DTN and DTP were similar in Mic, Pse, Bac and Rhi treatments. As shown in Fig. 2, the combination of the four denitrifying bacteria and plants all showed far greater removal of DTN and DTP than the single plants treatments or the single bacteria treatments. The combination of *R. rosettiformans* and plants had the highest average removal rates of DTN. Statistical analysis showed that the DTN removal rate in P-Rhi treatments was significantly higher than that in P-Pse treatments or P-Bac treatments, but not significantly different from that in P-Mic treatments. Although the amount of nitrogen removed by plant uptake increased under the influence of aerobic denitrifying bacteria, the plant uptake as a percentage of total nitrogen removal did not differ significantly from single plants treatments and remained at 25.02%-36.69%. As with DTN, the combination of *R. rosettiformans* and plants had the highest average removal rate of DTP. According to

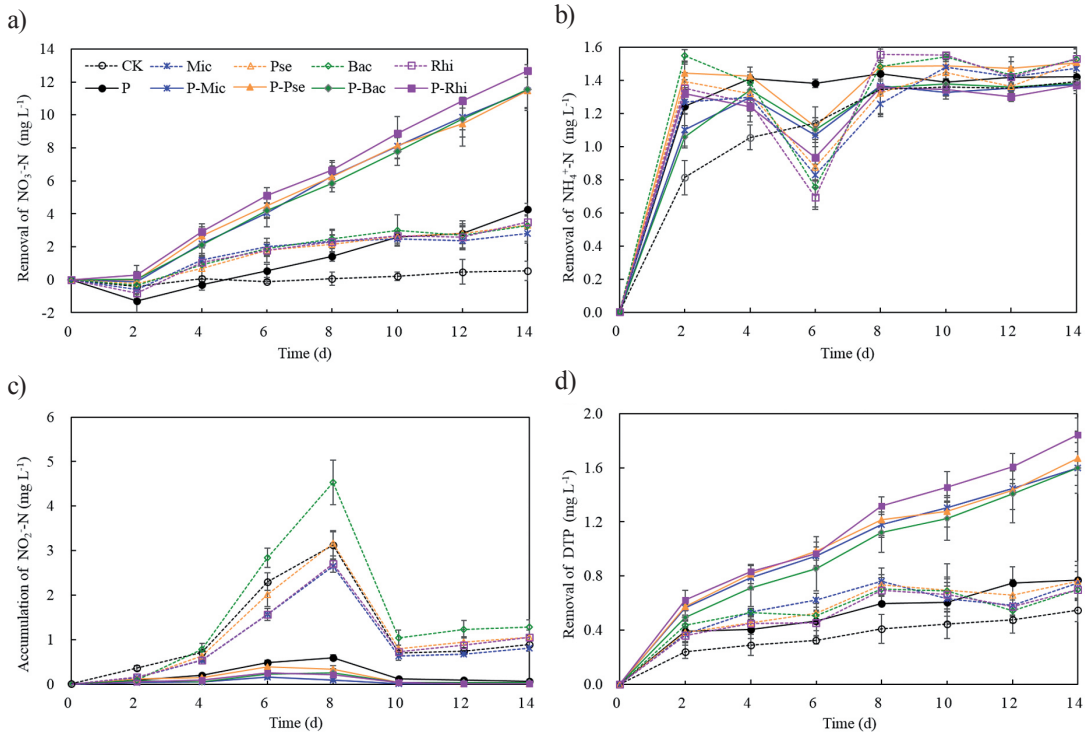

Fig. 1. Effects of plants and bacteria interactions on the removal of NO_3^--N a), NH_4^+-N b), NO_2^--N c) and DTP d) in water over time.

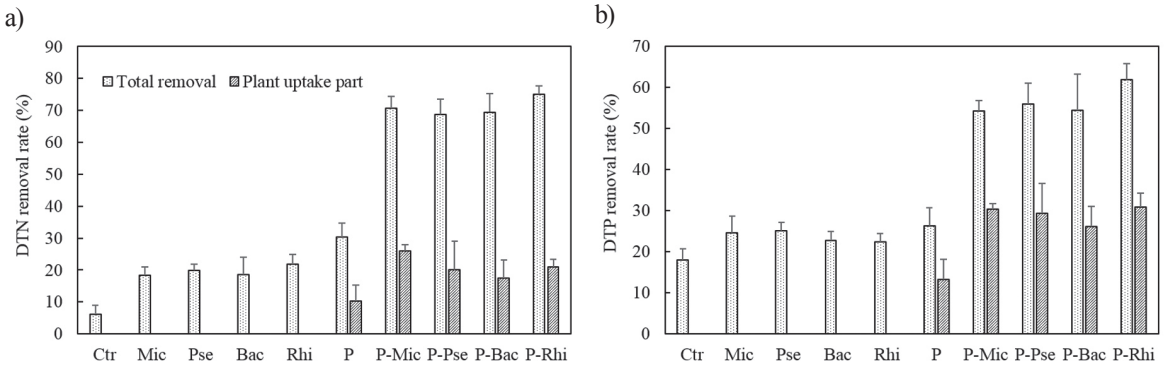

Fig. 2. Effects of plants and bacteria interactions on the removal rate of DTN a) and DTP b) after 14 days.

statistical analysis, the DTP removal rate in P-Rhi treatments was significantly higher than that in P-Mic treatments, but not significantly different from that in P-Pse treatments or P-Bac treatments. Although the amount of phosphorus removed by plant uptake increased in the presence of aerobic denitrifying bacteria, the plant uptake as a proportion of total phosphorus removal maintained constant at 47.94%-55.90% among treatments.

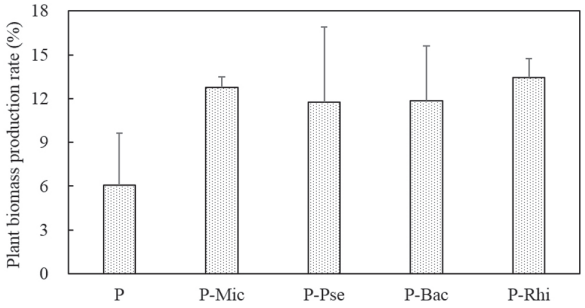

Fig. 3. The effect of denitrifying bacteria on the biomass production of *E. crassipes* after 14 days.

Effect of Bacteria on the Growth and Physiological Characteristics of Floating Plants

As shown in Fig. 3, the average biomass production rate of *E. crassipes* was greatly promoted by the use of these four bacteria inoculants. Statistical analysis showed that the biomass production rate of *E. crassipes* in P treatments was significantly lower than that in P-Mic treatments or P-Rhi treatments, but not significantly different from that in P-Pse treatments or P-Bac treatments. Correlation analysis revealed that the biomass production rate of *E. crassipes* showed a significantly positive correlation with the removal rate of DTN from water ($r = 0.743$, $p<0.05$) and the removal rate of DTP from water ($r = 0.631$, $p<0.05$).

The total-N content of *E. crassipes* was not affected by these bacteria (Fig. 4a). Compared to the single plants treatments, the total-N content of *E. crassipes* in all plants-bacteria treatments was not significantly different. Correlation analysis indicated that there was a significant and weak correlation between the total-N content of *E. crassipes* and the removal rate of DTN from water ($r = 0.218$, $p<0.05$). Different from total-N content, the total-P content of *E. crassipes* was affected by some bacteria (Fig. 4b). Statistical analysis showed that the total-P content of *E. crassipes* was significantly elevated by *M. oxydans*, *P. stutzeri* and *R. rosettiformans*, but not by *B. velezensis*. Significant positive correlation was found between the total-P content of *E. crassipes* and the removal rate of DTP from water ($r = 0.483$, $p<0.05$).

The root activity of *E. crassipes* increased significantly under the influence of *P. stutzeri* or *R. rosettiformans*, as illustrated in Fig. 5a). No apparent

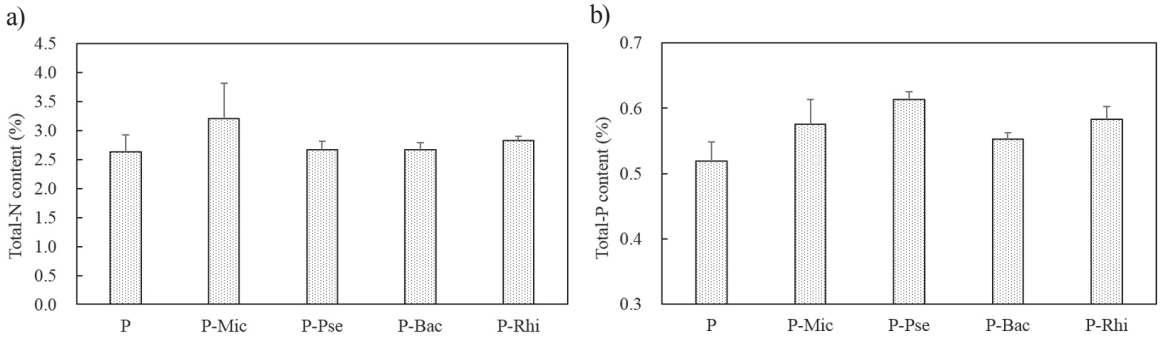

Fig. 4. The effect of denitrifying bacteria on total-N a) and total-P b) content of *E. crassipes* after 14 days.

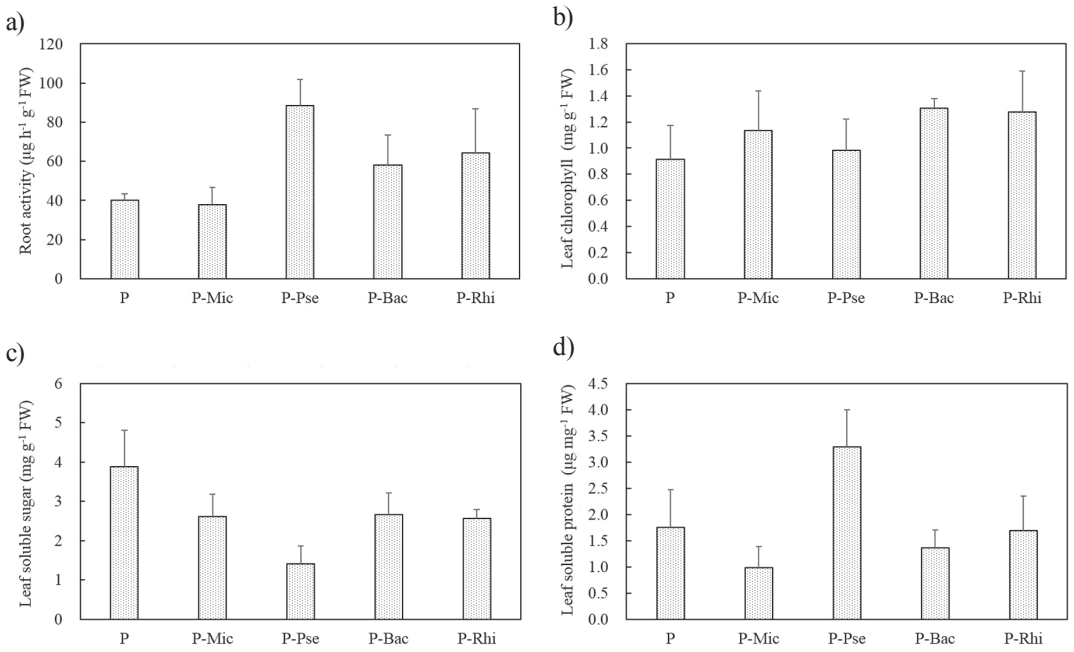

Fig. 5. The effect of denitrifying bacteria on root activity a), leaf chlorophyll content b), leaf soluble sugar concentration c) and leaf soluble protein concentration d) of *E. crassipes* after 14 days.

correlation was found between the root activity of *E. crassipes* and the removal rate of DTN from water, or between the root activity of *E. crassipes* and the removal rate of DTP from water. A significant positive correlation was discovered between the root activity of *E. crassipes* and the total-P content of *E. crassipes* ($r = 0.534$, $p<0.05$).

A significant increase in the leaf chlorophyll content was observed in both *E. crassipes* associated with *B. velezensis* and *R. rosettiformans* (Fig. 5b). Correlation analysis revealed that there existed a significantly but weakly positive correlation between the leaf chlorophyll content and the removal rate of DTN from water ($r = 0.295$, $p<0.05$), or between the leaf chlorophyll content and the removal rate of DTP from water ($r = 0.337$, $p<0.05$).

In contrast, the soluble sugar concentration in the leaves of *E. crassipes* decreased significantly only by the influence of *P. stutzeri*. But the average soluble sugar concentrations in the leaves of *E. crassipes* associated with all other three bacteria were more than 30% lower than that in the single plants treatments (Fig. 5c). Correlation analysis indicated that the leaf soluble sugar concentration of *E. crassipes* had significantly negative correlation with the removal rate of DTN from water ($r = -0.675$, $p<0.05$), the removal rate of DTP from water ($r = -0.688$, $p<0.05$), and the root activity of *E. crassipes* ($r = -0.703$, $p<0.05$).

As shown in Fig. 5d), a significant increase in the leaf soluble protein concentration was noticed in *E. crassipes* associated with *P. stutzeri*. No obvious correlation was found between the leaf soluble protein concentration of *E. crassipes* and the removal rate of DTN from water, or between the leaf soluble protein concentration of *E. crassipes* and the removal rate of DTP from water. But the leaf soluble protein concentration of *E. crassipes* had significantly positive correlation with the root activity of *E. crassipes* ($r = 0.603$, $p<0.05$), and the total-P content of *E. crassipes* ($r = 0.608$, $p<0.05$)

Discussion

The association of aerobic denitrifying bacteria and floating plants were effective in enhancing the simultaneous removal of nitrogen and phosphorus from eutrophic water with limited carbon sources. The limited carbon source, especially the low C/N ratio has proved to play a major role in the appearance of residual nitrites during bacterial denitrification and slow the denitrification process [26]. As the result of our experiment, the carbon source was not sufficient to ensure the elimination of nitrites in the single bacteria treatments during the denitrification process. When floating plants *E. crassipes* were present in the system, the residual nitrite gradually disappeared. *E. crassipes* was reported to secret dissolved organic carbon into water at 3.74-47.97 μg L^{-1} h^{-1} g^{-1} FW [27]. The molecular weight <1 kDa fraction of natural organic carbon matter was found to be suitable for bacterial growth and was further beneficial for aerobic denitrification [9], while the organic carbon released by plant roots was largely composed of low-molecular-weight compounds [28]. Therefore, carbon sources became a material basis for cooperation between aerobic denitrifying bacteria and floating plants.

The four aerobic denitrifying bacteria in this experiment respectively belonged to the class *Actinobacteria, Gammaproteobacteria, Alphaproteobacteria* and *Bacilli.* Our data indicated that they were all compatible with *E. crassipes* and promoted the absorption of nitrogen and phosphorus by plants. Plant growth promotion was the most common feature of bacteria that assisted in the phytoremediation of organic pollutants and heavy metals [20, 29]. Plant growth-promoting rhizobacteria have been verified to be able to induce the development of root hairs and increase absorption efficiency [30]. These four aerobic denitrifying bacteria in this experiment also promoted the growth of *E. crassipes* and increased the phosphorus content of plants at varying degrees. Just as the previous report, root activity was seldom significantly correlated with pollutant removal [31]. It was consistent with our result. No apparent correlation was found between the root activity of *E. crassipes* and the removal rate of DTN or DTP from water. This was probably due to the fact that root activity could only reflect the metabolic status of root development to a certain extent [32] and it had an indirect effect on pollutant elimination. However, a significant correlation was found between the leaf soluble sugar content of *E. crassipes* and the removal rate of DTN or DTP from water. This might be due to the complex and important role of soluble sugars in the whole plant. Soluble sugars not only serve as metabolic resources and structural constituents of cells, but also serve as signals that regulate plant growth and development processes [33]. Among the traits of plant growth-promoting rhizobacteria, the indole-3-acetic acid (IAA) synthesis was considered as one of the most important mechanisms for stimulating plant growth [34]. Interestingly, these four aerobic denitrifying bacteria were also detected to have the ability to produce IAA (data not shown). It turned out that these bacteria were multifunctional. But the comprehensive effects on the root activity, leaf chlorophyll content, leaf soluble sugar content and leaf soluble protein content of *E. crassipes* differed in these four aerobic denitrifying bacteria. It was possible due to their different IAA production capacity and the different carbon catabolite control. The synthesis of secondary metabolites, such as IAA, could be influenced by the carbon source-mediated regulation [35], while the organic carbon released by *E. crassipes* was complex.

Plants were highlighted to contribute to the TN and TP removal [12]. Plant overall uptake accounted for 25.9-72.0% of nitrogen removal and 24.1-91.5% of phosphorus removal [36-37]. Our data were consistent with the previous reports. Although the amount of nitrogen and phosphorus removed by plant uptake was greatly increased under the influence of aerobic denitrifying bacteria, it was interesting that the plant uptake contributed to the total nitrogen and phosphorus removal in a similar proportion with the single plant control. This indicated that while the aerobic denitrifying bacteria promoted the uptake of plants in the remediation, the plants were likely to bring a corresponding promotion in the activity of the bacteria for both nitrogen and phosphorus removal. Many aerobic denitrifiers like the genus *Pseudomonas* and *Bacillus* were found to drive the denitrification process by coupling phosphorus uptake [38-39]. From the view of the simultaneous improvement in the nitrogen and phosphorus removal in our experiment, *M. oxydans*, *R. rosettiformans*, *P.stutzeri* and *B. velezensis* were very likely to have the denitrifying phosphorous removal pathway. However, whether this was achieved only by increasing the number of denitrifying bacteria or also by increasing the denitrification efficiency coupling phosphorus uptake per unit of bacteria remained to be further studied.

During the remediation process of these four aerobic denitrifying bacteria, a certain amount of NH_4^+-N was found to release back into water in our experiments. NO_3^--N could reduce into NH_4^+-N by assimilatory nitrate reduction or dissimilatory nitrate reduction to ammonium (DNRA) [40]. But no NO_2^--N overflow appeared in the process of assimilatory nitrate reduction [41]. And usually, DNRA and denitrification pathways rarely coexisted in one microbe [42]. The release of NH_4^+-N back into water have been reported in the aging of *P. mosselii* [43] and *P. pseudoalcaligenes* [44]. During our experiment, the water turbidity was observed to obviously decrease on day 6 compared with the day 4. So it was speculated that the release of NH_4^+-N was related to the decline of bacteria. But during the dead phase of *P. mosselii* [43] and *P. pseudoalcaligenes* [44], the release of NH_4^+-N was continuous in the rest of experimental time, whereas the released NH_4^+-N was removed again as our experiment went on. It might be related to our cultural mode. Glucose was supplemented in all treatments every day to maintain the low-carbon environments and the root exudates were released continuously in the P, P-Mic, P-Bac, P-Pse, P-Rhi treatments. This was somewhat similar to a fed-batch cultivation. The fed-batch cultivation was useful to extend stationary phase of bacteria [45]. But we didn't assay the changes of bacteria densities during our experiment. This needed to be further investigated.

Based on the combined effects of these four aerobic denitrifying bacteria and *E. crassipes*, the combination of *R. rosettiformans* and *E. crassipes* has the best removal efficiency on nitrogen and phosphorus. This was a useful attempt to further enhance the simultaneous removal of nitrogen and phosphorus from eutrophic water by collaborating aerobic denitrifying bacteria and floating aquatic plants. Further work was required to screen the optimal combination of plants and bacteria suitable for applications at different temperatures and different eutrophic levels. Long-term management strategies for the bioaugmentation of plants and bacteria in the remediation of eutrophic water, including maintaining the effective abundance of added bacteria, also needed to be studied in detail.

Conclusions

The synergistic effects of four aerobic denitrifying bacteria and *E. crassipes* were demonstrated to effectively enhance the simultaneous removal of nitrogen and phosphorus from eutrophic water with limited carbon sources. The root organic exudates of *E. crassipes* helped aerobic denitrifying bacteria to accelerate the denitrification process and eliminate the accumulation of nitrites. From the insight into the plant growth stimulation and physiological attributes modulation, these aerobic denitrifying bacteria were multifunctional. The amount of nitrogen and phosphorus removed by plant uptake was greatly increased under the influence of aerobic denitrifying bacteria, due to the promotion of plant growth and phosphorus content in plants. The release of NH_4^+-N from aerobic denitrifying bacteria might be beneficial to improve the nitrogen uptake by *E. crassipes*. But the plant uptake as a percentage of total nitrogen removal or total phosphorus removal was not affected by the aerobic denitrifying bacteria. *E. crassipes* was likely to bring a corresponding promotion in the activity of the bacteria for both nitrogen and phosphorus removal. Among the aerobic denitrifying bacteria we used, the cooperation between *R. rosettiformans* and *E. crassipes* showed the best performance both in nitrogen removal and phosphorus removal. This research provided an effective strategy for enhancing the remediation of eutrophic water with low C/N ratio.

Acknowledgments

This work was supported by the Jiangsu Agriculture Science and Technology Innovation Fund [Grant No. CX(20)2027] and the National Key R&D Program of China [Grant No. 2021YFD1700805-02].

Conflict of Interest

The authors declare no conflict of interest.

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