Environ Sci Pollut Res (2014) 21:9617–9625
DOI 10.1007/s11356-014-2987-4

RESEARCH ARTICLE

Enhancement of nitrogen and phosphorus removal from eutrophic water by economic plant annual ryegrass (*Lolium multiflorum*) with ion implantation

Miao Li · Guo-ping Sheng · Yue-jin Wu · Zeng-liang Yu · Gary S. Bañuelos · Han-qing Yu

Received: 18 July 2013 / Accepted: 30 April 2014 / Published online: 10 May 2014

Abstract Severe eutrophication of surface water has been a major problem of increasing environmental concern worldwide. In the present study, economic plant annual ryegrass (*Lolium multiflorum*) was grown in floating mats as an economic plant-based treatment system to evaluate its potential after ion implantation for removing nutrients in simulated eutrophic water. The specific weight growth rate of *L. multiflorum* with ion implantation was significantly greater than that of the control, and the peroxidase, nitrate reductase, and acid phosphatase activities of the irradiated *L. multiflorum* were found to be greater than those plants without ion implantation. Higher total nitrogen (TN) and total phosphorus (TP) removal efficiencies were obtained for the *L. multiflorum* irradiated with 25 keV 5.2×10^{16} N^+ ions/cm^2 and 30 keV 4.16×10^{16} N^+ ions/cm^2, respectively ($p<0.05$). Furthermore, the nitrogen and phosphorus contents in the plant biomass with ion implantation were also greater than those in the control and were positively correlated with TN and TP supplied. *L. multiflorum* itself was directly responsible for 39–49 and 47–58 % of the overall N and P removal in the experiment, respectively. The research results suggested that ion implantation could become a promising approach for increasing phytoremediation efficiency of nutrients from eutrophic water by *L. multiflorum*.

Keywords Eutrophication · Phytoremediation · Ion implantation · Economic plants · Ryegrass · *Lolium multiflorum*

Responsible editor: Philippe Garrigues

M. Li
Key Laboratory of Agri-Food Safety of Anhui Province, School of Resources and Environment and Plant Protection, Anhui Agricultural University, Hefei 230036, China

M. Li · G.-p. Sheng · H.-q. Yu (✉)
The Environmental Engineering Laboratory, School of Chemistry, University of Science and Technology of China, Hefei 230026, China
e-mail: hqyu@ustc.edu.cn

M. Li (✉) · Y.-j. Wu · Z.-l. Yu
Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences, Hefei 230031, China
e-mail: miaoli@ustc.edu.cn

G. S. Bañuelos
Agricultural Research Service, United States Department of Agriculture, Parlier, CA 93648, USA

Introduction

Severe eutrophication of water bodies in the aquatic ecosystem has resulted in significant impairment of surface waters and groundwater quality, due to economical constraints in reducing point sources and to a high proportion of non-point sources of nutrients (Smith 2003). One ecological method used for the remediation of eutrophic water containing high nutrients of nitrogen and phosphorus is bioremoval and phytoremediation processes (Li et al. 2009; Lu et al. 2010). Phytoremediation technologies are increasingly employed for purification of wastewater because of positive greenhouse results and for being relatively low cost and energy efficient (Jayaweera and Kasturiarachchi 2004; Pilon-Smits, E. 2005; Huang et al. 2012). The high productivity and nutrient removal capability of economic plants have created substantial interest in their use for phytoremediation of wastewater and resource recovery (DeBusk et al. 1995; Abe and Ozaki 1998). The application of economic plants for wastewater phytotreatment has mainly involved constructed wetlands (CWs), hydroponic nutrient film technique (NFT) system, and floating mat economic plant-based treatment systems (FMETSs) (Vaillant et al. 2003; Sooknah and Wilkie 2004; Konnerup et al. 2009).

FMETSs, as a new developing ecological technique used for phytoremediation of wastewater, have promising potential for removing and recovering nutrients from wastewater. Hence, there is increasing use of economic plants in the biosystems of the FMETS to phytoremediation of wastewater (DeBusk et al. 1995; Sooknah and Wilkie 2004). In addition to the advantages cited by Abe and Ozaki (1998) for purification of wastewater, the FMETS systems have the following positive attributes: (1) non-invasive nuisance; (2) abundant in economic plant species and cultivars; (3) high economical value of economic plants relative to some aquatic macrophytes; and (4) fast growing, large biomass, and high productivity of many economic plants. Additionally, harvested biomass of economic plants can potentially be used for composting, soil amendments, anaerobic digestion with methane, and volatile fatty acid (VFA) production and be processed for animal feed. Furthermore, harvested economic plant biomass can be mixed with separated manure solids to increase the amount of nutrients available for exporting off wastewater (DeBusk et al. 1995; Sooknah and Wilkie 2004).

Among the economic plants, annual ryegrass (*Lolium multiflorum*), also known as Italian ryegrass, as a closely related and interfertile species with perennial ryegrass (*Lolium perenne*), is an important economic grass weed of winter crops. Both are grown all over the world as key for most widely used cool-season annual forage grasses. They are among the most palatable and highly digestible grasses for livestock. This plant species exhibits luxuriant growth and produced large amounts of aboveground biomass and has the potential to be used as a source of pasturage and biomaterials as well as bioenergy (Kim et al. 2003). Moreover, it may be useful in phytotreating wastewater and soil pollution because it grows quickly. The species does not die back in winter, has little weed potential and grows in adverse situations, and has been researched at laboratory to evaluate for nutrients or toxic heavy metals and organic contaminant removal from water and contaminated soil (Abe and Ozaki 1998; Merini et al. 2009; Xian et al. 2010).

However, the nutrient removal efficiency and adversity resistance of economic plants in the FMETS system are limited by genetic and environmental factors. Thus, it is essential to improve the nutrient removal efficiency and resistance ability under unfavorable environments exposed to the economic plants in the FMETS system with various approaches, e.g., plant breeding. Previous research has also found that low-energy ion implantation on plants has significant mutation or irritation effects (Yu 2006). Different energy and doses of ion implantation on plant can cause obvious stimulation effects, including hereditary and physiological effects, and induce damage and inhibit physiological effects (Feng et al. 2006). Furthermore, charge deposition by ion implantation can also irritate plant growth and development (Yamaguchi et al. 2003; Phanchaisri et al. 2012). Thus, a low-energy ion implantation, as an efficient physical mutagen in crop and microbe breeding method, could be such a promising new approach to improve the nutrient removal efficiencies of economic plants in the FMETS system.

Therefore, the aim of the present study was to explore N^+ ion implantation for improving the growth and nutrient removal of N and P efficiency by economic plant annual ryegrass (*L. multiflorum*) in eutrophic water. In this work, the suitability of utilizing ion implantation in combination with the FMETS system for improving water quality was examined, and the activities of peroxidase (POD), nitrate reductase (NR), and acid phosphatase (ACP) enzymes of *L. multiflorum* with and without ion implantation were measured.

Materials and methods

Plant materials

The experiments were performed with annual ryegrass (*L. multiflorum*) obtained from the Institute of Horticulture Sciences of Anhui Academy of Agricultural Sciences, Hefei, China. One cultivar of "gulf" was selected as test plants. The size, weight, and coat thickness of seeds were about 5–6× 1 mm, 2 g per 1,000 seeds, and 0.6 mm, respectively. Seeds of *L. multiflorum* without ion implantation were used as the control, while other seeds were treated with N^+ ion implantation at the Key Laboratory of Ion Beam Bioengineering, Chinese Academy of Sciences, China, and then sown into soil. The seedlings were grown in soil to a height of 12–16 cm prior to the experiments.

Low-energy heavy ion implantation

The procedure of ion implantation in *L. multiflorum* is shown in Fig. 1. N^+ ion beams at low energies of 20, 25, and 30 keV and current of 20 mA were chosen for ion implantation on *L. multiflorum* (Yu 2006). For each ion implantation, the seeds were fixed onto an irradiation dish (diameter of 90 mm) arrayed with the hila upward being exposed to the beam incidence direction. The pulse treatment technique was used (Li et al. 2007; Yu 2006). The pulse time was 10 s with an interval of 50 s. With each pulse, the applied dose D_0 was 2.6×10^{13} ions/cm^2. The dosages applied were counted as accumulating data (i.e., 0, 1,000, 1,200, 1,600, 2,000, 2,500, and 3,000 units) multiplied by D_0. Pulsed beam modes were adopted using periodical beam sweeping across the exposure holes of the sample holder, with each pulse bombarding the target to a dose of 0, 2.6, 3.12, 4.16, 5.2, 6.5, and 7.8×10^{16} N^+ ions/cm^2. The operating pressure in the target chamber was fixed at approximately 10^{-3} Pa, while the operating temperature was kept at 0 °C or lower. Three replicates were used for

individual species in each experiment, while the experiment for the cultivar was repeated at least three times.

Floating mat economic plant-based treatment systems

The floating mat economic plant-based treatment systems (FMETS) were set up as follows: Each planted floating mat was made of polyethylene foam and had a size of 33×32×3 cm (in width×length×height). On the bottom of the floating mats, there were three rows of holes with a diameter of 1 cm, through which the roots of plants could elongate into water. The holes were filled with sponge to provide support to the plants.

The floating mats were then placed in rectangular plastic containers (46×33×24 cm in length×width×height, respectively) filled with simulated eutrophic water (described later) and planted with young seedlings grown from the seeds with and without ion implantation. After measuring the initial fresh weights, nine plants were transplanted evenly in the corresponding holes of each floating mat. In order to avoid the medium dilution by rainfall and reduce evaporative losses by strong solar radiation, all containers were covered by shade cloth. A constant water level in the containers was maintained during the experimental period by irrigating weekly with distilled water to compensate for evaporation and evapotranspiration loss. The dilution effect of adding distilled water on the nitrogen and phosphorus concentration was recorded and used to calculate the actual removal by the plants.

Simulated eutrophic water

The simulated eutrophic water was made by dilution of a liquid fertilizer Aquasol (Fengle Agricultural Chemical Co., Hefei, China). Aquasol contains nine macronutrients, essential for plant growth, and other micronutrients, e.g., B, Mo, Mn, and Zn. Desired amounts of modified Aquasol were dissolved in distilled water and diluted to the desired total nitrogen (TN), NO_3-N, NH_4-N, and total phosphorus (TP), PO_4-P concentrations. The initial TN and TP concentrations of 4.60 and 0.55 mg/l were used for experiments according to the evaluation standards for Chaohu Lake eutrophication (Tu et al. 1990).

Growth of annual ryegrass

The FMETS was used to investigate the potential for the *L. multiflorum* to remove nitrogen and phosphorus from eutrophic water. The *L. multiflorum* plants exposed to ion implantation and the unirradiated controls were maintained in the standard Hoagland mineral medium for acclimation prior to the experiments. Six days later, the young plants were transferred to the growth chamber in the FMETS system. There were nine plants in each floating mat, six plant treatments with different ion implantation dosages, and two controls. There were three replicates for each treatment and control. Plants without ion implantation were used as the unirradiated controls. Non-plant control experiment sets contained only simulated eutrophic water without any plant.

The experiments were performed outdoors with a natural photoperiod and temperature of 8–19 °C from February to March in Hefei, China. After 28 days of growth, the experiments were terminated.

Sampling and analysis

The initial fresh plant weights were measured at onset of experiment. Water samples were collected every 2 days from each container during the course of the experiments. At the end of the experiments, plants were carefully removed from the floating mats and gently washed with tap water. They were blotted with absorbing paper, and the fresh weights were measured. After drying at 80 °C for 48 h, the dry weights and water contents were recorded. Plant analysis was performed at initial and final stages of the experiment. The concentrations of TN and TP in the culture solutions were measured according to standard methods (APHA 1998). The POD activity was determined according to the method of Scalet et al. (1995), whereas the NR and ACP activities were measured based on the method of Rai et al. (1998) and Shih and Kao (1998), respectively. The N content of plant materials was determined by the semimicro Kjeldahl method (Jensen 1991), and the P content was measured following the method of Christensen and Wigand (1998).

Statistical analysis

The removal of TN and TP in the FMETS system can be described by the first-order kinetic model (Sooknah and Wilkie 2004). The first-order reaction rate constants were calculated from linear regressions using the following integrated rate equation:

$$\ln(C_0/C_t) = kt \quad (1)$$

where C_0 (mg/l) is the initial concentration of a parameter in plant culture, C_t (mg/l) is the concentration at time t (days), and k is the first-order reaction rate constant (per day).

The data are expressed as the means of at least three replications ± standard errors. One-way ANOVA and Tukey's comparison of means were performed with Minitab software (Minitab Inc., PA, USA) to determine the significant differences. The significance level was set at $p<0.05$.

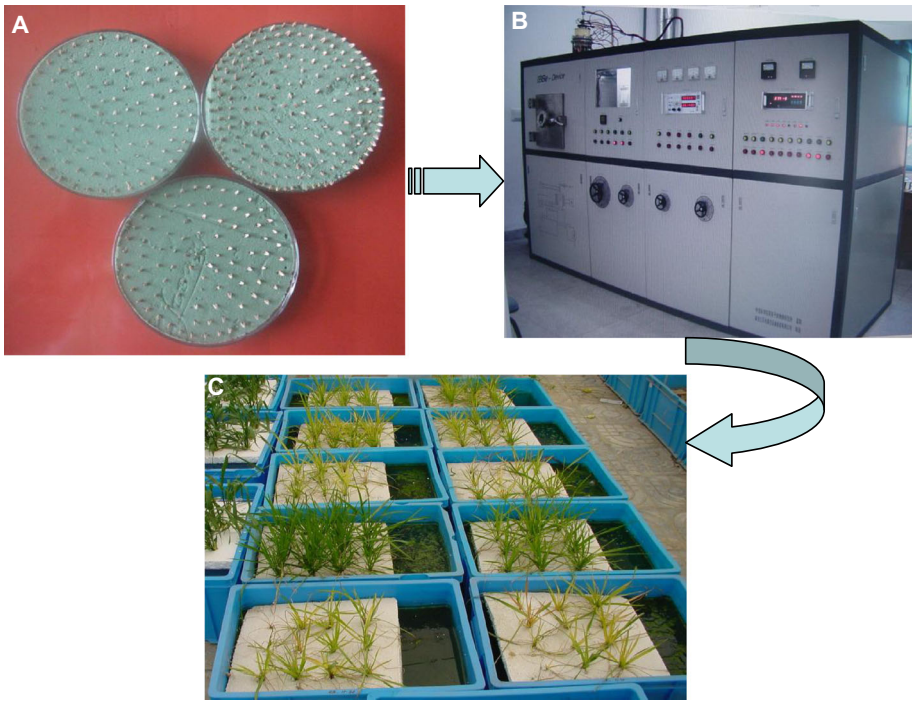

Fig. 1 The procedure of ion implantation in *L. multiflorum*. **a** Irradiation dish, **b** IBBe device for ion implantation, **c** floating mats of *L. multiflorum* plant-based treatment systems

Results and discussion

Biological effect of *L. multiflorum* with ion implantation

The growth performance of *L. multiflorum* with and without ion implantation is shown in Table 1 and Fig. 2. *L. multiflorum* exhibited different responses to the varied energies and doses of N^+ ion implantation. The germination percentage was 86 % for the control, but it was 90 and 92 % for the seeds exposed to 20 keV 4.16 and 5.2×10^{16} N^+ ions/cm^2, respectively. The irradiation at 25 keV 5.2×10^{16} N^+ ions/cm^2 and 30 keV 4.16×10^{16} N^+ ions/cm^2 had a more significant stimulation effect on the germination percentage than that at the control ($p<0.05$). Furthermore, the germination curves of the three doses showed the similar tendency. This indicates that irradiation at a low dose had no significant effect, and that irradiation at a moderate dose there was a stimulation effect on the germination of *L. multiflorum*, while a high dose had inhibition effect.

The survival percentages of *L. multiflorum* irradiated with different energies and doses showed the same patterns as the germination percentages (Fig. 2). The N^+ ion implantation affected significantly the growth and development of *L. multiflorum*. Moreover, after ion implantation, the *L. multiflorum* showed a significant "stimulation effect" for enhancing plant growth and resulting in an increase of biomass, plant root length, and leaf area (Table 1). Thus, *L. multiflorum* treated with 20 keV 5.2×10^{16}, 25 keV 5.2×10^{16}, and 30 keV 4.16×10^{16} N^+ ions/cm^2 was selected and cultivated. They were later grown on floating rafts to remove N and P from the eutrophic water. Plants exposed to other dosages of ion beam were excluded from further investigation in the work because they showed negligible stimulation growth or slight inhibitory effects on growth.

Growth characteristics of *L. multiflorum* cultured in water

The growth characteristics of *L. multiflorum* cultured in water are shown in Table 2. After 28-day exposure to hydroponic culture, the plant height, leaf length, leaf width, tillering number, fibrous root length, root range, and fresh weight of *L. multiflorum* with ion implantation increased greatly (Table 2). The best growth performance occurred at 25 keV 5.2×10^{16} N^+ ions/cm^2, followed by 30 keV 4.16×10^{16}, and 20 keV 5.2×10^{16} N^+ ions/cm^2, respectively. Compared to control, plants exposed to the three dose groups had greater root range, fibrous root length, plant height, tillering number, and fresh weight (shown in Table 2). However, the growth characteristics of *L. multiflorum* did not differ significantly among different dose groups (Table 2). Under hydroponic culture condition, the differences in the growth characteristics between the ion beam treatments and controls were significant for the cultivar. These results demonstrated that proper energy and dose ion beam treatment enhanced and maintained the growth potential of *L. multiflorum* under hydroponic culture condition.

N and P removal

The TN removal profiles from the simulated eutrophic water by the tested cultivar are shown Fig. 3. Compared to control, the three dose treatments had the higher TN removal

Table 1 Effect of N^+ ion implantation applied at different energies and dosages on the growth characteristics of *L. multiflorum* before transplanted into the water (means ± standard error, n=3)

Growth characteristics	N^+ ion implantation dosages[a]			
	Control	20 keV	25 keV	30 keV
Plant height (cm)	7.8±0.4	8.9±0.8	9.6±0.6	9.0±0.3
Fresh weight (g)	0.18±0.05	0.24±0.06	0.28±0.04	0.25±0.05
Leaf length (cm)	8.6±0.14	9.8±0.15	10.5±0.18	10.1±0.17
Leaf width (cm)	0.46±0.006	0.52±0.017	0.58±0.019	0.54±0.013
Tillering number	4.6±1.2	5.8±1.4	6.9±1.5	6.1±1.3
Fibrous root length (cm)	9.3±0.56	10.4±0.62	11.9±0.58	10.8±0.64
Root range (cm)	1.4±0.12	2.3±0.15	2.8±0.14	2.5±0.17
Fibrous root number	9.4±0.50	11.6±0.80	12.7±0.90	11.9±0.60

[a] 20 keV=20 keV 5.2×10^{16} N^+ ions/cm^2, 25 keV=25 keV 5.2×10^{16} N^+ ions/cm^2, 30 keV=30 keV 4.16×10^{16} N^+ ions/cm^2, control = non-ion-irradiated treatment

efficiency. Furthermore, the TN removal efficiency was found to be in the order of 25>30>20 keV among the three dose treatments (Fig. 3). For the removal efficiencies for TP, the cultivar had the same order (Fig. 3). In addition, the removal efficiencies of the *L. multiflorum* treated with ion beam were greater than those of the controls. Statistical analysis revealed significant variation (p<0.05) between the ion beam treatments and controls. There were small differences in the TN and TP removals by the *L. multiflorum* treated with various ion beam dosages. All treatments showed similar changing patterns in the concentrations of nitrogen and phosphorus ions.

Biomass production and enzymatic activities

A net increase in the biomass of *L. multiflorum* treated with 30 keV 4.16×10^{16} and 20 and 25 keV 5.2×10^{16} N^+ ions/cm^2

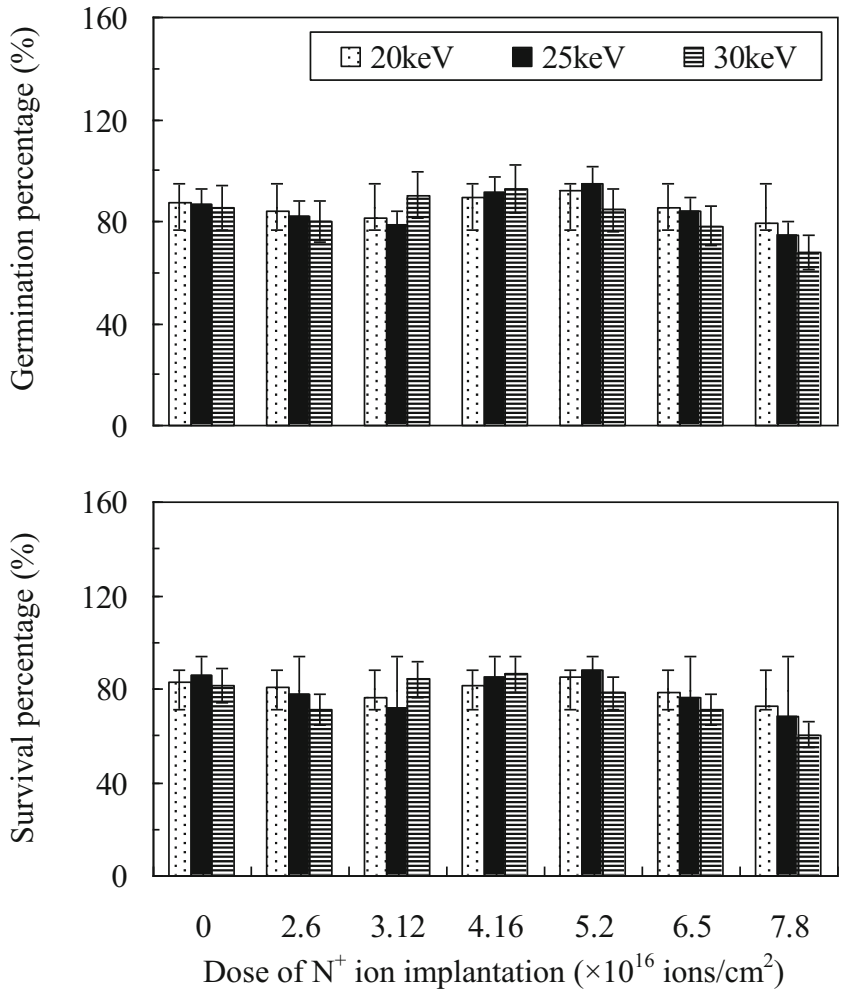

Fig. 2 Germination percentage and survival percentage of *L. multiflorum* seeds after N^+ ion implantation. Data were averaged from three independent experiments with 300 seeds

Table 2 Effect of N^+ ion implantation applied at different energies and dosages on the growth characteristics of *L. multiflorum* after 28-day batch growth in the water (means ± standard error, n=3)

Growth characteristics	N^+ ion implantation dosages[a]			
	Control	20 keV	25 keV	30 keV
Plant height (cm)	18.4±1.0	19.5±1.2	21.7±1.4	20.9±1.3
Fresh weight (g)	1.44±0.01	1.79±0.03	1.95±0.02	1.87±0.01
Leaf length (cm)	8.9±0.7	9.1±0.6	9.2±0.8	9.1±0.7
Leaf width (cm)	0.51±0.02	0.54±0.04	0.59±0.06	0.56±0.03
Tillering number	2±0.6	3±0.4	4±0.5	3±0.5
Fibrous root length (cm)	17.5±1.6	18.7±1.8	19.6±2.1	19.2±1.5
Root range (cm)	2.3±0.2	2.7±0.5	3.1±0.7	2.9±0.4
Fibrous root number	16±1.8	19±1.5	22±1.9	20±1.3

[a] Repeat the same as in Table 1

is shown in Table 2. *L. multiflorum* with ion implantation showed an increase in plant biomass, and the yield was always higher than that of the controls and greatest when *L. multiflorum* was treated with 25 keV 5.2×10^{16} N^+ ions/cm^2.

In the experiment, the POD activity of *L. multiflorum* treated with 25 keV 5.2×10^{16} was greater than that treated with 20 keV 5.2×10^{16} and 30 keV 4.16×10^{16} N^+ ions/cm^2, respectively, while the POD activities of the samples treated with 25- and 30-keV ion implantation were

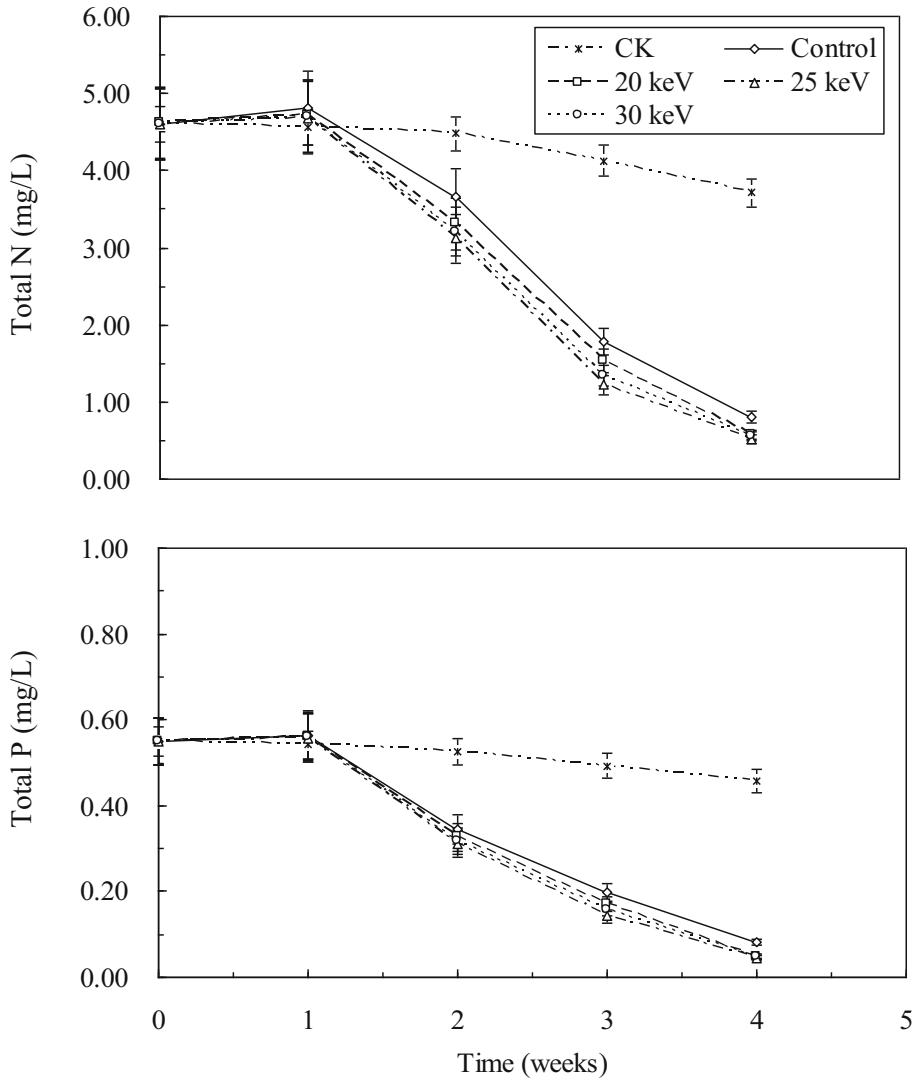

Fig. 3 The TN and TP concentration profiles in the water. *CK*=non-plant control

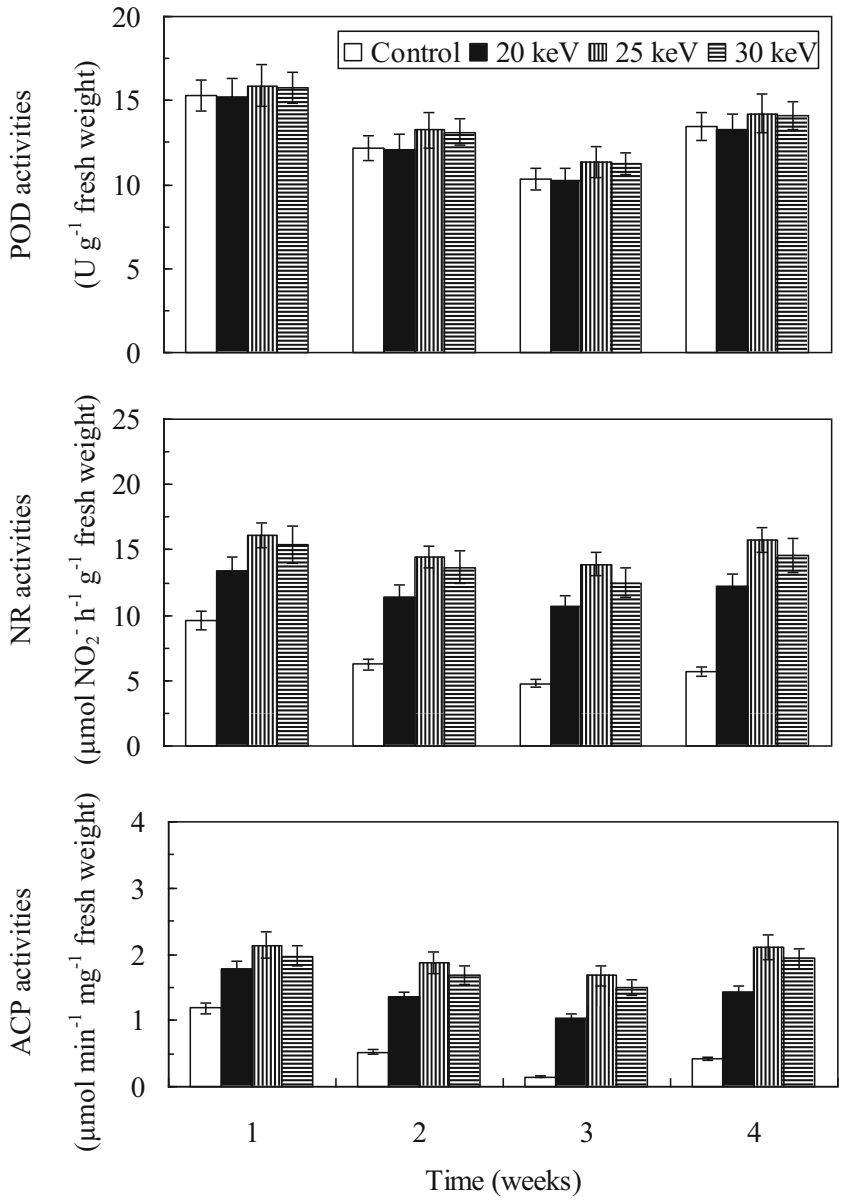

Fig. 4 The peroxidase (POD) activities (U/g fresh weight), the nitrate reductase (NR) activities (μmol NO_2^-/h/g fresh weight), and the acid phosphatase (ACP) activities (μmol/min/mg fresh weight) of *L. multiflorum* with N^+ ion implantation grown in the water (means ± standard error, n=3)

greater than those of the controls (Fig. 4). The NR and ACP activities of all the samples treated with ion implantation were greater than those of the controls (p<0.05) (Fig. 4). Furthermore, the POD activity of the plants decreased significantly in the third week, and a higher POD activity was found in the next week. A similar phenomenon was observed for the NR and ACP activities of *L. multiflorum* (Fig. 4).

Table 3 Nutrient content present (% dry weight ± standard error, n=3) in the tissue of *L. multiflorum* with ion implantation grown in the water for 28 days

Nutrient content of plant tissue (%)	N^+ ion implantation dosages[a]			
	Control	20 keV	25 keV	30 keV
Initial N	1.12±0.04	1.16±0.05	1.19±0.06	1.17±0.08
Final N	1.28±0.07	1.41±0.09	1.46±0.12	1.43±0.11
Initial P	0.15±0.03	0.19±0.05	0.21±0.06	0.19±0.04
Final P	0.21±0.02	0.33±0.03	0.39±0.07	0.35±0.04

[a] Repeat the same as in Table 1

Table 4 Nutrient storage (% N/m^2 and % P/m^2) in plant biomass of *L. multiflorum* with ion implantation grown in the water after 28 days

Nutrient storage in plant biomass[a] (%/m^2)	N^+ ion implantation dosages[b]			
	Control	20 keV	25 keV	30 keV
N	1.05	1.65	1.78	1.71
P	0.40	0.92	1.19	1.05

[a] Nutrient storage was calculated using mean nitrogen and phosphorus content of whole plant for each planted treatment

[b] Repeat the same as in Table 1

N and P bioaccumulation and nutrient storage in plant biomass

The N and P contents in the biomass of ion-tested *L. multiflorum* were significantly greater than those of the unirradiated controls, in the following order: 25>30>20 keV (Table 3). Under the ion beam treatment conditions, the order of N and P bioaccumulation rates was 25>30>20 keV. Similarly, the nutrient (N and P) storage in plant biomass of ion-tested *L. multiflorum* as compared to inflow was significantly higher than that of the unirradiated controls, and the order of nutrient storage in plant biomass rates was 25>30>20 keV (Table 4). Correspondingly, the N and P removal rates were also increased.

Comparison for reduction rate constants

The TN and TP followed a first-order decay model, and all the rate constants for the regression fits were significant (Table 5). Differences between the first-order rate constants for the non-plant control (CK) and the plant in the water culture treatments were all highly significant ($p<0.05$). The rate constants for the ion beam treatments were significantly greater than the corresponding rate constants for the irradiated control ($p<0.05$) (Table 5).

In contrast, the plant culture treatments exhibited significantly ($p<0.05$) greater first-order rate constants than the non-plant control (CK) for all parameters (Table 5). The treatment of 25 keV also exhibited the highest reduction rates for all parameters in the experiment compared to other ion beam treatments, in the following order: 25>30>20 keV (Table 5).

Table 5 First-order rate constants for removal of nitrogen and phosphorus in the water during 28-day batch growth of *L. multiflorum* with and without ion implantation

Treatments[a]	k_{TN} (per day)	k_{TP} (per day)
CK	0.008±0.001	0.007±0.003
Control	0.062±0.002	0.068±0.001
20 keV	0.075±0.003	0.086±0.001
25 keV	0.078±0.003	0.089±0.002
30 keV	0.076±0.002	0.087±0.002

[b] Repeat the same as in Table 1

Evaluation to the potential of economic plants with ion implantation for phytoremediation

The capacity of economic plants to remove N and P from water is usually limited by genetic or environmental factors. Low-energy ion implantation might be an ideal approach to enhance such a capacity because of its significant mutation or irritation effects (Yamaguchi et al. 2003; Feng et al. 2006). Dose of N^+ ion beam implantation into plants can cause considerable contemporary hereditary and physiological effects, and it also affects the growth and development of plants (Yu 2006; Phanchaisri et al. 2012). As the first approach, this study examined the N and P removal of *L. multiflorum* with N^+ ion implantation and demonstrated the feasibility of applying ion beam biotechnology to phytoremediation and bioremediation.

The experimental results also show that *L. multiflorum* with ion implantation was very effective in combination with the FMETS system for removing N and P from water. First, the productivities of *L. multiflorum* with N^+ ion implantation were higher than those of the controls (Table 2). Second, the N and P contents in the tissue of *L. multiflorum* treated with ion beam were also greater than those of the controls (Table 3). Third, the N and P nutrient storage in plant biomass of *L. multiflorum* treated with ion beam was also greater than that of the controls (Table 4). As a result, the floating mats planted to *L. multiflorum* with ion implantation showed a promising N and P removal potential under the tested conditions. Finally, the *L. multiflorum* treated with N^+ ion implantation performed a greater N and P bioaccumulation rate.

However, several questions still remain to be solved before this process is applied in practice: (1) the capacity of nutrient removal by *L. multiflorum* with ion implantation from real eutrophic lake water, (2) The genetic stability in the improvement of growth and nutrient metabolism abilities of *L. multiflorum* with ion implantation should be explored, and (3) The possibility of rapid spread of *L. multiflorum* with ion implantation. This possibility is of serious concern because of the ecological risk and economical consequences of such modification of *L. multiflorum*. This study raises such questions that warrant further research.

Conclusions

In conclusion, the N and P removal capacity of the *L. multiflorum* with N^+ ion implantation was examined, and the feasibility of applying ion implantation to phytoremediation was demonstrated in the study. *L. multiflorum* with N^+ ion implantation in combination with

the FMETS system was found to be effective for rapid removal of N and P from simulated eutrophic water. The productivities of *L. multiflorum* treated with N^+ ion implantation were greater than those of the control, while the N and P contents in these plants were also greater than those in the control. The floating mats planted with *L. multiflorum* with N^+ ion implantation showed a promising N and P removal potential from eutrophic water.

Acknowledgments The authors wish to thank Drs. Lixiang Yu and Hang Yuan (Institute of Plasma Physics, Chinese Academy of Sciences) for conducting their ion implantation. We also thank Dr. Zhiqing Lin who comes from Southern Illinois University Edwardsville for his useful comments and suggestions on the language and structure of our manuscript. The work was partially supported by the National Natural Science Foundation of China (50949038), China Postdoctoral Science Foundation (20060400722), National Hi-technology Development 863 Program of China (2006AA06Z340).

References

Abe K, Ozaki Y (1998) Comparison of useful terrestrial and aquatic plant species for removal of nitrogen and phosphorus from domestic wastewater. Soil Sci Plant Nutr 44(4):599–607

APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC

Christensen KK, Wigand C (1998) Formation of root plaques and their influence on tissue phosphorus content in *Lobelia dortmanna*. Aquat Bot 61(2):111–122

DeBusk TA, Peterson JE, Ramesh Reddy K (1995) Use of aquatic and terrestrial plants for removing phosphorus from dairy wastewaters. Ecol Eng 5(2–3):371–390

Feng HY, Yu ZL, Chu PK (2006) Ion implantation of organisms. Mater Sci Eng R 54(3–4):49–120

Huang JC, Passeport E, Terry N (2012) Development of a constructed wetland water treatment system for selenium removal: use of mesocosms to evaluate design parameters. Environ Sci Technol 46(21):12021–12029

Jayaweera MW, Kasturiarachchi JC (2004) Removal of nitrogen and phosphorus from industrial wastewaters by phytoremediation using water hyacinth (*Eichhornia crassipes* (Mart.) Solms). Water Sci Technol 50(6):217–225

Jensen ES (1991) Evaluation of automated analysis of ^{15}N and total N in plant material and soil. Plant Soil 133(1):83–92

Kim TH, Jung WJ, Lee BR, Yoneyama T, Kim HY, Kim KY (2003) P effects on N uptake and remobilization during regrowth of Italian ryegrass (*Lolium multiflorum*). Environ Exp Bot 50(3): 233–242

Konnerup D, Koottatep T, Brix H (2009) Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with Canna and Heliconia. Ecol Eng 35(2):248–257

Li M, Wu YJ, Yu ZL, Sheng GP, Yu HQ (2007) Nitrogen removal from eutrophic water by floating-bed-grown water spinach (*Ipomoea aquatica* Forsk.) with ion implantation. Water Res 41(14):3152–3158

Li M, Wu YJ, Yu ZL, Sheng GP, Yu HQ (2009) Enhanced nitrogen and phosphorus removal from eutrophic lake water by *Ipomoea aquatica* with low-energy ion implantation. Water Res 43(5): 1247–1256

Lu Q, He ZL, Graetz DA, Stoffella PJ, Yang XE (2010) Phytoremediation to remove nutrients and improve eutrophic stormwaters using water lettuce (*Pistia stratiotes* L.). Environ Sci Pollut R 17(1):84–96

Merini LJ, Bobillo C, Cuadrado V, Corach D, Giulietti AM (2009) Phytoremediation potential of the novel atrazine tolerant *Lolium multiflorum* and studies on the mechanisms involved. Environ Pollut 157(11):3059–3063

Phanchaisri B, Samsang N, Yu LD, Singkarat S, Anuntalabhochai S (2012) Expression of OsSPY and 14-3-3 genes involved in plant height variations of ion-beam-induced KDML 105 rice mutants. Mutat Res 734(1–2):56–61

Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39

Rai UN, Gupta M, Tripathi RD, Chandra P (1998) Cadmium regulated nitrate reductase activity in *Hydrilla verticillata* (L.f.) Royle. Water Air Soil Pollut 106(1–2):171–177

Scalet M, Federico R, Guido MC, Manes F (1995) Peroxidase activity and polyamine changes in response to ozone and simulated acid rain in Aleppo pine needles. Environ Exp Bot 35(3):417–425

Shih CY, Kao CH (1998) Methyl jasmonate induces acid phosphatase activity in rice leaves. J Plant Physiol 152(4–5):358–362

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ Sci Pollut Res Int 10(2):126–139

Sooknah RD, Wilkie AC (2004) Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecol Eng 22(1):27–42

Tu QY, Gu DX, Yin CQ (1990) Study on Chaohu lake eutrophication. University of Science and Technology of China Press, Hefei, China

Vaillant N, Monnet F, Sallanon H, Coudret A, Hitmi A (2003) Treatment of domestic wastewater by an hydroponic NFT system. Chemosphere 50(1):121–129

Xian QM, Hu LX, Chen HC, Chang ZZ, Zou HX (2010) Removal of nutrients and veterinary antibiotics from swine wastewater by a constructed macrophyte floating bed system. J Environ Manage 91(12):2657–2661

Yamaguchi H, Nagatomi S, Morishita T, Degi K, Tanaka A, Shikazono N, Hase Y (2003) Mutation induced with ion implantation in rose. Nucl Instrum Meth Phys Res B 206:561–564

Yu ZL (2006) Introduction to ion beam biotechnology. Springer, New York, USA