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Original Research

Enhanced Purification of Eutrophic Water by Microbe-Inoculated Stereo Floating Beds

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Abstract

This study was conducted to investigate the feasibility of using floating beds of tall fescue plants (*Festuca arundinacea*) inoculated with denitrifying polyphosphate accumulating microorganisms (DPAOs). In the presences of DPAOs, tall fescue showed greater removal of NH_4^+-N, NO_3^--N, total N (TN), and ortho-phosphate (ortho-P) after a 20-day treatment. The average removal rates were 86.32%, 93.60%, 90.12%, 72.09%, and 84.29%, respectively, for NH_4^+-N, NO_3^--N, TN, total P (TP), and ortho-P. Tall fescue plants grew well on the floating beds and the average growth rate was approximately 5.4 g DM $m^{-2}\cdot d^{-1}$. Tall fescue plants, being rich in crude potein, Ca, Mg, Fe, and Mn, can meet the daily feed requirements of livestock and poultry and were found safe to be used as animal feed.

Keywords: stereo floating bed, nutrient removal, tall fescue, mobilized DPAOs, animal feed

Introduction

The number of countries experiencing water scarcity has increased worldwide, particularly among developing countries. The quality of available freshwater also is deteriorating due to pollution, in addition to scarcity of freshwater in various regions and countries, accelerating the shortage of quality water [1]. More than five million people die annually from illnesses due to drinking of poor-quality water [2]. With increasing water eutrophication in lakes, ponds, and reservoirs of both developed and developing countries [3], water shortages due to quality degradation are more obvious. Therefore, efficient and cost-effective approaches are urgently needed for remediating eutrophic water.

Ecological engineering is an emerging field dedicated to the design and construction of sustainable ecosystems that ensure a balance of natural and human values [4]. Macrophytes have been widely used in ecological engineering for the remediation of surface water and wastewater due to their efficacy in assimilating nutrients and creating favorable conditions for the microbial decomposition of organic matter [5]. Restoration using floating, floating-leaf, emergent, and submersed macrophytes is considered crucial to regulating lake biological structure, as aquatic

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Table 1. Baseline values of water quality in the experiment.

Items	pH	DO	EC	TN	NH_4^+-N	NO_3^--N	TP
Unit	—	ppm	$\mu S\cdot cm^{-1}$	$mg\cdot dm^{-3}$	$mg\cdot dm^{-3}$	$mg\cdot dm^{-3}$	$mg\cdot dm^{-3}$
Average value	7.74	5.42	377.33	8.33	1.72	1.84	1.20
Standard deviation	0.12	0.41	10.65	0.55	0.13	0.12	0.13

DO, EC, TN, NH_4^+-N, NO_3^--N, and TP, respectively, stand for dissolved oxygen, electrical conductivity, total nitrogen, ammonia nitrogen, nitrate nitrogen, and total phosphorus.

plants limit algal growth by competing for nutrients and sunlight, and can also increase herbivorous fish biomass by providing food and refuge [6].

The floating plant treatment system offers an alternative and is perhaps more efficient in removing dissolved forms of nutrients from water [7, 8]. It differs from conventional constructed wetlands in that the microbes and plants grow on the beds, and within the floating beds the plants extend roots into the water where they take up nutrients hydroponically, and are capable of adapting variable water depths [9]. Floating beds of emergent plants (wetland or terrestrial) have been used widely in eutrophic water treatments in recent years, and there are many potential economic opportunities for beneficial use of biomass of terrestrial plants and hydrophytes [10].

Although many researchers have investigated the effectiveness of floating beds [11-13], these reports focus on hydrophytes, and the selected plants cannot grow well in autumn and winter. Moreover, it is an urgent problem to handle the huge biomass from ecological engineering. For this study a perennial cool-season grass, tall fescue, was chosen as a test plant. The study was aimed at enhancing purifying of eutrofic water using stereo floating beds with microbe-inoculated tall fescue.

Experimental Materials and Methods

Eutrophic Water

The eutrophic water used in this experiment was obtained from an inland river in the city of Hangzhou, China. The baseline values of water quality are presented in Table 1.

Plant Seedlings, Floating Beds and Microbial Materials

Tall fescue plant (*Festuca arundinacea*, var. "Bingo") was selected for this study due to its strong resistance to low temperatures. The seeds of tall fescue were disinfected with warm water (75-80ºC) for 10 min and allowed to soak for 5-6 h, then germinated at 30ºC in the thermautostat. After sprouts were visible, they were transferred into 40 mesh nylon rhizo-bags containing vermiculite and peat (v: v = 1:1). When the plants grew to 13±0.5 cm, the seedlings were transplanted to floating beds.

The floating beds were constructed of plastic trays with 12 holes filled with mixed media in rhizo-bags. Two trays were placed on a plastic tank and snugly covered the whole tank (Fig. 1). Moreover, two strains of denitrifying polyphosphate-accumulating microorganisms (DPAOs) were screened in the laboratory [14] and used in this study. In order to standardize the inoculum, the consortium was reconstituted by mixing the strains at equal volumes and optical density units.

Experimental Design and Methods

This study consisted of four treatments, each with three replicates:
(1) blank, without plants and floating beds
(2) floating beds without plants (FB)
(3) floating beds planted with tall fescue (FB+TF)
(4) floating beds with tall fescue and inoculated with immobilized microbes of DPAOs (FB+TF+IM); 2 g beads of DPAOs were added into the mixed media.

The experiment was performed in a water remediation platform with a glass roof to facilitate natural photoperiods while excluding the effects of rainfall from March 4 to March 24, 2010. A plastic tank 64 cm long and 43 cm wide was filled with eutrophic water (100 L). A mark line was made at the initial water level. Water loss due to evaporation and evapotranspiration was replenished to the marked line by adding deionized water every other day.

Immobilized Beads of DPAOs

Microbial beads were prepared by mixing bacterial solution (10 ml) with an equal volume (1:1, v/v) of sodium

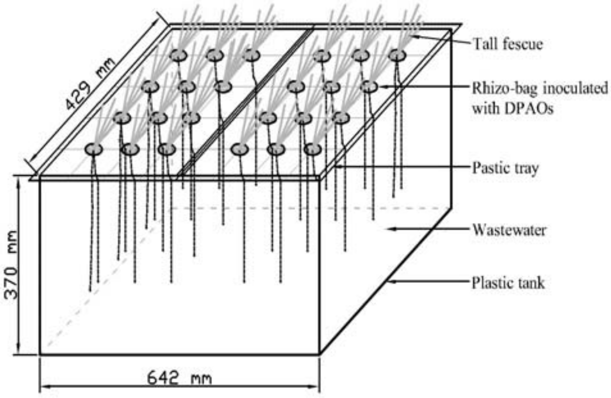

Fig. 1. Schematic diagram of stereo floating beds inoculated with denitrifying polyphosphate-accumulating microorganisms (DPAOs).

alginate solution (2%) and then stirred for 5 min. The solution was placed in a cylindrical reservoir and dropped into a well-stirred sterile $CaCl_2$ solution (3.5%) using a syringe. The alginate drops solidified upon contact with $CaCl_2$ and formed beads that encapsulated the microbes. The beads were left to harden for 30 min at 37ºC and then washed with sterile saline solution to remove excess calcium ions and unencapsulated cells [15].

Sample Collection and Physico-Chemical Analysis

Water samples were taken from the water column in the tanks between 8 and 10 a.m. every 6 days, and analyzed within 48 hours for ammonia (NH_4^+-N), nitrate (NO_3^--N), and total phosphorus using a Skalar San^{++} Automated Ion Analyzer (Skalar Co., The Netherlands). Total nitrogen was measured by potassium persulfate ultraviolet spectrophotometry [6]. The pH of water at sampling sites was monitored on-site and readings were recorded at 10 a.m.

Plant weights at initial and finial experimental stages were measured. Plant samples were carefully removed from the floating beds, gently washed with tap water, blotted with absorbing paper, and then fresh weights were measured. Plant samples were dried at 80ºC for 72 h until they attained a constant weight and then dry weights were recorded. The oven-dried plants were ground to pass a 60-mesh sieve. The plant tissue samples were then acid-digested with 5 ml of concentrated HNO_3 and 1 ml $HClO_4$ in closed Teflon vessels at 170ºC, and phosphorus in the biomass samples were determined by inductively coupled plasma optical emission spectrometry (Thermo Scientific ICAP 6000 Series, Thermo Fisher Scientific Inc., USA). Nitrogen contents in plant samples were determined with a CNS analyzer (Vario MAX CNS Macro Elemental Analyzer, Germany).

Data Analysis

Data were statistically analyzed using SPSS Base 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Differences in variables between treatments were calculated by one-way analysis of variance (ANOVA), and means were compared with following the least significant difference (LSD) method. The figures were generated using the software SigmaPlot 10.0.

Results

Effects on N Concentrations of Different Forms in Water

The concentrations of NH_4^+-N, NO_3^--N, and TN in eutrophic water were significantly reduced by the treatments, but to a varied extent (Fig. 2). The concentrations of N and P declined in the order: FB+TF+IM < FB+TF < FB < blank. Floating bed planted with tall fescue resulted in a greater decrease in the concentrations of NH_4^+-N, NO_3^--N, TN, and ortho-P than unplanted floating bed treatments. Inoculation of DPAOs enhanced the removal of NO_3^--N, TN and ortho-P from eutrophic water.

Regardless of DPAO inoculation, floating beds planted with tall fescue (FB+TF) rapidly decreased NH_4^+-N concentration in water, from 1.7 mg·dm^{-3} to 0.3 or 0.2 mg·dm^{-3}

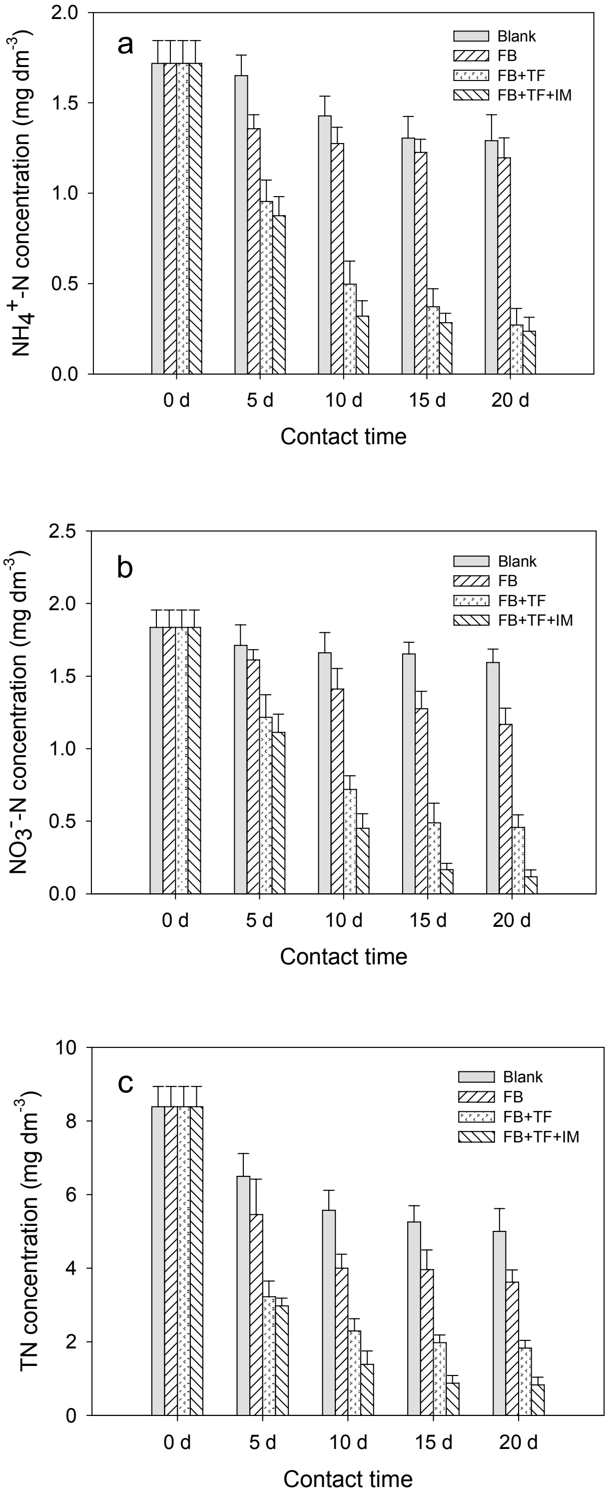

Fig. 2. Concentration changes of different forms of nitrogen (NH_4^+-N, NO_3^--N, and TN) with contact time in the water columns for different treatment systems. Values are means ± standard deviation of 3 replicates.

(Fig. 2a), which were significantly lower than those of blank and FB treatments (P ≤ 0.05). Similarly, nitrate concentration in the treatment water was reduced with increasing contact time, and most reduction in nitrate concentrations occurred in the FB+TF+IM treatment, i .e. lowered from 1.9 $mg{\cdot}dm^{-3}$ to 0.1 $mg{\cdot}dm^{-3}$ (Fig. 2b). Theoretically, NH_4^+ uptake is vigorously more efficient than NO_3^- [16], but there were no differences in concentration reduction between NH_4^+-N and NO_3^--N with the tall fescue floating bed system. The treatment with tall fescue was more efficient in TN removal, as compared to blank. Moreover, TN removal rate was increased further with the addition of immobilized microbes (Fig. 2c). The treatment of FB+TF+IM decreased TN concentration from 8.38 $mg{\cdot}dm^{-3}$ to 0.83 $mg{\cdot}dm^{-3}$ in 20 days. There was a significant difference in TN concentrations between the treatments of FB, FB+TF, and FB+TF+IM (P ≤ 0.05).

Effects on Concentrations of ortho-P and TP in Water

TP concentrations in all treatment systems rapidly declined during the first five days (Fig. 3a). FB+TF+IM accomplished a greater TP removal rate (72.1%) than FB+TF (60.5%), FB (53.3%), or blank (33.6%). The ortho-P concentration sharply decreased throughout the experimental period (Fig. 3b), which was similar to the trend of TP. The treatment of FB+TF+IM was most effective in decreasing ortho-P concentration in the eutrophic water, from 0.94 to 0.17 $mg{\cdot}dm^{-3}$, with a removal rate of 84.3%, significantly higher than those of other treatments (P ≤ 0.05). Although ortho-P change over time was similar to TP, a greater decrease in ortho-P was accomplished by the treatments of FB+TF and FB+TF+IM as compared to TP.

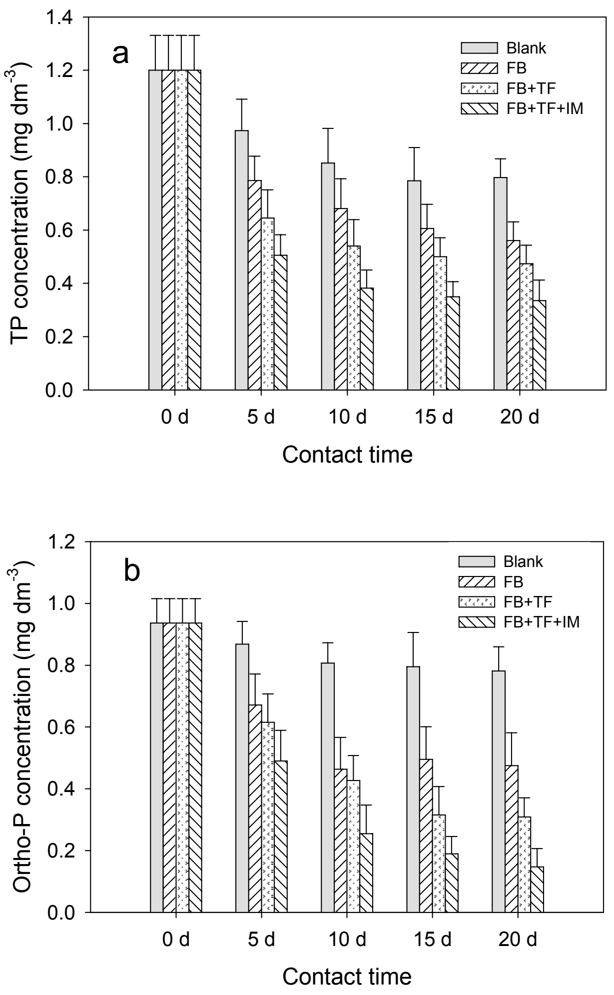

Fig. 3. Concentration changes with contact time of ortho-P and total P (TP) in the water columns for different treatment systems. Values are means ± standard deviation of 3 replicates.

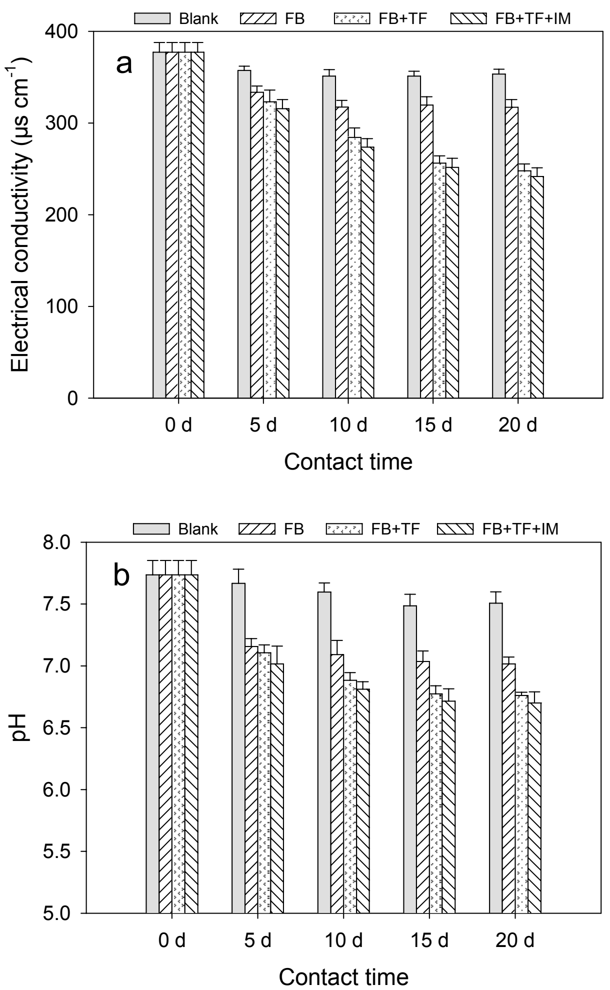

Fig. 4. Electrical conductivity (EC) and pH change with contact time in the water columns for different treatment systems. Values are means ± standard deviation of 3 replicates.

Effects on Electrical Conductivity (EC) and pH

As shown in Fig. 4a, although floating beds tended to decrease the EC of the tested water during the 20-day contact time, significantly lower EC occurred only in the treatment of FB+TF and FB+TF+IM systems. Except for blank, pH of the treatment water was significantly decreased over treatment time, especially in the first 5 days (Fig. 4b). The pH of the water in FB+TF and FB+TF+IM systems decreased to 6.7 from 7.7, as compared with 7.5 for the blank.

Table 2. Chemical compositions of tall fescue plants growing on floating beds and maximum tolerable levels (MTL) in animal feed.

Species	Crude protein	Crude fiber	Ca	Mg	Fe	Mn	Zn	Cu	Pb	Cr	As
	g·kg^{-1}						mg·kg^{-1}				
Aboveground	118.1 ±11.2	130.6 ±15.4	18.8 ±2.3	5.3 ±1.1	1.0 ±1.2	1.2 ±0.3	1.3 ±0.2	0.249 ±0.050	0.040 ±0.005	0.058 ±0.012	0.006 ±0.002
Root	78.4 ±8.5	206.8 ±20.6	290.6 ±24.1	124.5 ±14.5	19.7 ±2.5	10.1 ±0.9	2.1 ±0.2	0.884 ±0.107	0.144 ±0.010	1.034 ±0.207	0.012 ±0.003
MTL in China	NG [e]	NG	NG	NG	NG	NG	250 [a]	25-200 [b]	5.0-40 [c]	10-200 [c]	2.0-10.0 [c]
MTL in USA [d]	NG	NG	20.0	8.0	1.0	1.0	500	50	30	1000	50

[a] Agricultural Standard of China (NY 929-2005).
[b] National Standard of People's Republic of China (GB 26419-2010).
[c] Hygienical Standard for Feeds of China (GB 13078-2001).
[d] NRC (2000).
[e] Not given.

Biomass Production of Tall Fescue

After 20 days of treatment, tall fescue in the treatments of FB+ TF and FB+TF+IM grew well (Fig. 5). The dry matter (DM) of aboveground biomass increased to 14.1 g for FB+TF and 15.0 g for FB+TF+IM. Underground biomass of FB+TF+IM was significantly greater than that of FB+TF ($P \leq 0.05$). The average growth rate was about 5.4 g DM m^{-2}·d^{-1}, which was in the range of reported wetland species (Table 3). Nitrogen content of aboveground biomass ranged from 29.8 g·kg^{-1} in the FB+TF to 30.46 g·kg^{-1} in the FB+TF+IM (Fig. 6a), which were equivalent to those of emergent species [17]. Although there were no significant differences between treatments, N content of underground was significantly lower than that of aboveground ($P \leq 0.01$). A similar trend in P content was found for different treatments and plant tissues (Fig. 6b). Phosphorus contents of underground of tall fescue were in the range of 0.837 g·kg^{-1} (for the FB+TF) to 0.842 g·kg^{-1} (for the FB+TF+IM), which were significantly lower than those of aboveground (1.35-1.37 g·kg^{-1}).

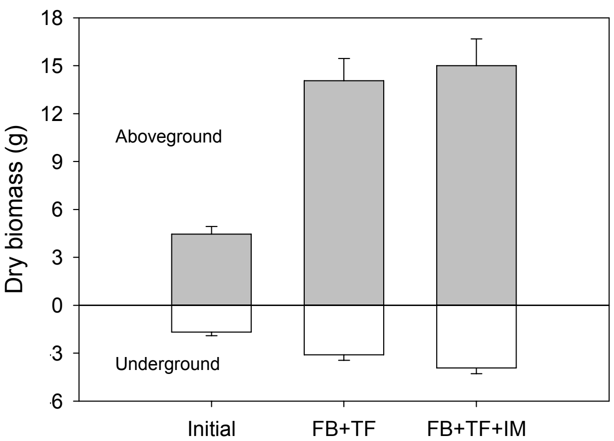

Fig. 5. Biomass increases of tall fescue plants (*Festuca arundinacea*) in aboveground and underground parts during the water purification experiment. Values are means ± standard deviation of 3 replicates.

Table 3. Comparison of the growth rates of tested plant species with reported data in literature.

Species	Growth rate g DM m^{-2}·d^{-1}	Reference
Water primrose	18.5	[23]
Water hyacinth	13.2	[23]
Sedge	2.5	[32]
Water couch	1.4-13.1	[8]
Parrot feather	1.8-7.1	[8]
Waterbuttons	4.8-10.9	[8]
Alligatorweed	6.3	[23]
Frogbit	2.1	[23]
Water Chestnut	9.0	[23]
Water spinach	4.2-6.3	[11]
Ryegrass	2.6-6.7	[33]
Tall fescue	4.50	This study

Utilization of Biomass as Animal Feeds

If biomass from ecological engineering is used for animal feeds, it could be beneficial to both water purification and feed production for poultry and livestock [18]. As shown in Table 2, crude protein content of tall fescue in aboveground tissue was 118.1 g·kg^{-1}, which was higher than Grade I (90 g·kg^{-1}) of quality classification for gramineous hay (NY/T 728-2003, China). Crude fiber was 130.6 g·kg^{-1}. The Ca, Mg, Fe, Mn, and Zn content in aboveground tissues were 5.295 g·kg^{-1}, 9.992 g·kg^{-1}, 1.243 g·kg^{-1}, and 1.325 g·kg^{-1}, respectively, all of which were lower than those in roots. Although Zn level of harvested biomass cannot meet the requirement, Ca, Mg, Fe, and Mn can meet the daily requirements of livestock and poultry at different growth stages according to the National Research Council (NRC, 2000) and China Feed Database (2009). The accumulation of toxic trace elements in aboveground and roots of tall fescue were below the standard of NRC (2000) and Hygienical Standard for Feeds in China (GB 13078-2001).

Discussion

Nitrogen and phosphorus are the key nutrient elements causing eutrophication in rivers, lakes and reservoirs [3].Floating beds are widely used to remove nutrient elements from polluted water. The plants are enforced to acquire nutrients directly from the water column as they are not rooted in any substrate, which may improve the uptake of nutrients [6]. There are three major mechanisms often referred to regarding N removal mechanisms:
(1) bacterial nitrification and denitrification
(2) uptake by plants
(3) volatilization of ammonia [19]

Removal of P by sorption, precipitation, and assimilation into microbial and plant biomass [20].

Phyto-uptake of plants is dependent on their growth rate and nutrient accumulation. In this study, the average

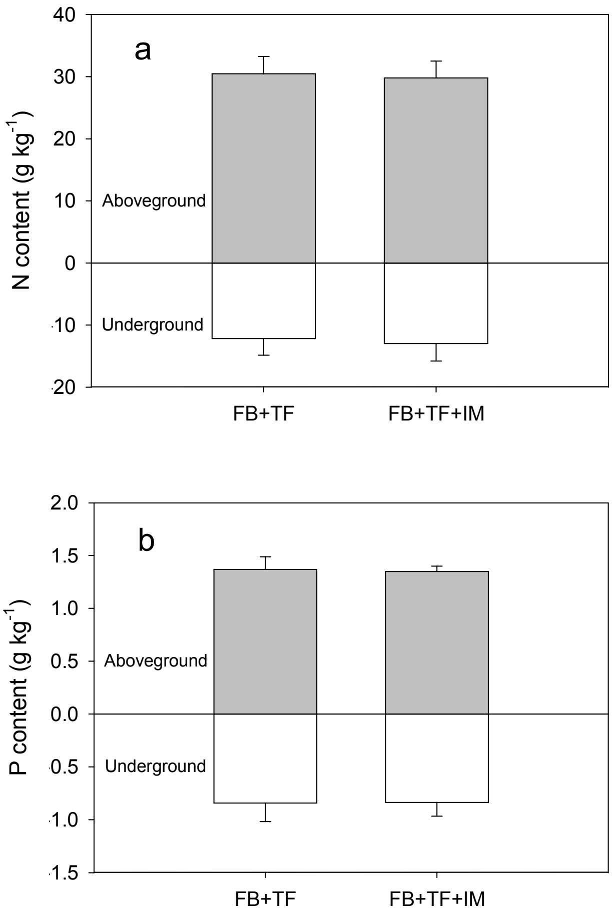

Fig. 6. Nitrogen (N) and phosphorus (P) content of tall fescue, aboveground and underground. Values are means ± standard deviation of 3 replicates.

Table 4. Comparison of nutrient retention by various plant species in floating beds.

System	Nutrient element	Retention mg·m^{-2}·d^{-1}	Reference
Water hyacinth	nitrogen	(310)	[23]
Water primrose	nitrogen	(214)	[23]
Alligatorweed	nitrogen	(64)	[23]
Frogbit	nitrogen	(29)	[23]
Water Chestnut	nitrogen	(64)	[23]
Tall fescue	nitrogen	137 (55.8)	This study
Water hyacinth	phosphorus	371 (243)	[34]
Duckweed	phosphorus	234 (87)	[34]
Azolla	phosphorus	128 (33)	[34]
Planted floats	phosphorus	(43-86)	[8]
Water hyacinth	phosphorus	(60)	[23]
Water primrose	phosphorus	(35)	[23]
Alligatorweed	phosphorus	(6.0)	[23]
Frogbit	phosphorus	(1.8)	[23]
Water Chestnut	phosphorus	(7.7)	[23]
Tall fescue floating bed	phosphorus	16 (2.5)	This study

Figures in parentheses represent the removal rates due to plant uptake only.

growth rate (5.4 g DM m^{-2}·d^{-1}) was in the range of reported wetland species (Table 3), which varies with plant species and is affected by habit conditions and plant physiology and morphology [21, 22]. The phyto-uptake of tall fescue was 55.8 mg·m^{-2}·d^{-1} for N. Although the removal rate was less than those of water hyacinth and water primrose systems, tall fescue removed more N than frogbit and was comparable with alligatorweed and water Chestnut [23]. The phyto-uptake of tall fescue for P (2.5 mg·m^{-2}·d^{-1}) was greater than frogbit (Table 4). This was probably related to the lower growth temperature (about 18ºC) and was affected by nutrient concentrations in the tested polluted water.

Nitrogen removal is accomplished by a two-stage treatment, aerobic nitrification, and anoxic denitrification, whereas P removal is achieved through enhanced biological processes under alternating anaerobic-aerobic conditions using polyphosphate-accumulating organisms [24]. In most hydrophytic ecosystems such as floating beds and natural wetlands, the removal of N is limited by a slow growth rate of denitrifying bacteria, thereby resulting in low N removal efficiencies for short-term treatments [12, 13]. In the present study, tall fescue plants were transplanted into mixed media consisting of vermiculite and peat (v:v = 1:1). Vermiculite and peat can adsorb nutrients available to plants [25]. The results showed that FB accomplished a greater decrease in the concentrations of NH_4^+-N, NO_3^--N, TN, and ortho-P as compared to the blank.

Adding IM, stereo floating beds with tall fescue accomplished a greater removal rate of N and P. The DPAOs may have contributed to the removal of nutrients in the treatment systems, because a more anaerobic condition (dissolved oxygen <1.0 mg·dm^{-3}) was beneficial to N denitrification and P bioavailability. This present study indicated that adding IM enhanced the removal of NO_3^--N, TN, and ortho-P (Figs. 2 and 3). Some studies have indicated that microbial density, activity, and diversity are enhanced in the plant rhizosphere [26, 27]. Plant root mass provided a large surface area for attaching roorganisms and carbon sources [28, 29], which favors the break-down of organic matters and the entrapment of suspended solids [30]. Moreover, it was found that IM seemed to promote the growth of underground roots (Fig. 5), as underground biomass of FB+TF+IM was significantly greater than that of FB+TF ($P \leq 0.05$). Therefore, the use of DPAOs for biological nutrient removal is highly desirable as it allows simultaneous P uptake and nitrate removal [31].

Conclusions

The stereo floating beds planted with tall fescue and inoculated with DPAOs decreased pH and EC in the water column. The presence of tall fescue (*Festuca arundinacea*) and DPAOs enhanced the removal of NH_4^+-N, NO_3^--N, TN, TP, and ortho-P from the water column. Tall fescue plant biomass was rich in Ca, Mg, Fe, and Mn, and can meet the daily requirements of livestock and poultry. The results suggest the stereo planted floating beds with inoculated DPAOs proved to be a simple and cost-effective manner for treating eutrophic water, and have great potential for adoption on a large scale.

Acknowledgements

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