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Phytoremediation Potential of *Hymenachne acutigluma* in Removal of Nitrogen and Phosphorus from Catfish Pond Water

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Abstract

The study assessed the growth and nutrient uptake capacity of *Hymenachne acutigluma* (Steud.) Gillilland by cultivating plants in striped catfish (*Pangasianodon hypophthalmus*) pond water for 42 days. The experiment simulated varying degrees of nutrient enhancement by using fish pond effluent enriched with three levels of nitrogen (N) (2.1, 4.3 and 8.6 mmol/l) and three concentrations levels of phosphorus (P) (0.16, 0.32 and 0.64 mmol/l) using NH_4NO_3 and KH_2PO_4, respectively. The treatments were arranged in a completely randomized design with four replications. Raw striped catfish pond water was served as background growth solution which was spiked with NH_4NO_3 (as a N source) and KH_2PO_4 (as a P source) and was renewed weekly. Nitrogen supply significantly affected most tested parameters whereas P only affected biomass, P content and N : P ratio content and accumulation in the roots and the shoots. *H. acutigluma* had significant higher growth, biomass and nutrient uptake when N concentration increased but not for the case of P increment. *H. acutigluma* helped to remove 7.6-19% N and 2.1-5.2% P from total input N and P concentrations in the experimental treatment. It indicates that *Hymenachne* appears to be a promising phytoremediation agent in nutrients removal from aquaculture water.

Keywords: Aquaculture water, *Hymenachne acutigluma*, Mass balance, Nitrogen, Phosphorus, Phytoremediation, Uptake

1 Introduction

The Mekong Delta (8°33'–10°55'N, 104°30'–106°50'E) is by far the most productive region for aquaculture in Viet Nam. Striped catfish, *Pangasianodon hypophthalmus*, is currently the main aquaculture species in the delta with a total production of 687,000 and 1,094,897 tonnes in 2007 and 2008, respectively, and discharging 31,602-50,364 tonnes of nitrogen (N) and 9,893-15,766 tonnes of phosphorus (P) to the environment [1]. In addition, Phan *et al.* [2] estimated that for every ton of catfish produced, 6.4 Ml of water was used, releasing nutrients-rich effluents into the receiving water bodies and causing eutrophication [3] and long-term adverse effect on the environment and human health [4]. Removal of nutrients from aquaculture effluents using phytoremediation techniques is one of the environmentally friendly methods that can be used to improve water quality and to minimize adverse environmental impacts from the aquaculture sector [5].

Nitrogen and phosphorus are important mineral nutrients for plants and promote high biomass production [6, 7]. In a review on responses of plants to N and P additions across marine, aquatic, and terrestrial ecosystems, Elser *et al.* [8] showed that simultaneously adding both nutrients gave a much stronger response than either of them alone. However, Romero *et al.* [9] reported that N level in the growth solution significantly affected the relative growth rate and tissue concentrations of N and P in *Phragmites australis*, whereas P did not have a significant affect. They also concluded that the N : P ratio in the growth solution significantly affected the growth rate which was highest at N : P ratios between 10 and 33 on a molar basis. Furthermore, Martins *et al.* [10] reported that the highest biomass of *Polygonum hydropiperoides* was obtained at a N : P ratio of 16 on a molar basis.

Hymenachne acutigluma Steud. (Poaceae) is an emergent grass that grows abundantly in natural wetlands, irrigation channels, and fresh waterways. This species has potential to remove excess nutrients from domestic wastewater [11] and aquaculture wastewater [7]. It is also commonly cultivated in the field for fodder production [12]. *H. acutigluma* has also been recognized as a potential N and P hyperaccumulator when it grown in striped catfish pond water enriched with N and P [7]. *H. acutigluma* had very high growth and biomass at the high N concentration of 40 mg N/l in the growth solution, and the authors suggested that the growth of *H. acutigluma* may be even higher at higher N levels. However, high N concentration can also be toxic to plants [13]. There are, to our knowledge, no information on the effects of N and P availability on growth and nutrient allocation of *H. acutigluma*. The objective of this study was therefore to assess growth and nutrient allocation responses of *H. acutigluma* to different combination levels of N and P. The background growth solution was raw striped catfish pond water which was spiked with ammonium nitrate (as a N source) and potassium phosphate (as a P source). We hypothesize that *H. acutigluma* grows best and has the highest nutrient accumulation at the highest N and P concentrations leading to a better N and P removal rate.

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2 Materials and methods

2.1 Experimental set up

The experiment was set up in the campus of Dong Thap University (10°25'18 - 10°25'20N, 105°38'27' - 105°38'28E) in Dong Thap province in the Mekong Delta of Viet Nam. The climate of the region is influenced by the monsoon. The average temperature during the experimental period was 32-37°C and 22-25°C in the day and the night, respectively. The experiment was setup under a glass roof which was sheltered on its sides by plastic nets to prevent the incursion of insects and rain.

A complete factorial experimental design with three concentrations levels of N (2.1, 4.3 and 8.6 mmol/l) and three concentrations levels of phosphorus (P) (0.16, 0.32 and 0.64 mmol/l) (Table 1) in the growth solution was setup with four replicates giving a total number of experimental units of 3 x 3 x 4 = 36 arranged in a completely randomized design. Each experimental unit consisted of a 50-L plastic pot (diameter 0.38 m; height 0.45 m) containing 7 kg catfish pond sediment (48% dry weight) and 35 L of pond water. Each pot was planted with three young shoots of *H. acutigluma* obtained from drainage channels in the vicinity of the setup.

Table 1: Concentrations of N and P (mmol/l) and the corresponding N : P ratios in the water in the experimental treatment

Treatments	Added concentration (mmol/l)		N : P ratios
	N	P	
Low N; low P	2.1	0.16	13.3
Low N; intermediate P	2.1	0.32	6.6
Low N; high P	2.1	0.64	3.3
Intermediate N; low P	4.3	0.16	26.6
Intermediate N; intermediate P	4.3	0.32	13.3
Intermediate N; high P	4.3	0.64	6.6
High N; low P	8.6	0.16	53.1
High N; intermediate P	8.6	0.32	26.6
High N; high P	8.6	0.64	13.3

The pond water and sediment were collected from a commercial striped catfish farm in Dong Thap province. The average concentrations of N and P in the pond water were 0.61±0.01 and 0.017±0.005 mmol/l (n=6), respectively. The pond water was spiked with NH_4NO_3 (as N source) and KH_2PO_4 (as P source) to reach the experimental treatment N and P concentrations in the pots (Table 1). The water in the pots were renewed weekly throughout the 6-week experimental period and analyzed for water quality. The water pH was in the range of pH 6.4-7.7 and was not controlled during the experiment.

2.2 Plant biomass and nutrient allocation

Initially, the shoot and root length of the experimental plants were measured as well as their fresh weight before transplanting them into the pots. After 42 days, the plants were harvested, rinsed thoroughly in deionized water, and then fractionated into shoots (stalks, leaves, flowers) and roots to determine the fresh and dry mass after drying at 60°C till constant weight. The relative growth rate (RGR, g/gDW/d), based on total plant dry weight was calculated.

The dried plant fractions were ground (<1 mm particle size) and the concentrations of N and P in the plant fractions were analyzed using the Kjeldahl method and the Ascorbic Acid method, respectively [14]. The tissue concentrations of N and P were used to estimate the amounts of N and P extracted by the plants from the spiked pond water. The plant nutrient accumulation (mg/plant/d) were calculated from the nutrient concentration in the plant fractions initially and at harvest, and the biomass production during the 42-day experiment.

2.3 Water, sediment sampling and analysis

Water temperature, pH and dissolved oxygen (DO) were analyzed weekly using a portable meter (HORIBA W-2000S, Korea). In addition, water samples were collected weekly and analyzed for *ortho*-phosphate (PO_4^{3-}), total ammonium (TAN), nitrite (NO_2^-) and nitrate (NO_3^-) using ion chromatography (1100 Thermo, USA), and total phosphorus (TP) and total Kjeldahl nitrogen (TKN) following standard procedures [14].

The sediment in the pots was sampled initially and at the end of the study. Air-dried sediment samples were analyzed for N and P using the Kjeldahl method and the Ascorbic Acid method, respectively [14].

2.4 Nutrients mass balance

The N and P mass balances (g/pot) were calculated based on the total inputs and outputs of N and P in the pots. The total inputs were the N and P contents in the initial plant biomass and the sediment plus the amount of N and P added with the spiked pond water weekly. The total outputs were the N and P contents in the harvested plant biomass and the content in the sediment at the end of the experiment plus the amount of N and P in the water before weekly renewal.

2.5 Statistical analyses

All data were tested for normal distribution and variance homogeneity (Levene's test) and logarithmically transformed if necessary. Two-way analysis of variance (ANOVA using Type III sum of squares were used to identify interactive effects of the N and P treatments on plant growth and tissue nutrients content. Post-hoc Tukey Honestly Significant Differences (HSD) was used to identify significant differences between treatments at the 5% probability level. The software SPSS 22 (IBM SPSS Statistics V22.0, USA) was used for all statistical analyses.

3 Results and discussion

3.1 Plant growth and biomass allocation

Nitrogen and phosphorous are essential plant nutrients and critical determinants of plant growth and productivity. The plants grew well in all treatments and showed no toxicity symptoms, even at the highest nutrient concentrations. Nitrogen significantly affected most of the measured growth and biomass allocation parameters while P only affected shoot and root biomass production (Table 2). Shoot height of *H. acutigluma* was affected by N treatment, but the effect depended on the P level as indicated by the significant N x P interaction (Table 2). Both N and P treatments significantly affected root and shoot dry biomass ($p<0.05$; Figure 1B; Table 2). Both root and shoot biomass increased with increasing N levels whereas the biomass decreased with increasing P level (Figure 1B). Shoot to root dry biomass ratio was in the range of 2.7-3.7 and was the highest at the high N level meaning that *H. acutigluma* allocate more biomass to the shoot fraction at high N levels. The biomass was the lowest at the low N : P ratio of 3.3 showing the negative effects of an unbalanced nutrient supply (Figure 1B). Martin *et al.* [10] reported that *Polygonum hydropiperoides* produced the highest biomass at the molar N : P ratio of 16 and the lowest biomass at the molar N : P ratio of 2. They concluded that within the range of molar N : P ratios between 7.8 and 11.8 the biomass production of *P. hydropiperoides* were similar.

Table 2: Results of ANOVA (*P*-values) showing the effects of N and P on growth, biomass partitioning and nutrient accumulating of *H. acutigluma* after growth for 42 days at 9 treatment combinations of N and P

	Main Effects		Interaction
	N	P	N x P
Growth			
Shoot height	**0.018**	0.051	**0.045**
Root length	0.171	0.340	0.215
Shoot biomass	**0.000**	**0.034**	0.897
Root biomass	**0.002**	**0.044**	0.703
RGR	**0.000**	0.057	0.540
Nitrogen and phosphorus concentration			
N shoot concentration	**0.000**	0.542	0.779
N root concentration	**0.000**	0.118	0.149
P shoot concentration	0.306	**0.002**	0.425
P root concentration	**0.000**	**0.001**	0.853
N : P shoot ratio	**0.000**	**0.005**	0.086
N : P root ratio	**0.000**	**0.000**	**0.026**
Nitrogen and phosphorus accumulation			
N shoot accumulation	**0.000**	0.062	0.783
N root accumulation	**0.000**	0.072	0.868
P shoot accumulation	**0.000**	0.938	0.524
P root accumulation	0.670	0.403	0.811
N : P shoot accumulation ratio	**0.000**	**0.003**	0.073
N : P root accumulation ratio	**0.000**	**0.001**	0.056

Note: *P*-values in bold are significant at the 0.05 probability level.

The N concentration in the roots and shoots of *H. acutigluma* increased as N supply increased ($p<0.05$; Figure 2A) regardless of the P treatment. Hence, the amount of N accumulated in the roots and shoots increased concomitantly ($p<0.05$; Figure 3A) and reached the highest values (30-40 mg/plant/d) in the high N treatment. Thus, the current levels of P did not affect neither the tissue N concentrations nor the N uptake capacity of *H. acutigluma* [7]. Similar findings were reported for *Phragmites australis* by Romero *et al.* [9], who found that the concentrations of N and P in the plant tissues increased in concert with N supply, and not P supply.

Koerselman and Meuleman [15] suggested that an N : P ratio greater than 16 on a weight basis should indicate P limitation, whereas an N : P ratio less than 14 should be indicative of N limitation. In our study, only the treatment combination of N-high and P-low had a N : P ratio of 24 on a weight basis (i.e. 53.1 N : P molar ratio) and the rest of the treatment combinations had N : P weight based ratios lower than 14. Thus, overall our treatment combinations, except one, were indicative of N limitation, and although the tissue P concentration responded to the P levels in the water, the lowest tissue P concentration were found in the treatment combination N-high and P-low (Figure 2B).

The N treatment significantly affected the RGR of the plants while the P treatment had no effect, and there the effect of N was independent on the P-treatment as shown by the lack of a significant interaction term between N and P (Table 2). This is similar to the findings of Romero *et al.* [9], who found that the N level in the water significantly affected the RGR of *Phragmites australis* while the P level had no effect. The higher RGRs were achieved in the high N treatments regardless of P level and tended to decrease (although not statistically significantly) with P level within N- treatments (Fig. 1C). The RGR was particularly low at the low N : P ratio of 3.3 showing the negative effects of an unbalanced nutrient supply ($p<0.05$; Figure 1C). This shows that, within the concentration range tested, nitrogen was the main nutrient limiting the growth of *H. acutigluma*.

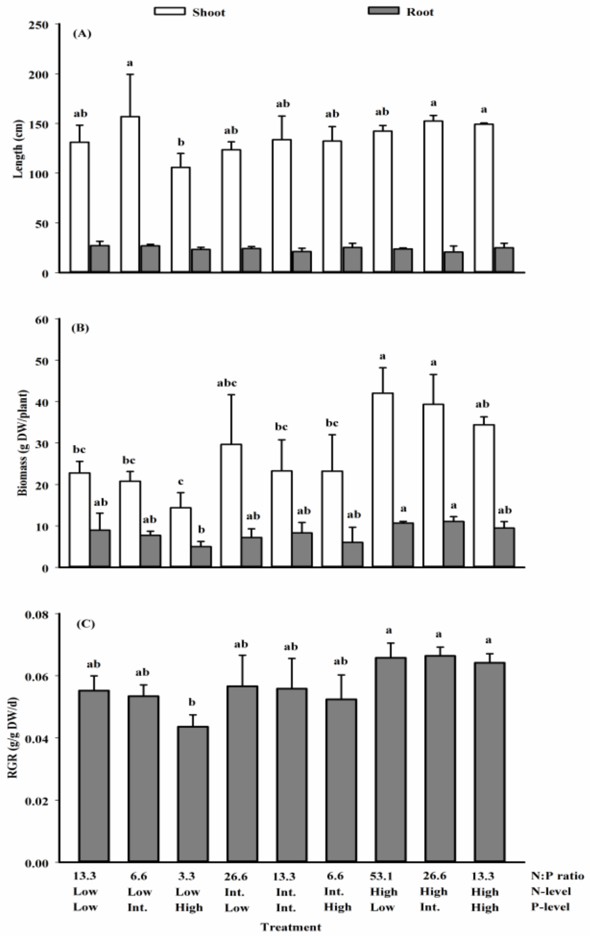

Figure 1: Mean (± 1 S.D., n=4) shoot height and root length (A), biomass allocation (B), and relative growth rate (C) of *H. acutigluma* grown for 42 days at three levels of N (2.1, 4.3 and 8.6 mmol/l) and three levels of P (0.16, 0.32 and 0.65 mmol/l). Identical letters above bars indicate groups of means that are not statistically significant different at the 95% confidence level based on a Tukey HSD test.

3.2 Nutrient content and accumulation

Both N and P treatments in the growth solution significantly affected the tissue N and P concentrations (%) and the N : P ratio except for the N concentrations in roots and shoots and the P concentration in shoots. There were no significant interaction terms in the ANOVA (Table 2).

The nutrient accumulation rate in the plant biomass (mg N or P per plant per day) was calculated for each treatment from the mean dry mass of each biomass fraction (shoot and root) multiplied by the mean N and P concentration of that fraction divided by the 42-day incubation time (Figure 3). The N accumulation rate in the shoots was five-folds higher than that in the roots (Figure 3A), because the shoot biomass production rate of *H. acutigluma* was 2-4 times higher than that of the roots (Figure 1B) and, in addition, the N concentration in the shoots was higher than the concentration in the roots (Figure 2A). Similarly, accumulated P in the shoots was 2-4 times higher than that of in the roots (Figure 3B). The accumulation of N and P in the shoots and roots' tissues largely reflected patterns of biomass allocation of *H. acutigluma*. Jiang *et al.* [16] also concluded that the N and P accumulations in plant tissues of 15 emergent wetlands species were significantly positively correlated with plant biomass. *H. acutigluma* produced an average of aboveground (i.e. the shoots) dry biomass of 40 g/plant in 42 days.

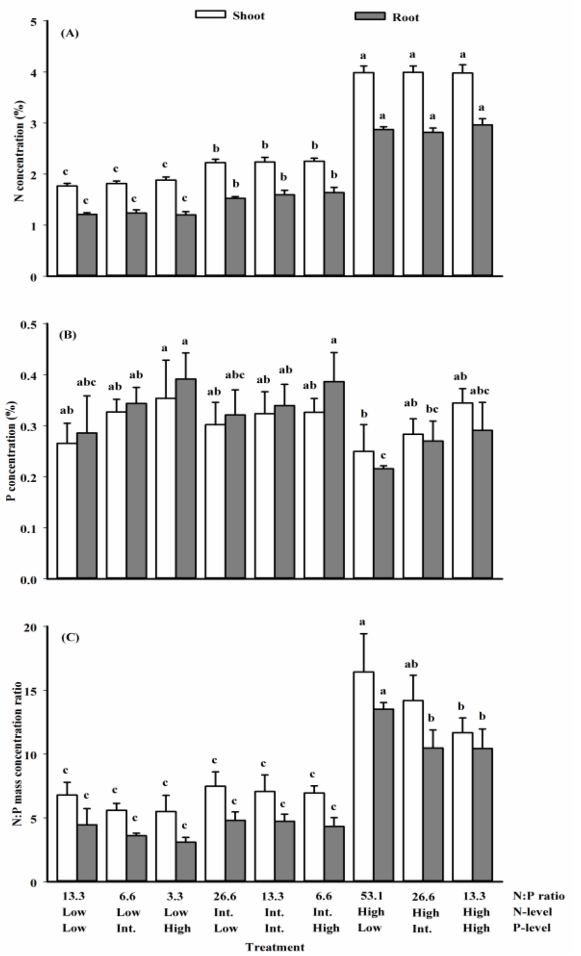

Figure 2: Tissue concentration (mean ± 1S.D., n=4) of (A) nitrogen, (B) phosphorus and (C) the N:P concentration mass ratio in plant tissue of *H. acutigluma* grown for 42 days at three levels of N (2.1, 4.3 and 8.6 mmol/l) and three levels of P (0.16, 0.32 and 0.65 mmol/l). Identical letters above bars indicate groups of means that are not statistically significant different at the 95% confidence level based on a Tukey HSD test.

The high growth rate and biomass production in concert with the high N and P tissue concentration at high nutrient supply rates indicates that harvesting the shoots of *H. acutigluma* may be possible way to remove N and P from polluted water [17] thus *H. acutigluma* may be a candidate species for use as a phytoremediator for aquaculture wastewater.

3.3 Nutrient mass balance

The N and P mass balance (g/pot) were calculated based on the total inputs and outputs of N and P per pot (Table 3). The total inputs were the N and P contents in the initial plant and sediment plus the sum of N and P contents added with the weekly renewal of the water. The total N and P inputs into each pot during the 42-day experiment were 11.9-30.8 and 7.2-10.4 g/pot, respectively (Table 3). The total outputs were the N and P contents in the harvested plant material and the sediment plus the sum of the remained N and P concentrations in the water before renewal. On average, 65-75% of N and 77-89% of P from the total inputs N and P in the water were removed, which is equivalent to 5.2-19.6 g N/pot and 1.0-3.6 g P/pot during the 42-day experiment. Only a fraction of this, namely 0.9-5.8 g N/pot and 0.2-0.4 g P/pot, were accumulated in the *H. acutigluma* biomass. Hence, the plants contributed to remove 7.6-19% N and 2.1-5.2% P from the total inputs (Table 3).

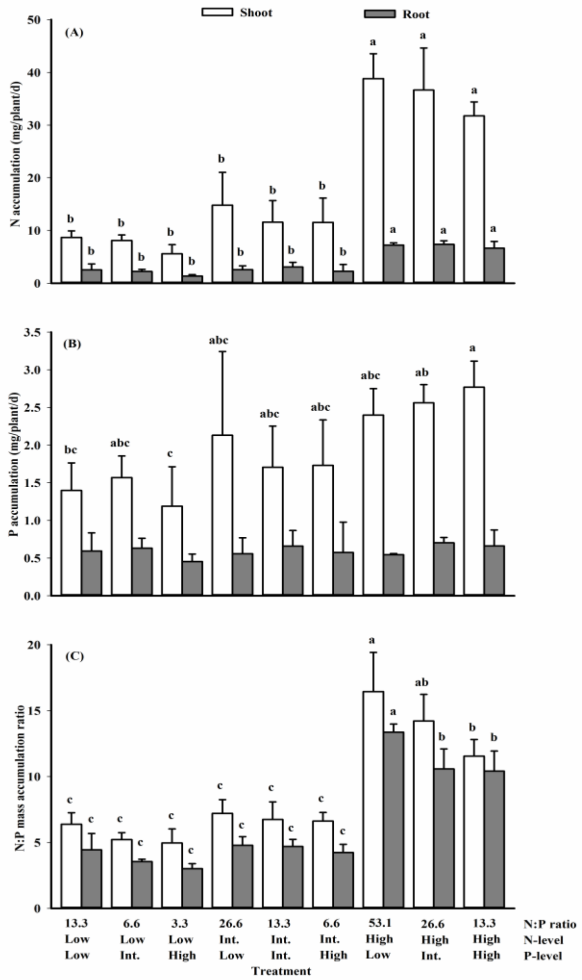

Figure 3: Mean nutrient accumulation rate (mean ± 1 S.D., n=4) of (A) nitrogen, (B) phosphorus and (C) the N : P mass accumulation ratio in plant tissue of *H. acutigluma* grown for 42 days at three levels of N (2.1, 4.3 and 8.6 mmol/l) and three levels of P (0.16, 0.32 and 0.65 mmol/l). Identical letters above bars indicate groups of means that are not statistically significant different at the 95% confidence level based on a Tukey HSD test.

Previous studies reported that N and P assimilation in plant biomass accounted for 30% N and 39% P with *Typha orientalis* [18], 76% N and 86% P with *Phragmites australis* [19], 11% N and 3% P with *Baumea articulata* [20]. The sediment was an important sink and accumulated 6.0-8.8% of N and 7.0-27.6% of P from the total inputs (Table 3). It can be explained by the accumulation of algae and the remained water in the sediment due to weekly renewal, which made up an increment of N and P in the sediment. Mayo *et al.* [21] concluded that denitrification and net sedimentation were the major N removal mechanisms in a water hyacinth pond accounting for 81.9% and 13.1% of removed nitrogen, respectively. Van der Steen *et al.* [22] reported that 18% of influent N was recovered by duckweed and 8% settled in sediment whereas El-Shafai *et al.* [23] found that 80% N was taken up by duckweeds (*Lemna gibba* and *Lemna minor*), 5% was settled in sediments and 15% N was unaccounted-for.

Table 3: Mass balance (mean ± S.E., n=4) of N and P (g/pot) in the treatments for the 42 days experiment

Treatment N	Treatment P	Input (g/pot) Water(1)	Input Plant(2)	Input Sediment(3)	Total input	Output (g/pot) Water(4)	Output Plant(5)	Output Sediment(6)	Total output	Unaccounted for
Nitrogen										
Low	Low	8.06	0.09 ± 0.003	3.77	11.92	2.63 ± 0.21	1.53 ± 0.14	4.48 ± 0.10	8.64 ± 0.14	3.28 ± 0.14
Int.	Low	14.36	0.09 ± 0.004	3.77	18.22	3.63 ± 0.07	2.31 ± 0.44	4.93 ± 0.09	10.88 ± 0.40	7.34 ± 0.39
High	Low	26.96	0.09 ± 0.005	3.77	30.82	7.31 ± 0.13	5.93 ± 0.31	5.71 ± 0.10	18.95 ± 0.22	11.87 ± 0.23
Low	Int.	8.06	0.09 ± 0.003	3.77	11.91	2.79 ± 0.14	1.39 ± 0.07	4.82 ± 0.17	9.00 ± 0.15	2.92 ± 0.15
Int.	Int.	14.36	0.08 ± 0.004	3.77	18.21	3.93 ± 0.34	1.96 ± 0.31	5.16 ± 0.07	11.05 ± 0.49	7.16 ± 0.49
High	Int.	26.96	0.09 ± 0.003	3.77	30.82	7.88 ± 0.67	5.66 ± 0.50	6.09 ± 0.08	19.63 ± 0.60	11.18 ± 0.60
Low	High	8.06	0.09 ± 0.005	3.77	11.92	2.78 ± 0.21	1.00 ± 0.13	4.69 ± 0.08	8.47 ± 0.25	3.45 ± 0.24
Int.	High	14.36	0.09 ± 0.005	3.77	18.22	4.02 ± 0.26	1.86 ± 0.37	4.86 ± 0.09	10.74 ± 0.58	7.48 ± 0.57
High	High	26.96	0.09 ± 0.004	3.77	30.81	7.98 ± 0.51	4.95 ± 0.17	5.95 ± 0.08	18.89 ± 0.34	11.93 ± 0.34
Phosphorus										
Low	Low	1.16	0.0058 ± 0.0002	6.07	7.24	0.14 ± 0.02	0.26 ± 0.03	6.69 ± 0.04	7.09 ± 0.06	0.15 ± 0.06
Int.	Low	1.16	0.0060 ± 0.0003	6.07	7.24	0.17 ± 0.03	0.35 ± 0.08	6.58 ± 0.08	7.10 ± 0.03	0.14 ± 0.03
High	Low	1.16	0.0062 ± 0.0003	6.07	7.24	0.13 ± 0.02	0.38 ± 0.02	6.70 ± 0.03	7.21 ± 0.00	0.02 ± 0.00
Low	Int.	2.15	0.0056 ± 0.0002	6.07	8.23	0.50 ± 0.03	0.28 ± 0.02	7.14 ± 0.06	7.92 ± 0.04	0.31 ± 0.04
Int.	Int.	2.15	0.0054 ± 0.0002	6.07	8.23	0.45 ± 0.06	0.31 ± 0.04	7.27 ± 0.11	8.03 ± 0.08	0.20 ± 0.08
High	Int.	2.15	0.0058 ± 0.0002	6.07	8.23	0.34 ± 0.08	0.42 ± 0.01	7.38 ± 0.15	8.13 ± 0.00	0.09 ± 0.00
Low	High	4.31	0.0057 ± 0.0004	6.07	10.39	0.86 ± 0.06	0.22 ± 0.04	8.86 ± 0.17	9.94 ± 0.08	0.45 ± 0.08
Int.	High	4.31	0.0057 ± 0.0003	6.07	10.39	0.86 ± 0.05	0.30 ± 0.06	8.63 ± 0.29	9.79 ± 0.08	0.60 ± 0.08
High	High	4.31	0.0056 ± 0.0003	6.07	10.39	0.73 ± 0.05	0.44 ± 0.03	8.94 ± 0.19	10.12 ± 0.09	0.27 ± 0.09

Notes: (1) Sum of N and P content in the renewed water; (2) N and P content in initial plant biomass; (3) N and P content in initial sediment; (4) the remained N and P content in the growth solution (sampling weekly); (5) N and P content in the harvested plant biomass; (6) the remained N and P content in sediment at harvest.

For N, the unaccounted-for component in a balance system can usually be considered as the quantity loss to the atmosphere through denitrification whereas P as a conservative element and all input P is theoretically countable within the growth system partitions [18]. In the present study, 24-41% N and 0.3-5.8% P from the total inputs were unaccounted-for (Table 3). The amount of P unaccounted-for in our system was assumed to be the amount of P in the sediment and the water loss during weekly renewal and sampling. Different nutrient loading rates and plant species were responsible for different nutrient uptake by plants in various studies [24]. The high plant uptake proportion was due to the rapid biomass growth and influent quality [25]. Altering nutrient availability in the growth solution can change productivity and potential nutrient uptake of *H. acutigluma*. In general, *H. acutigluma* grew well and accumulated high N and P contents in the plant tissues. The average N and P accumulated in the harvested *H. acutigluma*'s biomass were 17-64 and 38-79 times higher than in the initial plant biomass, respectively. Therefore, harvesting biomass is a good strategy to remove N and P from wastewater treatment system [17].

4 Conclusions

The results of the study support the general impression that *H. acutigluma* is a well-adapted species for growth in nutrient-rich environments. The plant is N limited under the experimental condition. After 42 days, *H. acutigluma* removed 7.6-19% N and 2.1-5.2% P from the total inputs. The results indicated that *Hymenachne* is an effective accumulator plant for phytoremediation of nutrients in aquaculture water.

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