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Nitrogen Phytoremediation by Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms)

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Abstract The phytoremediation potential of water hyacinth, *Eichhornia crassipes* (Mart.) Solms, was examined in two independent studies under nitrogen (N) rates of 0, 40, 80, 100, 150, 200, and 300 ppm. A modified Hoagland solution was added to ponds containing water hyacinths which were rated and measured weekly for 4 weeks. The hyacinths accounted for 60–85% of the N removed from solution. Net productivity, as measured by dry matter gain, increased with an increase in N rate until 80 ppm. Above that level dry matter productivity was similar. Tissue N increased linearly with dry matter gain, but total nitrogen removal from the water increased exponentially with net dry matter gain or with an increase in canopy cover. The relation between total N in plant tissue and N removal from the water was similar for the two experiments.

Keywords Assimilation · Biofiltration · Nutrient removal · Phytoremediation

1 Introduction

One of the most common environmental issues is nutrient pollution of surface waters, mainly from nitrogen (N) (NOAA 2000). Nitrogen enters surface water primarily through runoff and leaching from agricultural and urbanized areas, but also from precipitation, N_2 fixation in water and sediment, and N release from decomposing aquatic plants and animals. Excess N degrades water quality, poses risks to humans and livestock, threatens rare habitats and ecosystems, and accelerates the natural eutrophication process in aquatic systems. High levels of N from tributary waters have caused large hypoxic (oxygen depleted) zones in the Chesapeake Bay (Chesapeake Bay Foundation 2006) and the Gulf of Mexico (NOAA 2000) with subsequent adverse economic impacts.

In the USA in 1972, the Clean Water Act mandated clean water in lakes, rivers, streams, and aquifers. The increasingly stringent regulations governing water quantity and quality have resulted in the extensive use of stormwater retention ponds in most areas of

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agriculture and commercial and urban horticulture to manage runoff velocity, quantity, and quality. Some stormwater ponds have secondary benefits such as irrigation water storage and recreational uses. While strong emphasis has been placed on using stormwater ponds, relatively little effort has been directed towards their long term maintenance and management. Consequently, many ponds have experienced accelerated eutrophication, a process where water bodies receive excess nutrients through runoff. The nutrients stimulate excessive plant growth, and the aquatic ecosystem becomes unbalanced. Decaying vegetation negatively impacts water quality by reducing dissolved oxygen levels and releasing nutrients which fuel more vegetative growth. Organic matter that is not decomposed adds to the bottom sediments which accumulate until the pond no longer effectively manages runoff volume. The resultant flooding leads to issues of public health, liability, economic loss, and environmental problems. Pond renovation adversely impacts the surrounding environment and is very expensive. An inexpensive, site-adaptable phytoremediation system that uses floating water hyacinth (*Eichhornia crassipes* (Mart.) Solms) to remove N from runoff as it enters stormwater ponds is being evaluated as a method for slowing the eutrophication process and extending the life expectancy and functionality of stormwater ponds.

Phytoremediation is the process of using plants (*phyto*) to clean up (*remediate*) polluted soil or water. Aquatic macrophytes are able to remove a variety of nutrients from polluted water (Boyd and Vickers 1971; Moorhead et al. 1988; Reddy and DeBusk 1985), including the major agricultural pollutants N and phosphorus (P). The aquatic macrophyte, floating water hyacinth, has been of particular interest for water remediation. While water hyacinth is considered one of the world's most noxious weeds (Gopal 1987; Sculthorpe 1967), the characteristics that make it weedy also make it a good plant for remediation. The plant is adaptable to a wide range of environmental factors including pH, electrical conductivity (EC), and temperature (Desougi 1984; El-Gendy et al. 2004; Mitsch 1977). The dense fibrous root system provides an extensive surface area for absorption, adsorption, and for micro-organism attachment. Water hyacinths spend the majority of their lifecycle in a vegetative state and rapidly reproduce by vegetative propagation. Increased biomass leads to increased filtering capacity.

The plant absorbs and stores N in excess of what it requires for growth (hyper-accumulation or luxury uptake) (Alves et al. 2003; Reddy and Tucker 1983; Reddy and Reddy 1987). In the 1970s and 1980s water hyacinth was used in numerous waste water treatment systems. Many studies have evaluated the efficacy of water hyacinth, however, results differ widely on the amount of N removed (Dunigan et al. 1975; Ower et al. 1981; Tucker 1981).

The objective of this study was to assess water hyacinth phytoremediation potential of nitrogen, a common pollutant in stormwater runoff collected in urban stormwater retention ponds. Because of water hyacinth's invasive nature, and in order to complete the nutrient recycling cycle, subsequent studies were conducted to address the critical issues of containment, harvesting, and composting.

2 Materials and Methods

2.1 Location

This study was conducted at Virginia Polytechnic Institute and State University's Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA, USA.

2.2 Ponds

Sixty four ponds were constructed inside a polyethylene film covered greenhouse and arranged in four blocks, each with two parallel rows of eight ponds. Each pond had dimensions of 1.2 m × 1.2 m × 17.8 cm. Pond frames consisted of concrete block covered with a cushion layer of woven geotextile SI 200 ST followed by an impervious layer of 40 mm Very Flexible Polyethylene (VFPE) (ACF Environmental, Richmond, VA, USA). Each pond was filled with 189 l of water from a well (Table 1), and the water level was marked on the side of each pond.

2.3 Treatment

The study was conducted twice; study A 14 April through 12 May and study B 30 August through 27 September, 2005. Treatment rates were based on 2 years of water analysis data from golf course and commercial nursery ponds in Southeastern Virginia

Table 1 Component concentrations (mg l^{-1}) for pond fill water and nutrient stock solution

Compound	Concentration (mg l^{-1})	
	Fill water	Stock solution
Ammoniacal N (NH_4)	NA[a]	74
Nitrate N (NH_3)	2	226
Phosphate (P_2O_5)	1	71
Potash (K_2O)	3	300
Calcium (Ca)	17	71
Magnesium (Mg)	6	22
Boron (B)	0.03	0.23
Copper (Cu)	NA	0.15
Iron (Fe)	NA	1.50
Manganese (Mn)	NA	0.84
Molybdenum (Mo)	NA	0.15
Zinc (Zn)	NA	0.24
Na–EDTA	NA	9.6

[a] Values not assessed or below detection limit

(data not shown) and literature (Jayaweera and Kasturiarachchi 2004; Ower et al. 1981; Teager et al. 1993). A commercial fertilizer, Scotts Champion Water Soluble Fertilizer 17-4-17 with 4% Ca, 1.25% Mg (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA), was used to approximate a Hoagland's solution (Hoagland and Arnon 1950) (Table 1). Treatment rates were based on parts per million N, and included treatments of 0, 40, 80, 100, 150, 200 ppm in both study A and B, and 300 in study B. Ratios of the macro nutrients were 4.25 N:1 P:4.25 K:1 Ca:3 Mg with corresponding amounts of the micro nutrients. Aliquots from a 200 ppm N (study A) or 300 ppm N (study B) stock solution were used to produce the different treatments, which were applied once at the initiation of the study. Because the fertilizer contained Mg, Cu, Mn, and Zn in water soluble form, Na–EDTA (Table 1) was added to each pond to prevent Fe from precipitating out of solution. An initial batch of hyacinths was purchased and grown in greenhouse ponds in Hoagland's solution at 100 ppm N for 5 weeks prior to each experiment to establish a uniform stock population. From that stock population four (study A, average individual initial fresh weight 27 g) or five (study B, average individual initial fresh weight 72 g) uniformly sized water hyacinths with no attached progeny were pulled and placed in each pond. Deionized (DI) water was added on a weekly basis to keep individual pond water volume constant.

2.4 Data

Study duration was 28 days, and the study was completed before flower initiation. Environmental, water, and plant data were collected.

2.4.1 Environment

Air temperature and photosynthetically active radiation (PAR) (micromole per square meter per second) readings were taken hourly by a weather station placed inside the greenhouse (HOBO Micro Station Logger, Onset Computer Corporation, Cape Cod, MA, USA). The greenhouse sides were removed and a 50% shade cloth was added over the top of the greenhouse to offset higher summer temperatures and radiation levels during Study B.

2.4.2 Water

Pond water temperature was recorded hourly (HOBO Water Temp Pro v1 data logger, Onset Computer Corporation, Cape Cod, MA, USA). Individual pond pH and EC were also recorded at the same time of day once weekly (Oakton pH/CON10 pH/Conductivity/°C hand-held waterproof meter, Oakton Instruments, Vernon Hills, IL, USA). Water samples were collected from each pond at the initiation and end of the study for nutrient analysis which included Total Kjedahl Nitrogen (TKN) (copper catalyst EPA Method 351.2), nitrate/nitrite (Nitrite EPA Method 353.2) and ammonia (EPA Method 350.1).

2.4.3 Plant

For base line data, 30 whole plant samples were collected at the beginning of the study from the same propagation pond as the experiment plants. Plant canopy coverage was measured on a percent basis every seven days using the dot grid assessment method (Swiecki and Bernhardt 2001). Total plant number (including four original plants in study A and five in study B) and total biomass (whole plants) weight for each pond were collected at the end of the study. A young water hyacinth plant was counted if it had begun to extend from the parent plant, had visible roots, and at least two leaves. The fresh weight data (not shown) was used as a check for other data. Pond biomasses were air dried to a constant weight then dry matter weight was measured for each biomass. A representative whole plant sample was taken from each pond biomass and ground to particle size ≤3 mm. Whole plant samples were used because the hyacinths would not be treated as partitioned in the subsequent composting (recycling) process. Tissue samples were analyzed for Total Kjedahl Nitrogen (TKN) (copper catalyst EPA Method 351.2), nitrate/nitrite (Nitrite EPA Method 353.2) and ammonia (EPA Method 350.1). Total N uptake was calculated by adding TKN and nitrate/nitrite N. All analyses were performed by a US EPA certified laboratory.

2.5 Experimental Design and Data Analysis

The study was a randomized complete block (RCB) with six (study A) and seven (study B) treatments and eight replications. Data were analyzed using SAS (SAS version 9.3, Cary, NC 2005). Regression analysis and Tukey's test for mean separation ($P=0.05$) were performed. Data points of individual experimental units were graphed in some figures in order to observe data cluster patterns. Mean data values were graphed in other figures to show relationships.

3 Results and Discussion

Research conducted on N uptake by water hyacinth is generally divided between two types of systems; plants cultured in ponds with or without sediments. The culture solution is generally either a prepared nutrient solution such as Hoagland or polluted or waste/effluent water. In the present studies the ponds contained no sediment and a commercial water soluble fertilizer was used to simulate a Hoagland's solution.

Daily maximum and minimum air temperatures ranged from 9°C to 34°C (study A), and 16°C to 34°C (study B), respectively. Daily minimum and maximum water temperatures followed a similar pattern and ranged from 10°C to 32°C (study A) and 19°C to 35°C (study B). PAR readings were higher for study A than for study B as expected due to the early season timing and no shade cloth on the greenhouse. Total PAR for study A was 222,756 μmol/m^2/s. Total PAR for study B was 76,997 μmol/m^2/s, which was lower given the late season timing and addition of shade cloth to the greenhouse. Ponds in study B required significantly more DI water to maintain a constant volume than ponds in study A (Fig. 1). This was a result of the later season higher average air and water temperatures and more aggressive vegetative growth. At the initiation of both studies the pH of all solutions (Fig. 2) was close to neutral. After 2 weeks, pH increased for 0 and 40 ppm N treatments and decreased for all treatments over 80 ppm N. Average EC values (Fig. 2) showed the clear differences between treatments for both studies.

In both studies water hyacinths grew and reproduced in all treatments. Water hyacinth biomass increased with increasing N level as expected, with a very close relationship evident between total number of plants and total biomass (data not shown). A significant growth effect was observed at N levels greater than 80 ppm (Table 2). While water hyacinths grew faster in study B and produced more biomass, the

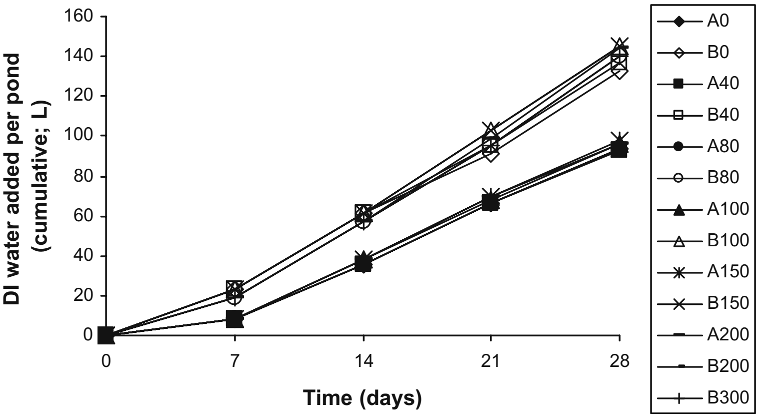


Fig. 1 Cumulative DI water added per pond weekly to maintain constant water volume. Legend symbols represent studies A and B and treatments in parts per million N

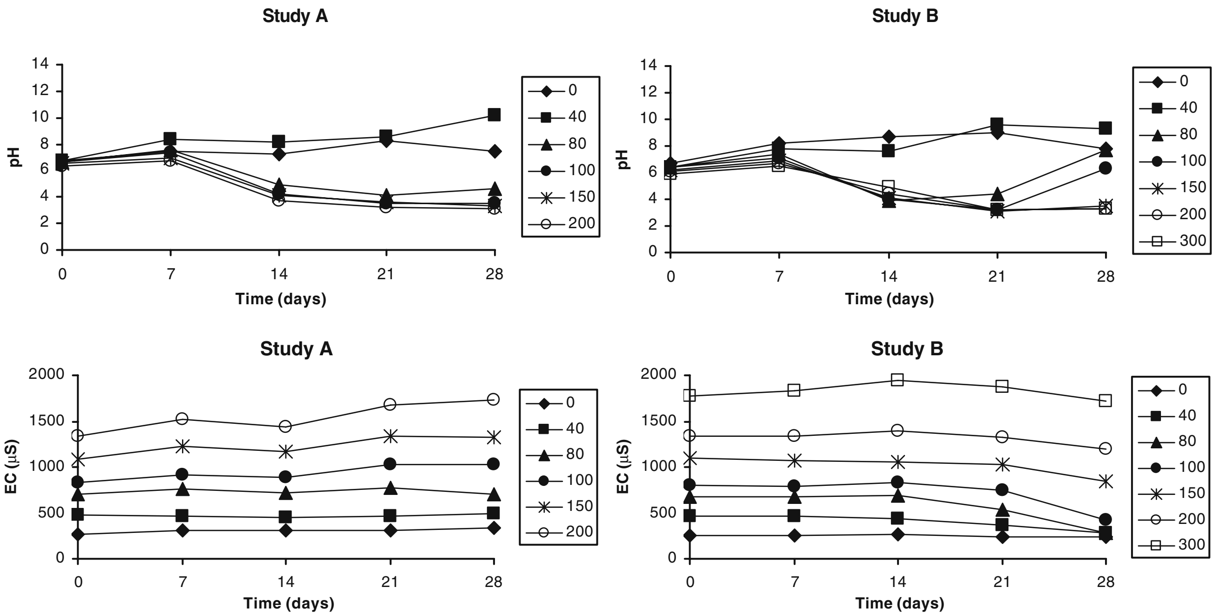


Fig. 2 Average pH and EC values by treatment over time for Study A and Study B

5-fold increase in dry matter from lowest to highest treatment was consistent across the two studies. Total N accumulation in the water hyacinth tissue was significantly greater for the 80 ppm N and higher treatments, but it was not significantly different between those treatments in either study. Total nutrient depletion of the culture solution did not occur in any treatment in either study A or B, but the N removal from the water significantly increased with an increase in the N level over the entire range (study A) or until 100 ppm (study B) (Table 2).

The correlation between N concentration in plant tissue and plant dry matter of vegetative crops is well-documented in literature pertaining to agricultural crops (Biemond and Vos 1992). This same relationship has also been well documented in water hyacinth (Mitsch 1977; Reddy et al. 1989; Shiralipour et al. 1981). Total N concentration in the water hyacinth

Table 2 Final dry matter (DM), total nitrogen (N) in plant tissue, and N removal from water; means and separations for studies A and B

Study A							Study B						
TRT (ppm) N	Means						TRT (ppm) N	Means					
	DM (g)		Total N tissue (g)		N removal water (g)			DM (g)		Total N tissue (g)		N removal water (g)	
0	22.5	b[a]	0.28	c	0.47	f	0	32.5	c[a]	0.53	c	0.31	c
40	29.1	b	0.62	b	5.69	e	40	85.9	b	2.66	b	2.35	bc
80	101.9	a	3.49	a	9.31	d	80	181.8	a	6.10	a	5.45	ab
100	128.3	a	3.84	a	11.86	c	100	172.8	a	6.57	a	9.01	a
150	117.8	a	4.29	a	16.97	b	150	172.1	a	6.50	a	9.05	a
200	122.9	a	5.48	a	22.13	a	200	180.3	a	6.84	a	4.84	bc[b]
	$P<0.0001$		$P<0.0001$		$P<0.0001$		300	188.0	a	7.88	a	9.26	a
								$P<0.0001$		$P<0.0001$		$P<0.0001$	

[a] Mean separation based on Tukey's test at $P<0.05$

[b] Initial values of treatment 200 were between initial values of treatments 100 and 300, and so were the final values; but the differences between final and initial values had a high coefficient of variation and on average were outlying

tissue correlated well with the final dry matter in both studies, as seen in the tight clustering of the data points and the R^2 values (Fig. 3). The factors that influenced final dry matter also influenced total N concentrations suggesting that N was removed through multiple mechanisms. A strong linear correlation was evident between the net increase in water hyacinth tissue N and the net dry matter increase (Fig. 4). The slope of the linear regression lines was similar for the two studies, suggesting a consistent nitrogen concentration in the tissue over studies.

Nitrogen added to each pond ranged from 40 mg/l (7.56 g) to 300 mg/l (56.73 g) (Table 2). Fifty-nine to 75% of the N was removed from the pond solution in study A, while only 13–48% was removed in study B (Fig. 5). Water hyacinths accounted for up to 60% of the N removed from the pond solution in study A and 85% in study B (Table 2).

Power regression curves confirm that when water hyacinth dry matter increased, N removal from the pond solution increased. Initially N removal from the water corresponded closely to dry matter increase, but this did not hold at the higher level N treatments, especially in study A. Although there was no additional growth or corresponding biomass increase, there was still variation in N removal from the pond solution. The relationships between water hyacinth canopy cover and N uptake from the pond solution (Fig. 6) for both studies was similar to their N uptake dry matter relationship in Fig. 5. Canopy cover is an indicator of N uptake, and the power relationships for both studies in both Figs. 5 and 6 clearly show the developmental effect on the water hyacinths as N is removed from solution.

While water hyacinths are known hyperaccumulators of nutrients, and they removed significant amounts of N, they did not account for the total amount of N removed from the pond solution. Processes other than plant uptake appeared to influence the N removal from the ponds, and plotting total N uptake in tissue against total N removal from water (Fig. 7) confirms this. The regression equations for both studies strongly deviated from the 1:1 line and the slope of the curves decreased with increasing total N removal. In study A (with lower temperatures but much higher light levels on the canopy and the water), the discrepancy between total N uptake in plants and total N removal from water was much larger than in

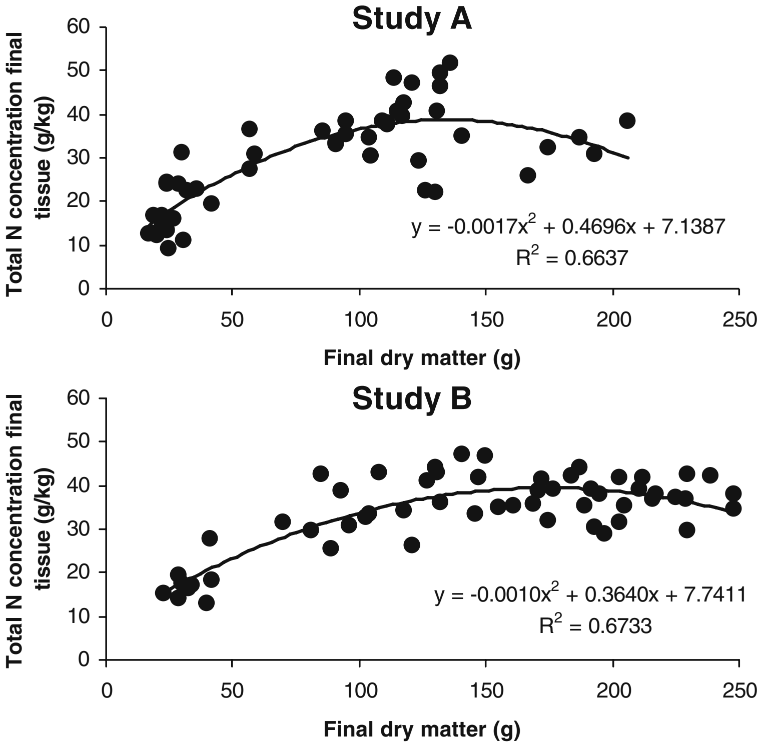
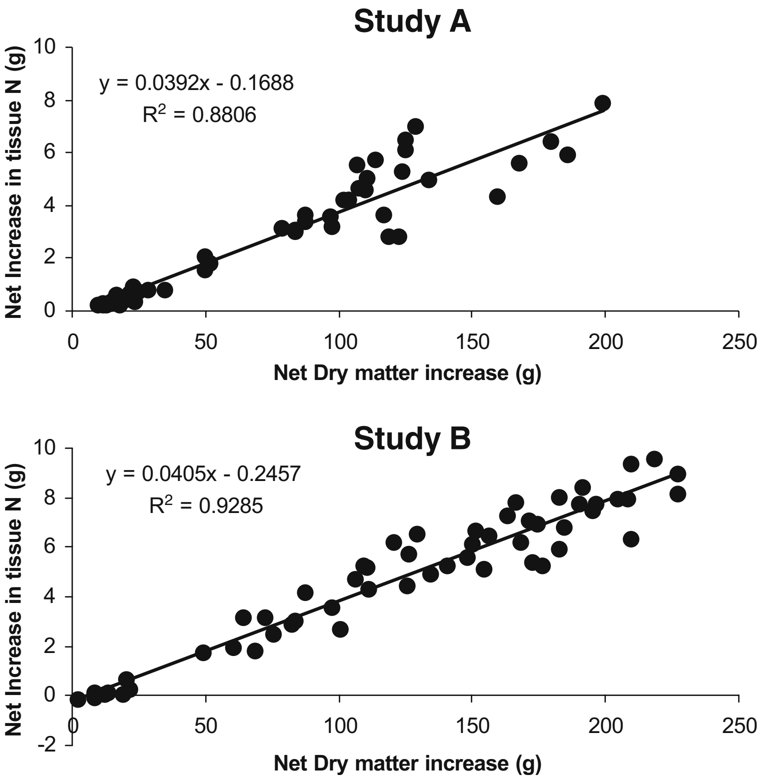


Fig. 3 Total nitrogen concentration in the final water hyacinth tissue versus final water hyacinth dry matter for study A and study B

Fig. 4 Increase in tissue nitrogen versus dry matter for study A and study B



study B. In addition to plant uptake, nitrification, denitrification, volatilization, and assimilation processes impact N removal from pond solutions (Schwarz et al. 1999). Since these studies were hydroponic, N loss from the denitrification process associated with sediment-based systems was not a factor. El-Gendy et al. (2004) reported that above pH 6.0, nitrogen transformations through nitrification and ammonia volatilization occur. In our studies, pH decreased for the higher N level treatments, eliminating nitrification and ammonia volatilization as significant sinks for the N removed in excess of what was observed in the water hyacinth tissue. These processes could have contributed to the N removal from the pond solution in the lower parts per million N treatments, though, where the pH was consistently

Fig. 5 Total nitrogen removed from pond solution versus net dry matter increase means for study A and B

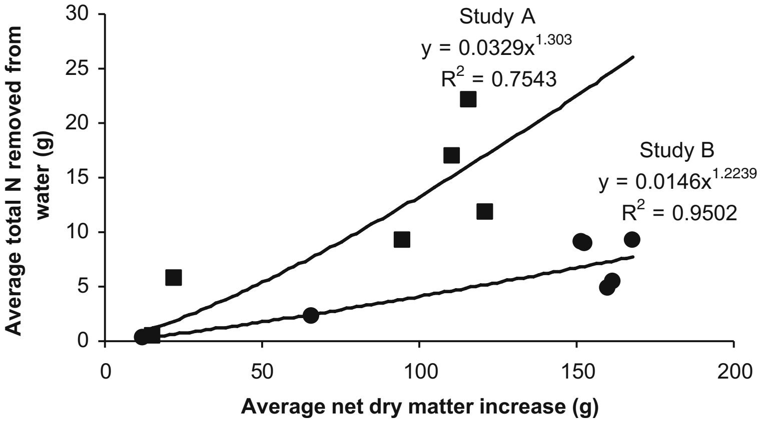
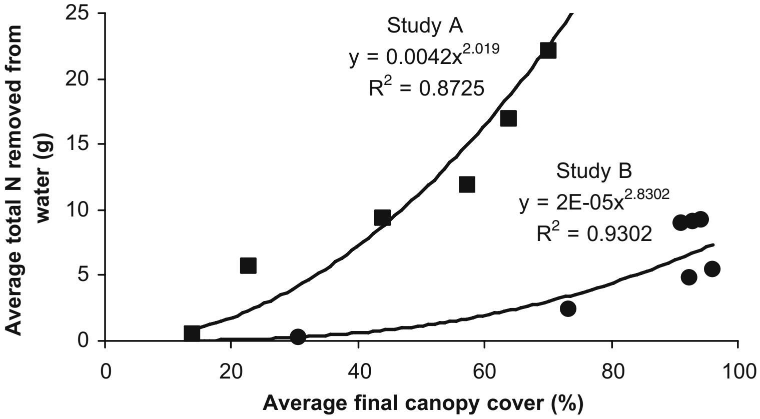


Fig. 6 Total nitrogen removed from pond solution versus final canopy cover means for study A and B

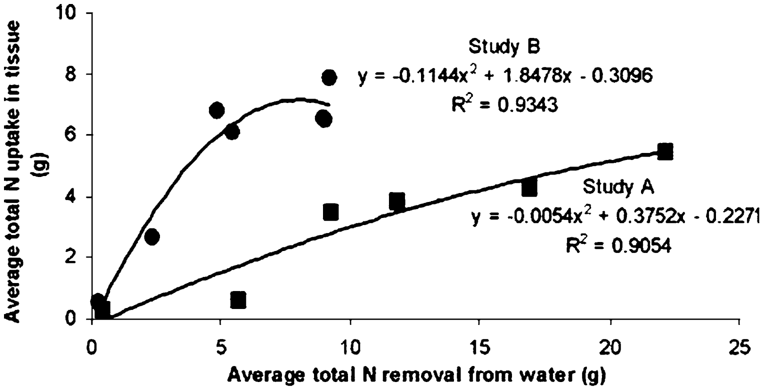


above 6.0. Reddy and Reddy (1987) report that N assimilation by algae and aquatic macrophytes can contribute to significant losses of N from aquatic systems. Algae infested all treatments and replications in both studies. Denser algal growth was observed within the higher N treatments until hyacinth canopy coverage of pond surface area was almost complete. Algal growth was therefore considered a significant component in N removal in these studies, and a major factor in explaining the discrepancy between total N taken up by the plants and total N being removed from the water. The most likely mechanism to explain this discrepancy is denitrification enhanced by oxygen consumption and organic matter production by the algae as seen in study B, which showed the smallest discrepancy, but where the water was replenished more frequently.

4 Conclusions

While information in the literature varies on the amount of N removed by water hyacinths, studies including this one show that these aquatic macrophytes are effective and efficient at nutrient phytoremediation. Other processes such as nitrification, denitrification, and volatilization also impact N removal in dynamic aquatic systems. How effectively and efficiently N is removed is relative to the individual system and end goals for nutrient removal. Nutrient removal from stormwater runoff can be achieved using water hyacinth in a contained system; however, water hyacinths alone cannot remediate 100% of the N in solution. Given that water hyacinths are invasive they should be used with caution and in conjunction with other practices under controlled conditions.

Fig. 7 Total nitrogen uptake in hyacinth tissue versus total nitrogen removed from pond solution means for study A and B



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