Phytoremediation of Nutrient-Controlled Water using Duckweed and Water Fern

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ES 375 Capstone
5/7/10

Abstract

Phytoremediation is a way to use plants to remove nitrogen and phosphorus from water bodies to reduce negative impacts of eutrophication. This study used duckweed, water fern, and their combination to analyze nutrient removal from water environments with varying levels of these two elements. For these environments, most of the N concentrations were within the range of a eutrophic water body, while P concentrations ranged from oligotrophic to eutrophic water bodies. Nitrogen concentrations decreased by up to 87% in the duckweed treatment. Possible synergistic effects between the two species were observed, as the combination treatment reduced nitrogen levels to 11% below expected values determined by the calculated average of the two individual plant treatments.

Introduction

Currently, 1.1 billion people do not have access to safe drinking water (World Health Organization 2005). The UN estimates that within fifteen years, three-fourths of the world's population could live in these conditions (Frey *et al.* 2006). In many instances, even when quantities of water are adequate, water is made unusable by contaminants. Contamination of water supplies comes in many forms, ranging from inorganic heavy metal pollutants, to pathogenic organisms, to elemental and organic pollutants that alter ecosystem structure and function.

A significant contributor to worldwide water contamination is pollution by nitrogen and phosphorus. These elements are critical to plant growth and are used in such processes as cell replication and protein formation, but they can be toxic and cause drastic ecosystem changes at elevated concentrations. Additions of nitrogen and phosphorus to water occur naturally over thousands of years (Darrin Fresh Water Institute 2010) as part of their respective cycles, contributing to the gradual eutrophication of a water body (Smith *et al*. 1999; Frey *et al*. 2006). As a result, plant growth increases, which is a primary factor in the gradual transformation of ponds to marshes, a process that occurs regularly in nature (Folke *et al.* 1994; Frey *et al*. 2006). Plant growth is dependent not only on absolute concentrations of N and P but also on the ratio of nitrogen to phosphorus. This ratio has been shown to be predictive of plant growth and ecosystem health (Bulgakov and Levich). While eutrophication is a natural part of a pond's life cycle, humans can accelerate and alter the cycles of water bodies in a process referred to as cultural eutrophication (Sagrario *et al*. 2005). Intensive fertilizer use, industrial effluents and increased areas of impervious surface are common sources of cultural eutrophication. Anthropogenic actions can increase the rate of eutrophication by up to a factor of four (Wang 2006).

Eutrophication has several consequences, including reduced oxygen availability in water (Smith *et al*. 1999; Frey *et al.* 2006). The added plant material that develops due to nutrient additions eventually falls out of the water column and decomposes (Smith *et al*. 1999; Frey *et al*.

2006). The organisms that perform this decomposition deplete oxygen from the water column, which can alter the community composition and structure of the ecosystem can change. Eventually, only certain species tolerant of anoxic conditions are able to survive (Frey *et al.* 2006). The growth of the plant material itself can be harmful to a pond ecosystem, as certain plants begin to dominate and block sunlight from penetrating to greater depths (Frey *et al*. 2006). Eutrophication can cause unpleasant smells and water colors and in some cases, a coating of foam on the surface of the water (Frey *et al.* 2006). Additionally, when added nitrogen is converted to ammonia, it is toxic to fish (El-Bestawy *et al.* 2005). Decreases in fish populations can have mild to disastrous effects on local fishing economies (Hunt *et al*. 2006). Increased rates of eutrophication can cause food web alterations (Qin 2009), hinder establishment of communities that would normally be parts of succession in that location or cause loss of biodiversity. Furthermore, in high enough concentrations, both nitrogen and phosphorus can be toxic to humans. Nitrates can be converted to nitrites and combine with hemoglobin in the blood, depleting oxygen levels and causing "blue-baby syndrome" in infants, and can be transformed into cancer-causing nitrosamines inside the human body (Frey *et al.* 2006, El-Bestawy *et al.* 2005).

Eutrophied water bodies may become no longer useful as water sources for humans and other organisms. In light of population pressures (El-Bestawy *et al*. 2005) reversing the ecosystem changes that we have caused is desirable. One approach is to pump water from a lake and treat it off-site with physicochemical methods. However, this can be expensive or environmentally harmful (Frey *et al.* 2006).

Bioremediation is an alternative that is less expensive and makes use of natural processes. It is defined as the use of any living organisms to degrade waste (Litchfield 2005). In the broadest sense, the process of bioremediation has been occurring since human beings have disposed of their trash and relied on natural systems to convert it to organic matter (Litchfield 2005). In more recent times, the process has been used in more intentional ways. During the late nineteenth century, wastewater treatments plants were developed, and along with them the first intentional application of biological processes to treat waste and wastewater (Litchfield 2005).

During the 1990s, phytoremediation became an established technique to clean polluted sites. (Litchfield 2005). Plants have a diverse range of applications in remediating polluted sites, with a capacity to hyperaccumulate metals and take up large quantities of organic "pollutants" such as nitrogen and phosphorus that they not only accept but require for their biological processes. Harvesting plants used in remediation efforts removes the nutrients contained in the biomass from the water body, decreasing the concentration of those nutrients in the ecosystem.

Duckweed (*Lemna minor*) and water fern (*Azolla sp*.) have been used successfully in phytoremediation applications. Duckweed, in particular, is commonly used in the United States to phytoremediate municipal, industrial and septic waste (Iqbal 1999). Many small-scale phytoremediation efforts are found in other locations and can be non-mechanized. For example, in one village in Bangladesh, duckweed, cultivated on raw sewage, is fed to fish (Iqbal 1999). For our study in phytoremediation, we chose to use both duckweed and water fern based on their growth patterns, nutrient uptake rates and the fact that they are native to the study region. Due to space constraints, we were interested in plants that grow primarily outward rather than upward. Duckweed, a prolific aquatic plant, has a life cycle of several weeks; an individual frond may produce ten generations of progeny over a period of ten days to several weeks (Skillicorn *et al*. 1993). It has been shown to double in mass every two days (Skillicorn *et al.* 1993) and can remove 75% of total phosphorus and nitrogen in a eutrophied water body (Cheng *et al*. 2002).

Water fern is common in many parts of the world and is used as a fertilizer and livestock feed. It has a unique potential for remediation because of its association with nitrogen-fixing cyanobacteria called *Anabaena azollae* Strasb.(Forni *et al.* 2001). Its fast growth rate is also amenable to phytoremediation applications; it can produce approximately 18 kg/m^2/yr of plant material (Sela *et al*. 1989).

The use of plants for nutrient uptake is especially valuable because following site remediation, it is possible to identify practical and value-added uses for the plant material. These could include conversion of plant biomass to energy, animal feed, or further breakdown of the material by using fungi. In particular, five duckweed species have been shown to be a valuable additive for animal fodder because of its high protein and low fiber and lignin contents (Vermaat and Hanif 1998). Between fifteen and forty percent of its dry weight is protein (Cheng *et al*. 2002; Alaerts *et al*. 1995).

One site that could benefit from nutrient removal is located in the Saratoga Lake watershed in Saratoga County, New York. Most water bodies in the watershed are not severely impacted by the effects of cultural eutrophication. The region is monitored by several water resource protection organizations, a fact which contributes to the relative health of the major water bodies. However, one pond, located at Daniels Road adjacent to the Skidmore College horse stables, has nitrogen levels characteristic of a eutrophied water body. A likely source of some of the nutrients present in this pond is horse manure from the stables, and from which runoff can enter the nearby pond. The pond is formed in a low-lying area between Daniels Road, a set of railroad tracks, and a hill leading up to the stables property. The pond is about 360 m^2 and contains abundant duckweed across almost the entire surface. The edges contain a diverse assemblage of plants and fungi including cattails and some woody species. Duckweed and other aquatic plants are abundant.

Much research has been conducted regarding these plants' abilities to remediate nutrient-rich water when both nitrogen and phosphorus are present in high concentrations. However, ponds vary in N and P concentrations and in N:P ratios (Sterner and Elser 2002). Furthermore, anthropogenic inputs to water bodies (sewage, fertilizer, etc.) vary in their compositions and proportions of N and P. The result of these two factors is that eutrophied water bodies can have varying amounts of N and P. Moreover, it is possible that some of these water bodies could have levels of one of these nutrients present in low enough concentrations so as to limit plant growth (Sagrario *et al*. 2005). Often phosphorus limits duckweed and water fern growth (Wagner 1997), and consequent nutrient uptake, and this is likely to be the case in the pond described above. In this study, we tested duckweed and water fern and their combination in environments where phosphorus was present in low concentrations and nitrogen while nitrogen was present in relatively higher concentrations.

Furthermore, we were interested in testing for synergistic effects that could arise due to the presence of multiple plant species in a water environment. Certain plant physiological traits allow for different nutrient uptake patterns and rates and could contribute to a broader range of nutrient environments in which remediation is effective. As a result, remediation using combinations of species may be more effective than using only one species. Furthermore, certain species could have positive synergistic effects on pollutant uptake. We tested the effectiveness of combining two aquatic plant species in nutrient removal in laboratory conditions.

Methods

Collection of Water Sample from Field Site

We collected a water sample at the field site near the Skidmore horse stables (henceforth referred to as the Stables site) in February 2010 and stored it in a Nalgene container. The sample was filtered through 0.7 micron Milipore filters and tested in April for filtered total nitrogen using a turbidimetric persulfate oxidation method, and for filtered total phosphorus using a colorimetric persulfate oxidation method. These laboratory nutrient tests were performed at Darrin Freshwater Institute, Bolton Landing, NY. Total nitrogen (TN) was determined to be 1.59 mg/L and total phosphorus (TP) was 11.9 ug/L. This nitrogen value fell within the N range of eutrophic water, which is greater than 0.65 mg N/L. In terms of P, the Stables water fell at the lower end of the mesotrophic classification, which consists of P levels between 10 ug/L and 30 ug/L.

Laboratory experimentation to assess the nutrient uptake abilities of duckweed and water fern in different water environments

Samples of water fern, duckweed and pond water were purchased through Carolina Biological Supply. We chose to order pond water to ensure more constant water composition than if we had used field-collected water. In order to determine nutrient uptake patterns of individual species, we cultivated duckweed and water fern individually in different water environments. We used the water (TN = 1.96 mg/L; TP = 76.4 ug/L), diluted to the following concentrations with de-ionized (DI) water to create seven environments: 100% (no dilution), 75%, 50%, 25%, and 0% (pure DI water). For example, the “75%” environment consisted of 75% pond water and 25% DI water. We created two additional water environments with elevated nutrient concentrations by adding 0.46 g/L NH4Cl and 0.033 g/L KH2PO4 to one environment, referred to as “A” and 0.92 g/L NH4Cl and 0.066 g/L KH2PO4 to another, referred to as “B.” These water environments were all created in clear plastic 15 cm x 14 cm containers filled to 800 mL. Plants were applied to the surface of the water in amounts of 0.4030 g +/- .0015 g. To test for any synergistic interactions between duckweed and water fern we used a third treatment consisting of a combination of the two plants. For this trial we used 0.2015g +/- .0015 g each of duckweed and water fern. All plants were cultivated at room temperature with a 16-hour photoperiod. To control for nutrient flow between water and air, for each water environment we established a control consisting of one container of appropriately diluted water, with no plants. In total we had 28 containers.

After 8 days, approximately 300 mL remained in each container; the rest had evaporated or been transpired by plants. We added 100 mL of water to each container, and then took water samples which we tested for filtered total N and P using the analytical methods described for the Stables water (a turbidimetric persulfate oxidation method for filtered TN, and a colorimetric persulfate oxidation method for TP). We compared the N and P in each of the 21 treatment containers to those of their respective controls. We divided the amount of N remaining in each treatment water environment by the N in the control. The same analytical procedure was followed for P. We calculated the final N:P ratios in all treatment containers.

Root length

In each container we measured plant root length after 24 days. To do this we stirred the contents and randomly selected 20 individuals, measuring with calipers the longest root from each individual. For the combination treatment, however, we measured 10 duckweed individuals and 10 water fern individuals. (We hereafter refer to these two groups as duckweed in combination and water fern in combination.) Some individuals selected did not have any measureable roots, and we did not include them in the data analysis. In these instances, the sample size is less than 20 or 10. We compared the root lengths for each of four groups (duckweed alone, duckweed in combination, water fern alone, and water fern in combination). Different species of plants, or plants grown in the different treatments, could have different maximum growth potentials. Therefore, we normalized each average root length by the highest average root length in each of the four groups to directly compare the plants of different species and subjected to different treatments.

pH

We used Accumet Basic AB15 pH meters to determine pH of water in each of the containers after 29 days.

Results

Water nitrogen concentration decreased compared to the control (the corresponding water environment without plants) in all duckweed and combination treatment water environments except DI water. The amount of N remaining in each water environment, for each treatment group, is shown in Figure 1. This amount is expressed as the ratio of the N remaining in each treatment water environment, to the N remaining in the corresponding control water environment. For example, for duckweed grown in the 75% environment, the ratio was calculated by dividing the amount of N remaining in that container by the amount remaining in the 75% control container. This normalization by the initial amount of N in each water environment allows for direct comparison of these N values. The ratio is less than one which, on a logarithmic scale, translates to a negative value. Therefore its change in N is shown as negative on the graph.

In the water fern and combination treatments, the DI water environment shows increased final N concentration compared to the control. Higher percent N removal occurred when plants were in lower initial water concentrations, as illustrated by the smaller bars on the graph at higher initial concentrations. This trend does not hold for water fern. Instead we see no correlation between proportion N removal and initial water concentration.

We calculated an expected theoretical value of final nitrogen for the combination treatment by averaging the final water nitrogen values in each water environment for duckweed cultivated alone and water fern cultivated alone. Expected values determined by this method are shown in Table 1. We compared this theoretical value to observed nitrogen values in the combination treatment, also shown in this table. Expected and observed N values for the combination treatment were averaged for all water environments. There was an insignificant difference between these means (p=0.09, paired t-test for means). These differences were then used to calculate a proportion difference. There is an average 11% reduction in N beyond what the expected performance of the combination treatment.

Using the procedure as for N, above, we compared the means of the expected and observed P concentrations for the combination treatment, shown in Table 2. We determined that there was an insignificant difference between the two (p=0.13, paired t-test for means). There was an average 4% *increase* in N compared to what would be expected for the combination treatment (Table 2).

While N concentrations were, overall, decreased compared to the control, there is no analogous trend regarding P concentrations. In all plant treatments, the P concentration in DI water approximately quadrupled. For the other six water environments, change in P showed either negative or positive changes with no apparent trends.

For the duckweed and combination treatments, the N:P ratio was reduced in all water environments. The N:P ratios of the water fern treatment generally followed those of the corresponding control environments except in the 0% and 75% environments, where the N:P ratio was reduced. Duckweed reduced the N:P ratio the most, and the combination treatment N:P ratios were intermediate between those of duckweed and water fern. There was an increase in N:P ratios in all plant treatments in the 100% water environment. In the elevated concentrations, N:P ratios for all plant treatments were similar to those of the controls.

For plant roots in duckweed, water fern and combination treatments, there is a trend of increasing root length with higher percentage water environments until the 75% environment. After this point there is a decrease. Elevated water environments show a significant decrease from 100%. Duckweed died in three of four elevated water environments in which it was cultivated. In general, root lengths of duckweed grown by itself or in combination with water fern were not significantly different from each other. Root lengths of water fern grown in combination with duckweed were longer than those of water fern grown alone in all water environments except the most nutrient-rich.

Root lengths standardized by absolute maximum root length are illustrated in Figure 5. This allows for a comparison of the effects of the different water environments on the two plants. There is a consistent trend among all plant treatments of increasing maximum root length until 75% and a subsequent overall leveling off. Water fern alone and in combination had less variability in root length than duckweed. This shows that, in terms of root length, the two species and plants in different treatments respond similarly to different water nutrient environments.

Final levels of pH water environments between 25% and 100% inclusive ranged from 5.49 to 7.42 and were conducive to growth of both duckweed and water fern (Table 3). The pH of the elevated concentrations A and B ranged from 3.18 to 4.39 (Table 3).

Discussion

Cultivating duckweed and water fern in different initial water environments resulted, to varying degrees, in a changed nutrient environment. Most of this change was due to remediation of nitrogen, which resulted in a more nitrogen-poor and phosphorus-rich setting, relative to the initial proportion of those nutrients. The duckweed treatment showed an up to 87% decrease in N (Figure 1). Greater N decreases were observed in the lower concentration water environments, at concentrations up to and including 100%. This could be explained by the limited amount of time (8 days) given for nutrient removal to occur, and the limit to the absolute amounts of nutrients that plants can absorb in that time.

Water fern showed smaller decreases in N water concentration, with a maximum decrease of 26%. This lesser removal of N can probably be attributed to its ability to fix N from the atmosphere, thus reducing its N demand from the water (Forni *et al*. 2001). Furthermore, it could be that water fern suffered from P deficiency. Several water fern individuals in almost all containers showed a red color. This is a symptom of, among other things, P deficiency (Wagner 1997).

We hypothesized that N concentration decreases would be more pronounced for the combination treatment than for either duckweed or water fern alone, due to hypothesized synergistic effects. In this experiment, the combination of the two species instead showed an intermediate decrease, between that of duckweed and water fern, decreasing N up to 77%. Even so, synergistic effects are suggested by the fact that the N decreases in the combination treatment were, on average, 11% greater than the theoretical decrease expected from the average N removal of the two species cultivated alone (Table 1). As with duckweed, the combination treatment shows a trend of greater N removal in lower concentration water environments. Again, this could have resulted from a limit in the absolute amount of nutrients able to be removed by plants. From visual observation, it appears that plant die-off of duckweed and water fern played a major role in increased nitrogen concentrations for the combination treatment relative to the control in the 0% (DI water) environment. The plants in this environment simply did not have enough nutrients to sustain growth, and some plants died. Plant decomposition releases the nutrients contained in the plant biomass, thus increasing N concentration.

We expected that a similar removal pattern as observed for N would occur with phosphorus. Interestingly, phosphorus did not follow these patterns. Instead, there is no discernable trend in P removal (Figure 2).

The marked increase in net phosphorus concentration in the 0% water environments for all treatments is considered to be a result of plant decomposition. Increases in phosphorus relative to controls occurred in all treatments in the 75% water environment. Decomposition of plants is not concluded to be a cause for this increase because plants in these water environments appeared healthy. It is unclear what might have caused this increase in phosphorus. Consistent decreases in phosphorus across treatments occurred, however, in the 25% and 100% water environments.

Given that phosphorus often limits growth of duckweed and water fern (Wagner 1997; Lumpkin and Plucknett 1980), and that levels of P below 930 ug/L have been found to be stressful for water fern (Wagner 1997). Duckweed was shown to be able to survive at P levels as low as 10 ug/L (Song *et al*. 2009). It is surprising that phosphorus was not efficiently removed from the water for incorporation into phosphorus-demanding plants.

One explanation for this is that the plants, while removing N from their environments, were not actually growing. In this case it would not necessarily be surprising to find a lack of P removal from the water. Two possibilities could account for N removal with simultaneous absence of any plant growth. Duckweed is known to accumulate N beyond what is needed for physical growth of the plant. This luxury consumption of N allows the plant to store its excess N as protein, accounting for the plant’s high protein content. If plants were performing luxury consumption during this experiment, that would account for the observed decrease of N in the water. The second possibility is that N could be being removed from the water not from the plants but from denitrifying bacteria living on the surface of the plant fronds. Nitrogen compounds would, in this case, be removed from the water and converted to atmospheric N_2.

This would allow N removal to occur with no associated plant growth, which, in turn, could explain the lack of P removal by plants.

The combination of decreases in water N with little change in P results in an altered N:P ratio in the water. The ratio of nitrogen to phosphorus in a water body has implications for ecosystems and organisms (Bulgakov and Levich). Duckweed and combination treatments showed a decrease in N:P ratio (Figure 3). Duckweed preferentially took up N, thereby reducing the N concentration. The N:P reduction for the combination was intermediate between the reductions for duckweed and water fern.

The shift in N:P ratio in the presence of plants has significance with regards to the characteristics of a water body's ecosystem. Unaltered environments, such as a water body that has not been impacted by humans, have typical N:P ratios, which change with anthropogenic pollutant inputs. If we see plants shifting the N:P ratio of their environment in the direction of the "natural" value, we see them changing that environment to resemble more closely its pre-altered condition. In our study, the N:P molar ratio for the duckweed treatments were reduced (almost consistently, with the exception of the 100% water environment) from upwards of 140:1 to less than 35:1. The latter ratio falls in the range of river water (Sterner and Elser 2002). General leafy plant biomass N:P ratios cluster around 6:1 to 18:1 (Downing and McCauley 1992). It has been determined that there the N:P ratios of marine plant life are consistent globally, and that this N:P ratio coincides with that of many marine water environments. The similarity and commonality of this ratio suggests that the plant life may play a role in regulating the nutrient regime of its environment. In our study we see duckweed, in particular, reducing the N:P ratio to a value closer to its own. This may suggest its ability to modify its environment.

All water environments started with N concentrations that fell within the eutrophic range, except the DI environment, which was oligotrophic and the 25% environment which was mesotrophic. For nitrogen, water is classified as oligotrophic when levels are below 0.35 mg/L, mesotrophic when levels are between 0.35 mg/L and 0.65 mg/L, and eutrophic when they are greater than 0.65 mg/L. For P, the DI, 25%, and 50% environments fell within the oligotrophic range, the 75% and 100% environments fell within the mesotrophic range, and only the elevated concentrations could be classified as eutrophic. For phosphorus, water is oligotrophic when levels are below 10 ug/L, mesotrophic when levels are between 10 ug/L and 30 ug/L, and eutrophic when levels are greater than 30 ug/L.

The elevated concentrations A and B present another part of the story. These initial water environments, with N and P concentrations characteristic of sewage sludge, may have been toxic to duckweed and water fern. No significant nutrient decreases were observed; changes in both N and P relative to the controls were slightly negative or positive. The N:P ratios for the elevated concentrations A and B were all not much lower than those of the controls (Figure 3). This can be attributed, in part, to the greater absolute amounts of nutrients in the higher nutrient water environments; plants taking up the same amount of nutrients in the higher concentrations caused less of an impact on the remaining N:P. In addition, plant die-off might have contributed to obscuring nutrient removal that may have occurred. In all elevated nutrient environments, duckweed began to die, while water fern showed evidence of chlorosis and plant reddening, both of which are symptoms of plant stress. Dead plants removed no nutrients, and also contributed some to the environment through decomposition.

It is possible that plants cultivated in the elevated A and B concentrations suffered nutrient or pH toxicity. For these water environments, the initial N and P concentrations used in the present study fall at the high end of nutrient ranges used in similar studies (Cheng *et al*. 2001, Vermaat and Hanif 1998), supporting the explanation of toxicity. Root lengths measured in the present study decreased significantly in the elevated concentrations (Figure 4). Additionally, final pH levels clustered around and below 4, levels which can be toxic to plants directly. The pH range at which duckweed growth is not inhibited is between 5 and 8 (Caicedo *et al*. 2000). Water fern can survive between pH values of 3.5 and 10 but has optimum growth at pH values between 4.5 and 7. One potential explanation for low pH levels in elevated nutrient environments, with or without plants added, is the significant additions of NH_4Cl and KH_2PO_4. These cause dissociation of H+ ions, which causes decreased pH values.

In this study, cultivation of duckweed and water fern yielded a net removal of N in conditions in which N was present in relatively higher concentrations, while P was present in low concentrations. The success of duckweed, and moderate success of water fern, in surviving and removing nitrogen from water with low levels of phosphorus has direct implications for the field site near the Skidmore horse stables. The water at the Stables site, with TN concentration of 1.59 mg/L and a TP concentration of 11.9 ug/L, can be categorized as eutrophic in terms of N and at the boundary of oligotrophic and mesotrophic in terms of P. Our results suggest that duckweed might be well-suited to remediate this pond, given its ability to survive and also remove N in low-P environments that could be stressful to other plants. However, the synergistic effects observed were not as significant as expected, suggesting that water fern might not be an ideal candidate for phytoremediation in combination with duckweed at this site.

Furthermore, the fact that nutrient removal decreased in the elevated nutrient water environments A and B suggests a diminished ability of these plants to survive in those conditions. During certain times of the year with high runoff, additional nutrients may enter the pond via runoff. This could shift the nutrient concentrations of the pond to higher levels, which may or may not approach levels toxic to duckweed and water fern growth.

Root length patterns of plants grown in 0% to 100% concentrations show an increase in root length, peaking at 75% and leveling off at 100%. This trend was the same regardless of species-specific maximum root length. It could be the case that plants will continue to lengthen their roots as long as nutrients are present in adequate amounts. In this case the leveling off of root length at concentrations higher than 75% could suggest that in the 100% water environment plants might have begun to shift their metabolic activities to reproduction or other activities besides root growth. Further research would be necessary to make more confident determinations about this issue.

Further investigation of nutrient removal abilities of duckweed and water fern would be essential to understanding the processes guiding the N and P fluxes observed in this study, and would be helpful in identifying the best phytoremediation strategy for the Stables pond. In particular, studies of plant growth, rather than nutrient removal alone, would help quantify the effect luxury consumption of N and possible denitrification by bacteria. To address potential P deficiency, especially of water fern, similar N:P ratios could be studied but at higher absolute levels of P. Furthermore, it would be informative to collect water samples for nutrient testing at multiple points throughout the experiment to observe changes and determine when nutrient removal rates might stabilize. Additionally, to paint a more accurate picture of N and P transformations through their different forms it could be useful to examine not only TN and TP but also the concentrations of specific N and P species. Additionally, to inform phytoremediation efforts at the Stables site, studies would need to be conducted using water environments representative of the Stables N and P concentrations, light photoperiods, pH environments, and concentrations of other micronutrients, during different times of the year. Such studies would be useful not only for the Stables site but also as a model for phytoremediation efforts of other water bodies with similar nutrient regimes.

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Acknowledgements

We would like to thank Josh Ness, our advisor, and also Karen Kellogg, for guiding us through this process. Much thanks to Cathy Gibson for all of her water and nutrient expertise, and also to Cathy Domozych, David Domozych, and Sue Van Hook for help in their respective fields. Thanks to the Student Opportunity Fund and the ES Program for their financial sponsorship that allowed us to conduct extensive water testing. We would also like to thank the folks at Darrin Freshwater Institute for helping us out with water testing. Our thanks go also to our Capstone friends for their excellent feedback in reviewing initial drafts of this project.

Tables and Figures

Table 1. Comparison of Expected and Observed Final N Concentrations in Combination Treatment

Water environment	Observed N (mg/L)	Expected N (mgL)	Observed N – Expected N (mg/L)	Proportion Difference
0	0.18	0.16	0.02	0.13
25	0.21	0.445	-0.235	-0.53
50	0.65	0.87	-0.22	-0.25
75	1.33	1.175	0.155	0.13
100	1.97	2.02	-0.05	-0.02
A	167	190.5	-23.5	-0.12
B	300	337.5	-37.5	-0.11
			Average	-0.11
			Standard error	0.09

Table 2. Comparison of Expected and Observed Final P Concentrations in Combination Treatment

Water environment	Observed P (ug/L)	Expected P (ugL)	Observed P-Expected P (ug/L)	Proportion Difference
0	21.5	15.25	6.25	0.410
25	13.3	12.65	0.65	0.051
50	19.5	16.8	2.7	0.161
75	41.5	30.55	10.95	0.358
100	29.7	34.8	-5.1	-0.147
A	9098	21588.5	-12490.5	-0.579
B	26904	25790	1114	0.043
			Average	0.04
			Standard error	0.13

Table 3. pH of Water Environments of Control and Treatment Groups

	Water environment						
	0%	25%	50%	75%	100%	A	B
Control	7.06	6.50	6.61	6.56	7.42	3.57	3.70
Duckweed	4.93	5.49	6.03	6.39	6.64	3.79	3.66
Water Fern	4.79	5.15	5.92	6.27	6.53	3.18	3.23
Combination	4.93	5.53	6.15	6.36	6.64	3.41	4.39

Figure 1. Change in N Represented by the Ratio of Final N in the Remediated Water to the Corresponding Final N in the Control, in Each Water Environment

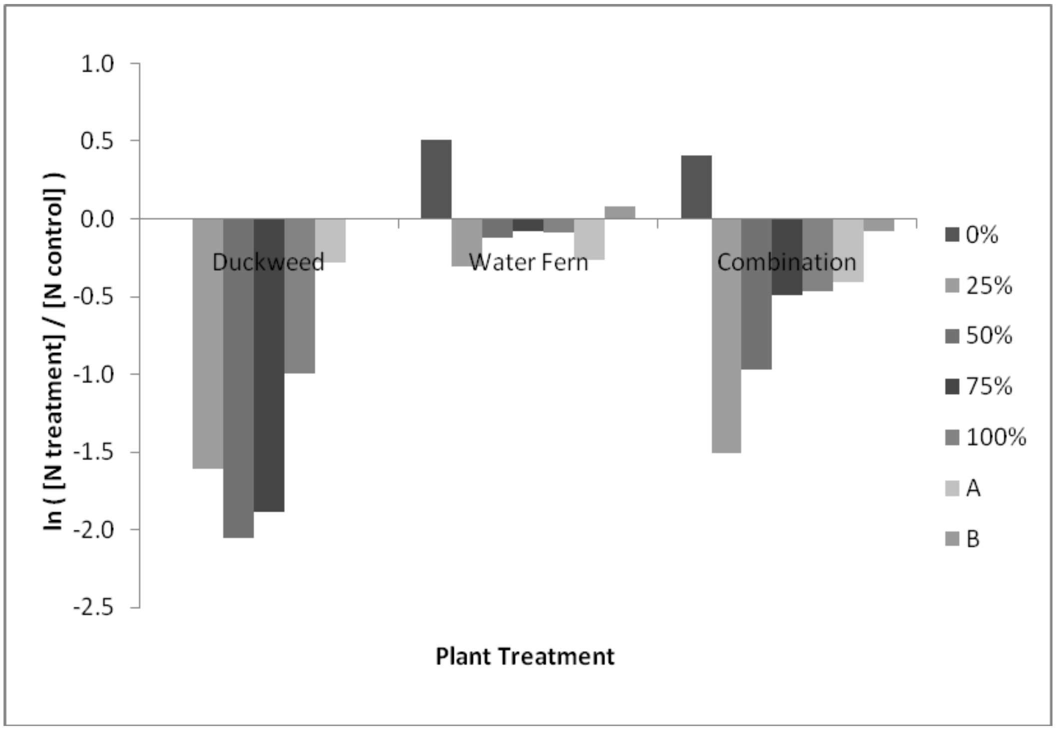

Figure 2. Change in P Represented by the Ratio of Final P in the Remediated Water to the Corresponding Final P in the Control, in Each Water Environment

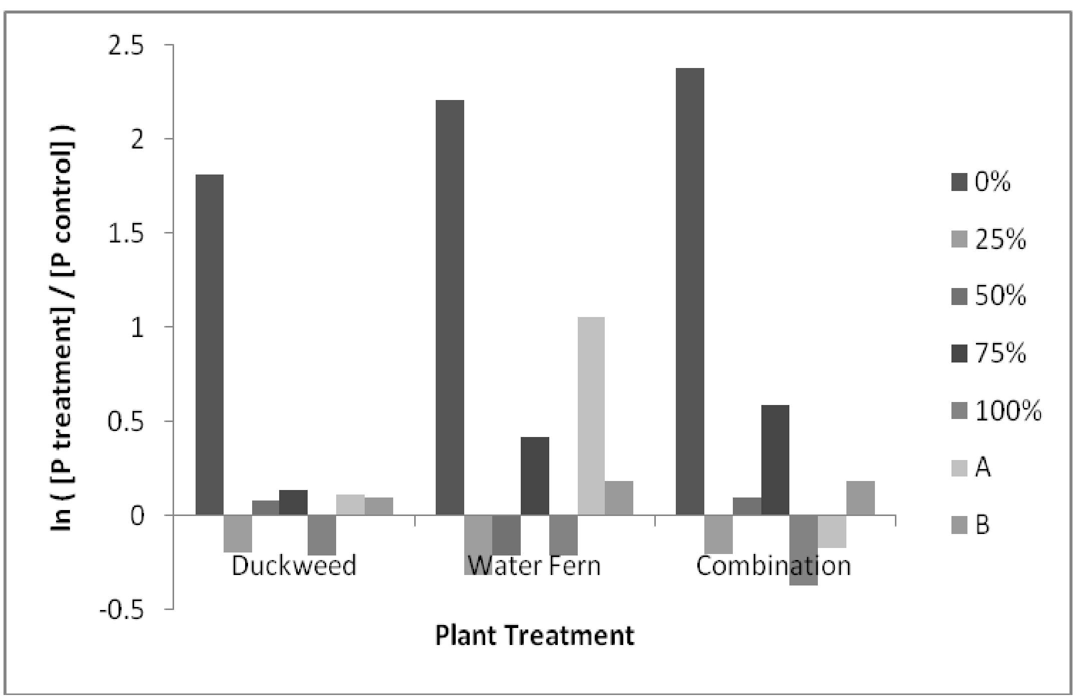

Figure 3. Depression of Average N:P Ratios

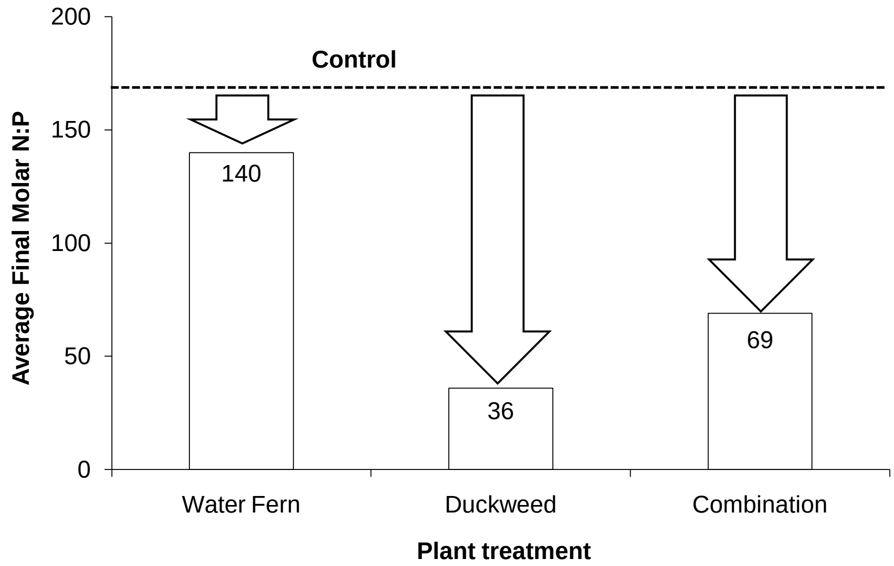

Figure 4. Average Length of Longest Root

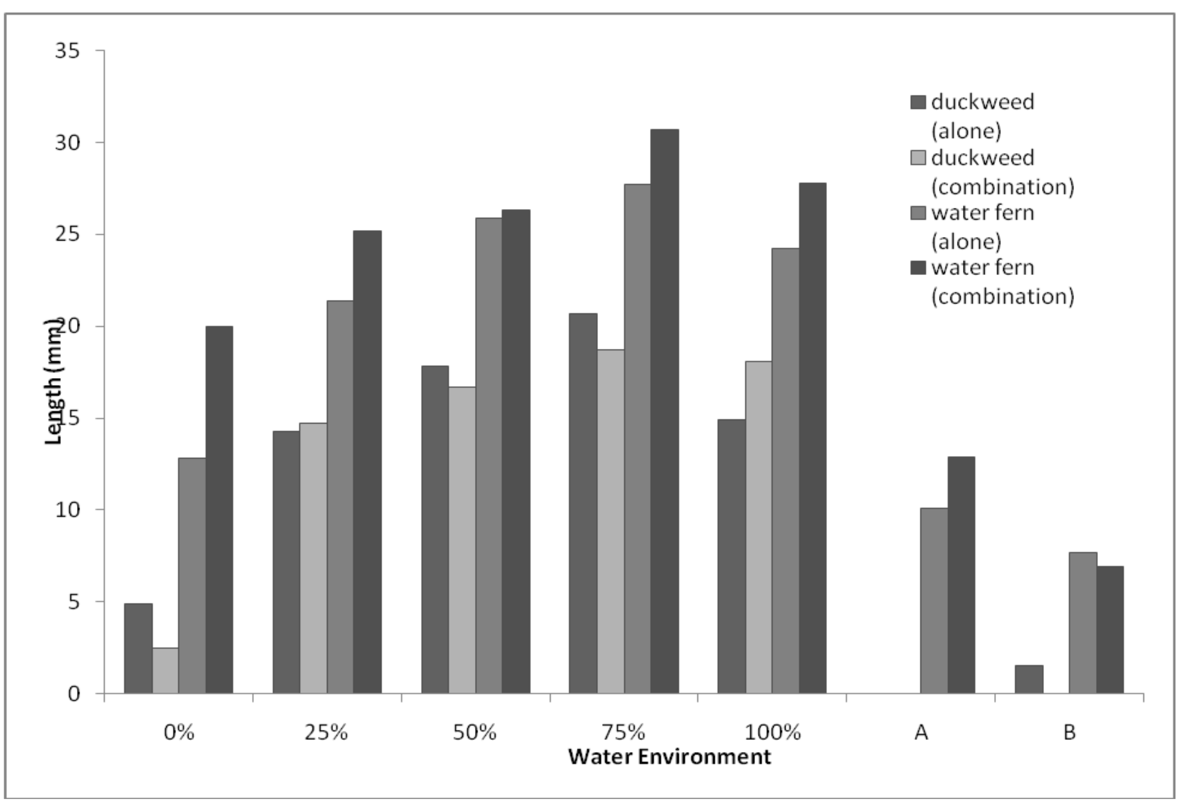

Figure 5. Standardized Maximum Root Length