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The Performance of Simple Artificial Floating Wetland Communities and Their Effects on Aquatic Nutrient Levels and Algal Abundance

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THE PERFORMANCE OF SIMPLE ARTIFICIAL FLOATING WETLAND COMMUNITIES AND THEIR EFFECTS ON AQUATIC NUTRIENT LEVELS AND ALGAL ABUNDANCE

by

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CERTIFICATE OF APPROVAL

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ABSTRACT

Harmful algal blooms are exponential increases in autotrophic microorganisms that proliferate in such a way that the surrounding environment, the local economy and the health of regional populations are negatively affected. Among the causes of these blooms are anthropogenic inputs of excess nitrogen and phosphorus into the environment through overfertilization. Floating treatment wetlands (FTW) have emerged as a novel method of reducing the negative impacts of these nutrient inputs by using artificial rafts to float normally emergent wetland plants on the surface of water bodies to assimilate excess nutrients. Because their use is so new, only limited research has been performed on their effectiveness. This mesocosm-level study evaluated the performance of a FTW consisting of a community of yellow canna (*Canna flaccida*), blue flag iris (*Iris hexagona*) and bulltongue arrowhead (*Saggittaria lancifolia*) in simulated stormwater of varying nitrogen and phosphorus concentrations. The community of plants displayed nitrogen limitation, while the cyanobacteria-dominated algal community that developed displayed phosphorus limitation, leading to the conclusion that in order for this community of macrophytes to limit algal growth, nitrogen must be present to support their growth and concurrent assimilation of the algae-limiting nutrient phosphorus. Canna and iris were found to significantly outperform arrowhead in terms of biomass gains. The study also showed that the size of the plants may be of great importance in the ability of FTWs to limit algal development. Despite the fact that the community of plants in this study were unable to limit the development of algae, the use of FTWs remains promising and further research should be done to continue to enhance our understanding of their strengths and weaknesses.

INTRODUCTION

Harmful algal blooms (HABs) are exponential increases in autotrophic microorganisms that proliferate in such a way that the surrounding environment is negatively affected (Heisler et al. 2008). These deleterious impacts can come in the form of chemical toxins, as well as development of hypoxia/anoxia, light deprivation, and indiscriminate killing of aquatic life after algal blooms reach high densities. Excessive, sudden proliferations of cyanobacteria, dinoflagellates, diatoms, green algae, or a number of other organisms can form HABs. Algal blooms affect the environment, the local economy and the health of regional populations. Many areas of Florida have been affected by HABs, including the Indian River Lagoon (Phlips et al. 2010), Florida Bay (Goleski et al. 2010), Tampa Bay (Badylak et al. 2007) and the Lower St. Johns River (Malecki et al. 2004).

During blooms, dense algal populations shadow the benthos, reduce light levels and cause the death of submerged aquatic vegetation (Hauxwell et al. 2004, Malecki et al. 2004, Oakey et al. 2004). Death of the vegetation reduces the dissolved oxygen availability because of the lowered photosynthetic rates. As the bloom grows, it depletes available nutrients, and the death of the algal community begins. Dead algae sink to benthos causing a bloom of saprophytic bacteria. As those bacteria decompose the algae, they consume most of the available dissolved oxygen (Paerl et al. 1998, Bricker et al. 1999). The loss of available dissolved oxygen results in massive fish kills and an anoxic dead zone. Moreover, HABs may also produce toxins that are harmful to other organisms, such as the neurotoxic diatom *Pseudo-nitzcschia* spp., the hepatotoxic *Microsystis* spp. of blue-green algae, and the neurotoxic dinoflagellate *Karenia brevis* (Cronberg et al. 1999, Smayda 1997, Heisler et al. 2008, Lelong et al. 2012, Pierce et al. 2003).

In addition to the biological harm caused by HABs, these blooms have a significant effect on many facets of the economy of the United States including public health, commercial fisheries, recreation/tourism as well as having monitoring/management costs. It has been estimated that harmful algal blooms can have an annual economic impact of over $620,000,000.00 (estimate from 1987-1992 period, adjusted for inflation to 2014 dollars; Anderson et al. 2000). Toxic blooms of cyanobacteria can affect human health by reducing the quality of potable drinking water (Fisher et al. 2009), and blooms of *Karenia brevis* may result in airborne toxins that can cause respiratory distress in local populations as well (Pierce et al. 2003). It is imperative, therefore, that these blooms are controlled or at least ameliorated.

High nutrient levels and increased population and development have been shown to directly correlate with increased occurrence and intensities of HABs (Phlips et al. 2006, Heisler et al. 2008). For example, in Puget Sound (Washington), the average decadal maximum amount of paralytic shellfish toxin (average annual maximum toxin level for each decade), which has been monitored since the 1950's, increased from 500 to 5000 (a ten-fold increase) as the population rose from 1 million to 3.5 million (only a 3.5-fold increase; Trainer et al. 2003). The use of fertilizer has also increased, and this increase in its use corresponds closely with the number of HABs. Indiscriminate and overfertilization causes the nutrient levels in waterways to increase as stormwater runoff flushes the excess fertilizers into them. It has even been hypothesized that nutrient-rich groundwater can leach into marine waters and trigger blooms in the Gulf of Mexico (Hu et al. 2006). The “Dead Zone” at the mouth of the Mississippi River, caused by eutrophication from agricultural fertilizers used in the Midwest, has been well documented (Dagg and Breed 2003). Strong correlations between nitrogen-loading (2.5-fold increase from 1940’s-1990’s) and long-term chlorophyll levels in Chesapeake Bay have been shown as well (Kemp et al. 2005).

It is not simply the amount of nutrients in the water that influences HABs, but also their proportions (Heil et al. 2007). While low nitrogen:phosphorus (N:P) ratios resulting from P-loading eutrophication from concentrated animal operations has been associated with dinoflagellate blooms on the West Florida Shelf of the Gulf of Mexico (in the vicinity of Tampa Bay), eutrophication composed of moderate N:P ratios just to the south result in dominant blooms of cyanobacteria (vic. Charlotte Bay), and eutrophication displaying N:P ratios greater than 48, found even further south, result in diatom-dominated blooms. It is clear from this that the composition of the nutrient loading is as important as the quantity, and efforts must be made to reduce eutrophication of any type.

Removal of nutrients has been shown in many cases to significantly reduce HABs in freshwater (e.g. Lake Washington), and in estuarine environments (e.g. Mumford Cove, CT) (Edmondson 1970, Vaudrey et al. 2010). One way to remove nutrients from water bodies and prevent their transmission downstream is to take advantage of stormwater control structures, such as wet detention ponds, which temporarily store stormwater runoff (SJRWMD 2012). Historically, littoral macrophytes have been planted at the edges of stormwater ponds as a best management practice to help manage high nutrient levels in stormwater (DB Environmental 2005). However, one characteristic of stormwater detention ponds is that the water level tends to fluctuate dramatically. As the planted macrophytes are fixed in position, they will be submerged in high water conditions and above the water line in low water conditions.

Floating treatment wetlands (FTWs) enable those normally littoral macrophytes to float in mats on the surface of the water and change elevation with the water level. The root systems of these plants are therefore always in the water, and the plants can continuously take up nutrients from the water. In addition, floating the plants in lieu of planting them on the shores increases the surface area of the plant's roots that are exposed to the nutrient-rich water (Stewart et al. 2008). Much of the nutrient-assimilating strength of FTWs comes from the interaction between the plants and the microbes that live on and among the plants and mats, and floating the plants in the water as opposed to planting them on the shore of the pond provides much more surface area for processes such as nitrification, denitrification and phosphorus adsorption to take place (Tanner and Headley 2011, Wang and Sample 2014). Other advantages of floating treatment wetlands include the fact that they do not require modification to existing detention ponds in order to be employed, they do not require additional land area for treatment, and they do not take away from the requisite storage volume of detention ponds (Winston et al. 2013). As opposed to constructed treatment wetlands, in which the plants senesce and reintroduce the assimilated nutrients back into the system, FTWs provide for easy harvesting of the plants so that they can be easily removed and composted in another location for removal of the assimilated nutrients from the system

(White 2008). Finally, FTWs are aesthetically pleasing so they can be employed in public places where other unsightly equipment might not be feasible. Drawbacks of the FTW systems include some initial cost and annual maintenance, as the plants need to be harvested to prevent the nutrients from re-entering the pond when the plants senesce. If employed in excess, the system would shadow the benthos and could prevent growth of aquatic plants; while this could be managed by moving the system periodically, but that adds to the maintenance costs.

There are a number of types of mats including buoyant fibrous matrix mats and closed-cell foam mats. Each has its own benefits and drawbacks. The fibrous matrix mats provide better environment for beneficial microbes, while the foam mats provide for customization of shape (for aesthetics), easier harvesting and reuse of the mats. FTWs can be used to control nutrient levels in stormwater, wastewater and agricultural runoff, and can take up heavy metals as well (Headley and Tanner 2006, Ladislas et al. 2013).

There have been relatively few studies performed on floating treatment wetlands (Chen et al. 2009, Tanner and Headley 2011, Winston et al. 2013). Chen et al. (2009) tested the nitrogen and phosphorus uptake ability of individual plant species while growing the plants in a nutrient recycling system. They found that Australia canna (*Canna generalis*) outperformed Golden Fleece iris (*Iris pseudacorus*), arrow arum (*Peltandra virginica*), dwarf papyrus (*Cyperus haspan*), pickerelweed (*Pontederia cordata*), bulltongue arrowhead (*Sagittaria lancifolia*) and calla lily (*Zantedeschia aethiopica*) in removing nitrogen and phosphorus directly from the water column. However this study only focused on nutrient assimilation by individual species. As floating treatment wetlands typically consist of a community of plants rather than a monoculture, it is important to assess the performance of plants in that polyculture. As well, that study did not assess the impacts of the plants on the development of algae.

Chang, et al. (2012, 2013) studied the effect of FTWs on water nutrient levels at multiple scales including microcosm, mesocosm, and full-lake. They showed that FTWs composed of soft rush (*Juncus effusus*) and pickerelweed (*Pontederia cordata*), deployed over 8.7% of a wet detention pond's surface, were capable of removing over 15% of total nitrogen, 20% of nitrate/nitrite-nitrogen, 51% of ammonia-nitrogen and 47% of total phosphorus. However, their study did not assess the growth of algae. In a separate study, a FTW consisting of a community of *Juncus effusus* and *Canna flaccida* was shown to reduce effluent nitrogen and phosphorus concentrations in a simulated stormwater pond by 58%-83% and 45%-75%, respectively, with the *Juncus* accumulating both more nitrogen and more phosphorus than the *Canna* (White and Cousins 2013). Winston et al. (2013) performed a field study which involved monitoring nutrient levels in two stormwater detention ponds both before and after application of a floating treatment wetland community. They found that while the FTW was able to improve the nutrient removal capability of the detention pond, the reduction was not always significant.

It has been suggested that species mixtures may be even more effective than monocultures at removing excess nutrients, and more research needs to be done to evaluate this proposal (White 2008). As well, further research needs to be done to evaluate the performance of individual species and communities under varying nutrient loading rates (White and Cousins 2013).

The goals of the study were to investigate the response of a three-species floating treatment wetland community to stormwater of varying nutrient compositions, that community's potential effectiveness in removing excess nutrients from the stormwater and its potential ability to limit algae growth. The project will also provide the opportunity to investigate how varying nutrient levels impact the development of algal communities.

METHODS

The project was conducted in two phases, both in the greenhouse on the fourth floor roof of the Biology Building at The University of North Florida. The first phase was designed to assess the response of the floating treatment wetland community to water of varying nutrient compositions. The second phase was designed to assess the ability of the plants themselves to reduce nutrient levels and to limit the development of algae under high nutrient loading conditions. Each phase was designed to assess the nutrient removal capability of the community, as well as the development of the algal community. Phase one was conducted from 6 September 2013 thru 31 December 2013. Phase two was conducted from 22 April 2014 thru 2 June 2014.

Phase I involved fifteen 1.15 m diameter mesocosms (0.17 m depth; 123 L total capacity) consisting of five nutrient treatments with three replicates of each (Figures 1 & 2). Each pool contained a floating treatment wetland community that consisted of two individuals of each of three species of common and readily available ornamental wetland plants (six total individuals). Plants that formed the community under investigation in this study included yellow canna (*Canna flaccida*), blue flag iris (*Iris hexagona*) and bulltongue arrowhead (*Saggittaria lancifolia*). All are wetland plants indigenous to Florida (Tobe et al. 1998). All have been used and studied individually in conjunction with FTWs (i.e. Chen 2009, Chang 2012, White and Cousins 2013). The plants are ornamental, and the supplier (Beeman's Nursery©, New Smyrna Beach, FL) stated that these were some of the most used ornamental plants in their Beemat© floating treatment wetlands (Beeman F, 2013, personal communication). In addition, the SJRWMD recommends these plants for aquascaping because they are slow growers, do not require tremendous maintenance and will not spread uncontrollably, overtake the surrounding area and stifle diversity (SJRWMD 2012). Two individuals of each of these three species were floated in each pool, for a total of six plants in each.

Five nutrient treatments were established as follows: high nitrogen + high phosphorus (HNHP), low nitrogen + low phosphorus (LNLP), high nitrogen + low phosphorus (HNLP), low nitrogen + high phosphorus (LNHP), low nitrogen + low phosphorus (LNLP) and zero nitrogen + zero phosphorus (0N0P) (Table 1). The levels of nitrogen and phosphorus were established from unpublished data provided by the Florida Department of Environmental Protection related to its development of the Numeric Nutrient Criteria for Florida's Waters that recently went into effect (Frydenborg R, personal communication, 26 July 2013). That data came from one hundred lakes throughout Florida which had been sampled in each of the four seasons in one year, for a total of 291 lake*years. The LN and LP treatment levels were set as the averages of the total nitrogen (TN) & total phosphorus (TP) values for these lakes (1.27 mg L^{-1} & 0.08 mg L^{-1}, respectively), while the HN and HP treatments were set three standard deviations above those levels (3.69 mg L^{-1} & 0.46 mg L^{-1}, respectively). As it was not feasible to transport actual stormwater to the greenhouse for use, the water for the pools was municipal water that was allowed to off-gas for 24 hours prior to the nutrients and any plants being added. Relative amounts of ammonium-nitrogen and nitrate-nitrogen were set at 1:1 based on the findings of a similar ratio in a review of seven colored lakes along the Upper St. Johns River, by the St. Johns River Water Management District (SJRWMD) (Fisher 2009). Nutrient levels and ratios similar to the HNHP treatment employed in this experiment have been used in previous studies when simulating high-nutrient stormwater (i.e. DB Environmental 2005). Nitrogen was provided in the form of ammonium nitrate (NH_4NO_3) and phosphorus was provided in the form of monopotassium phosphate (KH_2PO_4), both common inorganic fertilizers. Nutrients were provided in an initial pulse, and no additional nutrients were provided over the course of the study. Mesocosms were distributed in a randomized block design with each of the five nutrient treatments randomly distributed in each of three single rows. Water level was maintained to within 2.5 cm of the initial volume throughout the study by supplementing the mesocosms with local municipal water.

The floating treatment wetland system that was employed was the Beemat© system, produced in New Smyrna Beach, FL. Each system consisted of a 1 cm thick closed-cell foam mat that provided buoyancy, with 7.5 cm diameter holes spaced 12 cm apart in which specially designed perforated cups could be installed to secure the plants. Perforations in the cups both allowed water to enter the cup and allowed the roots of the plant to extend unimpeded outside the cup. Plants were secured in the cups by first wrapping the plant in coconut fiber to provide substrate, then securing that assembly in the cup with a specially designed clip, which also acted to secure the cup in the mat.

Mats were cut such that each individual mat held three plants in a triangular arrangement. Each of these individual mats held one each of the selected species, and two of these mat-plant assemblies were allowed to float freely in each mesocosm.

Phase II was designed to assess the ability of the plants themselves to reduce nutrient levels and to limit the development of algae under high nutrient loading conditions (Figures 3 & 4). It consisted of a single nutrient treatment (HNHP) in nine of the same mesocosms used in phase one. A complete floating treatment wetland community (the same community used in phase one) was floated in three of the mesocosms (“planted”). In three other mesocosms, the complete system *less plants* was floated (mats, cups, coconut fiber) to act as the control (“unplanted”). In the final three mesocosms, only nutrients were added (“blank”). The treatments were distributed in a completely randomized design. Phase II was conducted from 22 April 2014 thru 2 June 2014.

Initial and final water testing was conducted with a LaMotte SMART 2 Colorimeter to measure the NO_3^--N, NH_x-N & PO_4^{3-} levels. Nitrate-nitrogen was tested using the cadmium reduction method, ammonia/ammonium-nitrogen was tested using the Nesslerization method and phosphate was tested using the ascorbic acid reduction method. All tests had a detection range of 0 - 3.0 mg L^{-1}, and all test methods have been approved by the U.S. Environmental Protection Agency (40 CFR 136).

To assess the relative success of each species of plant in the various nutrient environments, initial and final wet biomass was measured for each plant. Because plants were acquired with their roots having already grown into the coconut fiber substrate, it was not possible to separate the plant from the fiber to measure the initial biomass of the plant alone without damaging the root system. As such, both initial and final biomass measurements included the coconut fiber substrate. The units were allowed to drain for approximately 12 hours prior to weighing to eliminate as much water as possible.

A number of analyses were used to assess algal development, including algal cell counts, chlorophyll-*a* levels and ash-free dry mass. Samples of algae for cell count were taken by denuding a randomly selected 21 cm^2 area of the bottom of the each mesocosm (one area in each pool, eliminating any area influenced by FTW shadowing). Samples were preserved in 2.5% glutaraldehyde and refrigerated. Algae were identified and quantified at the group level (bacillariophyta, chlorophyta and cyanobacteria). Biovolume was quantified by applying known geometric sizes and shapes to the cell count of each group (Wetzel and Likens 1991, Hillebrand 1999). Samples for determination of ash-free dry mass (afdm) were taken by denuding all algae from each mesocosm, suspending the denuded algae in the full volume of water in each pool, and drawing a 150 mL sample of this suspension. Those samples were immediately vacuum filtered onto glass fiber filters and dried at 70°C until a consistent mass was achieved (dry mass). Dry samples were then combusted at 500°C for two hours and reweighed (ash mass). Ash-free dry mass was calculated as the difference between the dry and ash mass (Zhu and Lee 1997). Samples for chl-*a* determination were taken at the same time as samples for ash-free dry mass were taken. Algae were mechanically suspended in the mesocosm water samples and chl-*a* measurements of whole, non-acidified cells were immediately taken using a Turner TD-700 Fluorometer (Wetzel and Likens 1991). Biovolume, ash-free dry mass and chlorophyll-*a* were all reported as per [pool] surface area concentrations (cm^{-2}).

In Phase I, the performance of the floating treatment wetland community (in terms of total wet biomass increase) was compared between each nutrient treatment level. Performance of individual species (biomass increase) between nutrient treatment levels were also compared, as were comparisons between species across all nutrient treatments. The ability of the FTW to reduce nutrient concentration at each nutrient treatment level was compared as well. Algal development (ash-free dry mass, chlorophyll-*a*, cell-count data) was also compared across nutrient treatment levels. All of these comparisons were made using one-way ANOVA (with Tukey's HSD).

In Phase II, the effect of the plants in the floating treatment wetland on the nutrient levels was assessed by comparing nutrient levels in the three treatments (planted, unplanted and blank) by one-way ANOVA (with Tukey's HSD). The development of algae (ash-free dry mass, chlorophyll-*a*, biovolume) was assessed by comparing the development of algae in the planted treatments with the development of algae in the non-planted control treatments through a t-test (there was no algae to assess in the blank treatments). All statistical tests were performed using IBM SPSS Statistics v.22.

RESULTS

PHASE I

Nutrients – With respect to final nutrient concentrations, no significant differences were found in NH_x-N ($F_{4,8}$ = 1.299, P = 0.348, log transformed), NO_3^--N ($F_{4,8}$ = 0.973, P = 0.473) or PO_4^{3-}-P ($F_{4,8}$ = 2.378, P = 0.138) concentrations of any treatment. Across all treatments, final nutrient levels were: NH_x-N (0.13 ± 0.02 mg L^{-1}), NO_3^--N

(0.10 ± 0.01 mg L^{-1}) and PO_4^{3-}-P (0.10 ± 0.01 mg L^{-1}) (Table 2; data reported throughout is mean ± sem, unless otherwise noted). In the high nitrogen treatments, 94.17% (±0.79%) of available NH_x-N and 93.50% (±0.61%) of available NO_3^--N was assimilated, and in the low nitrogen treatments 78.64% (±2.54%) of available NH_x-N and 78.50% (±3.11%) of available NO_3^--N was assimilated. In the high phosphorus treatments, 94.7% (±0.67%) of available PO_4^{3-}-P was assimilated, while in the low phosphorus treatments 61.33% (±5.35%) of available PO_4^{3-}-P was assimilated. While the percentage data make it appear that the higher nutrient treatments resulted in a more complete reduction of nutrients, those higher percentages were simply a result of the higher initial concentration (e.g. a reduction from 10 mg L^{-1} to 1 mg L^{-1} is a 90% reduction, while a reduction from 3 mg L^{-1} to 1 mg L^{-1} is "only" a 33% reduction); all treatments, whether initially high- or low-concentration, ended up at statistically similar concentrations.

Community Biomass - When percent biomass increase of whole floating treatment wetland communities (combined growth of all plants in each replicate) was compared across nutrient treatment levels, the plant communities in the high nitrogen treatments showed a greater percent increase in biomass, but those increases were not significantly greater than the increases seen in the other treatments ($F_{4,8} = 2.255$, $P = 0.152$) (Figure 5). The biomass of HNHP and HNLP communities increased by 16.27% (±4.05%) and 19.24% (±2.34%), respectively, while the LNHP and LNLP communities only increased by 6.55% (±1.77%) and 3.69% (±3.76%). The communities in the ONOP treatment also increased in biomass by 10.00% (±6.22%), and their increase was between that of the high and low nitrogen treatments.

Interspecific Plant Comparison - When comparing the performance of individual plant species across all treatments to determine which are generally best suited for employment in a floating treatment wetland, significant differences were found ($F_{2,85}$ = 10.151, P < 0.001) (Figure 6). The metric of percent biomass was used to level the comparison between species because significant differences were observed in initial biomass between species ($F_{2,87}$ = 4.320, P = 0.016). A Tukey's post-hoc test showed that arrowhead performed significantly worse than both canna and iris in terms of percent biomass increase. The arrowhead showed a percent biomass increase of only 2.03% (±2.70%), while the canna and iris showed much greater mean percent biomass increases of 19.10% (±2.78%) and 14.67% (±2.78%), respectively. This low result from the arrowhead correlates with the high number of individuals (12) that experienced decreases in biomass, as compared with canna (2) and iris (4). In terms of absolute biomass, canna showed the greatest increase (15.53 ± 2.68 g), followed by iris (9.03 ± 1.49 g) and finally arrowhead (0.90 ± 1.88 g).

Intraspecific Plant Comparison - Within species, comparisons were made of absolute biomass changes between nutrient treatments. Prior to comparing biomass changes, the similarity of initial biomass (within species) between treatments was confirmed through one-way ANOVA. It was confirmed that there were no significant differences in initial arrowhead biomass between nutrient treatments ($F_{4,25}$ = 0.629, P = 0.646), and the same was true with canna ($F_{4,25}$ = 2.736, P = 0.051); homogeneity of variance could not be established for initial iris biomass, so a Kruskal-Wallis test was performed to confirm that initial biomass was uniform between treatments (H = 0.944, P > 0.05). As the initial biomass within each species was determined to be similar across all treatments, it was not used as a covariate. Canna was the only species which showed significant differences in biomass change between treatments ($F_{4,23}$ = 3.645, P = 0.019), with the high nitrogen treatments generally exhibiting a greater increase in biomass (Figure 7). Neither arrowhead ($F_{4,23}$ = 2.255, P = 0.094) (Figure 8) nor iris ($F_{4,25}$ = 1.082, P = 0.389) (Figure 9) showed significant differences in biomass change between nutrient treatments, although arrowhead experienced a mean biomass decrease in both low nitrogen treatments and those differences approached significance.

Algae - Significant differences were seen in algal ash-free dry mass (AFDM) between nutrient treatments ($F_{4,8}$ = 5.455, P = 0.020 [sqrt transformed]), with Tukey's post-hoc tests showing that both high phosphorus treatments developed significantly more algae than the HNLP and ONOP, and the LNLP treatment developed amounts similar to all treatments (Figure 10). The HNHP and LNHP treatments developed very similar amounts of algae in terms of AFDM, with means of 1.47 ± 0.05 mg cm^{-2} and 1.47 ± 0.17 mg cm^{-2}, respectively. The HNLP and ONOP treatments also developed very similar, albeit lower, amounts of algae, with means of 0.87 ± 0.06 mg cm^{-2} and 0.91 ± 0.15 mg cm^{-2}, respectively. The algae that developed in the LNLP treatment fell between these two groups, and was statistically similar to all treatments.

Patterns similar to the AFDW development were seen in final chlorophyll-*a* concentrations ($F_{4,8}$ = 4.414, P = 0.035 [log transformed]), although the significant groupings were not necessarily the same (Figure 11). In terms of statistical significance, the LNHP treatment (0.64 ± 0.27 µg cm^{-2}) showed a seven-fold increase in algae over the ONOP treatment (0.09 ± 0.04 µg cm^{-2}), with the other three treatments (HNHP, HNLP, LNLP) developing levels of algae between and statistically similar to both of these two treatments. In general, both ash-free dry weight and chlorophyll-*a* development was generally higher in the high phosphorus treatments than in the low and zero phosphorus treatments.

When algal development was measured in terms of total biovolume, significant differences were seen between groups ($F_{4,8}$ = 7.890, P = 0.007 [sqrt transformed]), but a different pattern was exhibited (Figure 12). In this case, Tukey's post-hoc tests showed that the high nitrogen treatments developed significantly more algal biovolume than the low and zero nitrogen treatments. Biovolume development in the HNHP treatment was 2.47 ± 0.86 mm^3 cm^{-2} and in the HNLP treatment it was 1.86 ± 0.60 mm^3 cm^{-2}. Much lower levels were seen in the LNHP (0.47 ± 0.21 mm^3 cm^{-2}), LNLP (0.22 ± 0.10 mm^3 cm^{-2}) and ONOP (0.18 ± 0.05 mm^3 cm^{-2}) treatments.

Algal community composition, determined by cell count, was dominated by cyanobacteria across all treatment levels, with only occasional appearances of chlorophyta (in one replicate each of LNHP and LNLP) and bacillariophyta (in one replicate each of LNHP and ONOP) at lower nutrient levels (Figure 13). Significant differences were found in the biovolume of cyanobacteria that developed between nutrient treatment levels ($F_{4,8}$ = 7.271, P = 0.009 [sqrt transformed]) (Figure 14). The high nitrogen treatments produced generally more cyanobacteria (HNHP = 2.47 ± 0.86 mm^3 cm^{-2}; HNLP = 1.86 ± 0.60 mm^3 cm^{-2}) than the low nitrogen treatments (LNHP = 0.403 ± 0.25 mm^3 cm^{-2}; LNLP = 0.22 ± 0.10 mm^3 cm^{-2}; ONOP = 0.16 ± 0.06 mm^3 cm^{-2}).

PHASE II

Nutrients - When final nutrient levels were compared between the three planting treatments in Phase II (planted, unplanted and blank), significant differences were found (Table 3). Final NO_3^--N levels were significantly ($F_{2,6}$ = 785.330, P < 0.001) lower in the planted (0.03 ± 0.01 mg L^{-1}) and unplanted (0.06 ± 0.02 mg L^{-1}) treatments than in the blank treatment (1.59 ± 0.05 mg L^{-1}) (Figure 15). While both the planted and unplanted treatments reduced the NO_3^--N concentration (by 98.67% ± 0.33% and 96.33% ± 1.20%, respectively) significantly more ($F_{2,6}$ = 4594.056, P < 0.001) than the blank treatment (1.67% ± 0.01%), no significant differences were found between the planted and unplanted treatments when compared with Tukey's post-hoc test. On the other hand, final NH_x-N levels were significantly ($F_{2,6}$ = 6.722, P = 0.029) lower in the blank treatments (0.01 ± 0.01 mg L^{-1}) than in the planted (0.04 ± 0.01 mg L^{-1}) and unplanted (0.04 ± 0.01 mg L^{-1}) treatments (Figure 16). The planted and unplanted treatments reduced the NH_x-N levels by 97.33% ± 0.67% and 97.00% ± 0.58%, respectively, while the NH_x-N levels in the blank treatment were reduced even beyond these levels (99.67% ± 0.33%). While significant ($F_{2,6}$ = 7.125, P = 0.026) differences were found between the unplanted and blank treatments in terms of NH_x-N concentration percent reduction, no significant differences were found between the planted and unplanted treatments when compared by Tukey's post-hoc test. Significant differences were also seen in PO_4^{3-}-P reduction between the treatments, both in terms of final concentration ($F_{2,6}$ = 1081.752, P < 0.001) and percent concentration reduction ($F_{2,6}$ = 2253.469, P < 0.001) (Figure 17). The planted treatment reduced the PO_4^{3-}-P levels by 93.67% ± 0.67% to 0.03 ± 0.00 mg L^{-1}, and the unplanted treatment reduced phosphorus levels by 92.00% ± 0.67% to 0.04 ±

0.01 mg L^{-1}; phosphate levels in the blank treatment remained high at 0.46 ± 0.01 mg L^{-1}, representing a decrease of only 3.33% ± 0.33%. Tukey's post-hoc test, however, revealed no significant differences in final phosphorus levels or percent reduction between the planted and unplanted treatments.

Algae - There were no significant differences in algal development by any metric between experimental (planted) and control (unplanted) treatments when compared by t-test, but development was slightly higher in experimental treatments than in control treatments; there was no algal development to assess in the blank treatments. Algal ash-free dry mass was 1.08 ± 0.10 mg cm^{-2} in the experimental treatments and 1.04 ± 0.11 mg cm^{-2} in the control ($t_4 = 0.245$, $P = 0.819$) (Figure 18). Chlorophyll-*a* levels in experimental treatments were 0.763 ± 0.133 µg cm^{-2} while they were 0.722 ± 0.156 µg cm^{-2} in control treatments ($t_4 = 0.203$, $P = 0.849$) (Figure 19). Finally, algal biovolume was 0.707 ± 0.188 mm^3 cm^{-2} in the experimental treatments and 0.384 ± 0.203 mm^3 cm^{-2} in the control ($t_4 = 1.166$, $P = 0.308$) (Figure 20). The population of algae that developed in all replicates was composed entirely of cyanobacteria.

DISCUSSION

PHASE I

Nutrients – The fact that final nutrient concentrations showed no significant differences between any of the various nutrient treatment levels indicates that plant and algal communities were able to assimilate available nutrients only up to a point where a common equilibrium baseline was reached, but were not able to completely eliminate all nutrients from the water. It is likely that nutrients remained in the water for a number of reasons. The algal community that developed was found to be dominated by cyanobacteria (esp. *Calothrix*), many taxa of which are able to fix nitrogen (Smith 1983). Cyanobacteria were found to be present in every replicate of every treatment level in the study, and this could explain why NH_x-N and NO_3^--N could still be detected at the end of the study. Phosphate was likely returned to the water through the death and decomposition of plant and algal tissue within the mesocosm (Mitsch and Gosselink 2007). The increase in nutrient concentrations seen in the ONOP treatments was likely due to the nutrients being imported with the nursery pond water that was carried by the coconut fiber and plant tissue; there is no other way that the PO_4^{3-}-P especially could have been brought into the mesocosm, as phosphorus does not exist in a gaseous phase at any point in its nutrient cycle (while nitrogen does). The increase in nitrogen in the ONOP treatments could also be explained by the presence of cyanobacteria. From the similarities in final nutrient levels it is evident that the there was not a high enough nutrient load placed on the macrophytes to show differences in nutrient removal efficiency between the treatments. While the levels used in this study were higher than in some other studies, this study ran over a much longer period. For example, Wang and Sample (2014) used actual pond water with 1.19 mg L^{-1} N and 0.15 mg L^{-1} P in a mesocosm study (compared to the 3.7 mg L^{-1} N & 0.45 mg L^{-1} P used in the present study). However in that study, the water in the mesocosms was replaced every seven days. Because one of the goals of the present study was to evaluate the development of algae, replacing the water was not an option. While differences in nutrient removal efficiency of the FTW between the treatments were not able to be elucidated, based on the similarities of the final nutrient concentrations it could be concluded that a lack of nitrogen did not limit the system's capacity to assimilate phosphorus, and that a lack of phosphorus did not limit the systems capacity to assimilate nitrogen.

White and Cousins (2013) performed a long-term evaluation of nutrient assimilation in floating treatment wetland communities of *Canna flaccida* and *Juncus effusus*, although that study was a "press" rather than a "pulse" experiment. They found that a 95% FTW cover, under a nutrient loading rate of 0.24 mg L^{-1} d^{-1} N and 0.02 mg L^{-1} d^{-1} P (approximately the same N:P ratio as the HNHP treatment in the present study), could reduce the concentrations of those nutrients by 88% & 81%, respectively with a 3 day hydraulic retention time (a second phase of that study also showed that an approximate doubling of that loading rate lowered the nutrient reduction performance of the FTW by approximately 8%). The present study provided an initial pulse of approximately 20 days worth of the nutrients supplied by White and Cousins, and ran the study over approximately three months. While that means that relatively less nutrients overall were supplied in the present study, the percent coverage of FTW was also considerably less (20%). Even though there were differences between the studies, both showed similar final nutrient levels following treatment by the floating wetlands. White and Cousins showed final effluent levels of 0.14 mg L^{-1} N and 0.02 mg L^{-1} P, and the present study found final nutrient levels (for the HNHP treatment) of 0.19 mg L^{-1} N and 0.03 mg L^{-1} P. This indicates that there may be a nutrient concentration floor below which nutrients can be assimilated.

There was a tradeoff that had to be made when conducting Phase I of the study. There were only enough funds available for limited water testing, which included only initial and final testing. The study also had to run long enough for a measurable amount of algae to develop and for a measurable amount of growth to take place in the plant community. As such, in the extended time that it took for the algae to develop and the plants to grow, the available nutrients in all treatments were reduced to equilibrium levels and differences between nutrient treatments were not able to be measured. Future studies should include intermediate water testing to track the reduction in nutrients. If additional water testing were available it would also afford the opportunity to change from the "pulse" perturbation experiment employed in the present study, which involves a single, instantaneous alteration of conditions, to a "press" perturbation experiment, which involves continuous and sustained alteration of conditions (Bender et al. 1984). The press experiment would have the advantage of maintaining nutrient levels at elevated levels to potentially distinguish differences in nutrient assimilation between treatments.

Plant Community - While the plant communities in the two high nitrogen treatments showed greater biomass increases than those in the other three treatments, the difference was not statistically significant. The lack of a statistically significant result was most likely due to the high level of within-group variation and small sample size. Both high nitrogen treatments had a community replicate that showed a much lower biomass increase than the other two replicates at that treatment level, and in both of these cases that low replicate was the only replicate in that treatment level in which an individual plant died (negative change in biomass). The loss of the individual from those treatments caused a substantial change in biomass increase for that replicate, and a subsequent substantial increase in the within group variance for that treatment level. The death of these individuals could have been caused by any number of reasons. Despite the lack of a statistically significant result, the trend was that nitrogen levels had more of an impact on plant growth than phosphorus levels. The increase in biomass of the ONOP communities could have been facilitated by nutrients imported in the nursery pond water that was carried by the coconut fiber husks, a conclusion that is supported by the fact that an increase in nutrients was also seen in these ONOP treatments. It could also be explained by the fact that one of the community replicates exhibited a 22% increase in biomass (the second highest biomass increase across all treatments), while the other two replicates at this treatment level only exhibited an average increase of 4%; the high average biomass increase seen for this treatment level could therefore simply be a result of a single treatment having a community of particularly robust individuals. While the biomass increases of the floating treatment wetland communities were not significantly different between treatments, these results indicate that this community of plants will have greater success in high nitrogen conditions, and that phosphorus levels will have less of an effect.

Plant differences – Canna was the most successful species across all treatments, showing an average biomass increase of almost 20% across all treatments. Canna showed significantly greater success when nitrogen and/or phosphorus was high, but less so when they were not, suggesting that this species has a high requirement for these nutrients. Iris also showed that it was a good candidate for employment in floating treatment wetlands with a mean biomass increase of 15% across all treatments. While there were no significant differences in iris performance across the treatments, this species seems to be less impacted by the differences in the nutrient combinations presented in this study. When comparing canna and iris, canna showed both a greater percent increase in biomass as well as a greater absolute increase, meaning that it could be able to assimilate more total N & P than iris. This information would be useful for those trying to design a floating treatment wetland with limited space. Arrowhead, however, did not perform well at all. Overall, 43% (13/30) of the arrowhead individuals showed either zero or negative changes in biomass over the course of the study, and every nutrient treatment level contained at least one of these individuals. This lack of success was especially evident in both low nitrogen treatments. Although arrowhead is an emergent wetland species that has adapted to wet soil, these results suggest that it may not be the best choice for use in a totally immersed condition such as floating treatment wetlands.

When compared to results of other studies, both similarities and differences can be seen. Chen (2009) tested similar species in a floating treatment wetland application (Australia canna [*Canna generalis*], golden fleece iris [*Iris pseudacorus*] and bulltongue arrowhead (*Sagittaria lancifolia*]), however he tested each species separately as opposed to testing them as part of a community. As in the present study, canna was found to be the most successful in terms of biomass increase, however differences were seen in the results of the iris and arrowhead. Chen found that arrowhead actually showed success in a FTW application, while the success of the iris was more measured. This result could be due to the much higher (c. 3x) concentration of nutrients employed in that study. It is possible that arrowhead has a higher nutrient requirement than iris does, and that it is very successful at higher nutrient concentrations while being very limited at lower nutrient concentrations; at the same time, iris could respond similarly across many nutrient conditions. Alternatively, the differences in these results could suggest that arrowhead is not as able to compete for nutrients when employed as part of a community;

this result could imply that arrowhead would not be a good choice for use in a floating treatment wetland when a community is preferred (i.e. when a high level of importance is placed on aesthetics), but it may perform better in a monoculture. Another aspect of the performance of macrophytes in FTW applications is the tissue nutrient concentration. While both Chen's study and the present study found that canna outperformed iris in terms of overall biomass increase, Chen found that iris had a higher tissue nitrogen content than canna (he found that canna and iris performed similarly in terms of tissue phosphorus content). Tissue nutrient concentrations vary widely between species. Tanner (1996) grew various wetland species (although none of the current subjects) under similar (albeit not floating) treatment wetland conditions and found tissue nitrogen contents between species ranging from 12-32 mg g^{-1} and tissue phosphorus concentrations ranging from 1-8 mg g^{-1}. These results suggest that both biomass increases and tissue nutrient content factors should be taken into consideration to provide a higher level of evaluation of macrophyte nutrient removal efficiency. In addition, even when the removal efficiency is known for a particular species under a certain set of conditions, designers and managers should remember that removal efficiencies can vary widely when grown under differing conditions (Brisson and Chazarenc 2009).

Algal concentration – When measured by ash-free dry mass and chlorophyll-*a*, phosphorus levels affected algal development more than nitrogen levels. As phosphorus is widely considered to be the limiting nutrient in freshwater ecosystems (Schindler 1974), it is understandable that the treatments with higher relative amounts of phosphorus would result in more algal development. However, this result does conflict with the results of a similar mesocosm study, in which a high phosphorus, low nitrogen treatment resulted in *lower* algal biomass than treatments with high nitrogen availability (Dunn 2007).

Algal community composition – Across all treatments, the algal community that developed was dominated by cyanobacteria. While diatoms are another predominant group of algae that form dense blooms in eutrophic freshwater lakes (Bellinger and Sigee 2010), the community of cyanobacteria that developed in this study is consistent with the community composition that would be expected given the N:P ratios that were tested. Smith (1983) reported that a N:P ratio of 29:1 is the threshold that delineates cyanobacteria-dominated communities; a N:P ratio of less than 29:1 typically results in a cyanobacteria-dominated community, while a ratio above 29:1 typically results in a non-cyanobacteria-dominated communities (i.e. domination by diatoms or other algal groups). In the present study, three of the four nutrient treatments tested had ratios of less than 29:1 (LNLP [16:1], HNHP [8:1] & LNHP [3:1]), while only one had a N:P ratio greater than 29:1 (HNLP [46:1]). It is likely that the community composition of algae that developed in this study was influenced by the types and quantities of algae that developed in ponds at the nursery and were imported with the plant specimens. This could explain why even the HNLP treatment (N:P = 46:1) developed an algal community that was dominated by cyanobacteria. As it was only a peripheral goal of the study to examine the algal community composition under different nutrient loading conditions, preference was given to establishing nutrient conditions that were representative of Florida lakes rather than nutrient conditions which would highlight differences in algal community development.

The fact that chlorophyll-*a* levels and ash-free dry mass data indicated that greater algal development occurred in the high phosphorus treatments rather than the high nitrogen treatment, while the cell-count and biovolume calculations showed the opposite trend is difficult to explain. Simple visual observation of the algae in the pools supports the conclusion that the high phosphorus treatments resulted in greater development of algae. It has been reported that environmental conditions such as nutrient ratios can affect cell growth. Caperon and Meyer (1972) reported that nitrogen limitation resulted in higher growth rates of multiple species of marine phytoplankton, but at lower carbon:volume ratios; this trend would result in nitrogen-limited treatments showing a falsely high biovolume reading if relying solely on application of a generic formula to simple cell counts. Overall, that finding suggests that a possible explanation for the discrepancy in the results of the current study could be that there was variability in the growth patterns of the algae between nutrient treatments. Some treatments could have resulted in fewer, larger cells while other treatments could have resulted in more smaller cells; as the biovolume was calculated by applying the same generic formula to all cells, without respect to possible differences in cell size between treatments, changes in growth patterns such as this would have skewed the results. However, while Caperon and Meyer specifically reported that there was a correlation between nitrogen limitation and growth pattern changes, that same correlation could not be found between N:P ratios and biovolume calculations in the present study when applying these findings. Despite there not being a parallel finding between these two studies, the possibility still exists that the different nutrient treatments in the present study could have altered the growth patterns of the algae, and therefore caused the discrepancy seen in the algae results.

An interesting discrepancy that was seen was that the FTW plant community showed generally, albeit not significantly, greater biomass increases in the high nitrogen treatments (indicating nitrogen limitation), while two of the three algal metrics showed that the algae experienced (again, not significantly) greater success in high phosphorus treatments (indicating phosphorus limitation). While cyanobacteria have evolved mechanisms that make them excellent competitors for phosphorus, such as the production of phosphatases and the ability to sequester "luxury phosphorus" (Coleman 1992, Reynolds 2006, Carey et al. 2012), these results show that they still exhibit susceptibility to phosphorus limitation. This is not surprising, given that freshwater systems are usually phosphorus limited (Schindler 1974). These results confirm that limiting phosphorus levels is more important than limiting nitrogen levels in order to effectively curb cyanobacteria blooms. A second conclusion is that in order for it to be possible for this community to be successful in limiting a cyanobacteria bloom, there needs to be sufficient nitrogen available to support plant growth; that growth would in turn facilitate increased uptake of phosphorus to compete with cyanobacteria and possibly limit their development. These results also indicate that FTWs composed of plants with a greater affinity for phosphorus might be more able to limit algal development by competing for that resource.

PHASE II

Nutrients – Surprisingly, no significant differences were seen in nutrient reduction between the planted and unplanted treatments. This study therefore showed that this community of plants did not contribute significantly to the nutrient reduction ability of the floating treatment wetlands. Results may be different however, if the plants were bigger. The plants that were employed in this study were juvenile – if larger, more mature plants were employed, then those would likely be able to assimilate more nutrients, and therefore have more of an impact on nutrient levels. The size and maturity of macrophytes employed in FTWs becomes even more important when one considers the finding by Pietro et al. (2006) that tissue phosphorus concentration of macrophytes can increase with increasing ash-free dry mass; while this might not be true for all macrophytes, this finding does indicate that some larger macrophytes could be much more able to outcompete algae for phosphorus both due to their larger size as well as the higher concentration at which they can sequester phosphorus in their tissue. Dunn (2007) showed that epiphytic algae was able to outcompete submerged aquatic vegetation in mesocosm experiments in which they had to compete for limited nutrients, showing that algae are vigorous nutrient competitors. The size and maturity of any plants employed in floating treatment wetlands should therefore be considered, with preference given to larger, more mature plants which will be more able to compete with the algal community for available nutrients. The present results indicate that small, immature plants compete poorly with algae for available nutrients, at least under the limited conditions of this study.

That the blank treatment showed NH_x-N being almost completely eliminated from the system without the presence of either plants or algae suggests that NH_4^+ could have left the system via deprotonation to NH_3 and subsequent volatile loss. While more common in urea-based fertilizers, all fertilizers containing ammonium are susceptible to loss via volatilization (Mikkelsen 2009). The high temperatures and constant air movement in the greenhouse, and the “flooded” nature of the pools all contribute to a greater likelihood of this type of loss occurring. Alternatively, the ammonium could have been converted to nitrate through nitrification, but no analogous increase in nitrate concentrations was seen. No assessment of an appropriate nitrifying bacterial community was made in this study, but is unlikely that a consortium of nitrifying bacteria could have established without the surface area provided by the root system of the floating macrophytes, with which these bacteria typically form symbiotic relationships (Sooknah and Wilkie 2004). Chen (2009) also saw that ammonium-nitrogen was undetectable at the conclusion of a similar 10-week study. As the same volatile loss of NH_x-N could have happened in the planted and unplanted treatments, it is difficult to be able to attribute the reduction in NH_x-N in those treatments to the plants or algae. The lack of living biomass in the blank treatments prevented any additional NH_x-N from being reintroduced into the water through death and decomposition, or through waste generation, so final NH_x-N levels were even below those of the planted & unplanted treatments where these processes were occurring. These observations from Phase II of the elimination of NH_x-N by mechanisms other than assimilation by algae or plants necessarily make the same NH_x-N results from Phase I inconclusive as well.

Despite this study showing no effect of the plants on nutrient removal, other studies have found that the living plant component of floating treatment wetlands plays an essential role in the system's ability to reduce nutrient concentrations in the water. In addition to taking up nutrients themselves, the plants are able to contribute to the system by providing increased surface area, oxidizing the rhizosphere and excreting bioactive compounds that supplement the important biofilm of microorganisms which also contributes to the nutrient removal effectiveness of floating treatment wetlands (Van de

Moortel et al. 2010, Tanner and Headley 2011). While no biomass information was available from Van de Moortel et al. (2010), plants evaluated by Tanner and Headley (2011) were far larger than those employed in the present study, and, as previously mentioned, this could potentially explain why the findings were different.

Algae – The plants were not able to limit the development of algae in the same way that they were not able to reduce the nutrient concentration. Consideration should again be given to the fact that the plants employed in this study were relatively small when compared to those employed in floating treatment wetlands in other studies (i.e. White and Cousins 2013). That the Beemat© system is able to support larger, more mature plants is important, as the size and maturity of the plants may be critical to the system's ability to outcompete algae for available nutrients. Future studies should employ larger, deeper mesocosms to investigate the ability of larger plants to compete for nutrients than smaller ones. Community composition of the algae in the planted and unplanted treatments was not different, with both being entirely composed of cyanobacteria – the plants therefore exhibited no observable effect on the types of algae that developed. Moreover, community composition of the algae that developed in Phase II was similar to that of Phase I; this was not surprising, considering that the plants came from the same nursery and likely imported the same algae from the same ponds.

General Conclusions - Floating treatment wetlands are a novel method of reducing nutrient concentrations in lakes and possibly limiting the development of harmful algal blooms. However, much more research is needed to determine exactly how these systems work, and how to employ them in the most effective fashion. One of the major conclusions of the present study was that the size of the plants in the FTW may be of great importance, as small plants are simply unable to assimilate enough nutrients to limit the development of algae. In addition, arrowhead was found to be much less successful when employed as part of this community and that it may perform better as a monoculture, while canna showed that it could remain successful as part of a community. The present study was unable to determine the ability of a floating treatment wetland system to remove NH_x-N because of the observed losses apparently via volatilization. Another conclusion was that this specific community of plants displayed some level of nitrogen limitation, and that it would require enough nitrogen in the system to facilitate the requisite uptake of phosphorus that would truly limit a bloom of cyanobacteria. Alternatively, a FTW composed of plants with a greater affinity for phosphorus might be more able to compete with algae for that resource and be more able to limit algal development. Despite the fact that the results of this study were relatively inconclusive with respect to the ability of floating treatment wetlands to limit the development of algae, their use remains promising and further research should be done to continue to enhance our understanding of their strengths and weaknesses.

APPENDIX I – TABLES

Table 1 Nutrient concentrations (mg L^{-1}) and ratios for each treatment level in Phase I

Tag	Description	Nitrogen concentration	Phosphorus concentration	N:P Ratio
HNHP	high nitrogen + high phosphorus	3.69	0.46	8:1
HNLP	high nitrogen + low phosphorus	3.69	0.08	46:1
LNHP	low nitrogen + high phosphorus	1.27	0.46	3:1
LNLP	low nitrogen + low phosphorus	1.27	0.08	16:1
0N0P	zero nitrogen + zero phosphorus	0.00	0.00	0:0

Table 2 Nutrient concentrations in floating treatment wetland mesocosms, by nutrient treatment in Phase I [mean (sem)].

	HNHP			*HNLP*		
	NH_x-N	NO_3^--N	PO_4^{3-}-P	NH_x-N	NO_3^--N	PO_4^{3-}-P
Nutrient Concentration						
Initial (mg L^{-1})	1.76 (0.05)	1.28 (0.15)	0.45 (0.02)	1.70 (0.06)	1.33 (0.08)	0.09 (0.00)
Final (mg L^{-1})	0.10 (0.01)	0.09 (0.01)	0.03 (0.00)	0.10 (0.03)	0.08 (0.00)	0.04 (0.01)
Change (mg L^{-1})	-1.66 (0.04)	-1.19 (0.15)	-0.42 (0.01)	-1.60 (0.07)	-1.25 (0.08)	-0.05 (0.01)
Change (%)	-95 (0)	-93 (1)	-94 (1)	-94 (2)	-94 (1)	-59 (11)

	LNHP			*LNLP*		
	NH_x-N	NO_3^--N	PO_4^{3-}-P	NH_x-N	NO_3^--N	PO_4^{3-}-P
Nutrient Concentration						
Initial (mg L^{-1})	0.51 (0.02)	0.51 (0.05)	0.46 (0.01)	0.52 (0.02)	0.57 (0.02)	0.09 (0.00)
Final (mg L^{-1})	0.13 (0.01)	0.12 (0.03)	0.02 (0.00)	0.09 (0.02)	0.11 (0.03)	0.03 (0.00)
Change (mg L^{-1})	-0.38 (0.02)	-39 (0.05)	-0.44 (0.1)	-0.43 (0.02)	-0.46 (0.03)	-0.06 (0.00)
Change (%)	-75 (2)	-76 (4)	-95 (1)	-83 (4)	-81 (5)	-63 (3)

	ONOP		
	NH_x-N	NO_3^--N	PO_4^{3-}-P
Nutrient Concentration			
Initial (mg L^{-1})	0.02 (0.01)	0.07 (0.01)	0.02 (0.01)
Final (mg L^{-1})	0.21 (0.06)	0.12 (0.02)	0.04 (0.01)
Change (mg L^{-1})	0.19 (0.05)	0.05 (0.02)	0.02 (0.01)
Change (%)	892 (110)	70 (30)	210 (145)

Table 3 Nutrient concentrations in floating treatment wetland mesocosms, by planting treatment in Phase 2 [mean (sem)].

	Planted		
	NH_x-N	NO_3^--N	PO_4^{3-}-P
Nutrient Concentration			
Initial (mg L^{-1})	1.60 (0.05)	1.66 (0.05)	0.47 (0.02)
Final (mg L^{-1})	0.04 (0.01)	0.03 (0.01)	0.03 (0.00)
Change (mg L^{-1})	-1.56 (0.06)	-1.63 (0.05)	-0.44 (0.02)
Change (%)	-97.3 (0.7)	-98.7 (0.3)	-93.7 (0.7)

	Unplanted		
	NH_x-N	NO_3^--N	PO_4^{3-}-P
Nutrient Concentration			
Initial (mg L^{-1})	1.56 (0.03)	1.68 (0.06)	0.50 (0.02)
Final (mg L^{-1})	0.04 (0.01)	0.06 (0.02)	0.04 (0.01)
Change (mg L^{-1})	-1.52 (0.03)	-1.63 (0.08)	-0.46 (0.02)
Change (%)	-97.0 (0.6)	-96.3 (1.2)	-92.0 (1.7)

	Blank		
	NH_x-N	NO_3^--N	PO_4^{3-}-P
Nutrient Concentration			
Initial (mg L^{-1})	1.62 (0.08)	1.61 (0.05)	0.48 (0.01)
Final (mg L^{-1})	0.01 (0.01)	1.59 (0.05)	0.46 (0.03)
Change (mg L^{-1})	-1.61 (0.08)	-0.05 (0.00)	-0.02 (0.00)
Change (%)	-99.7 (0.3)	-1.7 (0.7)	-3.3 (0.3)

APPENDIX II – FIGURES

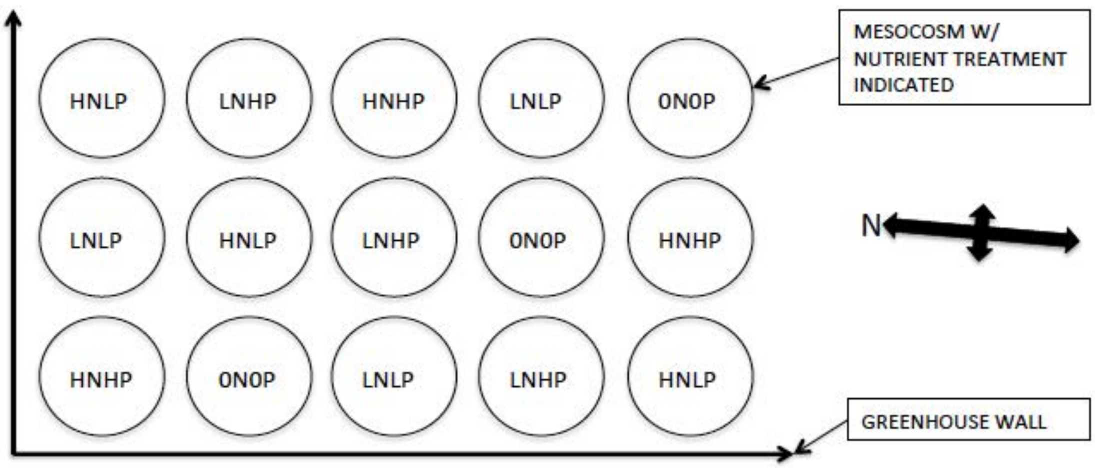

Figure 2 Layout diagram of Phase I.

Figure 3 Photograph of Phase I in progress.

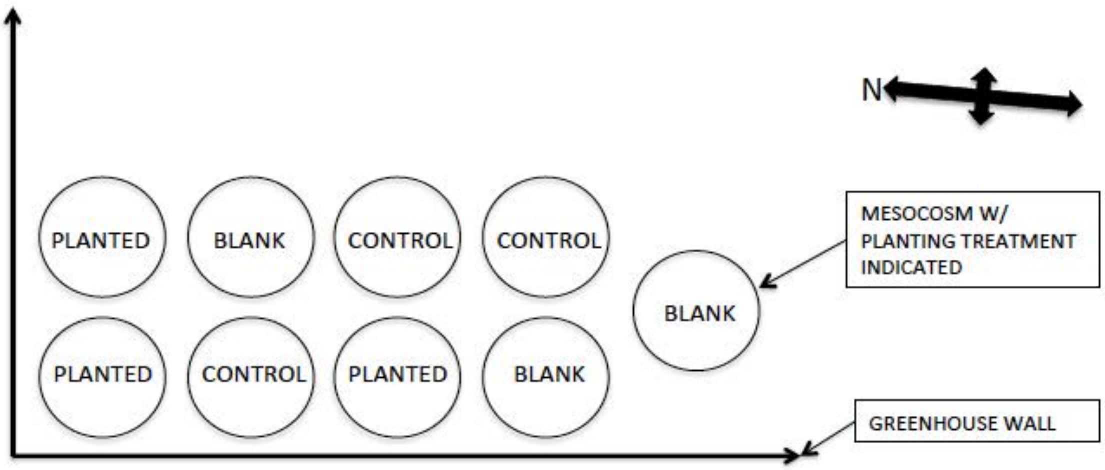

Figure 4 Layout diagram of Phase II.

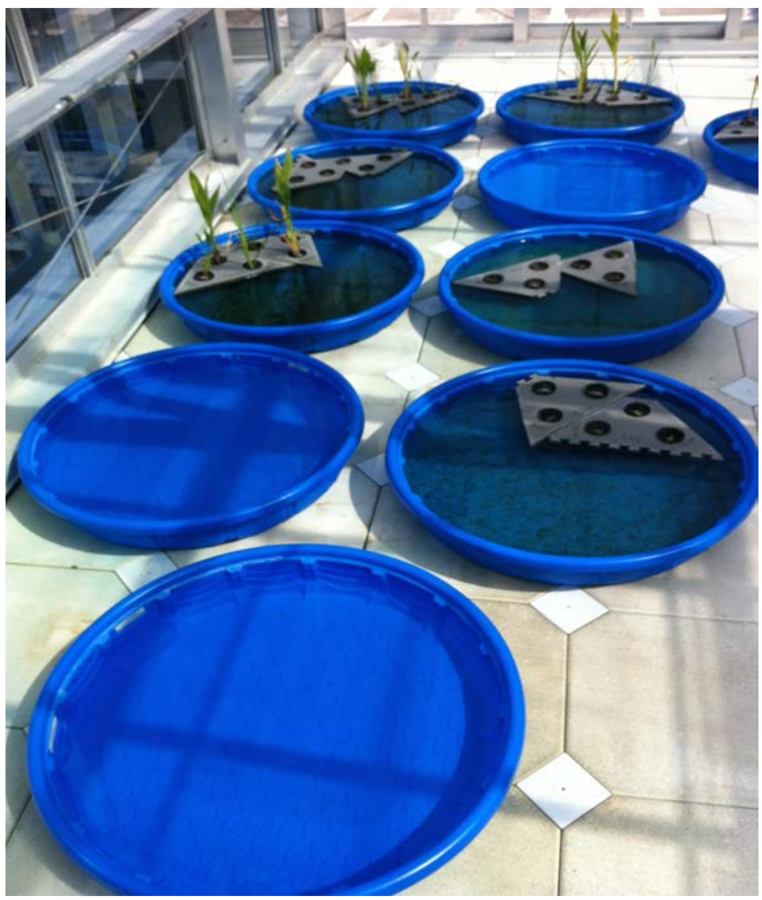

Figure 5 Photograph of Phase II in progress.

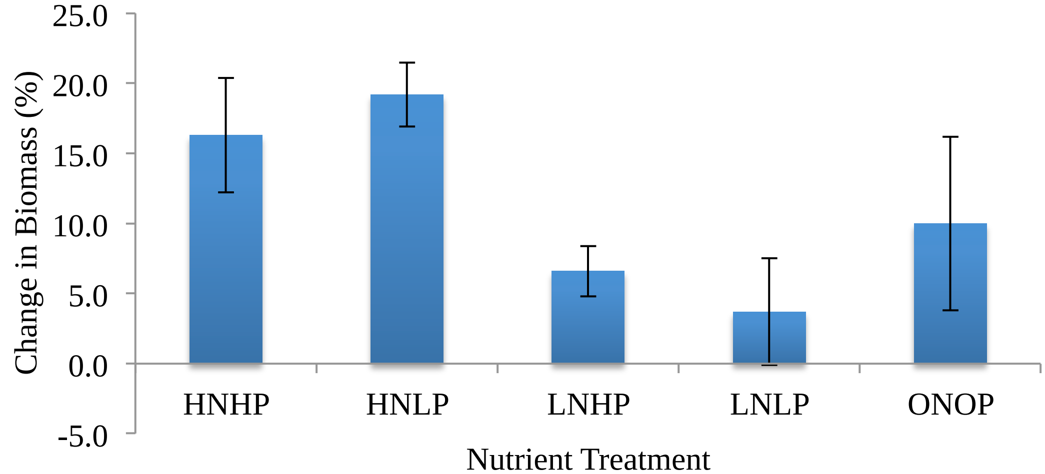

Figure 6 Percent change in total biomass of floating treatment wetland communities by nutrient treatment in Phase I (mean ± sem).

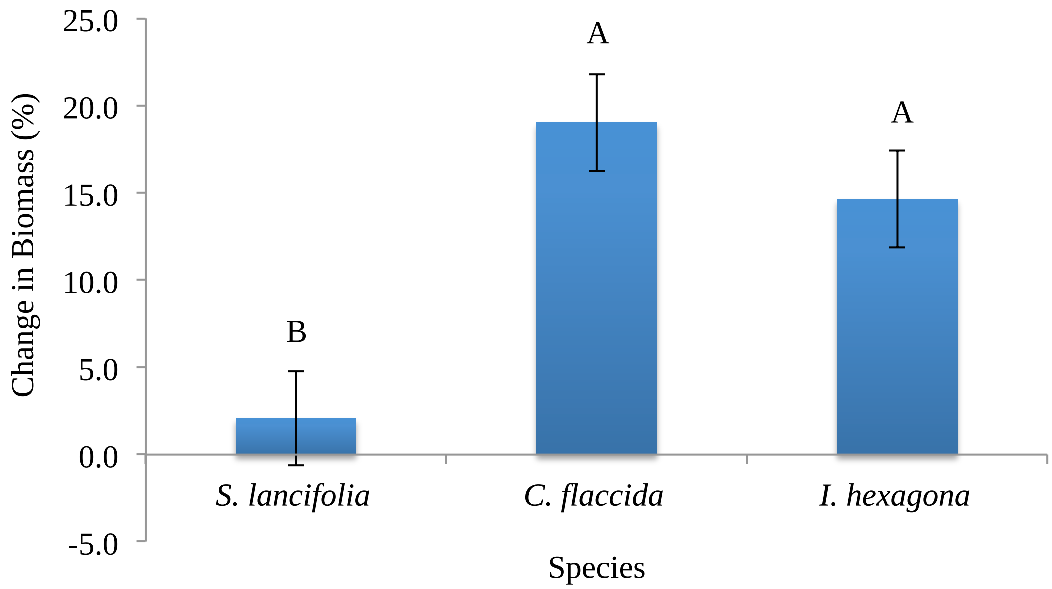

Figure 7 Percent change in biomass of the three plant species under investigation, across all nutrient treatment levels, in Phase I (mean ± sem).

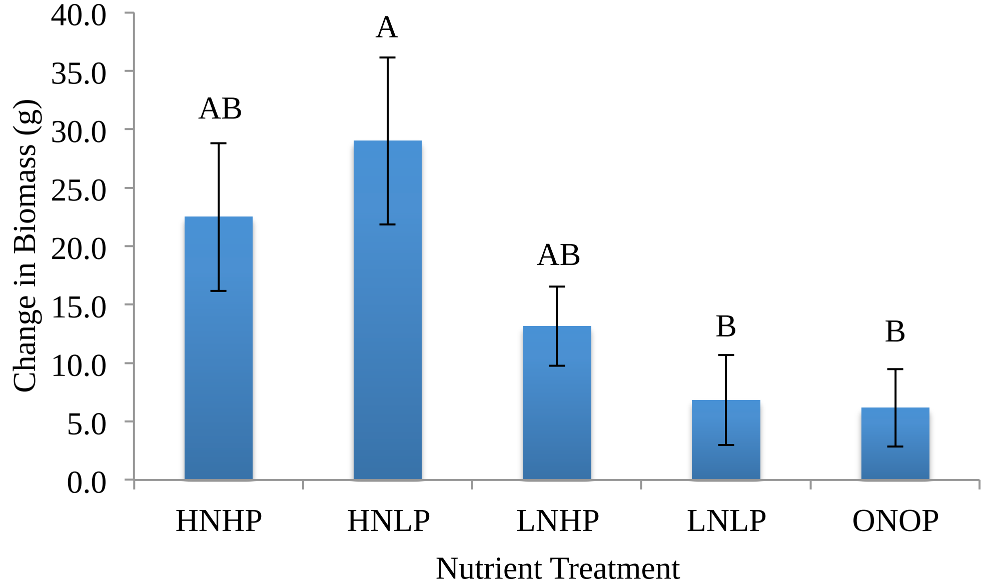

Figure 8 Change in biomass of *C. flaccida* by nutrient treatment level in Phase I (mean ± sem).

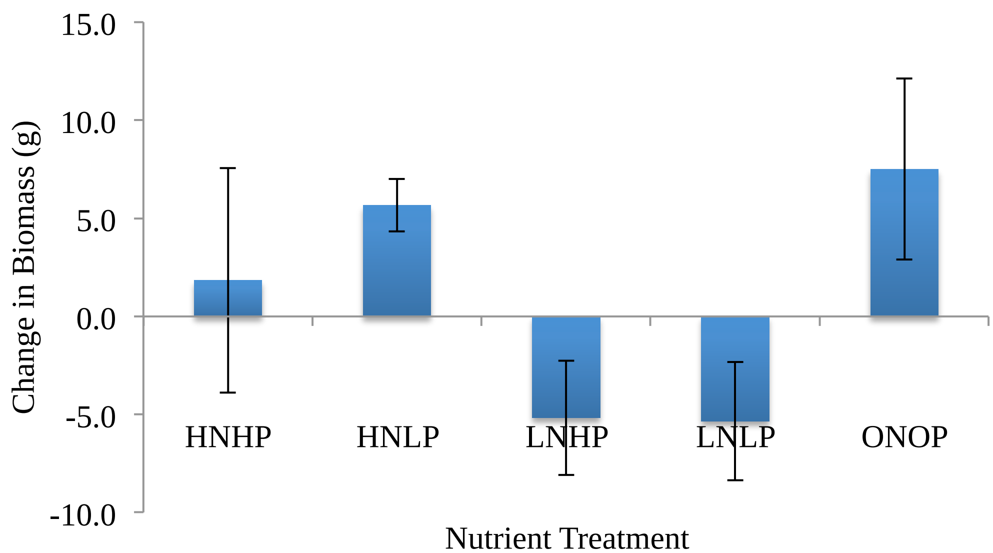

Figure 9 Change in biomass of *S. lancifolia* by nutrient treatment level in Phase I (mean ± sem).

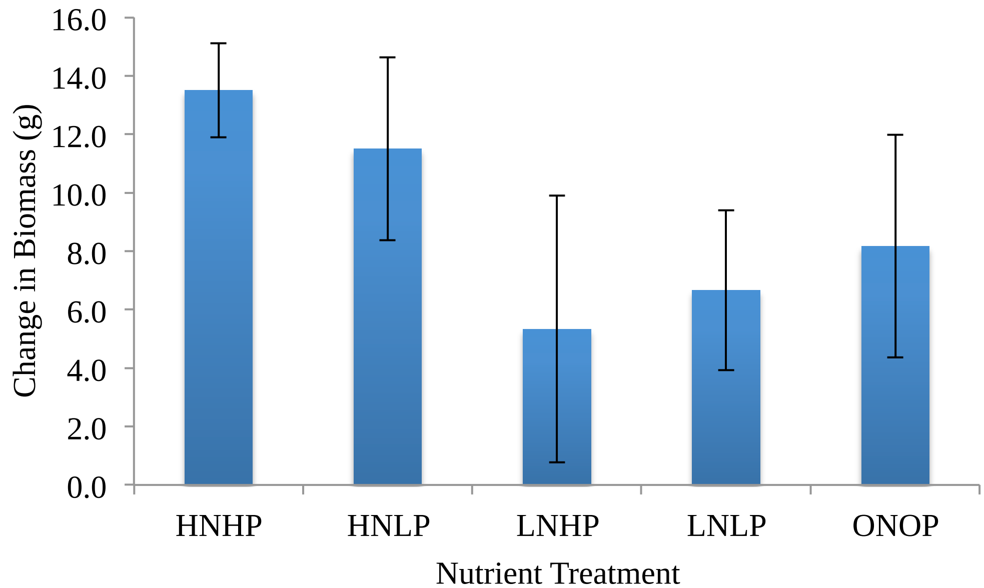

Figure 10 Change in biomass of *I. hexagona* by nutrient treatment level in Phase I (mean ± sem).

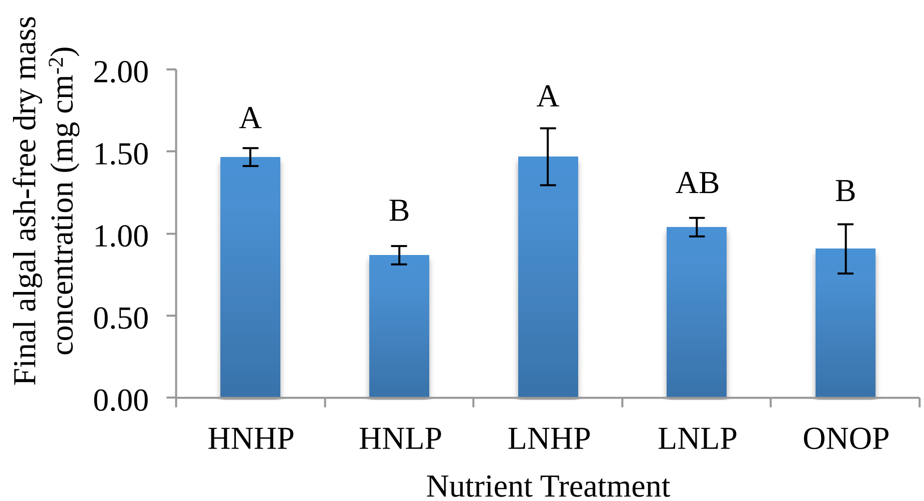

Figure 11 Final algal ash-free dry mass concentration (mg cm^{-2}) by nutrient treatment level in Phase I (mean ± sem).

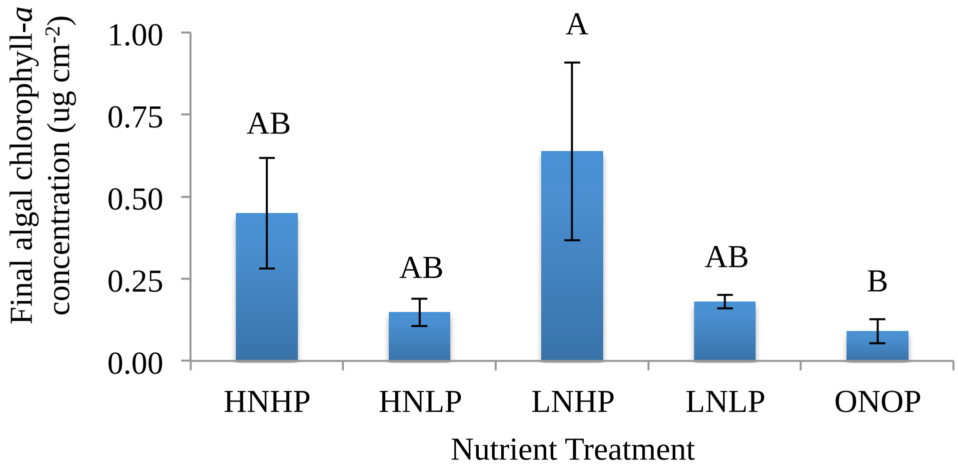

Figure 12 Final algal chlorophyll-*a* concentration (µg cm^{-2}) by nutrient treatment level in Phase I (mean ± sem).

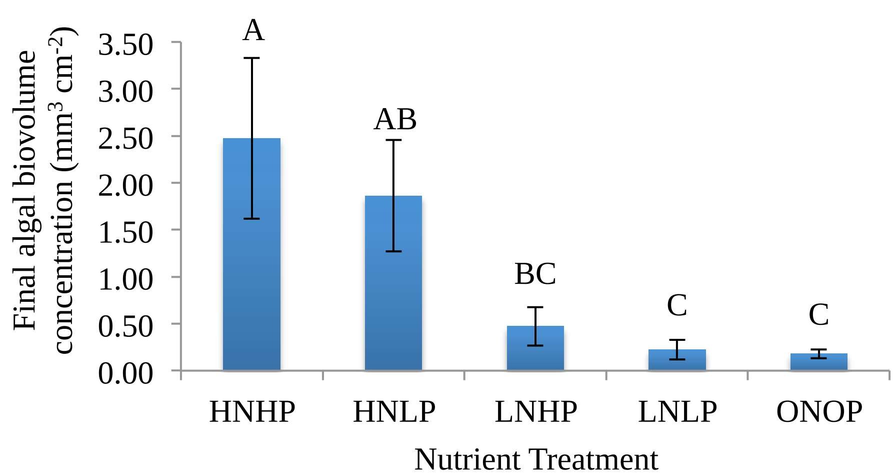

Figure 13 Final algal biovolume concentration (mm^3 cm^{-2}) by nutrient treatment level in Phase I (mean ± sem).

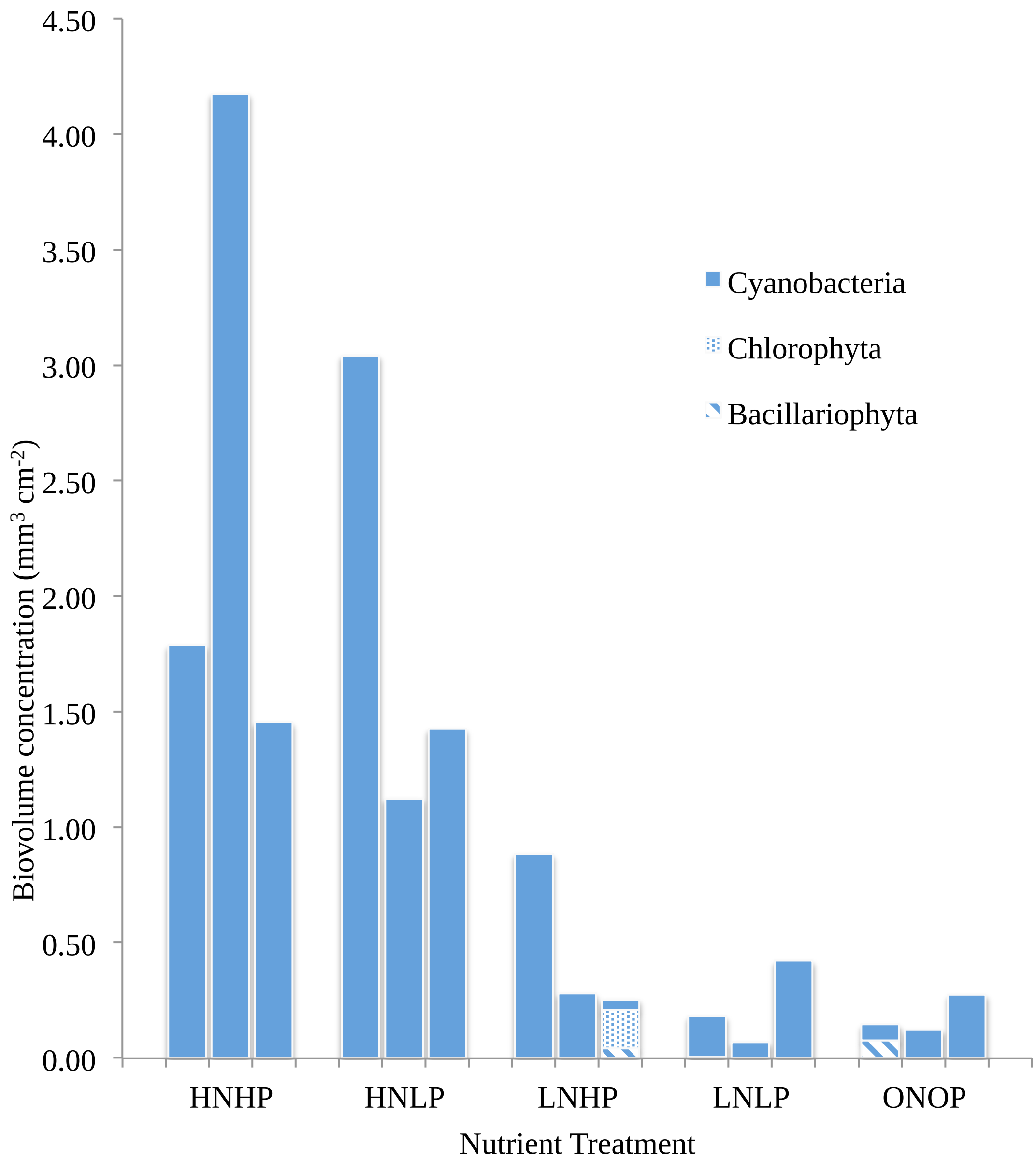

Figure 14 Algal community composition (biovolume concentration) for each replicate, by nutrient treatment level, in Phase I.

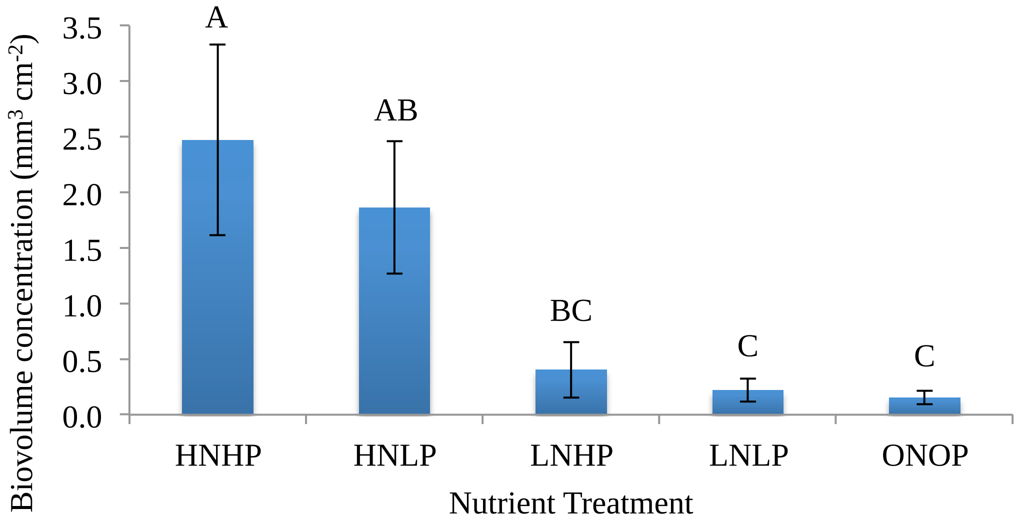

Figure 15 Cyanobacterial biovolume concentration (mm^3 cm^{-2}), by nutrient treatment level in Phase I (mean ± sem).

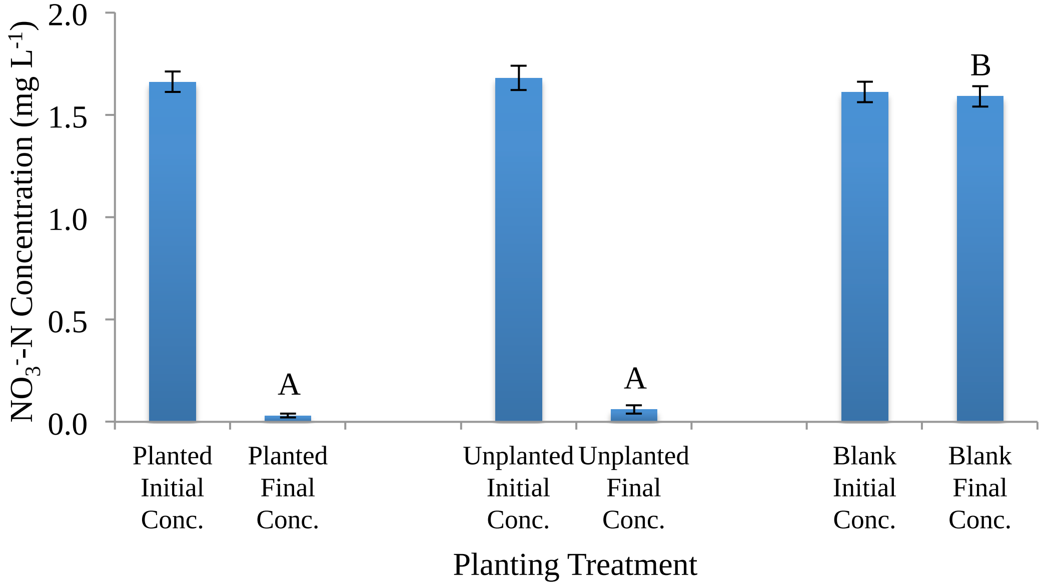

Figure 16 Initial and final NO_3^--N concentrations (mg L^{-1}), by planting treatment in Phase II (mean ± sem).

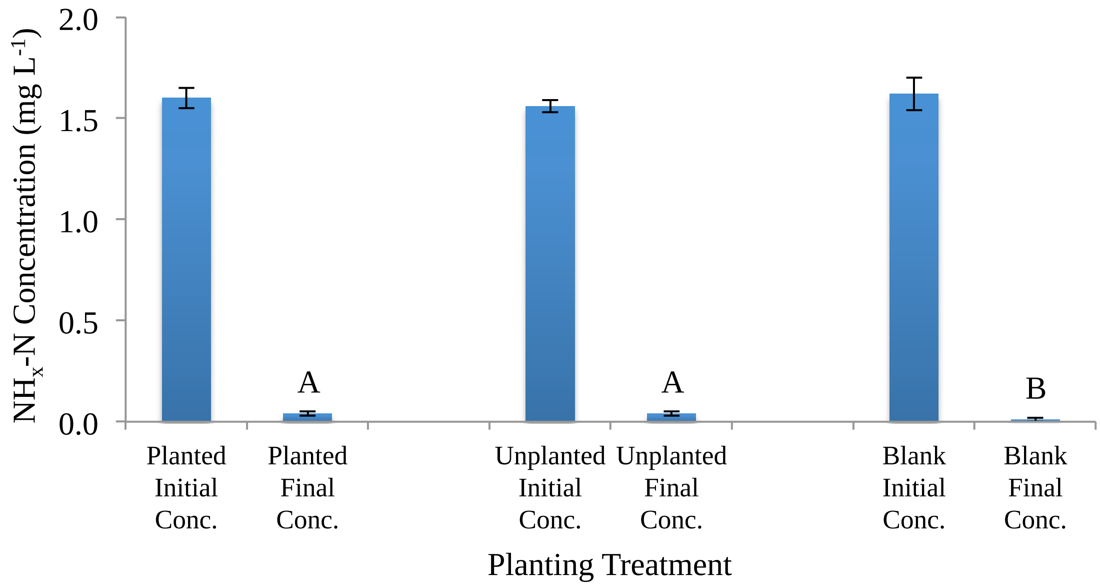

Figure 17 Initial and final NH_x-N concentrations (mg L^{-1}), by planting treatment in Phase II (mean ± sem).

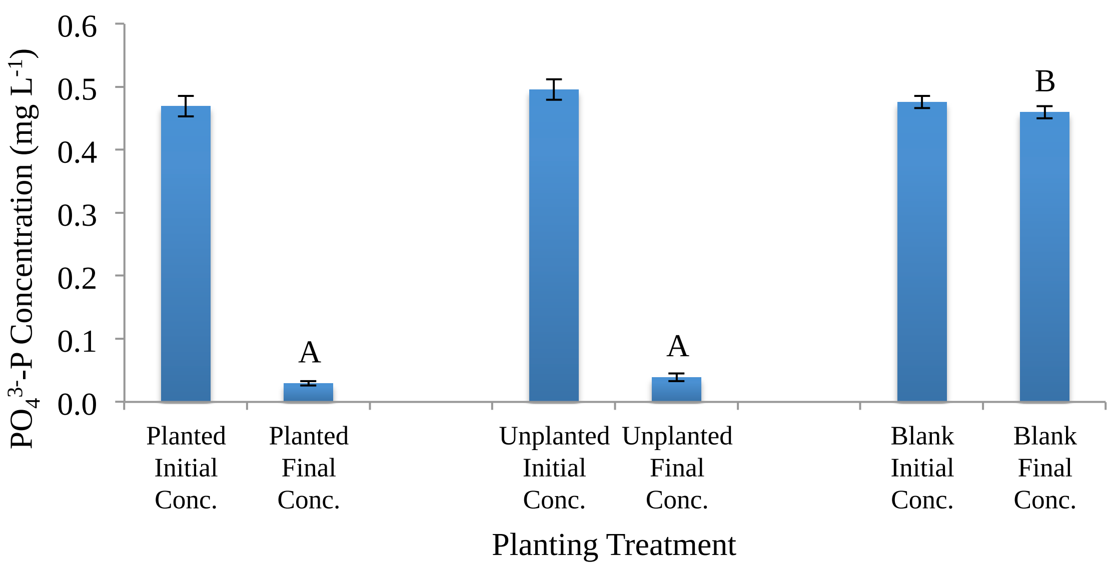

Figure 18 Initial and final PO_4^{3-}-P concentrations (mg L^{-1}), by planting treatment in Phase II (mean ± sem).

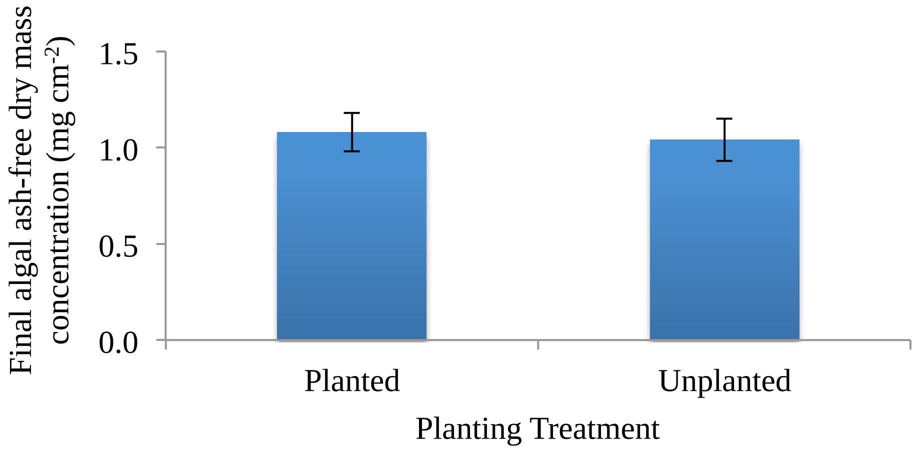

Figure 19 Final algal ash-free dry mass concentration ($mg\ cm^{-2}$) by planting treatment in Phase II (mean ± sem).

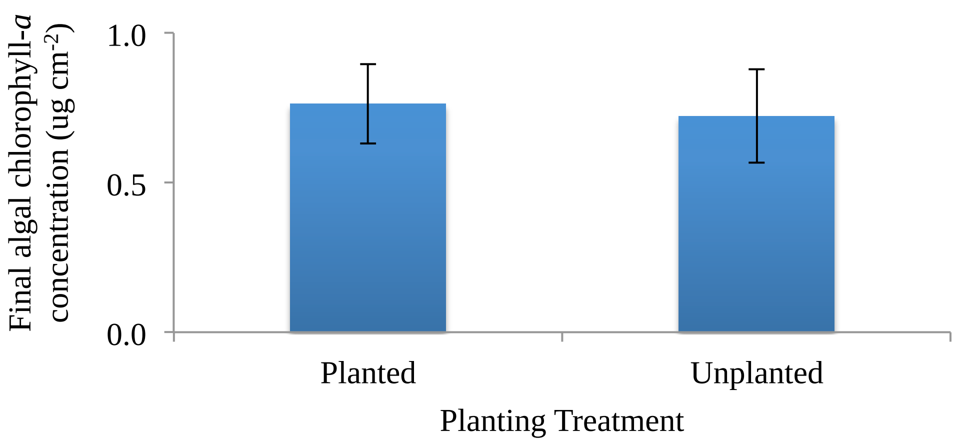

Figure 20 Final algal chlorophyll-*a* concentration ($\mu g\ cm^{-2}$) by planting treatment in Phase II (mean ± sem).

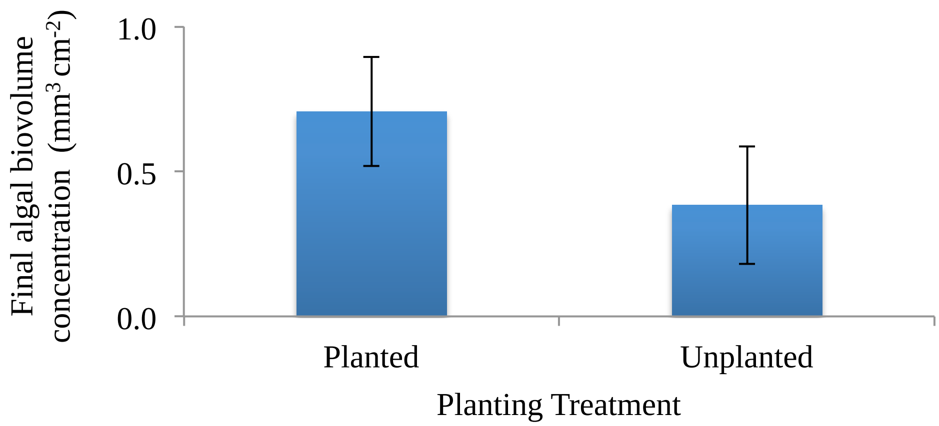

Figure 21 Final algal biovolume concentration ($mm^3\ cm^{-2}$) by planting treatment in Phase II (mean ± sem).

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