Floating Treatment Wetlands in a Northern Climate: Examination of Phosphorus and Nitrogen Removal

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Dedication

This thesis is dedicated to my mother. This thesis is also dedicated to my grandparents, whose hard work made the opportunity of a master's degree even possible.

Also, I would like to extend my gratitude to my family and friends who provided unconditional support in this endeavor.

Abstract

Excess phosphorus is the largest contributor to nutrient impairment in Minnesota waters. Floating treatment wetlands (FTWs) are a novel best management practice (BMP) to reduce excess nutrients in waterbodies. This study examines the nutrient reduction efficiency of floating treatment wetlands in a northern climate under agricultural loading conditions. A field-based, mesocosm study was completed to quantify the removal efficiency of total phosphorus, Orthophosphate-P, Nitrate-N, and Ammonia-N. The FTWs were each planted with wetland plants *Juncus effusus*, *Eleocharis acicularis*, and *Glyceria canadensis*. A system phosphorus budget was prepared to identify phosphorus sources and sinks within the BMP. Floating treatment wetlands had higher total phosphorus reduction efficiencies. *Eleocharis acicularis* had the fastest growth rate and highest removal efficiency of the three plants studied. Mesocosms with FTWs had statistically significant lower pH and dissolved oxygen concentrations. Further research areas and FTW design improvements are recommended based on findings from this experiment.

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1 Introduction and Research Overview

In Minnesota, 573 surface waterbodies are impaired for excess nutrients and eutrophication. The category “nutrient/eutrophication” impairment is the second largest water quality impairment in Minnesota (MPCA, 2014a). Excess total phosphorus (TP) in waters is the largest contributor to eutrophication in Minnesota lakes (MPCA, 2014b; Perry et al., 2004). Nonpoint sources carry the majority of phosphorus to surface waters during normal flow years. Agricultural lands comprise the largest nonpoint source of phosphorus to surface waters in Minnesota (Perry et al., 2004).

Phosphorus added to animal feed and applied to intensively managed fields is transported to surface waters via sorption to soil particles. Manure and fertilizers contain high percentages of bioavailable phosphorus which easily sorb with soils or bond to cations, calcium, aluminum, or iron (Busman, Lamb, Randall, Rehm, & Schmitt, 2002). Runoff from rain events or irrigation carries eroded soils into downgradient surface waters. Soluble phosphorous can also be flushed from agricultural lands to surface waters. Precipitation, agricultural practices and soil types, pH, and erodibility, are major determinants of phosphorus movement into surface waters (Cruse, Wang, Lee, & Chen, 2014).

Nitrogen is generally the secondary nutrient of concern for eutrophication. Nitrogen usually reaches agricultural lands through applied fertilizers. Nitrogen not used by plants is volatilized or transported with water (Cruse et al., 2014). Ammonia can easily vaporize from the water surface to the atmosphere (Kadlec & Wallace, 2009). Nitrate is highly soluble and can infiltrate into groundwaters (Sharpley, 2000). Nitrogen contained in organic material can be mineralized, usually into nitrate, and can contribute to eutrophication or water pollution (Cruse et al., 2014).

Elevated phosphorus concentrations, especially when paired with excess nitrogen, is considered the major cause of eutrophication in fresh waters (Serediak, Prepas, & Putz, 2013). Eutrophication driven by excess nutrients promotes the rapid development of primary production. When these abundant materials are decomposed, dissolved oxygen levels in the water column can drastically decline (Serediak et al., 2013). A concentration of dissolved oxygen (DO) below 4 mg/L can be fatal for aquatic invertebrates (EPA, 1986). Eutrophication of surface waters negatively impacts recreational uses, aquatic habitats, and potable water (Sharpley, 2000). Outside the state, excess nutrients exported from Minnesota and the Midwest into the Mississippi River contribute to hypoxia in the Gulf of Mexico (Cruse et al., 2014).

Several strategies exist to control and reduce the amount of nutrients entering surface waters. Best management practices (BMPs) to reduce excess phosphorus include nutrient management, conservation tillage, and filter strips. The former two involve in-field practices. Nutrient management is one of the top BMPs currently employed on agricultural lands in Minnesota. It involves the correct timing and amount of nutrients to make efficient use of applied phosphorus and reduce excess nutrients moving from soils. Conservation tillage is used on a little over one-third of row crop fields in Minnesota and is required on all highly erodible soils being planted. Conservation tillage reduces soil erosion and associated nutrient movement. However, it is not recommended on slowly draining soils and is rarely a standalone BMP. Filter strips slow runoff which allows for the deposition of sediments through infiltration and filtration However, filter strips may require transferring productive land into a BMP (Miller, Peterson, Lenhart, & Nomura, 2012). This land use change may be undesirable for some farmers.

Floating treatment wetlands (FTWs) are a novel approach for reducing excess nutrients in surface waters. “A FTW is a system with floating mats and associated ecological communities such as macrophytes, macroinvertebrates, zooplankton, and biofilms” (Wang & Sample, 2013). There are a variety of FTWs currently on the market. This study focuses on BioHaven FTWs. BioHaven FTWs are made of recycled plastics. Marine-grade foam is inserted into the porous, plastic media to help the BMP float. Anchoring ensures the FTW remains in place in both lentic and lotic waters. Holes are drilled within the islands to provide areas to plant macrophytes (Midwest Floating Island, 2016). Plant roots are designed to grow through the FTW and into the water column. Biofilms grow on the macrophyte roots and the FTW structure (Floating Island International, 2016b).

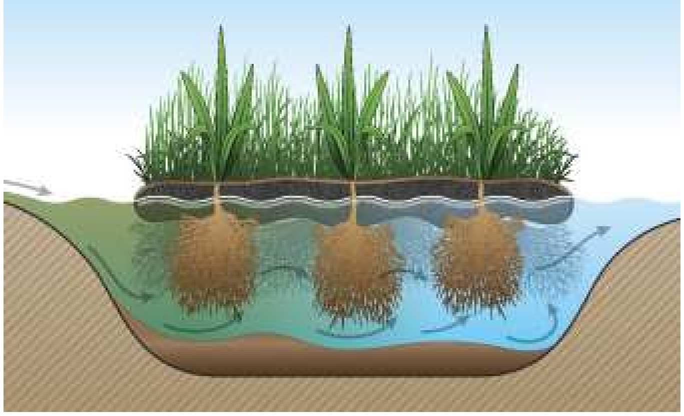

Figure 1. Side-view drawing of an installed FTW (Floating Island International, 2016a).

For BMP comparison purposes, it is useful to compare plant types to natural wetlands. In terms of currently used plants, FTWs are best represented by sedge meadows. Plants commonly growing in sedge meadows are from the Cyperaceae family. Genera include *Carex*, *Eleocharis*, *Scirpus*, and *Cyperus*. In some instances, *Calamagrostis* and *Juncus* can grow within sedge meadows (Eggers & Reed, 2011).

Floating treatment wetlands are a unique BMP in that they are installed within the impaired waterbody (Lynch, Fox, Owen, & Sample, 2015). Floating treatment wetlands were designed to improve water quality through many of the same processes of wetlands. The benefit of a FTW for water quality improvement is the smaller size compared to a treatment or restored wetland (Midwest Floating Island, 2016). Additionally, FTWs can effectively treat water at fluctuating levels through floatation and anchoring (Lynch et al., 2015). Lastly, the cost of FTWs may be less than other BMPs, depending on land prices and BMP installation costs (Wang, 2013).

Floating treatment wetlands also have their limitations. Due to cost limitations, the size of FTWs may be small compared to the surface area of larger impaired waterbodies. This may diminish their nutrient removal impact. Additionally, FTWs have several maintenance costs. Plant harvesting to prevent nutrient release during senescence, weeding, and geese fencing may need to be completed in order to maintain proper function of the BMP. Floating treatment wetlands can only treat impaired waters and do not mitigate nutrient loading issues from contributing landscapes. Therefore, nutrient loading may continue into waterbodies.

Previous research on FTWs has found similar phosphorus removal pathways to wetlands. First, phosphorus sorbs to soil particles. These particles then sink to the bottom of the wetland (Kadlec & Wallace, 2009; Lynch et al., 2015). However, this removal method is limited based on soil type, hydrology, and phosphorus water concentrations over time. Additionally, this sorbed phosphorus can be released from changes in pH, dissolved oxygen levels, or when exposed to water with lower concentrations of phosphorus (Kadlec & Wallace, 2009). Second, phosphorus can be assimilated by plants and microorganisms for growth (Kadlec & Wallace, 2009; Lynch et al., 2015). Only a small amount of phosphorus used by microorganisms is removed through microbial death and burial; the remainder is released as dissolved or particulate phosphorus. Plants also uptake phosphorus for their growth needs. The phosphorus uptake and release depends on the season and plant life cycle. A small portion of plant-assimilated phosphorus, generally 10-20%, is buried in residual plant material (Kadlec & Wallace, 2009). Floating treatment wetlands have one additional pathway of phosphorus removal: phosphorus has been found attached to root biofilms (Borne, 2014; Tanner & Headley, 2011; Wang & Sample, 2014). Researchers studying FTWs theorize that because macrophyte roots are suspended in the water column, phosphorus sorbed to soil particles becomes stuck to biofilms growing on plant roots (Borne, Fassman, & Tanner, 2013; Tanner & Headley, 2011).

For nitrogen, the two largest removal pathways for FTWs are denitrification and assimilation (Lynch et al., 2015). Denitrification is the process completed by microorganisms where nitrate is transformed into dinitrogen. The denitrification process involves conversion stages of nitric oxide and nitrous oxide. Microorganisms use nitrate for energy during anaerobic respiration; therefore, denitrification generally occurs in very low oxygen or anaerobic environments. Plants assimilate ammonia and nitrate for growth. Plant residuals that do not decompose and are added to the benthic material are long-term nitrogen stores (Kadlec & Wallace, 2009).

1.1 Existing research and research needs

To date, FTWs have been installed and studied in at least 17 countries in 5 continents in stormwater, livestock, and wastewater ponds, and natural systems such as lakes and rivers (Wang & Sample, 2013). Nutrient removal results have been mixed based upon experiment-specific conditions. The following experiments are lotic mesocosm systems, in batch-based experiments with seven-day hydrologic residence times.

Lynch et al. (2015) completed a mesocosm experiment on FTWs in Virginia Beach, Virginia. Water for the experiment was sourced from a pond surrounded by highly pervious land uses. Nutrient concentrations were similar to those in stormwater runoff, average TP was 0.22 mg/L and average TN was 1.00 mg/L. In an 18-week study, the FTWs removed less total nitrogen (TN) and TP than the control. The control was a mesocosm with equal shading to the area of FTWs studied. The FTWs covered approximately 65% of the water surface area and were planted with *Juncus effusus* (common rush). Average water temperature in the study was 27.2 °C. The FTWs removed 25% of TN and 4% of TP. The control removed 28% of TN and 31% of TP. However, researchers found removal rates of the FTWs improved in July through

September after a plant acclimation phase in the FTWs, 8 weeks into the experiment. After the acclimation period, the removal rate for TP was 0.008 g/m^2/day (2015).

Tanner and Headley (2011) found a much higher removal rate in a similar FTW mesocosm study in New Zealand. These mesocosms had one control with equivalent FTW shading and seven treatments: four planted FTWs with different plant species, an unplanted FTW, a FTW with only soil, and FTW with soil and artificial roots. The surface coverage was smaller at 36%. The mean water temperature was also lower at 17.5 °C. Macro and micro plant nutrient fertilizer was dissolved into the experiment water. The experiment was phosphorus limited; dissolved phosphorus was spiked to 0.1 mg/L. Starting nitrate levels ranged from 7 to 8 mg/L. FTWs planted with *Cyperus ustulatus* (coastal cutty grass) and *Carex virgata* (swamp sedge) had the highest TP percent reductions of all treatments, 20-50%. The FTW planted with *Carex virgata* had the highest removal rate at 0.0269 g/m^2/day. Plants were planted in the FTWs 10 months prior to the experiment state date (2011). The longer plant acclimation time and phosphorus limitation may have promoted the higher TP removal rate than found in Lynch et al. (2015)

Wang and Sample (2014) achieved higher TP removal rates in an outdoor experiment in Fairfax, Virginia. The longest experiment of the three discussed, it occurred over the full growing season (May to October). The experiment included a control with only open water and four treatments: a FTW planted with *Schoenoplectus tabernaemontani* (softstem bulrush), a FTW planted with *Pontederia cordata* (pickerelweed), a FTW with coir, and a mesocosm with equal shading. Plants were planted in FTWs 20 days prior to the experiment start date. Unlike other FTW mesocosm experiments, the plants were exchanged every four batches (28 days) to replicate each stage without plant acclimation and growth factors. FTWs covered 65% of the water surface. The mean surface water temperature was 23.09 °C. Mean starting concentrations of TP, Orthophosphate-P and Nitrate-Nitrate-N were 0.15 mg/L, 0.02 mg/L, and 0.05 mg/L, respectively. *Pontederia cordata* removed phosphorus more effectively at 68.6% removal of TP. The removal efficiency of both planted FTWs was 8.2% higher than the control in May through August.

Previous published studies in North America have occurred in Florida, Virginia, and North Carolina. One FTW study occurred in Montreal, Canada. However, the experiment focused on plant community dynamic effects on microorganism metabolisms. This experiment is unique in that it occurs in a cool, northern climate. Plant and microorganism uptake of phosphorus and nitrogen are dependent on seasonal cycles and temperatures (Kadlec & Wallace, 2009; L. Wang & D'Odorico, 2008). In cooler climates, plant uptake of phosphorus is highest in spring and autumn. In spring, plants begin their growth phase and use phosphorus. Again during the fall, plants store phosphorus in their roots for the next season's growth. During winter, in temperate climates, plants take up little to no phosphorus. This differs from warm climates where growth only slows, instead of pauses, during winter months (Kadlec & Wallace, 2009). For microorganisms, warmer temperatures stimulate growth and reproduction. Freezing temperatures during winter in the Midwest can kill microbial populations (L. Wang &

D’Odorico, 2008). Therefore, these nutrient removal processes may be affected in Midwest installations.

The above previous studies with similar designs to this experiment have produced inconsistent results. All three studies occurred in subtropical climates with stormwater nutrient concentrations. Percent coverages of FTWs ranged from 36% to 65%. Results from these studies indicate that removal efficiencies of FTWs may depend on the season and site-specific conditions. In both studies in Virginia, researchers found FTWs outperformed controls in different times of the growing season. Lynch et al. (2015) found higher phosphorus reductions of FTWs in July through September. However, Wang and Sample (2014) measured higher phosphorus reductions of FTWs in May through August. Removal rates were also higher in Wang and Sample (2013) even though both experiments had stormwater nutrient concentrations. Tanner and Headley (2011) had phosphorus removal rates between those published by Lynch and Wang and Sample (2014). Site-specific conditions such as plants used in the FTWs, water chemistry, and naturally-occurring microorganisms may explain these different findings. Further research is needed to discern what components make an efficient FTW nutrient removal system.

1.2 Research overview

This field experiment measures the removal efficiency of FTWs in a northern climate under agricultural loading conditions. Goals of the experiment were to:

1. Quantify the removal efficiency of total phosphorus, Orthophosphate-P, Nitrate-N, and Ammonia-N;
2. Quantify the contribution of plants in phosphorus removal; and
3. Prepare a phosphorus budget for the materials customarily used in FTWs to determine phosphorus sources and sinks.

Findings will aid those managing surface waters in the Midwest determine if FTWs may be an appropriate BMP for reducing excess nutrients. This research is especially important for watersheds impacted by agricultural influences.

1.3 Background

The experiment site was located on the University of Minnesota St. Paul campus in St. Paul, Minnesota, as displayed in Figure 1. The water for the experiment was sourced from the Sarita Wetland forebay. The forebay was constructed in 2004 as a pretreatment and settling area before water moves into Sarita Wetland. Through surface runoff and an underground pipe system, the forebay receives the stormwater from campus. Land uses on and around campus which drain into the forebay include the urban campus, farm and livestock areas, and the Minnesota State Fair Grounds.

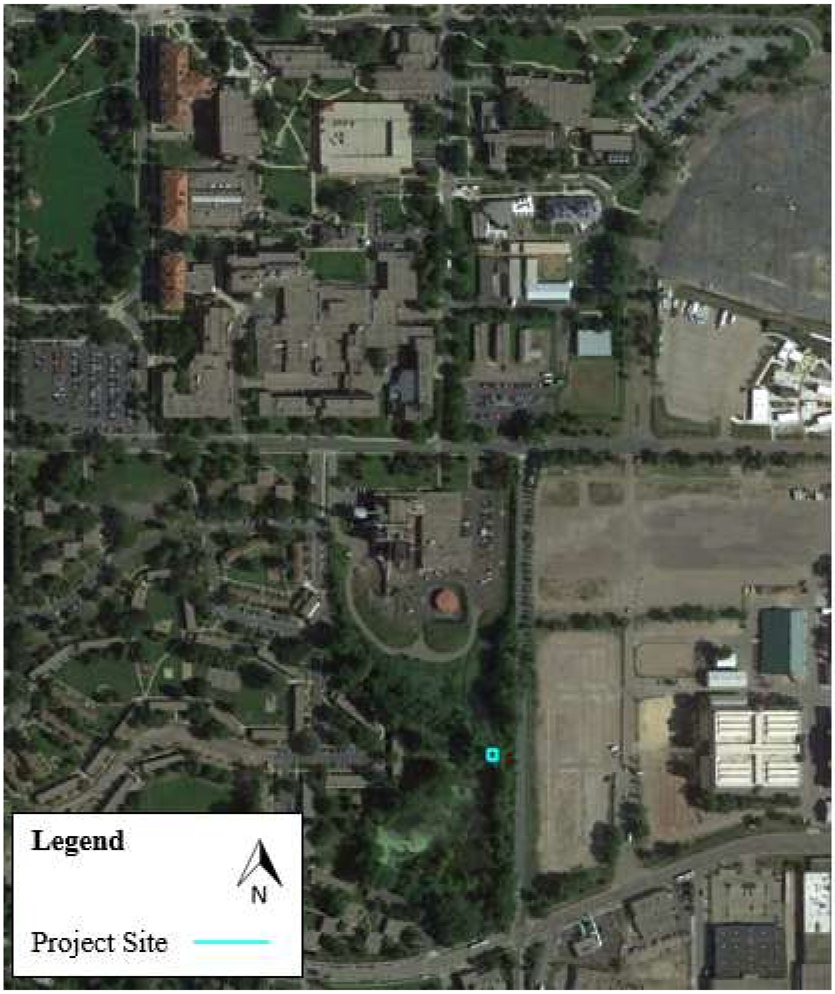

Figure 2. Location of floating treatment wetland experiment site.

The experiment was situated approximately 5 meters north of Sarita Wetland, and 15 meters south of the constructed wetland forebay. The tubs were placed in partial sunlight and arranged in two rows of five. The arrangement of control and experiment tubs was determined using Excel’s random number generator. All tubs were placed in previously-excavated, 30.5-centimeter-deep holes to mitigate overheating from direct sunlight on the tubs’ black exterior and to prevent experiment water from freezing during cold nights at the end of the growing season. This effort was made to simulate naturally-occurring water temperatures in the wetland forebay.

2 Methods and Materials

2.1 Experimental design

The experimental design consisted of ten 284-liter mesocosm tubs. Five tubs served as controls (containing only water) and five contained floating treatment wetlands. The mesocosm tubs were approximately 104 centimeters long, 81 centimeters wide, and 63.5 centimeters deep. Floating treatment wetlands were 33-centimeter-long by 30.5-centimeter-wide and 16.5 centimeters thick, and were centrally anchored in the five treatment tubs. The water surface area covered by the FTWs was approximately 15%. The top half of the FTWs were wrapped in coir to protect against ultraviolet light degradation. Within each FTW, a 2-centimeter-diameter by 33-centimeter-long PVC pipe was installed by the manufacturer for anchoring. The FTWs were lowered to the proper water depth, where the water surface met the bottom extension of the coir, and were anchored in place. Anchoring was achieved by looping fishing line through the center pipe of the FTW to a brick at the bottom of the experiment tub.

A central holding tank collected the experiment water for each experiment batch. The tank was approximately 2.4 meters wide by 2.4 meters long and 0.7 meters deep, and could hold a maximum of approximately 4,032 liters.

2.2 Experiment preparation

The central holding tank held approximately 1,893 liters of experiment water in preparation for each batch. The required volume for each batch was calculated prior to experiment execution. A 37.85-liter bucket was filled three times with a pump. The amount of time required to fill the bucket three separate times was recorded, and then the average was multiplied by 50 to calculate the amount of time to fill 1,893 liters. The central holding tank was then filled for the calculated time using the same pumping rate. The water line was recorded on the interior of the tank. To calculate pumping time for the tubs, the same average filling time from the buckets was used, but multiplied by 5 to equal the amount of time to pump 189 liters. One tub was filled for the calculated time, then the height of the water inside the tub was measured and used to mark the water line in the remaining 9 tubs.

Prior to the experiment, major constituents of experiment water from the Sarita wetland forebay were determined. Total elemental analysis on an unfiltered sample was completed at the University of Minnesota Research Analytical Laboratory (RAL) by Inductively Coupled Plasma (ICP) Atomic Emission Spectrometry, as described in Dahlquist and Knoll (1978). Samples were digested prior to analysis, as described in U.S. Environmental Protection Agency Method 200.7 (1983). Total element concentrations for major elements are listed in Table 1.

Table 1. Total element concentrations in the experiment water sourced from the Sarita Wetland forebay.

Element	**Total Element mg/L**
Al	0.048
B	0.028
Ca	9.095
Cd	< 0.01
Cr	< 0.01
Cu	0.02
Fe	0.187
K	< 0.30
Mg	1.24
Mn	< 0.01
Na	0.954
Ni	< 0.02
P	< 0.37
Pb	< 0.18
Zn	0.024

Three plant species were planted within each of the five floating treatment wetlands prior to the experiment. Three holes were drilled in each FTW by the manufacturer. Holes were 11-centimeters-deep and 5-centimeters in diameter. Within the drilled holes, approximately 3.7 centimeters were filled with ground coconut to allow water to infiltrate up through the floating treatment wetland matrix. On top of the ground coconut, approximately 7.3 centimeters of mixed compost soil and peat moss was placed to plant the experiment plants. Peat moss and soil were mixed to a three-part soil to one-part peat moss ratio by weight, per standard FTW practice (A. Boos, personal communication, June 11, 2015).

Three plant species were planted within the three pre-drilled holes (one plant per hole) on each FTW: *Juncus effuses*, *Eleocharis acicularis*, and *Glyceria canadensis*. Sprouts of each species were purchased from regional suppliers. Plants were planted within the FTWs 20 days prior to experiment commencement. The *Juncus effusus* planted on FTW 3 died two days prior to the experiment start date. It was removed and a healthy *Juncus effusus* was planted in its place.

The use of three different plant species was selected for two reasons. First, FTWs are generally planted with more than one plant species. Second, by comparing three plant families, sedge, rush, and grass, the experiment can identify which plant family may be more efficient in nutrient removal. Species were selected based upon the following criteria:

1. Species is native to Minnesota,
2. Plant is an obligate plant in the Midwest region as defined by the U.S. Army Corps of Engineers,
3. Plant is tolerant of disturbance, and
4. Considered a non-nuisance species.

Species-specific traits are summarized in Table 2.

Table 2. Plant trait matrix for species planted on FTWs in the experiment.

Trait	***Juncus effusus***	***Eleocharis acicularis***	***Glyceria canadensis***
Family	Rush[1]	Sedge[6]	Grass[15]
Duration	Perennial[1]	Perennial[6]	Perennial[11]
Form	Herbaceous[14]	Herbaceous[6]	Herbaceous[16]
Growth Form	Bunch[17]	Rhizomatous[18]	Bunch[19]
Growth Rate	Moderate[17]	Moderate[18]	Moderate[19]
Growth Period	Spring[17]	Spring, summer, and fall[18]	Spring[19]
Lifespan	Long[17]	Moderate[18]	Moderate[19]
Photosynthetic Pathway	C_3[3]	$C_3 + C_4$[5]	C_3[13]
Sunlight Requirements	Full to partial sun[2]	Full sun[8]	Full sun[12]
Water Depth Tolerance	Less than 12 inches[2]	Refer to situation-specific information below.	Unknown
Flood/Inundation Tolerance	4 days[2]	Refer to situation-specific information below.	Refer to situation-specific information below.
Anaerobic Tolerance	Medium[17]	Medium[18]	Medium[19]
Root Type	Stout rhizomes[2]	Slender, branching rhizomes and stolons[7]	Adventitious, fibrous[13]

Root Length	6 to 10 inches[1]	Refer to situation-specific information below.	Unknown
Typical height minimum	2 feet[2]	1 cm[6], 2 cm[7]	2 feet[12]
Typical height maximum	4 feet[2]	60 cm[6], 15 cm[7]	3 feet[12]
Preferred Soils	Finely textured[1]	Clays and silts[10]	Muck, wet sand or silt, or peat[12]
Preferred pH	5.5 to 8.8[17]	3.9 to 6.9[4] 4.5 to 7.0[18]	5.0 to 8.5[19]
Nutrient Requirements	Moderate[2]	Found in habitats with low concentrations of dissolved nutrients[9]	Refer to situation-specific information below.
Competitor Strength	Weak competitor in early development.[2] Mid-successional species. [20]	Guerrilla competitor.[9] Tend to dominate areas where they are found.[9]	Early- and mid-successional species.[20]
Frost Free Days (minimum)	120 days[17]	110 days[18]	100 days[19]

[1] (USDA NRCS Plant Materials Program, 2002)
[2] (Shaw & Schmidt, 2003)
[3] (Kadlec & Knight, 1996)
[4] (Morton & Keeley, 1990)
[5] (Keeley, 1999)
[6] (Jepson Flora Project, 2014)
[7] (Hamel et al., 2001)
[8] (Prairie Moon Nursery, 2015)
[9] (McCreary & Carpenter, 1987)
[10] (Schein & Kroh, 1981)
[11] (Natural Resources Conservation Service, n.d.-a)
[12] (Department of Natural Resources, 2011)

[13](USDA Forest Service, 2009)
[14](Kadlec & Knight, 1995)
[15](Kellogg, 2015)
[16](Vernescu, Coulas, & Ryser, 2005)
[17](Natural Resources Conservation Service, n.d.-d)
[18](Natural Resources Conservation Service, n.d.-b)
[19](Natural Resources Conservation Service, n.d.-c)
[20](Annen et al., 2009)

Current research has not identified a range of root lengths for *Eleocharis acicularis*. However, some observational data is available. A 1981 study conducted in acid-mine polluted and non-polluted lentic and lotic surface waters found *Eleocharis acicularis* roots within the top 8 centimeters of soil. Additionally, over 80% of roots, by mass, were growing within the top 4 centimeters of soil (Schein & Kroh, 1981).

There is not a consensus on the minimum and maximum height for *Eleocharis acicularis*. The Jepson Flora Project (2014) states that plant shoots range from 1 to 60 centimeters. While the plant identification manual from the Washington Department of Ecology lists the plant as between 2 and 15 centimeters tall (Hamel et al., 2001). This discrepancy may be due to differences in climate and growing conditions.

Eleocharis acicularis water fluctuation tolerances are also not well researched. For water depth preferences, the species has been found in shallow, oligotrophic lakes of Wisconsin and Michigan in a 1987 study by McCreary and Carpenter (1987). Researchers posit that *Eleocharis acicularis* may prefer fluctuating water tables. Fluctuation allows for the deposition of silt, which is the preferred soil for the species (Schein & Kroh, 1981).

There is a dearth of information on *Glyceria canadensis*' flood tolerance and nutrient requirements. A 2005 study of wetlands along Lake Huron during fluctuating lake levels found *Glyceria canadensis* could not survive a 46 centimeter rise in water levels over a one-year period (Gathman, Albert, & Burton, 2005).

In terms of nutrient requirements, *Glyceria canadensis* is found in nutrient-poor wetlands in the Northeast United States (Calhoun & DeMaynadier, 2008). This may suggest they are able to effectively use nutrients or do not have a high nutrient requirement for growth.

2.3 Experiment execution

The floating treatment wetland mesocosm study was carried out from July 29, 2015 to October 22, 2015. The experiment was run in batches, with water being exchanged every seven days. “Day 0” was the first day of each experiment batch. Batches were completed on “Day 7.” A total of twelve batches were completed during the experiment. The same FTWs were used throughout the experiment.

On Day 0 of each batch, water for the experiment was sourced via pump from the Sarita Wetland forebay. The source water hose was set at a depth of approximately 15 centimeters above the forebay bottom, when water levels were sufficient. The source water hose had a fine sediment screen affixed to the mouth to prevent damage to the pump and uptake of benthic material. Water was routed from the pump to the central holding tank.

Once approximately 1,893 liters were drawn, water within the central holding tank was sampled for nitrate and orthophosphate concentrations (refer to section 2.4.1 for water sampling methods). After initial nutrient concentrations were determined, the experiment water was spiked with $NaNO_3$ and KH_2PO_4 to attain the desired nitrate and orthophosphate concentrations for each batch. Refer to Table 3 below for nutrient spikes over the course of the experiment.

Table 3. Orthophosphate-P and Nitrate-N experiment water spikes over the 12 batch period.

Batches	Dates of Spike	Target PO_4-P Concentration (mg/L)	Target NO_3-N Concentration (mg/L)	Actual PO_4-P Concentration (mg/L)	Actual NO_3-N Concentration (mg/L)
1 – 6	07/29 – 09/10	0.10	2.0	0.10 ± 0.02	2.0 ± 0.1
7 – 9	09/11 – 10/01	0.33	6.5	0.36 ± 0.04	6.6 ± 0.4
10 – 12	10/02 – 10/22	0.33	3.1	0.33 ± 0.06	3.0 ± 0.1

Each of the three spiking levels were replicated three times to determine the nutrient removal capacity of the FTWs at different nutrient concentrations. The Orthophosphate-P and Nitrate-N spiking concentrations for batches 1 through 6 matched mean 2009 nutrient concentrations in the Cedar River Watershed District. The Cedar River Watershed District located in south-central Minnesota and is dominated by agriculture, which covers 88% of the land surface (Asmus et al., 2012). The Orthophosphate-P spike was increased to a targeted 0.33 mg/L for batches 7 through 12 to simulate a more nutrient-impaired water. All spikes were phosphorus-limited, according to the Redfield ratio (Table 4).

Table 4. Redfield ratios of three spiking levels.

Batches	Redfield Ratio (N:P)
1 – 6	45:1
7 – 9	41:1
10 – 12	20:1

Water was spiked with two compounds. The orthophosphate spike was achieved using potassium phosphate monobasic (KH_2PO_4). The nitrate spike was achieved using sodium nitrate ($NaNO_3$). The compounds were dissolved separately in excess central holding tank water used to measure initial nutrient concentrations. The bottles containing the compounds and excess experiment water were shaken vigorously for one minute. Contents were then emptied into the central holding tank and triple rinsed with central holding tank water. A 10-foot long paddle was used to stir the spiked central holding tank water for 10 minutes to ensure thorough incorporation.

Once the water in the central holding tank was spiked, it was tested for: TP, orthophosphate, nitrate, Ammonia-N, temperature, DO, conductivity, turbidity, and pH. Then 189 liters of the spiked water was pumped into each mesocosm tub. Seven days later, the physicochemical properties and nutrient concentrations of the water in the tubs was sampled. Additionally, on Day 7, water levels were recorded to evaluate water loss or gain due to evapotranspiration or precipitation.

After each batch, experiment water from the 10 tubs was emptied into Sarita Wetland, which is hydraulically down gradient from the forebay. All tubs were brushed and rinsed based on visual inspection to remove any periphyton that may have established on the tub walls.

2.4 Data collection

2.4.1 Water sampling and analysis

Experiment data were collected on Days 0 and 7 of each batch to determine the change in nutrients and physicochemical properties of water. On Day 0, nutrient samples were grabbed and physicochemical properties of water were measured approximately 15 centimeters from the bottom of the central holding tank. On Day 7, nutrient samples were grabbed and physicochemical water properties were measured from the center of each tub, approximately 15 centimeters from the bottom. All water was drawn and measurements were taken between 8:00 am and 11:45 am central time. Two exceptions occurred when a pump repair (batch 2) and a severe thunderstorm (batch 7) delayed sampling.

Nutrients measured were: TP, orthophosphate, nitrate, and Ammonia-N. Grab samples were taken in plastic bottles cleaned with hydrochloric acid, following the methodology of the United States Geological Survey's *National Field Manual for the Collection of Water Quality Data* (2004). Nutrient sampling and sample storage followed methods from the *Standard Methods for the Examination of Water and Wastewater* (Clesceri, Greenberg, & Eaton, 1999). When field technicians sampled, they donned nitrile, powderless gloves for the sampling of experiment water in the tank and each tub. Sample bottles were affixed to a plastic dipping pole which was rinsed with deionized water in between each tub to avoid cross-contamination. Orthophosphate samples were filtered through a 0.45 µm filter. All other nutrient samples were unfiltered. All samples taken in the field were immediately placed on ice within coolers.

Total phosphorus and Ammonia-N samples for batches 1, 2, and Day 0 sampling for batch 3 were preserved with sulfuric acid to a pH<2. Acidification of samples ceased after Day 0 sampling for batch 3 when it was determined acidification and subsequent lab neutralization was interfering with the lower detection limit of TP. Total phosphorus and Ammonia-N samples for the remainder of the experiment were preserved by freezing at -20 °C.

Total phosphorus was measured at the RAL using Lachat Instruments (Loveland, CO) following Lachat QuikChem method 10-115-01-4-B (2009).

Orthophosphate samples were analyzed by a DR/890 Hach Colorimeter. A subset were delivered to the RAL for quality control (QC) analysis. Orthophosphate samples analyzed by the Hach were completed following the PhosVer 3 (ascorbic acid) method as described in the *Hach DR/890 Colorimeter Procedures Manual* (Hach Company, 2013). Samples analyzed by the RAL were analyzed using Lachat Instruments (Loveland, CO) following Lachat QuikChem method 10-115-01-1-V (2008).

Water samples were analyzed for nitrate with a probe and QC samples were sent to the RAL. Water samples were measured for nitrate with a Hach Nitratax Plus SC and Hach Nitratax Clear SC. The Nitratax Plus SC was the primary probe used in the experiment. The Nitratax Plus SC was calibrated prior to the experiment start date and again in the fall following calibration procedures in the *Nitratax sc User Manual* (Hach Company, 2014). Throughout the experiment, both probes' accuracy was ensured through correct reading of deionized water blanks and nitrate spikes. Nitrate quality control samples were analyzed using Lachat Instruments (Loveland, CO) according to Lachat QuikChem method 10-107-04-1-A (2007).

Ammonia-N was analyzed at the RAL using Lachat Instruments (Loveland, CO), following Lachat QuikChem method 10-107-06-2-R (2009).

Using a YSI 6920 V2, physicochemical properties of the experiment water were measured: temperature (degrees Celsius), dissolved oxygen (percent saturation and mg/L), conductivity (mS/cm), turbidity (NTU), and pH. The YSI sonde was calibrated for pH and dissolved oxygen each day before sampling following the YSI calibration procedures as described in *6-Series Multiparameter Water Quality Sondes User Manual* (YSI Incorporated, 2012). The conductivity and turbidity probes were calibrated once a season, once on Day 0 of the first batch and again in the fall following procedures in the user manual.

A rain gauge was installed at the experiment site to measure orthophosphate and water volume inputs into the experiment. Orthophosphate samples from the rain gauge were filtered through a 0.45 µm filter and analyzed with DR/890 Hach Colorimeter or delivered to the RAL for analysis.

2.4.2 Quality control of water sampling and analysis

Blanks and replicates were taken to represent approximately 10% of the sampling events. Blanks were filled with deionized water, taken into the field, and analyzed with experiment samples. Replicate QC samples were collected at the same time as regular samples. Sample replicates customarily analyzed with the Nitratax probe or 890/DR Hach Colorimeter with available analysis at the RAL were submitted to the lab (orthophosphate and nitrate). Replicates with only analysis available at the RAL were submitted to ensure consistent lab readings (TP and Ammonia-N).

Spikes were created at a known level of the constituent in the Biosystems and Agricultural Engineering laboratory. Spikes helped determine lab analysis accuracy and percent recovery of TP and Ammonia-N samples. Spikes were taken to represent 5% of sampling events.

The RAL analysis was also quality controlled. One complete tub replicate was submitted to the RAL and the second was submitted to Pace Analytical to compare with the RAL's results. All blanks, spikes, and replicates were analyzed with samples from that day's batch.

Blank, spike, and replicate results are attached in Appendix I.

2.4.3 Plant sampling and analysis

Prior to the start of the experiment, all plants were measured for biomass. Plants were removed from their potting matrix, rinsed with deionized water, patted dry, and weighed. Following biomass measurements, one randomly chosen plant from each three species had their dry weights taken. Plant roots and shoots were separated and dried at 65 degrees Celsius until a constant weight was reached. Specimens were ground through a 20 mesh sieve. Specimens were analyzed for TP concentrations via the Miller Digest, Total Elemental Microwave Technique at the RAL as described in Gavlak, Horneck, and Miller (1994) and Miller and Kotuby-Amacher (1996). The samples from the roots and shoots were not mixed.

At the culmination of the experiment, post-experiment biomass measurements were compared to pre-experiment biomass measurements. Plants were removed from the FTWs via dissection of the plastic matrix. Some roots, especially fine roots of *Glyceria canadensis*, were unable to be fully recovered from within the matrix. As before, plants were rinsed with deionized water, patted dry, and weighed for biomass. Additionally, three randomly selected plants from each three species were selected for post-experiment analysis. As with pre-experiment plants, post-experiment plants were separated from roots and shoots, dried at 65 degrees Celsius until a constant weight was reached, and had their dry weights taken. Dried plant roots and shoots samples were ground through a 20 mesh sieve, and analyzed for phosphorus content at the RAL using the Miller Digest, Total Elemental Microwave Technique. The remaining six plants not submitted to the lab for analysis had dried weights of roots and shoots measured.

2.4.4 Potting material and floating treatment wetland sampling and analysis

Potting material was measured for TP at the RAL by the ICP-OES Microwave Procedure as described in Tadon, Cuescas, and Tyner (1968). Pre-experiment soil was well-mixed prior to planting. A representative sample was submitted to the lab. Post-experiment soil was collected via the grab method from one of the three FTW plant holes.

Ground coconut, peat moss, and coir from the FTWs were analyzed for TP by Inductively Coupled Plasma dry ash method at the RAL as described in Munter, Halverson, and Anderson (1984) and Dahlquist and Knoll (1978). Ground coconut was well-mixed prior to planting and a representative sample was submitted to RAL. Post-experiment coconut samples were collected as grab samples from FTWs 1, 2, and 4 where a sufficient amount of coconut powder was available for analysis. The pre-experiment coir sample was cut from extra coir provided by the manufacturer to avoid damage to the experiment FTWs. Post-experiment coir samples were taken from FTWs 1, 2, and 3, as selected by Excel's random number generator. Peat moss was well-mixed prior to incorporation into the soil used for planting. A representative pre-experiment sample of peat moss was submitted to the lab. A post-experiment analysis of peat moss was not possible due to its thorough incorporation into the soil matrix.

2.5 Data analysis

2.5.1 Calculation of nutrient removal and plant uptake rates

Areal nutrient removal rates ($\mathrm{R_{nutrient}}$) were calculated using Equation 1, where $\mathrm{N_{Day\ 0}}$ is the initial concentration of the nutrient (mg/L) on Day 0, $\mathrm{V_{Day\ 0}}$ is the initial volume in each mesocosm (189 L), $\mathrm{N_{Day\ 7}}$ is the median nutrient concentration (mg/L) on day 7, $\mathrm{V_{Day\ 7}}$ is the median mesocosm water volume taking into account changes from evaporation and rain, A is the area of the FTW mat, and d is the number of days (7) in the experiment batch.

$$\mathrm{R_{nutrient}} = \frac{\left(\mathrm{N_{Day\ 0}} \cdot \mathrm{V_{Day\ 0}}\right) - \left(\mathrm{N_{Day\ 7}} \cdot \mathrm{V_{Day\ 7}}\right)}{\mathrm{A} \cdot \mathrm{d}}$$

Equation 1. Calculation of nutrient removal rates in the FTW experiment.

Shoot and root nutrient contents (g) were calculated by multiplying dry mass (kg) by the TP concentration (mg/kg) and converting to grams.

Plant phosphorus uptake ($\mathrm{P_{uptake}}$) was calculated using Equation 2, where $\mathrm{P_{initial}}$ is the initial phosphorus content (g) in the representative plant of the species, $\mathrm{P_{final}}$ is the mean post-experiment phosphorus content (g) of the same species, *A* is the area of the FTW mat, and *d* is the number of experiment days (86).

$$\mathrm{P_{uptake}} = \frac{\mathrm{P_{initial}} - \mathrm{P_{final}}}{A \cdot d}$$

Equation 2. Calculation of phosphorus uptake by plants from the FTW experiment.

2.5.2 Statistical analysis

The median percent reductions in Orthophosphate-P and Nitrate-N between experiment and control tubs were analyzed using Wilcoxon rank sum tests because the data were not normally distributed (Gibbons & Chakraborti, 2011). The Wilcoxon rank sum test was also employed to determine differences in median concentrations of TP and Ammonia-N. Both nutrients had concentrations below the detection limits (17% of TP samples and 8% of Ammonia-N samples); therefore, percent reductions could not be calculated. Differences between experiment and control tubs were tested at the 0.05 significance level.

Wilcoxon rank sum tests were used to analyze the difference in physicochemical properties of water because results were not normally distributed. Physicochemical differences between experiment and control tubs were tested at the 0.05 significance level.

The assimilation of phosphorus by the three different plant species were analyzed using the Kruskal-Wallis test. With the small sample size for each species (n=3), normality could not be assumed. The statistical software RStudio was used for all analyses.

3 Results

3.1 Physicochemical results

3.1.1 Day 0

Day 0 physicochemical properties of water measured in the central holding tank are summarized in Table 5. Percent dissolved oxygen concentrations had the highest variability. Conductivity varied the least throughout the experiment.

Table 5. Mean physicochemical properties of water within the central holding tank measured on Day 0.

Physicochemical Property	Minimum	Maximum	Mean ± SD
Conductivity (mS/cm)	0.078	0.208	0.122 ± 0.033
Dissolved oxygen (%)	40.4	114.8	71.2 ± 23.7
pH	7.01	8.50	7.56
Temperature (°C)	7.52	26.43	19.31 ± 5.41
Turbidity (NTU)	7.8	37.5	18 ± 8.2

3.1.2 Day 7

Conductivity, evaporation, temperature, and turbidity measurements for experiment and control tubs were similar (Table 6). Turbidity data in batch 9 was removed as an outlier because the sonde produced negative turbidity readings, indicating that the turbidity probe was not properly calibrated; other probes were not affected. All differences in these four physicochemical properties were not statistically significant at a 0.05 significance level in Wilcoxon rank sum tests.

Table 6. Mean physicochemical properties of mesocosm tub water measured on Day 7.

Physicochemical Property	Experiment Tubs Median ± MAD	Control Tubs Median ± MAD
Conductivity (mS/cm)	0.120 ± 0.014	0.117 ± 0.011
Evaporation (cm)	0.83 ± 0.75	0.75 ± 0.28
Temperature (°C)	17.76 ± 3.42	17.48 ± 3.72
Turbidity (NTU)	3.50 ± 0.60	4.20 ± 0.80

MAD: median absolute deviations.

3.1.2.1 pH

The average pH in experiment tubs was 7.69 ± 0.362. The average pH in control tubs was 8.43 ± 0.599. The differences in pH were statistically significant ($p = .005573$).

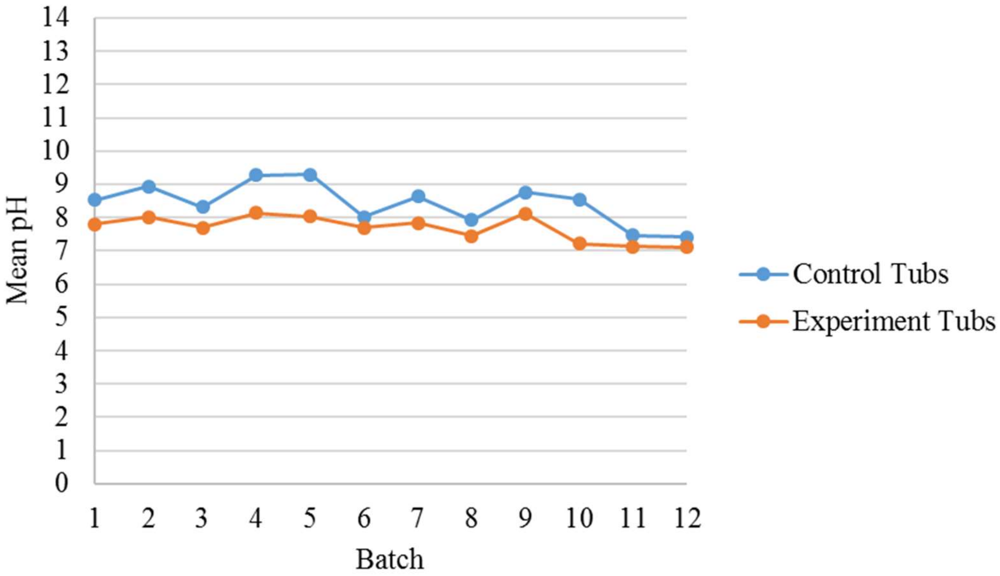

Figure 3. Average pH of control and experiment tubs.

3.1.2.2 Dissolved oxygen

The average dissolved oxygen percent saturation in experiment tubs was 68.66% ± 20.71%. Experiment tubs never exceeded 100% dissolved oxygen. Control tubs had a higher average dissolved oxygen percent saturation, 99.99% ± 23.61%. The differences in dissolved oxygen percent saturations were statistically significant (*p* = .001433).

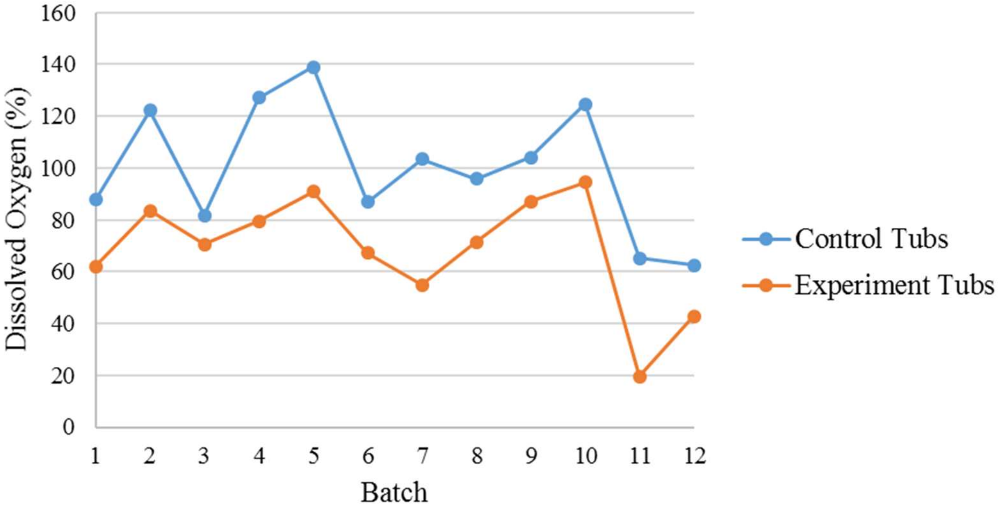

Figure 4. Average dissolved oxygen percent saturation in experiment and control tubs.

3.2 Nutrient reduction

3.2.1 Phosphorus

Median Orthophosphate-P reduction and median absolute deviations in experiment tubs were 47% ± 9.8% and 47% ± 17% in control tubs. Differences in percent reduction of Orthophosphate-P between the control and experiment tubs were found to be statistically insignificant at the 0.05 significance level based on a Wilcoxon rank sum test (*p* = .6678). The null hypothesis was there was no difference in percent reduction of Orthophosphate-P between the experiment and control; the alternative hypothesis was percent reductions in experiment tubs were higher than in control tubs.

Experiment tubs removed more Orthophosphate-P than control tubs during batches 7 through 12, where starting concentrations were targeted at 0.33 mg/L PO_4-P. Batch 10 is one exception; control tubs outperformed experiment tub reduction by 1%. During batches 1 through 6, where experiment water was spiked to 0.10 mg/L PO_4-P, control tubs outperformed experiment tubs. Batch 5 was one exception; experiment tubs outperformed control tubs (Figure 5). Orthophosphate-P reduction was not significantly correlated to water temperature, pH, or DO concentrations in linear regression analyses.

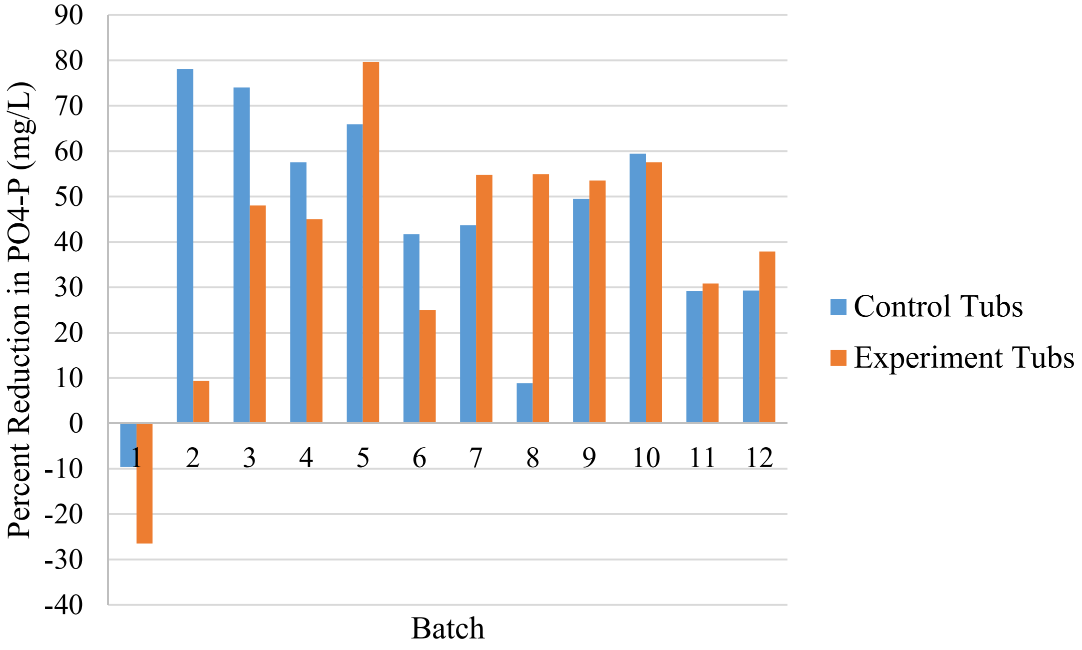

Figure 5. Median reduction efficiency of PO_4-P.

When FTWs generally removed more PO_4-P than the control (batches 7 – 12), the median removal rate was 0.05 g/m^2/day PO_4-P.

Total phosphorus percent removal in experiment tubs was equal to or higher than percent removal in control tubs. The median percent removal in experiment tubs was 51% ± 11%. The median percent removal in control tubs was 37% ± 8%. Since a percent reduction for TP could not be calculated for certain batches, median concentrations were statistically compared using Wilcoxon rank sum tests. Differences in median concentrations for TP were found to be statistically insignificant at the 0.05 significance level (p = .226). The null hypothesis was median concentrations were not different; the alternative hypothesis was median TP concentrations in experiment tubs were less than concentrations in control tubs.

Concentrations from batches 1 through 3 were all below the detection limit due to neutralizing acidified samples. The relationship between the concentrations of PO_4-P and TP was weak in a regression analysis. Therefore, TP concentrations for batches 1 through 3 could not be estimated by known PO_4-P concentrations. During batch 6, control and experiment tubs both achieved reductions below the method detection limit (MDL) (< 0.05 mg/L TP). Since the median reduction is unknown, a percent reduction could not be calculated for batch 6 (Figure 6). Total phosphorus reduction was not significantly correlated to water temperature or DO. Total phosphorus reduction was correlated to pH in linear regression analysis ($R^2 = 0.6849$) in control tubs; no significant relationship was detected in experiment tubs though the visual relationship was the same.

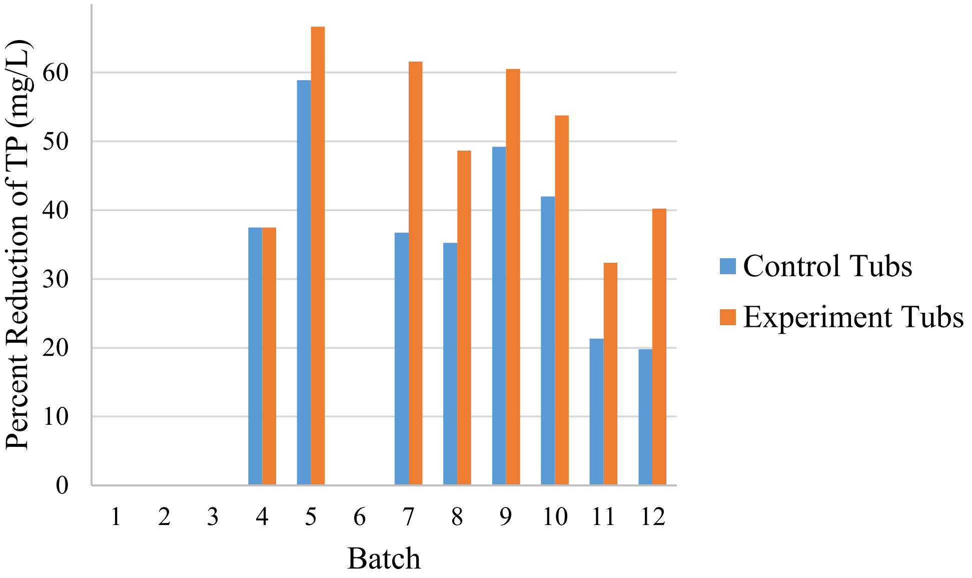

Figure 6. Median reduction efficiency of TP.

The median removal rate based on the size of the FTW in experiment tubs for known TP reductions (batches 4, 5, and 7-12) was 0.05 g/m^2/day TP. Experiment tubs had a higher median removal efficiency than control tubs. Total phosphorus removal based on tub size found experiment tubs removed 0.008 g/m^2/day TP and control tubs removed 0.006 g/m^2/day TP.

Percent Orthophosphate of TP increased slightly from Day 0 to Day 7 sampling. In batches 3 through 6 on Day 0, median Orthophosphate-P concentrations comprised 49% of TP. Median Orthophosphate-P on Day 7 of batches 3 through 6 comprised 58% of TP. In batches 7 through 12, with higher spiking concentrations, Orthophosphate-P made up a higher percentage of TP. In batches 7 through 12 on Day 0, median Orthophosphate-P made up 80% of TP. On Day 7 of batches 7 through 12, median Orthophosphate percentages of TP were at 82%.

Table 7. Phosphorus sources and sinks, on a per-tub basis.

Phosphorus Source	Phosphorus Added to Control and Experiment Tubs (g)	Phosphorus Added to Experiment Tubs (g)	Phosphorus Removed from Experiment Tubs (g)
Rain	0.02[a]		
Spike (batches 1-3, 6)	0.07[b]		
Spike (batches 4-5, 7-12)	0.57		
Soil		0.6[c]	
Coconut Powder		0.012[d]	0.018[d]
Plants			0.06[e]

[a] Phosphorus from rain water was measured as orthophosphate and converted after analysis to Orthophosphate-P. Therefore, rain phosphorus contributions may be higher due to unmeasured TP in rain.

[b] Total phosphorus concentrations are not available for batches 1-3, and 6. Therefore, Orthophosphate-P values were used for these batches. Phosphorus contributions are likely higher due to additional phosphorus present in the water not in orthophosphate form.

[c] Some phosphorus loss from soils may have been taken up by plants and microorganisms through assimilation. Therefore, phosphorus inputs to experiment tub water may be smaller than total measured TP loss from soils.

[d] Coconut powder was both a source and sink for phosphorus, depending on the FTW sampled.

[e] Includes all three plant species used in the experiment.

Phosphorus removal by coir could not be calculated due to unknown location of phosphorus uptake in coir. A representative sample of coir was taken from different parts of the FTW. Due to minimal contact of coir to mesocosm water, the phosphorus source is not definite.

3.2.2 Nitrogen

Median percent reduction of Nitrate-N in experiment tubs and control tubs was 33%. The percent removal was more varied in experiment tubs, with a median absolute deviation of 16%. Control tubs had a median absolute deviation of 11%. Differences in percent reduction of Nitrate-N between the control and experiment tubs were found to be statistically insignificant (*p* = .5115). The null hypothesis was percent reductions were not different; the alternative hypothesis was percent reductions in experiment tubs were higher than in control tubs.

Experiment tubs removed a higher percentage of nitrogen than control tubs in batches 7 through 12. Similar to Orthophosphate-P reduction in batches 1 through 6, batch 5 was exempt to the trend of control tubs removing more Nitrate-N than experiment tubs. During batch 5, both experiment and control tubs removed 51% of Nitrate-N. Nitrate-N reduction was not significantly correlated to water temperature, pH, or DO concentrations.

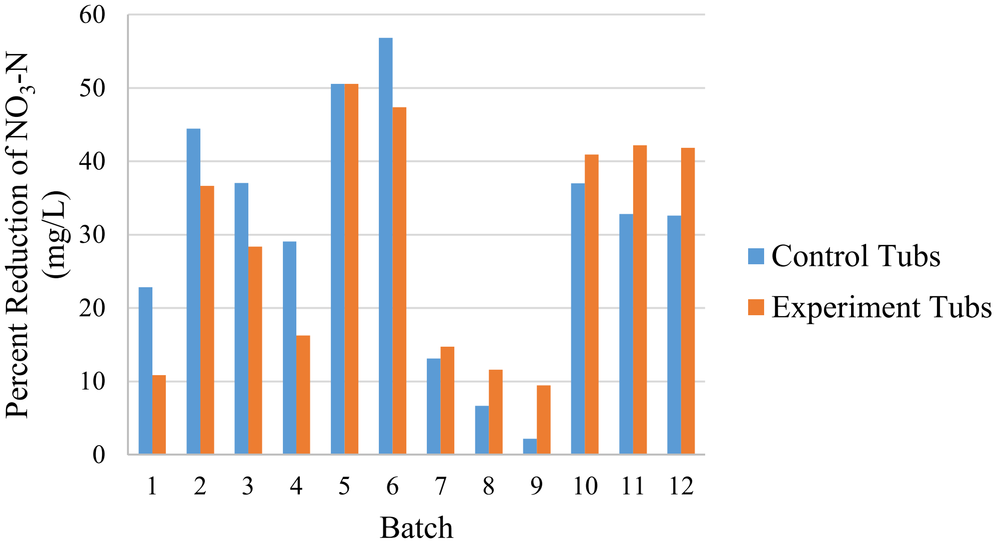

Figure 7. Median reduction efficiency of NO_3-N.

When FTWs removed more NO_3-N than the control (batches 7 – 12), the median removal rate was 0.33 g/m^2/day NO_3-N.

The median percent reduction of Ammonia-N in experiment tubs was 23% ± 69%. Control tub median reduction of Ammonia-N was 21% ± 66%. Since a percent reduction for Ammonia-N could not be calculated for certain batches, median concentrations were statistically compared using Wilcoxon rank sum tests. Differences in median concentrations for Ammonia-N were found to be statistically insignificant at the 0.05 significance level (*p* = .05799). The null hypothesis was median concentrations were equal; the alternative hypothesis was median Ammonia-N concentrations in experiment tubs were less than concentrations in control tubs.

Three batches reduced Ammonia-N to levels below the MDL (< 0.02 mg/L NH_3): batches 7 and 11 from experiment tubs and batch 8 from control tubs. In batches 3, 10, and 12, Ammonia-N concentrations increased drastically from starting concentrations. Ammonia-N reduction was not significantly correlated to water temperature, pH, or DO.

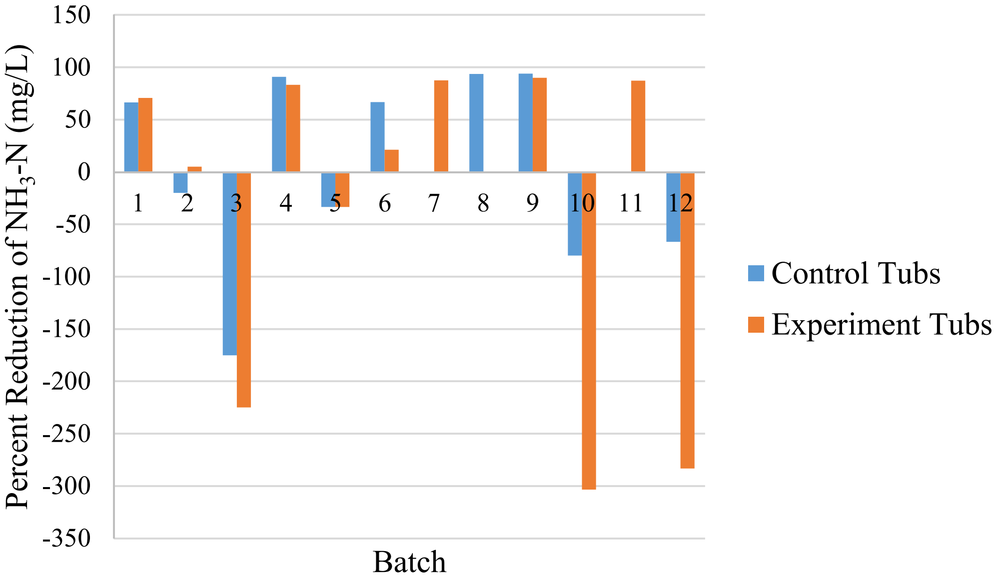

Figure 8. Median reduction efficiency of NH3-N.

Statistical results of nutrient differences in control and experiment tubs are summarized in Table 8.

Table 8. Statistical results from one-sided Wilcoxon Rank Sum Tests.

Parameter	H_0	H_a	*p*-value	Result of H_0
Orthophosphate-P Percent Reductions	E = C	E > C	.6678	Fail to reject
Total Phosphorus Median Concentrations	E = C	E < C	.226	Fail to reject
Nitrate-N Percent Reductions	E = C	E > C	.5115	Fail to reject
Ammonia-N Median Concentrations	E = C	E < C	.05799	Fail to reject

E: Experiment, C: Control.

3.3 Rain data

Rain volumes were measured from the installed rain gauge at the site. Data was quality controlled by comparing to recorded rainfall amounts at The National Oceanic and Atmospheric Administration climate observations station GHCND: USC00218450. The station is located approximately 0.53 mile from the experiment site.

Rain collected at the site had a mean Orthophosphate-P concentration of 0.17 ± 0.12 mg/L. One rain event of 12 total events was unable to be sampled because the thunderstorm knocked over the rain gauge. Additionally, one sample out of 11 had to be discarded due to contamination.

3.4 Plant growth and phosphorus uptake

Glyceria canadensis had the highest average wet biomass prior to the experiment. At the end of the experiment, *Eleocharis acicularis* had the highest average wet biomass (Table 9).

Table 9. Average weight of plant species before and after the experiment.

Species	Pre-Experiment Wet Weight Mean ± SD (g)	Post-Experiment Wet Weight Mean ± SD (g)
Eleocharis acicularis	8.4 ± 2.2	93.9 ± 31.9
Glyceria canadensis	35 ± 12	71.5 ± 28.1
Juncus effuses	3.2 ± 0.4	24.4 ± 18.3

The fastest growing plant in the experiment was *Eleocharis acicularis*, followed by *Glyceria canadensis*, and *Juncus effuses*. One plant had a negative growth rate in the experiment, *Glyceria canadensis* planted on FTW 1 (-2.8 g/m^2/day). With the negative growth rate removed from the average growth rate per plant species, *Glyceria canadensis* remains the second fastest growing species (Table 10).

Table 10. Average growth rate by species. Average growth rate includes negative growth rate of *Glyceria canadensis* planted on FTW 1.

Species	Growth Rate of Species Mean ± SD (g/m^2/day)
Eleocharis acicularis	9.9 ± 3.6
Glyceria canadensis	4.2 ± 4.5
Juncus effuses	2.4 ± 2.1

Glyceria canadensis and *Juncus effuses* both increased shoot biomass percentages of total plant dry weight from the start of the experiment to the culmination of the experiment (total of 86 days). *Eleocharis acicularis* had approximately the same percentage of shoot and root masses at the beginning and the end of the experiment. Only *Juncus effuses* had an average shoot biomass larger than half of the total biomass at the end of the experiment (Figure 9).

On the start of batch 3, approximately 66% of plants had roots grow through the bottom of the FTW matrix and access the water column. *Juncus effusus* and *Eleocharis acicularis* both had four plants with below-FTW root systems.

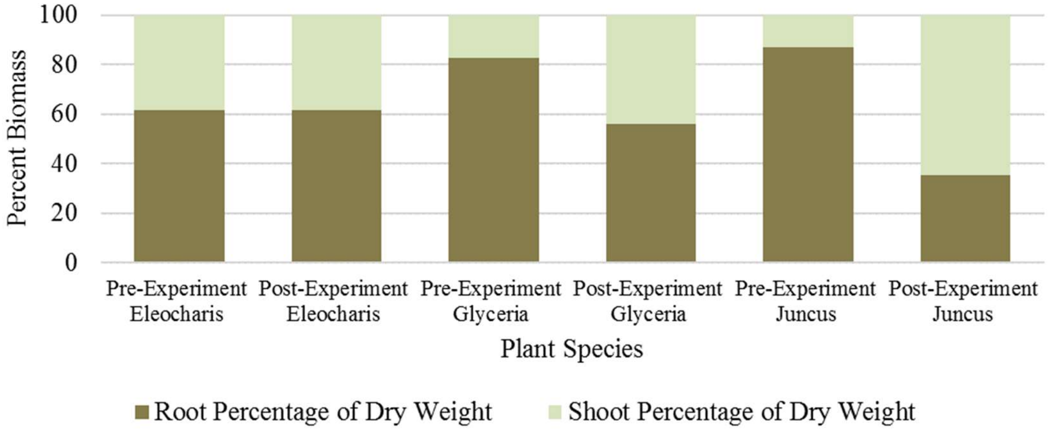

Figure 9. Percent of plant biomass in roots and shoots by dry weight. Pre-experiment percentages are from one representative plant. Post-experiment percentages are averaged from all post-experiment plants (n=5 per species).

Phosphorus comprised a higher percentage of biomass in shoot tissues than in root tissues during both pre- and post-experiment sampling periods. Phosphorus percentages in both root and shoot biomasses increased from the start of the experiment. *Eleocharis acicularis* showed a more even distribution in the percent of phosphorus in the root and shoot tissues during the post-experiment analysis (Figure 10).

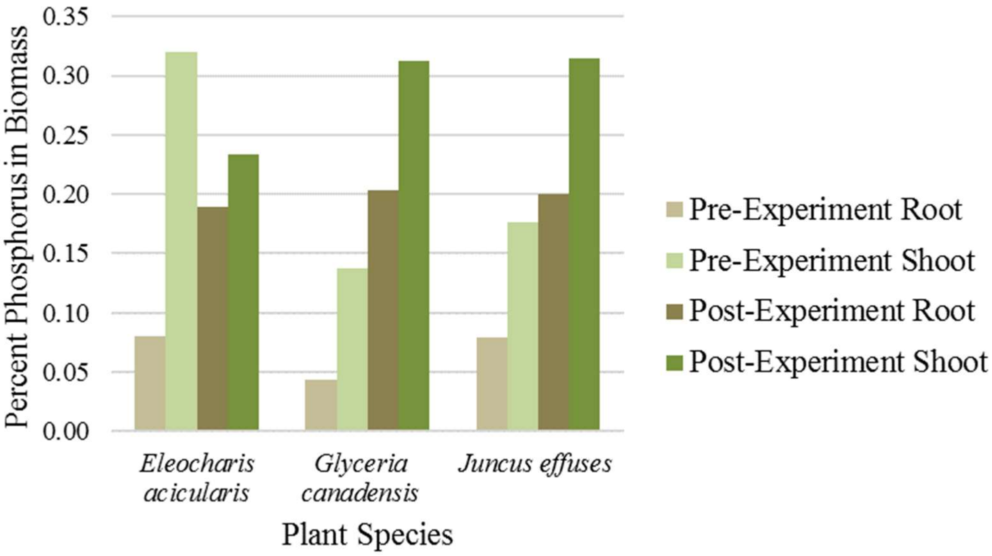

Figure 10. Percent of phosphorus in root and shoot tissues.

Eleocharis acicularis had the highest rate of phosphorus uptake, followed by *Glyceria canadensis*, and *Juncus effusus* (Table 11).

Table 11. Phosphorus uptake rate averaged over the length of the experiment.

Species	Phosphorus Uptake Rate ($g/m^2/day$)
Eleocharis acicularis	0.0030
Glyceria canadensis	0.0025
Juncus effuses	0.0015

There was not a statistically significant difference in the assimilation of phosphorus (g) by the three different plant species (H=1.5673, 2 d.f., $p = .4567$) or the phosphorus uptake rate ($g/m^2/day$) (H=1.4222, 2 d.f., $p = .4911$).

Macronutrients and micronutrients were also analyzed prior to the experiment and following the experiment completion. Concentrations of boron, calcium, copper, iron, and magnesium decreased in all species from prior to the experiment to post-experiment analyses. Potassium was the only element that increased in all species through the experiment (Table 12).

Table 12. Average concentrations of macronutrients and micronutrients in experiment plants.

Species	**Sample Time**	**B mg/Kg**	**Ca mg/Kg**	**Cu mg/Kg**	**Fe mg/Kg**	**K mg/Kg**	**Mg mg/Kg**	**Mn mg/Kg**	**Zn mg/Kg**
Eleocharis acicularis	Pre-Experiment	42.5	11,431.4	100.3	8,084.3	18,407.8	26,596.5	128.4	127.4
	Post-Experiment	9.4	4,682.3	12.1	504.0	22,661.5	1,685.6	166.4	66.1
Glyceria canadensis	Pre-Experiment	12.0	11,478.1	23.2	4,748.8	10,339.2	11,255.6	114.2	79.0
	Post-Experiment	8.9	8,456.9	19.4	468.0	21,576.6	2,586.2	83.4	91.3
Juncus effuses	Pre-Experiment	33.4	12,935.0	48.2	4,540.6	10,520.2	13,931.4	180.2	104.4
	Post-Experiment	18.2	4,589.1	10.9	1,408.8	25,185.7	1,958.1	210.9	118.1

3.5 Biofilms

Biofilms were first observed on a FTW during batch 5. Floating treatment wetland 5 was the first BMP to develop a biofilm. Biofilms formed on exterior sides of FTWs on inundated areas below the water surface. Biofilms were not equally distributed on all FTWs; some FTWs had larger, more readily observable biofilms.

Green and light brown biofilms were often found growing on the inner surface area of the control tubs during Day 7 sampling. On batch 6, it was observed that biofilms covered the majority of control tub surfaces below the water line; experiment tubs had less than 15% biofilm coverage on inner surfaces during this batch. The trend of higher biofilm surface area coverage in control tubs continued throughout the experiment.

3.6 Potting material and FTW materials

Total phosphorus decreased in all FTW soils. The average percent reduction of TP was 35.4% ± 9.2% (Table 13).

Table 13. Change in total phosphorus in soils planted in the FTWs.

Sample Time	Soil Sample	Total Phosphorus (mg/kg)	Percent Reduction of Total Phosphorus (%)
Pre-Experiment	Composite	2,416.2	
Post-Experiment	FTW 1	1,933.5	20.0
	FTW 2	1,529.3	36.7
	FTW 3	1,380.2	42.9
	FTW 4	1,655.6	31.5
	FTW 5	1,309.2	45.8

Total phosphorus in coconut powder decreased slightly, by 2.6% ± 17.0%. One sample, FTW 1, had an 18.4% increase in total phosphorus in coconut powder.

Total phosphorus increased in all coir samples at an average of 46.6% ± 35.3%. The large variation in the standard deviation is due to TP concentrations in FTW 1. The percent increase in FTW 1 (6.9%) was almost 6 times smaller than the next smallest coir sample from FTW 2 (40.3% increase in TP).

4 Discussion

This study quantified FTW reductions of TP, Orthophosphate-P, Nitrate-N, and Ammonia-N. Additionally, it measured the plant contribution to phosphorus removal and identified phosphorus sources and sinks in the FTW structure. Results from this study provide additional information on one of many excess nutrient-reducing BMPs. As a relatively novel BMP, this experiment lends data to a growing number of studies on FTWs. Additionally, as a study conducted in a northern, temperate climate it provides unique results, applicable to installations of FTWs in the Midwest.

4.1 Physicochemical properties of water

Dissolved oxygen was significantly lower in experiment tubs than in control tubs. This finding has occurred in previous floating treatment wetland studies (Lynch et al., 2015; Tanner & Headley, 2011; Wang & Sample, 2014). The lower DO in the experiment tubs is likely the result of two main factors, lower wind diffusion rates and higher respiration rates.

The structure of FTWs covers the water surface and slows winds which can decrease turbulent mixing and oxygen diffusion from the atmosphere into the water column (Ahn & Mitsch, 2002). In a similar experiment on Beemats, a FTW product that allows for whole-plant harvesting, the large percent cover of the Beemat precluded regeneration of dissolved oxygen from the atmosphere. The Beemat in the experiment covered 95% of the mesocosm water surface (White & Cousins, 2013). The effect of reduced wind turbulence and atmospheric occlusion in this experiment is likely smaller due to the smaller percent coverage of the FTW (15%).

The lower DO in experiment tubs also suggests higher respiration rates than photosynthesis rates from plants and biofilms. During aerobic respiration, oxygen is utilized and carbon dioxide is released in the process. Respiration uses the energy gained from photosynthesis to grow and maintain plant cells and relocate carbohydrates (Sage, 2008). In addition to naturally-occurring microorganisms that were pumped from the forebay into the control and experiment tubs, the experiment tubs also had plants and biofilms that could respire. Evidence from a similar mesocosm experiment suggested that planted FTWs favor respiration over photosynthesis. Tanner and Headley (2011) examined nutrient and heavy metal removal of seven FTW treatments: four planted FTWs with different plant species, an unplanted FTW, a FTW with only soil, and FTW with soil and artificial roots. The control mesocosm had equivalent FTW shading. Tanner and Headley found that low dissolved oxygen concentrations only occurred on planted FTWs. This finding indicates that biological processes are contributing to lower dissolved oxygen.

Respiration in experiment tubs may have been favored over photosynthesis due to microbial decomposition of organic matter (Tanner & Headley, 2011). Decomposers use the process of respiration to gain energy from breaking down organic compounds (L. Wang & D'Odorico, 2008). Vähätalo and Wetzel (2008) found that aquatic microorganisms can decompose a large fraction of dissolved organic carbon (DOC) from wetland sources, including recalcitrant DOC. They created leachate from *Juncus effusus* leaves and exposed the leachate to solar radiation and microbes. Using carbon isotope analysis, the researchers determined that microrganisms were responsible for over 50% of organic carbon decomposition (Vähätalo & Wetzel, 2008). With organic content from the FTW soils and plants in experiment tubs, there would be increased energy sources for microorganisms to decompose.

Plant roots may be a contributing factor to increased microbial respiration (Tanner & Headley, 2011). The surface of plant roots provide colonizing area for microorganisms. Plants roots also expell compounds, such as carbohydrates and amino acids, into the adjoining soil. Access to these resources aids microbial growth (Coleman et al., 2001). Plants also provide oxygen to organisms in the rhizosphere, which would allow for increased respiration (Neori, Reddy, Čížková-Končalová, & Agami, 2000).

Photosynthesis was likely not inhibited by decreased access to sunlight. As previously described in Tanner and Headley, shaded control tubs had black covers in the same dimensions of FTWs used in the experiment. Shaded control tubs did not have the low DO levels as observed in the planted FTWs (2011). Therefore, physical shading was not a factor for lower DO concentrations caused by decreased photosynthesis. Turbidity can also affect the amount of light penetrating through the water column, impacting photosynthesis rates (Grobbelaar, 2009). In this experiment, control tubs had higher turbidity levels in 9 of 11 batches. Therefore, suspended particles were more likely to decrease light penetration of the water column in control tubs. Lower turbidity was likely due to decreased wind-driven turbulence from the FTWs (Ahn & Mitsch, 2002; White & Cousins, 2013). Macrophyte roots also promote particle settling through physical reduction of water speeds (Vadeboncoeur, 2009).

The low dissolved oxygen concentrations found in experiment tubs can result in fatal or stressful conditions for aquatic life. A concentration of dissolved oxygen below 4 mg/L can be fatal for aquatic invertebrates (EPA, 1986). On Day 7 measurements, experiment tubs had mean DO concentrations below 7.0 mg/L on 7 out of 12 batches. Three of these were below 5.0 mg/L and one batch was below 4 mg/L DO. Mean control tub DO was always above 7.0 mg/L.

Dissolved oxygen can impact the nitrogen cycle. Low dissolved oxygen levels also favor denitrification. Certain microorganisms use nitrate for respiration, either strictly or facultatively when oxygen is low. Oxygen does not need to be completely absent for denitification. Dissolved oxygen concentrations between 0.3-1.5 mg/L have allowed for denitrification (Kadlec & Wallace, 2009). However, dissolved oxygen levels less than 2 mg/L were not observed in this experiment.

In a calcium-dominated, alkaline system, the phosphorus cycle is not as strongly impacted by changes in dissolved oxygen and redox potential. Changes in pH is a larger driver of phosphorus prepitation and dissolution with calcium. The influences of pH on phosphorus removal in a calcium-dominated system are discussed below.

Experiment tubs also had statistically lower pH levels than control tubs. The lower pH is likely due to two factors: increased respiration and acidic exudates from plant roots. In freshwaters, the proportion of dissolved inorganic carbon (DIC) is the largest factor in determining water pH. Natural, freshwaters are usually dominated by bicarbonate. Carbon dioxide increases in proportion to bicarbonate during respiration. As carbon dioxide is produced, H^+ ions increase and lower the pH of the water. For pH above 5.5, minor increases in carbon dioxide can have a large impact in water pH (Cole & Prairie, 2009). The starting pH of water within the central holding tank was approximately neutral, with a mean of 7.56. The lowest pH measured was 7.01. Therefore, the water in experiment tubs was highly susceptible to changes in pH from plant and biofilm respiration. Second, wetland plants have the ability to alter the pH of soil and water in their habitat (Titus & Urban, 2009). Plants exude organic acids and acidic enzymes from their roots. Typically, these exudates alter compounds into usable forms of iron and phosphorus (Koo, Adriano, Bolan, Barton, & Daniel, 2005; Neori et al., 2000). These acidic secretions from plants may have contributed to the statistically lower pH in experiment tubs.

Changes in pH impact phosphorus precipitation and dissolution with calcium (Reddy & DeLaune, 2008). As determined by the total elemental analysis of Sarita Wetland forebay, calcium is the dominate element in the water of elements that generally bind with phosphorus (Al, Fe, and Mg). The Day 0 experiment water was also alkaline (mean pH = 7.56). Phosphate forms in this pH range are HPO_4^{2-} and $H_2PO_4^-$. When dissolved calcium (Ca^{2+}) is available in alkaline conditions, it will bind with the major phosphorus forms, as shown below.

$$Ca^{2+} + 2H_2PO_4^- = Ca(H_2PO_4)_2$$

$$Ca^{2+} + HPO_4^{2-} = CaHPO_4$$

(Reddy & DeLaune, 2008)

4.2 Nutrient reduction

4.2.1 Phosphorus

The differences in TP, while not statistically significant, show a similar trend (Figure 6). For every known batch, except batch 4, the experiment tanks removed an average of 14% more TP. On batch 4, control tubs and experiment tubs reduced TP by the same percent, 38%. This higher TP removal is similar to other FTW mesocosm studies. Wang and Sample (2014) found TP removal was 8.2% higher on planted FTWs compared to the control. Since this trend was not observed for Orthophosphate-P, it is likely that a different process reduced more particulate-bound phosphorus in experiment tubs. The most likely cause of higher TP removal are plant root processes and the more neutral water pH.

In a stormwater retention pond FTW study, researchers discovered large amounts of total suspended solids attached to the biofilm of plant roots during post-experiment analysis

(Borne et al., 2013; Borne, 2014). This observation was also made during the post-experiment analysis of FTWs used in this experiment. It is possible that particulate-bound phosphorus, measured in TP concentrations, was reduced in the water column due to adherence on root systems (Borne et al., 2013; Borne, 2014). Plant root exudates may have enhanced biofilm growth, allowing for larger binding surface areas. Plant roots exudates can also break off phosphorus bound to calcium, iron, and aluminum. Plants then uptake the soluble phosphorus (Borne et al., 2013; Koo et al., 2005; Neori et al., 2000). This process would also reduce particulate phosphorus concentrations in experiment tubs.

The more neutral pH in experiment tubs can allow for the binding of phosphorus to soils. The same FTW in-pond study conducted by Borne (2014), examined the change in total phosphorus in the pond bottom material. In the pond with the FTW, phosphorus concentrations increased in pond soils during summer and fall. Phosphorus increased in the control pond as well, but at much lower levels. Researchers speculated that phosphorus was binding to clay particles and highly organic soils under the more neutral pH found in the FTW pond (mean pH 7.3); the control pond was more basic (mean pH 8.0) (Borne, 2014). In basic pH ranges soil particles lose H^+ protons and have a negative charge and therefore do not bind with negatively charged phosphates (Reddy & DeLaune, 2008).

Total phosphorus reduction in control tubs had a positive relationship with pH, as shown by linear regression analysis. Experiment tubs showed a similar relationship, with increasing pH and increasing total phosphorus reduction. This positive relationship between pH and TP reduction is likely due to soluble calcium binding to phosphate forms. The observed relationship may not be as strong in experiment tubs due to other TP removal factors, such as plant and biofilm uptake and sorption to coir.

An influx of Orthophosphate-P was observed in batch 1 of both experiment and control tubs. Leaching of phosphorus contained in the mesocosm tubs, such as from coatings or dust, are the likely cause. However, resin mesocosm tubs have been used in a previous FTW field experiment without evidence of phosphorus leaching (Lynch et al., 2015).

A clear pattern does not exist for median Orthophosphate-P removal in control versus experiment tubs. In general, removal rates of Orthophosphate-P in experiment tubs were slower to rise than control tubs in the beginning of the experiment. Removal rates in experiment tubs may have lagged due to plant establishment and growth patterns. Lynch et al. (2015), in an outdoor mesocosm experiment, found FTWs increased phosphorus concentrations during what they termed as a plant "establishment phase." The establishment phase consisted of the first 8 weeks of the 19-week experiment. During the following plant "growth phase," the FTWs removed more phosphorus than the control. This finding aligns with results in this experiment, experiment tub removal rates for Orthophosphate-P were less than control removal rates for 5 of the first 6 batches. In batches 7 through 12, removal rates in experiment tubs were higher than control tubs in 5 out of 6 batches. Additionally, removal rates in the final 6 batches were more consistent than removal rates in the first 6 batches. While macrophytes can assimilate more phosphorus during early growth and colonization, it is not true for every situation.

Individual species growing patterns and soil-available phosphorus affect the pace of plant uptake of phosphorus (Kadlec & Wallace, 2009).

Periphyton establishment may also explain the wide range in reduction patterns during batches 1 - 6. The time from periphyton establishment to maximum biomass can range from days to months. Resource availability impacts periphyton growth rates (Azim, 2009). The inconsistent reduction patterns in batches 1 – 6 may have been influenced by colonization, disturbance, and competition of periphyton in the Sarita forebay water and experiment tubs.

Another possibility for the lower Orthophosphate-P removal, are inputs of phosphorus from the FTWs. Tanner and Headley (2011), found control FTWs with soil and FTWs with artificial roots had increasing concentrations of TP and dissolved reactive phosphorus. They posited that phosphorus was leaching from the soil media in the unplanted FTWs or from the FTWs themselves. Total phosphorus levels in all FTW soils used in this experiment decreased over the experiment timespan. Wetland soils can release sorbed phosphorus when in contact with water of lower phosphorus concentration (Kadlec & Wallace, 2009). Assimilation or desorption are likely factors in the TP soil reduction. Attempts were made to locate a method and lab to determine phosphorus concentrations in the FTWs. However, a reliable method for determining phosphorus in the polyester-blend material could not be found.

Compared to similar FTW experiments, the 51% median removal rate of TP is close to other published values from Tanner and Headley (2011) and Chang et al. (2012). Tanner and Headley (2011) observed up to 50% reduction in TP from their best two planted FTWs (*Carex virgata* and *Cyperus ustalatus*). While starting TP concentrations were lower (ranged from 0.096-0.136 mg/L), dissolved reactive phosphorus comprised the majority of TP, similar to this experiment. Chang et al. (2012) achieved 49-52% removal of TP with 5% surface area coverage. The removal rate also included potential removal from sediments in the bottom of the mesocosms. However, the 51% reduction in this experiment is smaller than an in-pond FTW retrofit experiment with a similar FTW surface area coverage. Winston et al. (2013) retrofitted two ponds, one with 9% FTW coverage and the second pond with 18% coverage. The pond with 18% coverage had a statistically significant higher TP removal rate than TP removal before the retrofit. The FTW reduced TP by 88%. This may be due to in-pond processes that cannot fully be replicated by mesocosm experiments.

The removal rates in this experiment (0.05 $g/m^2/day$ TP and 0.05 $g/m^2/day$ PO_4-P) are higher than previously published rates. Tanner and Headley (2011) measured a 0.0269 $g/m^2/day$ removal rate of DRP. Lynch et al. (2015) reported 0.008 $g/m^2/day$ TP removal during plant "growth stage." In both of these experiments, the percent coverage was higher than this experiment but starting TP concentrations were lower.

4.2.2 Nitrogen

There were four occasions of drastic Ammonia-N concentration increases within the mesocosm experiment (batches 3, 5, 10, and 12). Increases in Ammonia-N concentrations were observed within both control and experiment tubs during these batches.

Ammonification of organic nitrogen is the likely cause of this ammonia influx. Ammonification is the transformation of decomposing organic matter into ammonia by microorganisms (Kadlec & Wallace, 2009). In these observations, experiment tubs had higher Ammonia-N concentrations in three of the four batches; in one batch, the Ammonia-N median concentration was the same. Decaying plant matter is likely the additional nitrogen source for ammonification in experiment tubs. Wang and Sample (2014) also observed higher Ammonia-N concentrations in mesocosms with FTWs. Reductions in Ammonia-N were likely achieved by biological uptake, nitrification, and physical volatilization to the atmosphere (Kadlec & Wallace, 2009; Wang & Sample, 2014). Plants prefer ammonia to nitrate for nitrogen growth requirements (Kadlec & Wallace, 2009). However, this experiment did not detect a clear relationship between Ammonia-N reduction and Nitrate-N concentrations.

The inconsistent removal rates of Nitrate-N in batches 1 through 6 may be due to FTW and plant and periphyton acclimation to the mesocosms, as discussed in section 2.4.1. Along with phosphorus, Lynch et al. (2015) measured increases in TN in mesocosms with Biohaven FTWs during the first eight weeks of the study. During this time period, the control outperformed the FTWs in TN removal. In the remainder of the experiment, weeks 9-18, FTWs had higher TN removal efficiencies than the control. Lynch and others (2015) believed the change in removal rates may be due to a plant acclimation period or early-experiment nutrient release from the peat moss planted in the FTWs.

Experiment tubs consistently removed more Nitrate-N in batches 7 through 12. The most likely route of Nitrate-N removal in experiment tubs was uptake and assimilation. Lower percent reductions in Nitrate-N were observed during batches 7 through 9. These batches contained the highest level of nitrate spiking in the experiment (28.8 mg/L NO_3). Plants and microorganisms may not have been able to uptake additional nitrogen as nitrate for their growth needs (Kadlec & Wallace, 2009). An additional factor may have been growth limitation caused by a limiting macronutrient or trace element needed for growth (Kadlec & Knight, 1996). These factors may have dampened the Nitrate-N removal rate.

Denitrification in the water column is not the likely explanation for the reduction in Nitrate-N due to observed oxic conditions during all batch measurements. One potential source for denitrification in the FTW system, are saturated soils in the FTWs. Anaerobic processes for denitrification can occur in anoxic soils, allowing converted nitrogen gases to be released. A second possible site for denitrification is within biofilms. Biofilms on plant root and FTW surfaces can denitrify nitrate in low dissolved oxygen conditions (Kadlec & Wallace, 2009).

Percent removal of Nitrate-N in this experiment (33%) was less than reported in other FTW studies. However, previous studies had lower or much higher starting Nitrate-N concentrations. In the study by Chang et al. (2012), FTW mesocosms without littoral plants removed approximately 70-90% of nitrate with 5% coverage. The larger coverage, 10%, removed less nitrate at 65% reductions. However, these mesocosms had filled, sediment bottoms, which may have allowed for nitrate loss through denitrification. Additionally, the mesocosms in Chang et al.'s experiment were spiked to a match stormwater runoff concentrations, which were nitrogen limited. Winston et al. (2013) had an 86% median reduction efficiency of nitrite-nitrate in the stormwater pond retrofitted with a 9% surface cover. The nitrogen reduction compared to the pre-retrofit was statistically significant in the study completed by Winston et al. In both of these studies, the starting concentrations of nitrogen were much lower than this experiment. Chang et al. (2012) spiked to 3 mg/L total nitrogen. The median concentration of influent water in the retrofit pond was 0.35 mg/L Nitrate-Nitrite-N (Winston et al., 2013). The starting Nitrate-N concentrations in this experiment were generally higher, ranging from 2.0 to 6.5 mg/L.

The removal rate in this experiment is also lower than experiments with higher starting nitrate concentrations. In a lab test Stewart et al. (2008) found FTWs removed approximately 96 g/m^2/day of nitrate. These experiments were spiked to 230 mg/L NO_3-N, had an added carbon source (molasses), were artificially heated to 27 degrees Celsius, and were aerated.

4.3 Plant growth and phosphorus uptake

Phosphorus content in plant dry weight in this experiment closely matches published ranges. Percent phosphorus in dry weight of emergent macrophytes have been found to range from 0.14 to 0.30% (Kadlec & Wallace, 2009). Reliable and comparable information for *Glyceria canadensis* and *Eleocharis acicularis* could not be obtained. *Juncus effusus* is a commonly occurring species and has been previously implemented in FTW experiments.

At the culmination of the Beemat versus the Biohaven FTW experiment, Lynch et al. (2015), found similar phosphorus content in *Juncus effusus* planted on both floating island products. Phosphorus in the shoots of the plant was 0.15% and 0.25% in the roots. This is similar to the findings of in this experiment, 0.20% phosphorus in shoots and 0.31% in roots for *Juncus effusus*. The slightly higher average phosphorus percentage in dry biomass is surprising because the Lynch et al. field experiment was conducted in Virginia, with higher average temperatures during the growing season, over a longer period of time (experiment started in May and ended in October). The higher phosphorus content in this experiment may be due to higher phosphorus concentrations in the spiked experiment water. The average TP in Lynch et al. mesocosm water was 0.22 mg/L. Macrophytes have been found to accumulate more phosphorus in shoot tissues when planted in nutrient-rich wetlands (Kadlec & Wallace, 2009). Hubbard et al. (2004), in nutrient-rich waters (8 mg/L P and 53 mg/L N), tested *Juncus effusus* growth and nutrient uptake. At the culmination of the one-year month experiment, phosphorus comprised 0.36% of total dry biomass.

The biomass for *Juncus effusus* was much lower than other FTW studies. *Juncus effusus* planted within FTWs grew to an average plant dry biomass of 59 g above ground and 7.9 g below the FTW (Lynch et al., 2015). Comparatively, *Juncus effusus* in this study grew to an average shoot dry biomass of 5.61 g and root dry biomass of 3.04 g. The larger biomass may be due to the longer experiment length and longer growing season at the study site in Virginia (Lynch et al., 2015). Similarly, Hubbard et al. (2004) had an average biomass of 275 g after the first two months of the experiment (August 20 through October 15). As before, the much larger biomass may be a product of the warmer average temperatures at the field site, Georgia, and longer growing time during the experiment (starting in June).

5 Research Limitations and Future Research Needs

This experiment attempted to quantify the nutrient-reducing potential of FTWs in a northern climate. For that reason, wetland soil was not placed in bottom of the mesocosms. Microorganisms and soil properties of added benthic material may have influenced nutrient cycling within the experiment, muddying the nutrient removal effect of FTWs. Microorganisms living in the soil may have used phosphorus or nitrogen for growth. Additionally, anoxic conditions in the bottom material may have allowed for phosphate release or denitrification. Phosphorus could also have bound to benthic soils. In a field experiment or application, phosphorus and nitrogen reduction rates may be impacted by soil type, antecedent soil nutrients, and microorganisms present in the soil. As previously described in Borne (2014), TP concentrations increased in pond sediments with the FTW in comparison to the control pond. The more neutral pH created by the FTW likely promoted phosphorus binding to clay particles and organic soils. In the long-term sediments would become burial sites of residual plant material containing phosphorus and nitrogen (Kadlec & Wallace, 2009).

The small scale of the mesocosm experiment may have affected dissolved oxygen concentrations. Ahn and Mitsch (2002) compared the physicochemical properties of water and nutrient concentrations of a constructed wetland and mesocosms mimicking the constructed wetland. Sampling from two growing seasons demonstrated that the small mesocosm scale reduced DO concentrations. The smaller fetch of the mesocosms may have reduced atmospheric mixing of oxygen into the water column. Low oxygen and anoxic conditions can encourage phosphorus release, which may not occur on a full-scale wetland.

Plant phosphorus growth and uptake rates should also be viewed with caution. In free water surface constructed wetlands, with high concentrations of phosphorus and where phosphorus is the limiting nutrient, early plant growth and uptake can be high. However, this accelerated growth and nutrient assimilation may only last for one to two years. Phosphorus removal will still occur through burial of dead plant material, albeit at a lower rate (Kadlec & Wallace, 2009).

In this study, it was also unclear the proportion of nutrient removal due to microbial activity. An investigation into the microbial communities inhabiting the FTW matrix would elucidate microorganisms' role in excess nutrient reduction. A small portion of previous research on FTWs have focused on microbial contribution to nutrient uptake. Li et al. (2011) studied the effect of denitrifying polyphosphate-accumulating organisms (DPAOs) on FTWs planted with two variations of *Lolium perenne* (perennial ryegrass), Top One and Respect. Removal rates were calculated for both plant variants, microorganisms on their own, and both plant variants with added microorganisms. Variant Respect showed the higher removal rate of two *Lolium perenne* species. However, the highest removal rate was achieved by variant Respect with added microorganisms, indicating a shared role in nutrient uptake. Li et al. suggested future research on DPAOs and FTWs focus on the succession of these microbial communities and the exact processes of nutrient reduction.

Lastly, “results from transient studies must not be construed as being representative of long-term patterns” (Kadlec & Wallace, 2009). Many FTW studies, including this one, have been completed over one growing season. More data over multiple growing seasons is necessary to determine the long-term nutrient removal efficiencies of this BMP. A two-year study completed by the Minneapolis Parks and Recreation Board on seven FTWs installed in Spring Lake (Minneapolis, Minnesota) identified multiple issues with long-term use of the islands. Invasive plant species colonized the FTWs in the first and second years. Over the winter, the FTWs’ structure and plantings were damaged by animals and ice. Similar to this study, water clarity improved, as measured by Secchi disc depths. Additionally, total nitrogen and TP increased. However, the Minneapolis Parks and Recreation Board could not determine if the FTWs or another process was responsible for the increase in nutrients (2013).

5.1.1 BMP design improvement

As a relatively new BMP, design alterations may improve its performance. A larger surface area to volume ratio may improve removal rates of biofilms. Biofilms were not observed on inner surfaces of the FTW matrix during the dissection of FTWs in post-experiment analysis. Microbial communities were found growing on exterior surfaces, mostly along the sides of the FTWs. Therefore, many small FTWs may be more beneficial than one large size FTW due to the extra surface area provided. Another option in the FTW construction process would be to inject less buoyancy material in the matrix. This alteration would lower the BMP, increase water contact area, and areas for biofilm growth. Additionally, less buoyancy foam may increase plant growth.

During FTW dissection, some pre-cut FTW holes were found to be placed directly above the marine-grade foam added to the FTWs. In these situations, plant roots of *Eleocharis acicularis* were unable to penetrate through the foam; roots were found balled on top of the foam, as seen in Figure 11. *Juncus effusus* and *Glyceria canadensis* roots were able to grow through and around the foam.


Figure 11. A root ball created by blockage of the marine-grade buoyancy foam.

5.1.2 Considerations for managing impaired waters

Those making management decisions for improving impaired waters should carefully consider the range of BMPs that treat excess nutrients. This and other FTW experiments have found reduced DO levels in waters treated with FTWs. This possible result of installation should be considered when macroinvertebrates or other aquatic life may be negatively impacted by low DO concentrations. In terms of nutrient cycling, low DO may be desirable for denitrification for nitrogen-impaired waters but undesirable for phosphorus-impaired waters because low DO can release bound phosphorus.

Results from this study indicate that robust microbial population associated with the plants on the FTW will improve nutrient removal. An assessment of the DPAOs already present in the impaired water may be useful to determine if powerful nutrient-reducing biofilm may grow on the FTW structure. Fast growing plants are also recommended for planting because they are able to assimilate larger amounts of nutrients more quickly.

Lastly, this research suggests FTWs may perform best in more impaired waters; batches 7-12 with higher starting nutrient concentrations had higher experiment tub removal efficiencies than in control tubs. Further research is needed, however, to determine if this result is due to plant and microbial acclimation on the FTW. Future, long-term field installations of FTWs in temperate climates waterbodies impaired by excess nutrients will help clarify the ideal setting for this BMP.

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Appendix I – Blank, Spike, and Replicate Results

1. *Blank Sample Analysis*

Four of seventeen reagent blanks had concentrations of orthophosphate over the MDL. In three of these four reagent blanks, the average concentration of the analyte detected was 0.02 mg/L PO_4 above the estimated detection limit (0.05 mg/L PO_4). The fourth contaminated reagent blank only appeared to affect one tub sample in the batch (tub 2); this sample had a replicate analyzed at the RAL. The RAL result matched the range of other samples from that batch. The contaminated sample was removed and the RAL replicate result was used in statistical analysis.

Two of eighteen reagent blanks had concentrations of TP over the MDL. The average concentration of the analyte detected was 0.02 mg/L TP above the MDL (0.05 mg/L TP).

Two of fourteen reagent blanks had concentrations of NO_3 over the Nitratax probes lower detection limits. One reagent blank was 0.01 mg/L NO_3 above the lower detection limit of the Nitratax Clear SC (0.5 mg/L NO_3). The second reagent blank was 0.05 mg/L NO_3 above the lower detection limit of the Nitratax Plus SC (0.1 mg/L NO_3).

Four of eighteen reagent blanks had concentrations of Ammonia-N over the MDL. The average concentration of the analyte detected was 0.01 mg/L NH_3 above the MDL (0.02 mg/L NH_3).

2. *Spike Sample Analysis*

Spikes were submitted to represent 5% of samples customarily analyzed at the RAL. Spiked concentration results of TP and Ammonia-N are summarized in Tables 14 and 15, respectively.

Table 14. Total phosphorus results from the RAL of prepared spikes.

Prepared Spike TP (mg/L)	RAL Result TP (mg/L)
0.08	0.07
0.16	0.14
0.25	0.25
0.33	0.29
0.65	0.63
1.00	1.00

Table 15. Ammonia-N results from the RAL of prepared spikes.

Prepared Spike NH3-N (mg/L)	RAL Result NH3-N (mg/L)
0.04	0.11
0.06	0.12
0.12	0.19
0.18	0.37
0.40	0.45
0.80	0.87

3. *Replicate Sample Analysis*

The differences between RAL and the Hach DR/890 orthophosphate replicates were found to be statistically insignificant in a paired, two-sided Wilcoxon rank sum test. Alpha was set to 0.05 and equal variances were assumed. The median difference between the RAL and Hach DR/890 results was 0.00013 mg/L. RStudio was used for the analysis.

The differences between RAL and the Nitratax Plus SC replicates were found to be statistically insignificant in a paired, two-sided Wilcoxon rank sum test. Alpha was set to 0.05 and equal variances were assumed. The median difference between the RAL and Hach DR/890 results was 0.3 mg/L. RStudio was used for the analysis.

Research analytical laboratory replicate samples were analyzed at Pace Analytical. Analytical nutrient results are summarized in Table 16.

Table 16. Results of replicate analysis for nutrients.

Nutrient	**RAL**	**Pace Analytical**
TP (mg/L)	0.33	0.23
Orthophosphate-P (mg/L)	0.14	0.15
Nitrate-N (mg/L)	1.8	1.9
Ammonia-N (mg/L)	0.11	ND

ND: Non-detect.

Analysis methods and method detection limits from Pace Analytical are summarized in Table 17.

Table 17. Analytical methods used at Pace Analytical and the method's MDL.

Nutrient	**Method**	**MDL (mg/L)**
TP	SM 4500-P E Preparation Method: SM 4500-P B	0.025
Orthophosphate-P	SM 4500-P E	0.0017
Nitrate-N	SM 4500-NO3 H	0.050
Ammonia-N	EPA 350.1	0.020

SM: *Standard Methods for the Examination of Water and Wastewater* (1997).