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AQUATIC PLANTS AND THEIR APPLICATION TO SUCCESSFUL FLOATING TREATMENT WETLANDS

by

KATHERINE MAY MAZANEC

A THESIS

Presented to the Graduate Faculty of the

MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

in

APPLIED AND ENVIRONMENAL BIOLOGY

2020

Approved by:

Dev Niyogi, Advisor
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ABSTRACT

This research aims to mitigate eutrophication of freshwater habitats affected by urban stormwater runoff. Two highly impacted urban ponds near the Missouri S&T campus in Rolla were the focus of this research on the application of floating treatment wetlands (FTWs). An FTW consists of a man-made floating mat that is planted with emergent or floating macrophytes. The plants grow on the mat and their roots extend into the water column below the mat. Plant tissues, especially roots in direct contact with the water, take up nutrients, act as biofilm growth sites, and may facilitate precipitation of nutrients. With urbanization, ponds receive enhanced fluxes of nutrients from runoff that can negatively impact the ponds and downstream ecosystems. By mitigating the inflows of nutrients, FTWs can help maintain water quality and biodiversity of these systems. My research objectives were to examine nutrient (nitrogen and phosphorus) removal rates from microcosms containing different plants. Simulated stormwater runoff was added to lab microcosms containing coir fiber medium and bare-root plants. The removal rate of N and P from the water was monitored by taking samples over time. Based on a one-way ANOVA, there was a significant difference among the plant treatments for the rate of uptake for soluble reactive phosphorus (SRP) for rates per microcosm ($P = 0.003$) but not for rates per mass of plant used ($P = 0.22$). ANOVA did reveal significant differences among plant treatments for uptake rates of N per microcosm ($P < 0.001$) and per biomass of plant used ($P < 0.001$). Microcosms planted with *Cladophora* and *Spirogyra* (algae) and *Scirpus atrovirens* (Bulrush) had higher uptake rates of N compared to most other plants (Tukey post-hoc comparison, $P < 0.05$).

ACKNOWLEDGMENTS

I deeply thank my advisor, Dev Niyogi for his continuous support and encouragement throughout my research. He made his expertise available in numerous ways, including collecting plants for research, running nitrate on the IC, and supplying a tired graduate student with the occasional Twix bar. I am grateful for Mark Fitch (co-advisor and committee member) for challenging me to think outside the box and adding useful data insight. I thank Robin Verble for her help reviewing my thesis and participating in discussions for further research ideas. I thank the Department of Biological Sciences for funding my education through a graduate teaching assistantship. This thesis could not have been written without Carla Campbell. She selflessly helped me with lab work, while encouraging and assuring me that everything would be okay when my data did not turn out as planned.

Undergraduates, Jeremy Mesa, Ethan Vinyard, Lydia Klenke and Emma Hannah were a great help taking late-night samples and working tirelessly in the lab to maintain our precious sample freezer space. I thank the women of GWIS-Rolla for supporting me and encouraging me through my journey as a graduate student. I am forever grateful for my husband, Kyle Summers, who spent some weekends helping me with field or lab work. On long lab days, I knew I could come home to a nice dinner and loving husband who always listened to my stories of the long day. This thesis would not be possible without the love and encouragement from Kyle. Lastly, I would like to thank my family for always letting me pursue my dreams and continue into the sciences. Almost 1000 miles away, I can always feel the love from my family.

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1. INTRODUCTION

1.1. THE PROBLEM: STORMWATER RUNOFF

Stormwater runoff in urban areas can contribute excess nutrients, sediment, and other pollutants along with high flows to downstream water bodies. Nutrient pollution entering waterways is an increasing problem caused by human development (Arnold & Gibbons, 1996). Urbanization around waterways leads to greater amounts of suspended and deposited sediment in streams. Flooding can also be an issue caused by an increase of impervious surfaces and loss of vegetative cover in urban watersheds (Anderson, 1970). The constant stress of pollutants coming into a freshwater system in urban landscapes can damage the quality of streams and other freshwater systems.

Non-point sources of pollutants can contribute nutrients, sediment, and other unnatural chemical compounds to urban watersheds (Loperfido, 2013). These urban pollutants lead to stress in urban streams, wetlands, ponds, and lakes (Feminella & Walsh, 2005). Excess nutrients can promote enhanced algal growth, which leads to eutrophication. Eutrophic waters can significantly affect aquatic life, such as fish populations (Willemsen, 1980). High algal growth and warm waters can trigger summerkill events, where lack of oxygen leads to fish death (Anderson, 2009). Eutrophic conditions can prevent autotrophic benthic communities from receiving sunlight by having excessive algae in overlying waters. This undesirable growth of algae can severely hurt ecosystem dynamics in freshwater communities. In addition, urban pollution can kill sensitive species that are unable to tolerate high concentrations of pollutants (De La Torre et al., 2005).

In some settings, environmental engineers and urban planners are creating more stormwater retention ponds and constructed wetlands to collect and treat stormwater runoff. These basins are commonly seen along highways, in suburban neighborhoods or around shopping centers. In some cases, the retention ponds are aerated to reduce algal growth and add oxygen to improve water quality for fish and sensitive biota in these ecosystems (Kuntz, 2015).

1.1.1. Eutrophication. Eutrophication is an increase in primary production in ecosystems, usually related to enhanced concentrations of nutrients. In ponds and lakes, high growth of the phytoplankton (suspended algae) can create issues with water quality as well as visual appearance of the ecosystems (Stoermer, 1978). Once the phytoplankton die, bacteria break down the dead biomass, using oxygen in the process. This can deplete the pond of oxygen, often causing fish kills (Burkholder et al., 1999). When nutrient loading occurs, phytoplankton can quickly take over a pond (Kalff & Knoechel, 1978). They reproduce fast and can thrive in poor water quality environments such a drainage ditches and heavily polluted water bodies. Algae can cover ponds as suspended phytoplankton, causing benthic plants to suffer from limited light availability. Harmful algal blooms (HABs) have also been found to produce toxins that can affect human health (Pearson et al., 2010). Hypoxic zones are especially dangerous because the loss of oxygen in a short period of time can quickly harm the entire ecosystem and lead to catastrophic fish kills (Burkholder et al., 1999).

Algae is a common problem that costs the United States millions of dollars every year (Hoagland & Scatasta, 2006). In a 2000 Annual Report from NOAA, they estimated that the average economic impact for HABs from year 1987 to 1992 period was over

$740 million dollars (Anderson et al., 2000). Algal blooms can affect drinking water quality, recreation/tourism, commercial fisheries, and the high monetary cost to monitor and manage HABs (Anderson, 2009). Lake Erie, for example, provides water for about 11 million people, but since the use of heavy fertilizers in the Midwest, the lake has been suffering from catastrophic HABs (Michalak et al., 2013). The EPA has invested millions of dollars in research, monitoring, and restoration of the Great Lake region (Russ, n.d.).

Algal blooms can lead to loss of fishing opportunities (Moore et al., 2019). For example, commercial fisherman on the Great Lakes are unable to create revenue when HABs are present (Wolf et al., 2017). Given that algal blooms can cause negative health affects (Center for Disease Control, 2018), restrictions are also put in place to prevent locals from enjoying recreation.

Eutrophication is usually related to common stormwater pollutants seen in urban areas: phosphate, nitrate, and ammonium (Barbosa et al., 2012). These nutrients come from a variety of sources. Phosphates can also occur naturally from the weathering of rocks, but in urban areas, it is most likely from fertilizers used on manicured lawns as well as animal waste (Carpenter et al., 1998). Nitrate can be found in lawn fertilizers and animal waste but it can also occur naturally from geologic deposits (McMahon et al., 2011). Lastly, ammonium is also commonly found in fertilizer and animal waste, which can easily make its way into freshwater environments through pet waste or from eroded sewage pipes in older cities (Misiunas, 2008). All these pollutants can cause problems when added to a freshwater source in large quantities. Loss of biodiversity, poor water quality, habitat degradation, and other negative impacts are common in such polluted waterways (Sansalone & Buchberger, 1997).

1.1.2. Water Quality. Poor water quality from non-point source pollutants is a common issue across the globe. Water is a key element for our lives from agricultural irrigation to our drinking water supply. As populations grow and as climate change intensifies, there is a higher demand for the use of fertilizers and pesticides (Tenkorang & Lowenberg-Deboer, 2009). When adding these to crops in large concentrations, the surrounding aquatic ecosystems are negatively impacted (Richter et al., 1997; Sharpley et al., 1994).

When regulations are not in place or rules are not followed, contaminants can easily make their way into drinking water. Flint, Michigan is an a example of a polluted water source affecting the lives of people with lead poisoning (Hanna-Attisha et al., 2016). There are many other locations that are currently being cleaned up under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) also known as the Superfund. As of 2019. There are over 1344 contaminated Superfund sites that span across the United States (U.S. Environmental Protection Agency, 2016).

Heavy metals are also a concern in many urban environments. Heavy metals can come from a variety of sources including agricultural, domestic, and sewage runoff (Akpor, 2014). In high concentrations, heavy metals can be deadly to aquatic life (Baby et al., 2011). One concern with heavy metals is bioaccumulation in the food chain (Chen et al., 2000).

1.1.3. Habitat Quality. Urban ponds seen in local parks and neighborhoods can serve multiple purposes, including stormwater storage or treatment. Ponds in the city usually have trails around them, and people appreciate their aesthetic value. People can take their dogs on a walk and enjoy the fresh air, while others may recreationally fish in the pond. However, many ponds that are not being effectively maintained or monitored by the community can easily become eutrophic. In the last 10 years, toxic cyanobacterial blooms in lakes and ponds across the U.S. have killed dogs (Backer et al., 2013). These areas have impacts beyond their ecosystem by potentially harming citizens' pets.

Not only will stormwater runoff cause nutrient loading to urban ponds, it will also affect the geomorphology and hydrological connectivity of the aquatic ecosystem (Bracken & Croke, 2007). Heavy rainfall often causes high water velocity and discharge in urban streams. This can result in heavily eroded stream banks and significant sediment deposition downstream when the water velocity slows (Chin, 2006). Sediment deposition is known to smother macroinvertebrates resulting in altered trophic systems (Gray & Ward, 1982).

1.1.4. Biodiversity Loss. The biodiversity of aquatic ecosystems can provide many different ecosystem services. Ecosystem services are broken down into categories which include provisioning, regulating, cultural and supportive services (Bolund & Hunhammar, 1999). For example, people can eat fish from clean aquatic ecosystems, thus benefitting from ecosystem provisioning service. Clean water systems can be used for recreation, which enhances their overall cultural service. A rich, biodiverse body of water has healthy nutrient cycling and healthy levels of primary productivity, thereby regulating the ecosystem.

1.2. THE SOLUTION: FLOATING TREATMENT WETLANDS (FTWs)

Floating islands have been documented to attract fish as early as 237 AD (Alcaraz, 2005). However, the scientific value of such systems has increased in the last three decades (Colares et al., 2020). There has been an exponential rise in publications for FTWs since 2006 (Colares et al., 2020). The top two countries studying this technique are China and the United States (Colares et al., 2020). FTWs used across the globe vary because of regional plant selection and different environmental factors (temperature, humidity, biotic region etc.). FTWs have varying treatment applications. They can treat wastewater, agricultural runoff, urban runoff and stormwater (Colares et al., 2020).

1.2.1. FTW Structure. Wetlands have excellent capabilities to retain nutrients and heavy metals. However, in an urban area, a constructed wetland may be neither viable nor affordable, if space or budget is an issue. FTWs have three components: an emergent macrophyte shoot, a floating base, and the roots of the selected plant(s) (Headley & Tanner, 2008). The size of FTWs can vary depending on the size of the pond. Many places implement multiple FTWs into their pond to remediate the nutrient loads (Winston et al., 2013a). FTWs can not only take up nutrients, like constructed wetlands, but they also provide the pond with habitats for aquatic organisms and contribute to food web support by supplying carbon (Knight et al., 2001).

Another benefit of FTWs is their visual appeal and uniqueness. Park visitors can experience the beauty of FTWs. However, FTWs are still a new technique and are not widely known or implemented in urban areas. Studies are lacking data for harvesting strategies and performance of specific plant species. The abilities of nutrient retention can vary based on plant selection and other environmental factors (Vymazal, 2007). FTWs can be configured in a variety of ways. Most scientists suggest a biodegradable floating mat (Z. Chen et al., 2016). The floating mat can encircle emergent hardy plants along with smaller macrophytes. FTWs can also be positioned in coconut fiber netting or hydroponic pots (Garcia Chance & White, 2018). Since there are so many options for FTWs, it is easy to gather materials and place them within a polluted freshwater system.

1.2.2. FTW Capability. FTWs have been successful in different environments including stormwater retention basins (Winston et al., 2013b), wastewater treatment areas (Van De Moortel et al., 2010), urban ponds (Tanner & Headley, 2011), lakes (Lu et al., 2018) and other freshwater systems. FTWs have been used all over the world and with a wide variety of plants.

Different plants have shown varying capabilities for taking up nutrients in FTW systems. *Juncus effusus,* a common tall grass sedge, has been successfully used to reduce nitrate and phosphate concentrations in a mesocosm study done in South Carolina (Garcia Chanc et al., 2019a). Also in South Carolina, *Canna flaccida* (native south-eastern U.S aquatic plant) and *Juncus effuses* after two seasons as a FTW were capable of significantly reducing N and P while also reducing temperature, pH, and dissolved oxygen (White & Cousins, 2013). *Iris pseudacorus,* yellow flag iris, can remove nutrients and heavy metals from simulated stormwater and constructed wetlands (Chen et al., 2009; Keizer-Vlek et al., 2014; Wang et al., 2015). *Pontederia cordata,* pickerel weed, has been shown to be successful for nutrient uptake in several studies (Chen et al., 2009; Garcia Chanc et al., 2019a; Wang et al., 2015). In a nursery runoff study, *Pontederia* did significantly better than *Juncus* at reducing TP and TN (Spangler et al., 2019). Bulrush, was also successful in an FTW study for removing N and P over a seasonal period (Wang et al., 2015). *Typha domingensis* , southern cattail, efficiently removed phosphorus while the highest concentrations of N and P remained in the plant biomass in an FTW study (Di

Luca et al., 2019). However, there are many aquatic plants that have yet to be tested for their nutrient uptake rates.

Hybrid FTWs include the addition of another facilitator that will improve the nutrient removal efficiency. For example, FTWs can also be inoculated with additional rhizospheric microbes to facilitate the nutrient uptake process (Shahid et al., 2020). Aeration is commonly used to disrupt the diffusion effect by promoting mixing (Garcia Chance & White, 2018) and breaking up large mats of algae while keeping the dissolved oxygen (DO) high. The addition of other plants, including emergent and floating macrophytes, can be beneficial for nutrient removal (Nahlik & Mitsch, 2006).

1.2.3. FTW Feasibility. In many urban areas, stormwater retention basins are restricted by space (Pavlowsky, 2016). If space was not an issue, many environmental engineers could construct wetlands or additional riparian forest near the outflow of a pond to naturally reduce the high concentrations of pollutants. However, in many cases there is no room for a large constructed wetland. FTWs can solve the problem of limited space and nutrient enrichment. Instead of using terrestrial space, FTWs can be an addition to an already existing stormwater retention basin.

1.2.4. Plant Selection and Physiology. Emergent macrophytes are plants that are rooted in water with plant mass above the surface. Floating macrophytes are aquatic plants that float on the surface of the water. All plant types retain nutrients that are essential for their growth and reproduction (Caldwell et al., 2005). Nutrients can be distributed throughout the plant based on current biological needs. In constructed wetlands, the nutrients are usually sequestered in plant biomass (Breen, 1990). Nutrients in plant biomass can be used for growth, reproduction, and homeostasis/regulation of cellular activities. Plants are capable of adjusting their metabolic processes when nutrients are scarce, resulting in larger root mass to interact with nutrient-rich soil (Hermans et al., 2006).

Plant senescence is one of the last developmental phases where the plant begins to degrade as a result of changing temperature (Woo et al., 2018). Plants in senescence show visible signs of chloroplast degradation with dis-colored leaves (Avila-Ospina et al., 2014). This phase is important because the plant is no longer investing energy into growth. During senescence the plant is investing energy into nutrient remobilization where nutrients will be used to develop organs and seeds (Roberts et al., 2012). Harvesting FTWs before senescence would be ideal to maintain high rates of nutrient removal (Garcia Chanc et al., 2019a).

1.3. GOALS AND HYPOTHESES

At Missouri University of Science and Technology (Missouri S&T), my research group has studied the issue of stormwater runoff and how to mitigate it using FTWs. The goals of this experiment were to measure nutrient uptake in microcosms with plants and mesocosms with FTW systems. With this research, decisions regarding local pollution in ponds can be made accordingly.

The hypotheses for my thesis were as follows:

- Selected aquatic plants (both floating and emergent macrophytes) for FTW applications reduce simulated stormwater pollutants in a controlled microcosm setting.

- Different aquatic taxa selected in this study have statistically different nutrient uptake rates.

Aquatic plants are the foundation of a FTW and by studying their ability to remove nutrients, it will strengthen the current scientific literature (Table 1.1). Urban planners can implement these ideas in storm water ponds. Engineers have options to create and design more ecologically viable FTWs that are biodegradable. These are just a few insights that can result from our experiments.

Table 1.1 Missouri plants used for this study with success shown in previous studies.

Selected Plant	Common Name	Native to Missouri	Previous Studies
Pontederia cordata	Pickerel Weed	Yes	(Garcia Chanc et al., 2019a) (Garcia Chanc et al., 2019b)
Iris virginica	Blue Flag Iris	Yes	(Chen et al., 2009)
Juncus effusus	Common Rush	Yes	(Garcia Chanc et al., 2019a) (Garcia Chance & White, 2018) (Garcia Chanc et al., 2019b)
Ceratophyllum demersum	Coontail	Yes	(Dierberg et al., 2002; Sung et al., 2015)
Eleocharis	Spikerush	*Eleocharis compressa* native in Missouri	(Sim et al., 2011)
Nasturtium officinale	Watercress	Yes, in Ozark Mountain Region	(Hoffmann et al., 2008; Vincent & Downes, 1980)
Scirpus validus	Bulrush	*Scirpus atrovirens*, native Missouri species	(Picard et al., 2005; Rycewicz-Borecki et al., 2017; Wu et al., 2011; Zhang et al., 2013)
Oscillatoria	Filamentous Cyanobacteria	Yes	(Chevalier et al., 2000; Suttle & Harrison, 1986)
Spirogyra or *Cladophora*	Filamentous Algae	Yes	(Adey et al., 1993; Havens et al., 1999; Kim et al., 2018)

2. METHODOLOGY

Rolla, Missouri has two main ponds within the same Burgher watershed (Figure 2.1), which is close to campus. Water samples were taken at the 14th and 16th street inlets of Frisco Lake, the western inlet of Ber Juan Pond, and the outlets of both ponds. Stormwater concentrations in the inflows to these ponds were about 200 µg/L of soluble reactive phosphorus (SRP), 2000 µg/L of nitrate-N, and 200 µg/L of ammonium-N (Figure 2.2).

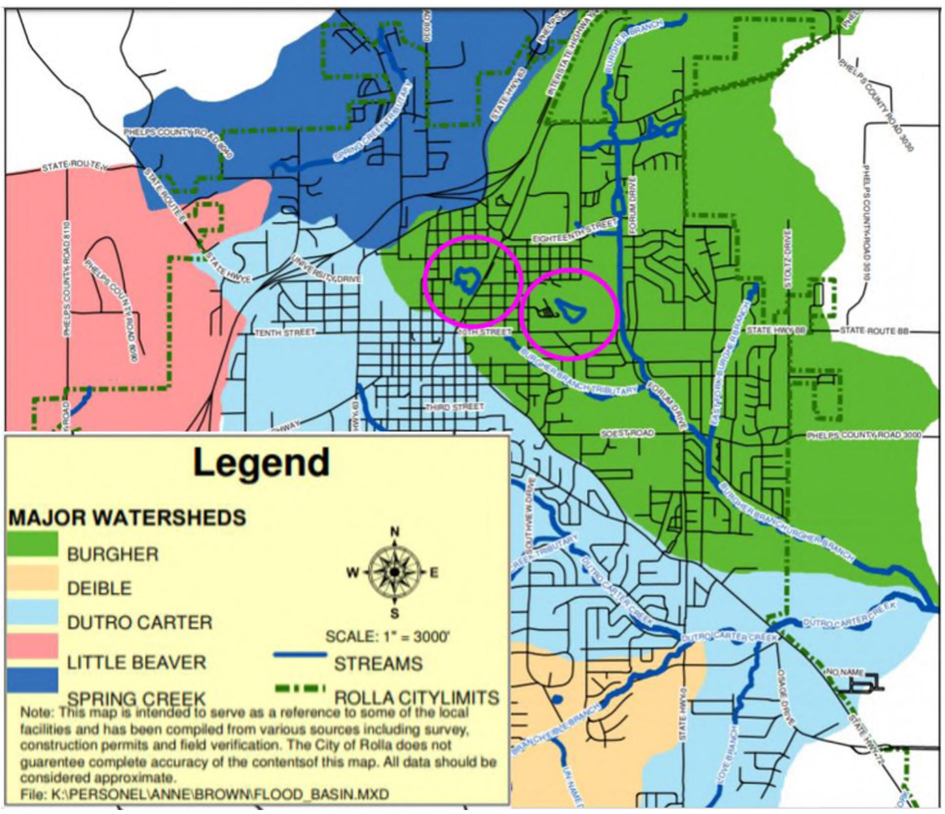

Figure 2.1 Rolla, Missouri Watersheds. Left circle Frisco Lake (Schuman Park) and right circle Ber Juan Pond. Image from the City of Rolla.

The first flush phenomenon explains that during the beginning of a storm event, the nutrient concentrations are the highest (Sansalone & Buchberger, 1997). Concentrations before a storm event in Ber Juan Pond inflow for all nutrients were higher suggesting the stormwater is diluting current nutrients in the inflows (Figure 2.2). This also could suggest that old sewer pipes are leaking into Ber Juan Pond.

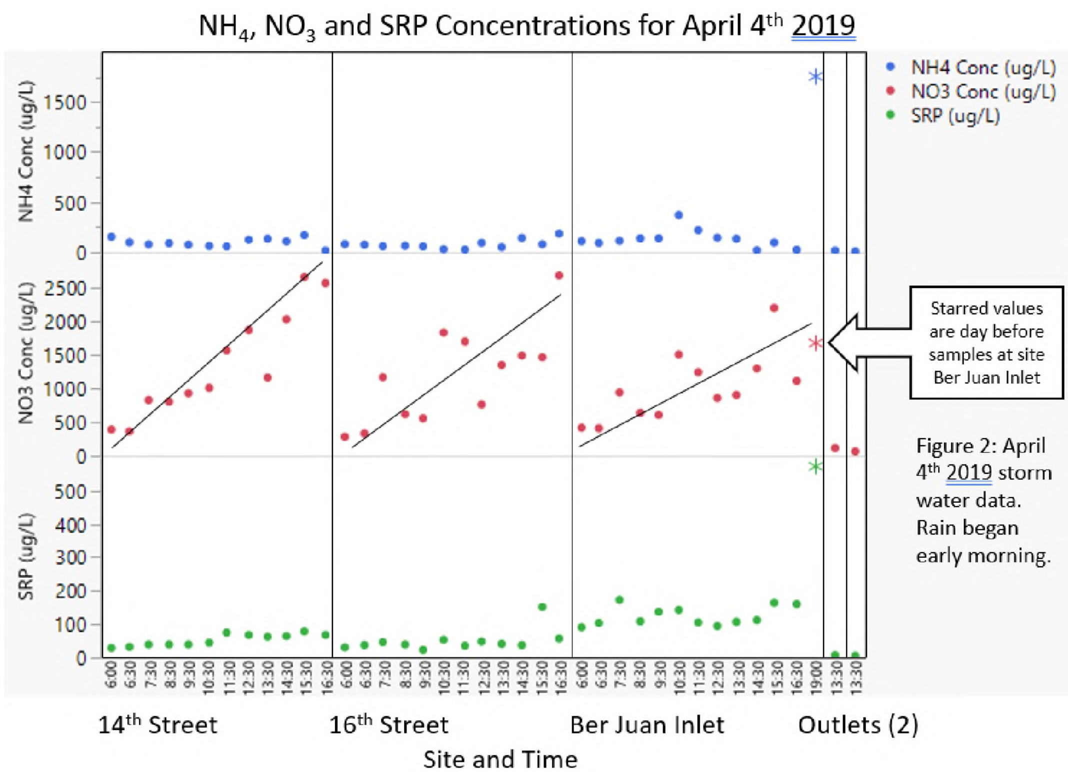

Figure 2.2 Strom water pollutant concentrations on April 4th 2019 storm.

2.1. MICROCOSM EXPERIMENT SET – UP

Large 17-L white plastic tubs were filled with 10 L of tap water and allowed to dechlorinate for 24 hours. Microcosm tubs were selectively spaced under equal amounts of LED illumination (under 4 ft. 16-watt LED Grow Shop light fixtures from Toggled).

Floating mats (made of Apache Mills, Inc. Anti-fatigue runner Gray Cast Vinyl Utility Runner) were cut to fit the microcosm tubs. Additionally, the mats were cut to allow a black plastic hydroponic pot to rest in the mat. These pots were used with pure shredded coconut fiber to stabilize emergent aquatic macrophytes. Microcosms (Figure 2.3) were aerated by use of aquarium pumps. Dissolved oxygen (DO) in microcosms ranged from 7.5 to 8.9 mg/L.

Figure 2.3 Experimental Chambers. Microcosm tubs with floating mat, coconut fiber and emergent macrophyte in hydroponic pot.

Microcosm controls were created to monitor any environmental changes that would result in unreliable data. Microcosm experiments had at least one positive and one negative control. A positive control tub was filled with the same amount of water and nutrients as the treatments in the study. A negative control simply had tap water only.

2.1.1. Experimental Chambers. Climate-controlled chambers in Butler-Carlton Hall at Missouri University of Science and Technology were used to study plants in a controlled setting. Climate chambers were temperature controlled. During this study, the temperature was set between 20-25 °C. The full-spectrum lights were on the entire time of study. Air flow and humidity were consistent in the chambers.

2.1.2. Plant Origins. Plants were selected based on their local availability, and their native origins. *Pontederia cordata* was collected from Ben Branch Conservation Area with permission from Missouri Department of Conservation. *Iris virginica*, *Eleocharis compressa*, and *Scirpus validus* was collected from Millpond Plants who specializes in Missouri natives. *Ceratophyllum demersum* and pond algae was collected from the Ozark Research Field Station. *Nasturtium officinale* was collected from Roubioux Spring in Waynesville, MO.

2.1.3. Microcosm Dosages. Each microcosm tub was dosed with 500 µg/L solution of nitrate-N (as sodium nitrate) and 500 µg/L solution of phosphate-P (as monobasic potassium phosphate) at the start of each trial. These concentrations were determined based upon typical stormwater concentrations of the pond inflows measured during our lab's preliminary research.

Plants were thoroughly washed with tap water to remove any soil, sediment, or macroscopic organisms. The wet mass of plants was determined with a scale before placing into each microcosm.

2.1.4. Sampling. After spiking microcosms with nutrients, samples were taken at regular intervals (every few hours for two days for short trials, every other day for two weeks for longer trials) to measure uptake rates. Microcosms were mixed gently with the needle of a 60-mL syringe and then sampled in 15-mL increments from each corner of the tub to acquire a composite sample representative of the entire microcosm. The resulting 60 mL of microcosm water was filtered with a glass-fiber filter (25 mm Whatman GF/F filter) into plastic bottles, which were frozen until analysis.

2.2. WATER ANALYSIS

Soluble reactive phosphorus (SRP) was measured by the ammonium molybdate-ascorbic acid colorimetric method (APHA, 2012). A basic linear regression was used to convert absorbances to concentrations based on readings from blanks and standards of SRP. Nitrate-N was measured using a Dionex DX-500 ion chromatograph. Peak area of nitrate was integrated based on standards and blanks to give concentration in µg/L. A basic linear regression was used to standardize the values. Nitrate-N and SRP concentrations were analyzed over time for each trial to determine nutrient uptake rates.

2.3. NUTRIENT UPTAKE RATE CALCULATIONS

Nutrient uptake rates were calculated two different ways. The first way the nutrient uptake rate was calculated was the uptake rate of nutrients per time per microcosm trial; microcosms usually had one plant per trial, although some plants (coontail, algae) were not individual plants. The change in concentration over time was then multiplied by 10 L to give the rate of nutrient mass removal in the 10 L of water in the microcosms. The units for this calculation were µg/hr.

For the second nutrient rate, the nutrient rate found in #1 was divided by the wet biomass for the plant in each replicate microcosm. The units for this rate were µg/hr/g of plant.

Each plant replicate for individual microcosm studies had their own nutrient uptake rates for both SRP and NO_3-N. The ranges used to determine uptakes rates usually included the entire sampling interval. However, in some cases I used a different interval that had a more linear pattern to the change in concentrations over time.

2.4. STATISTICS

A one-way ANOVA test using SigmaStat version 4.0 was used to compare rates of nutrient uptake with different treatments based on the plants tested. Uptake rates were log-transformed before ANOVA to meet assumptions of parametric statistics. If ANOVA found a significance difference in uptake rates among plant treatments, then a Tukey test was used to compare individual treatments.

3. RESULTS: AQUATIC PLANT SUCCESS

3.1. CERATOPHYLLUM DEMERSUM

Ceratophyllum demersum, commonly known as coontail, had an average nutrient retention rate of 42.7 µg/hr for SRP and 7.21 µg/hr for NO_3. The August 2019 experiment was done in a non-temperature-controlled chamber (lab setting) with average room temperature of 22°C. Results are show in Figure 3.1 and Figure 3.2.

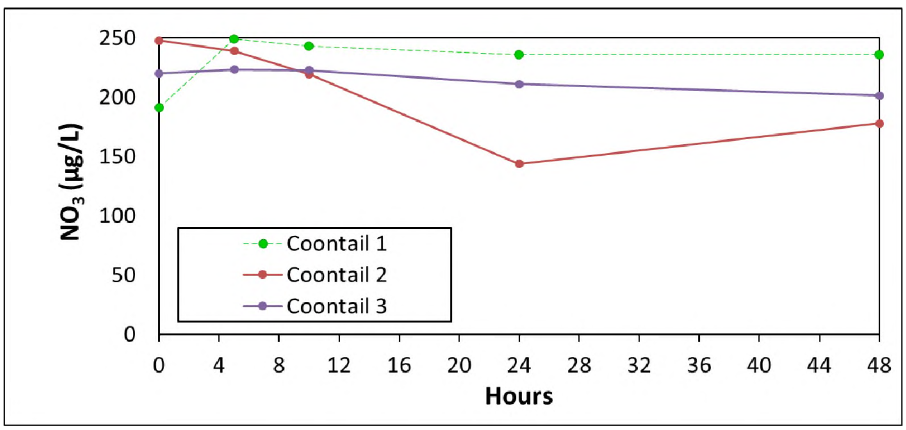

Figure 3.1 NO_3 concentration (µg/L) for August 28th 2019 study.

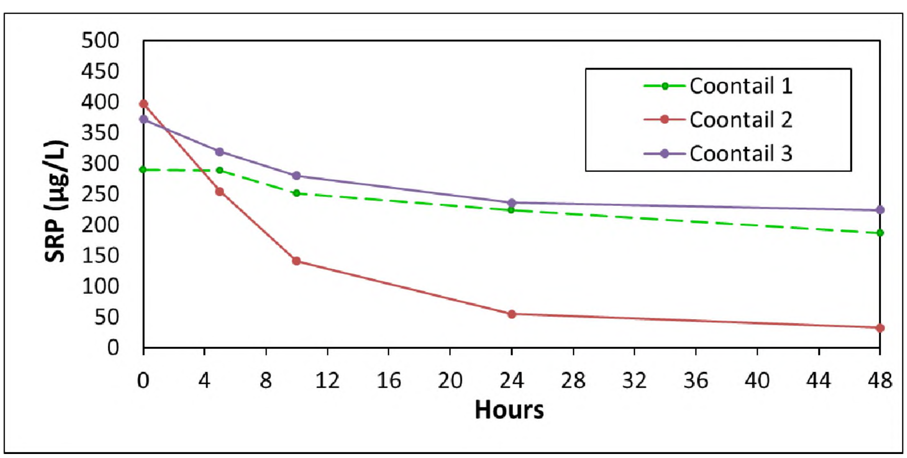

Figure 3.2 SRP concentration (µg/L) for August 28th 2019 study.

3.2. IRIS VIRGINICA

The blue flag iris had nutrient uptake rates of 16.7 and 46.6 µg/hr for SRP and NO_3, respectively. The September 4th 2020 study took place in temperature controlled chambers set to 20°C. Results for this study are shown in Figure 3.3 and 3.4.

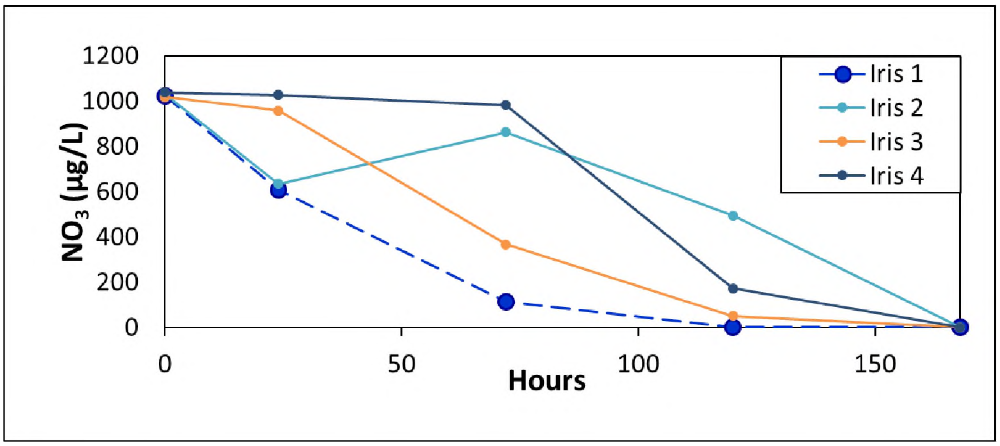

Figure 3.3 NO_3 concentration (µg/L) for September 4th 2020 study.

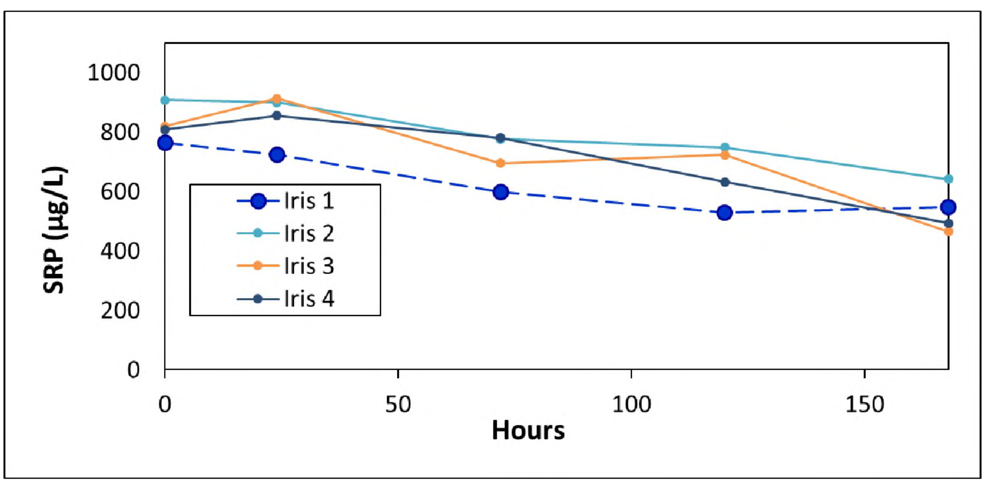

Figure 3.4 SRP concentration (µg/L) for September 4th 2020 study.

3.3. SCIRPUS ATROVIRENS

Bulrush has nutrient uptake rates of 6.52 µg/hr and 53.7 µg/hr for SRP and NO_3, respectively. On September 4th 2020, the temperature controlled chamber was set to 20°C. Results for this study are shown in Figure 3.5 and 3.6.

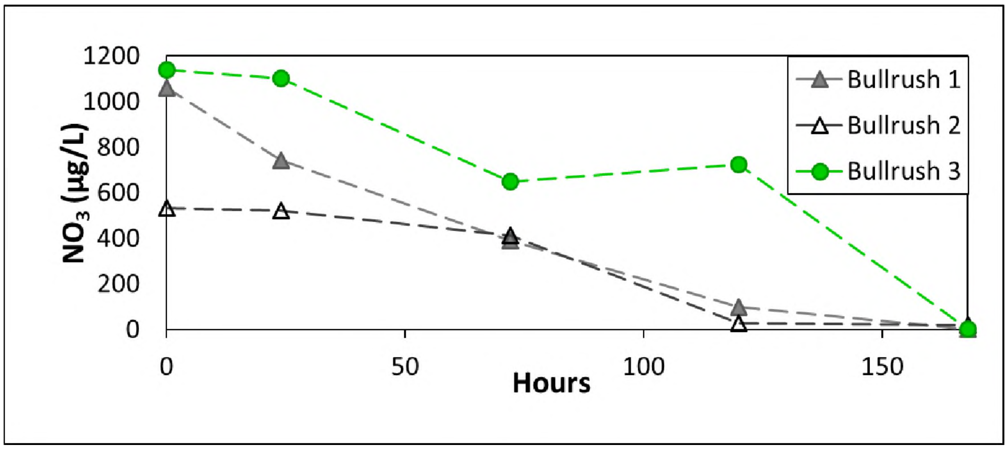

Figure 3.5 NO_3 concentration (µg/L) for September 4th 2020 study.

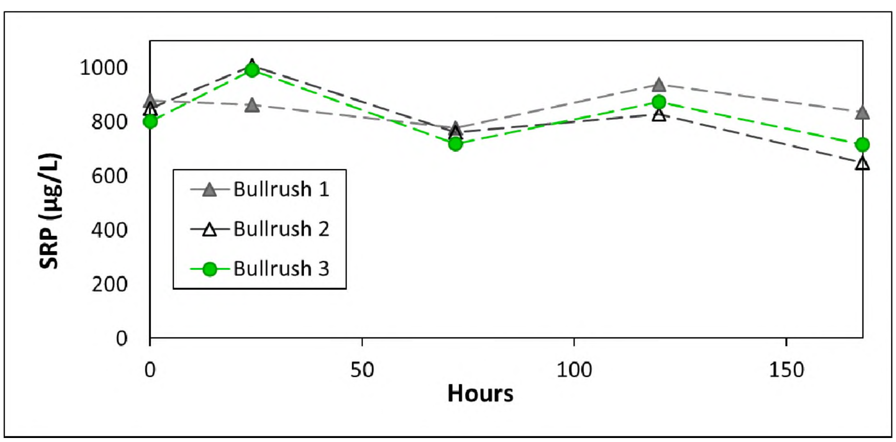

Figure 3.6 SRP concentration (µg/L) for September 4th 2020 study.

3.4. PONTEDERIA CORDATA

Pickerel weed had an average nutrient retention rate of 7.97 µg/hr and 48.61 µg/hr for SRP and NO_3 respectively for two microcosm studies with four total replicates. On July 21st 2020 and September 4th 2020, the temperature controlled chamber was set to 20°C. Results from this study are shown in Figure 3.7 through 3.10.

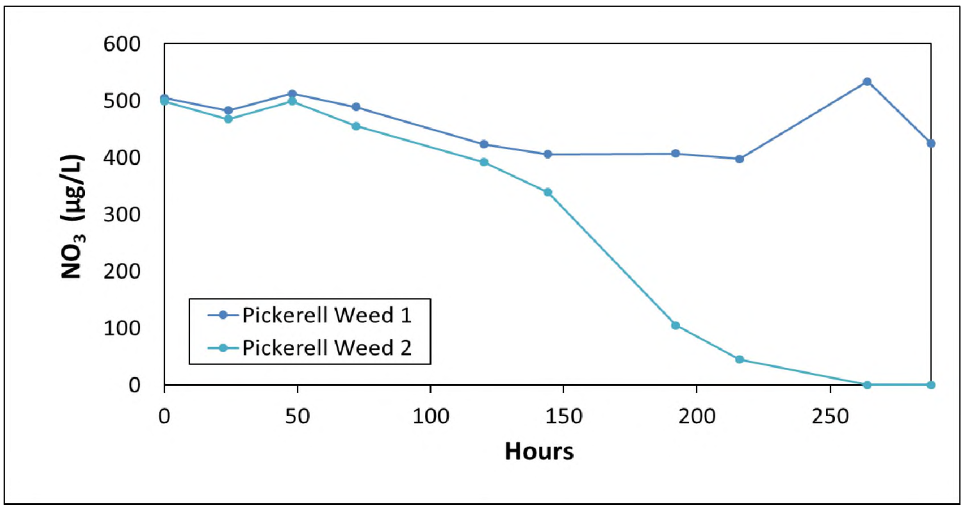

Figure 3.7 NO_3 concentration (µg/L) for July 21st 2020 study.

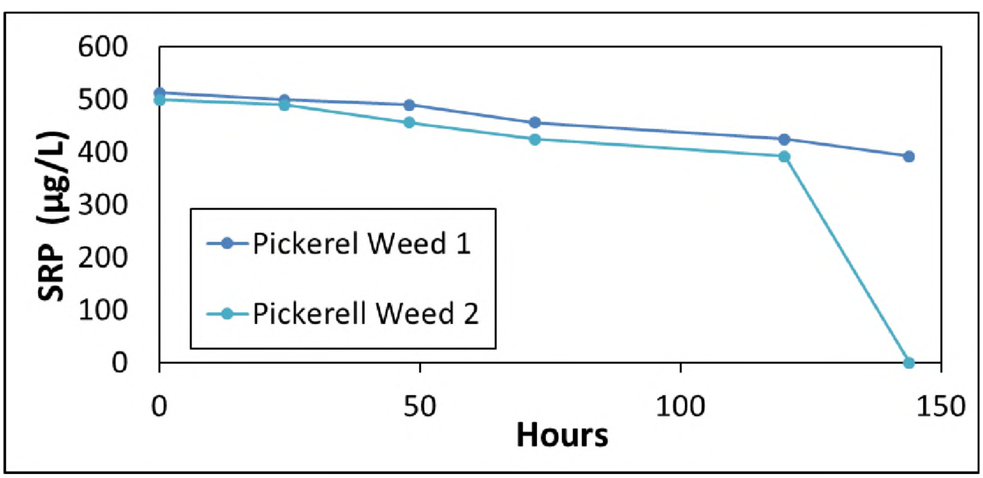

Figure 3.8 SRP concentration (µg/L) for July 21st 2020 study.

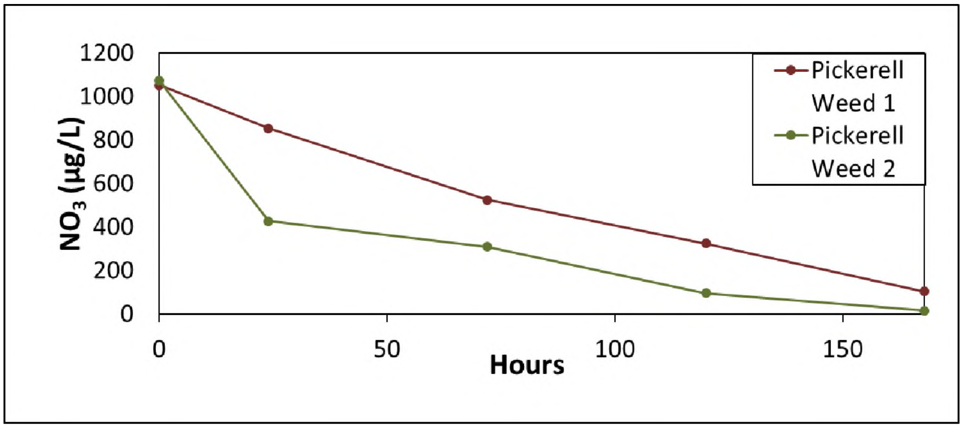

Figure 3.9 NO_3 concentration (µg/L) for September 4th 2020 study.

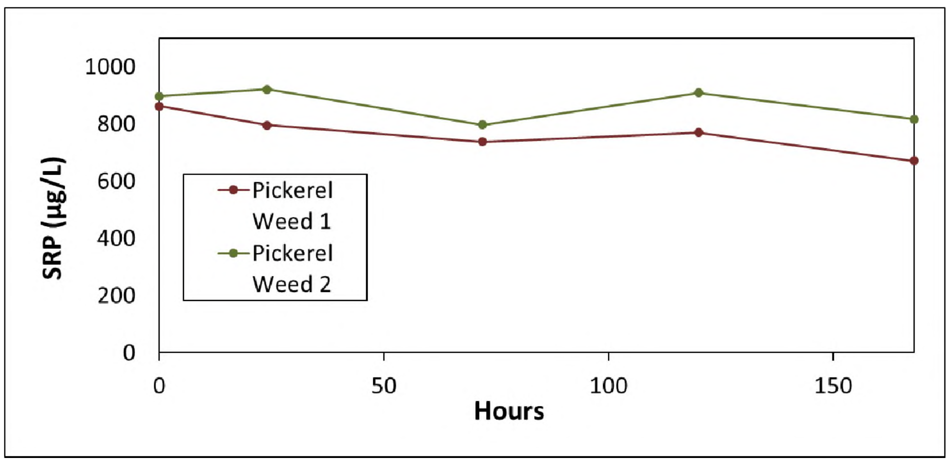

Figure 3.10 SRP concentration (µg/L) for September 4th 2020 study.

3.5. ELEOCHARIS COMPRESSA

*Eleocharis compressa (*Spike Rush) had nutrient removal rates of 2.51 µg/hr and 38.61 µg/hr for SRP and NO_3 that are an average of two microcosm studies. On September 4th and July 21st 2020, the temperature controlled chamber was set to 20°C. Results from this study are shown in Figures 3.11 through 3.14.

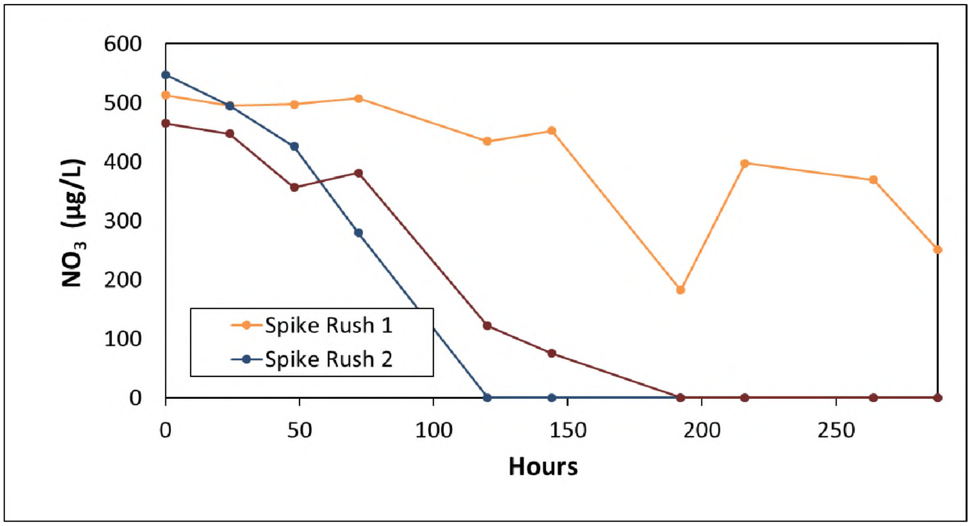

Figure 3.11 NO_3 concentration (µg/L) for July 21st 2020 study.

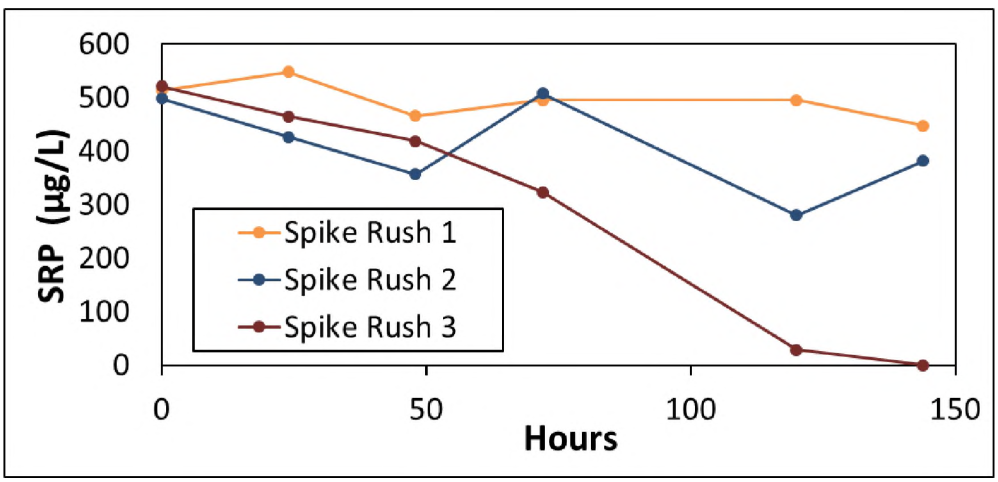

Figure 3.12 SRP concentration (µg/L) for July 21st 2020 study.

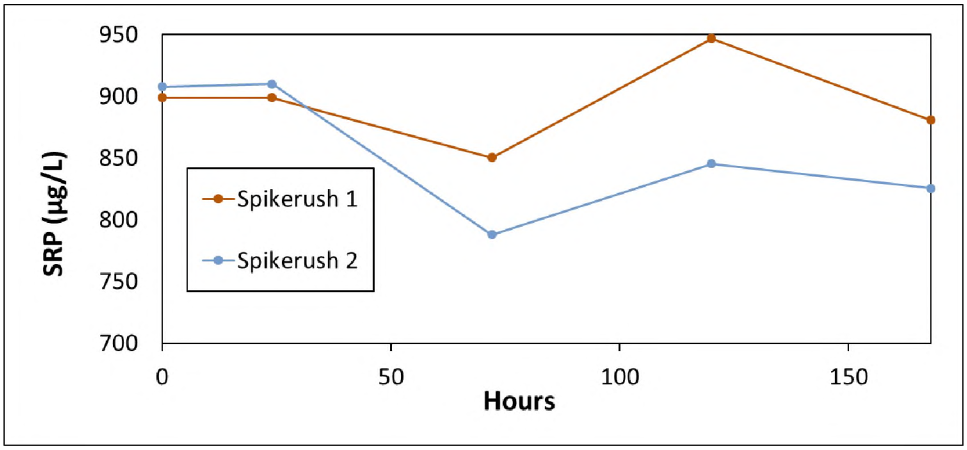

Figure 3.13 SRP concentration (µg/L) for September 4th 2020 study.

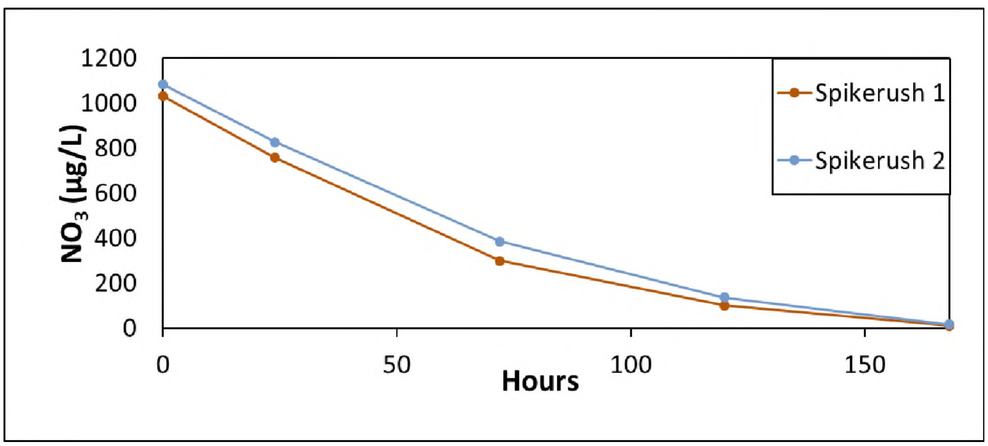

Figure 3.14 NO_3 concentration (µg/L) for September 4th 2020 study.

3.6. ALGAE (CLADOPHORA AND SPIROGYRA)

Algal samples for nutrient uptake studies consisted mainly of *Cladophora* and *Spirogyra.* Algae had nutrient uptake rates of 33.8 and 355.2 µg/hr for SRP and NO3. July 21st 2020 study was in a temperature controlled chamber set to 20°C. Algae results from this study are in Figures 3.15 and 3.16.

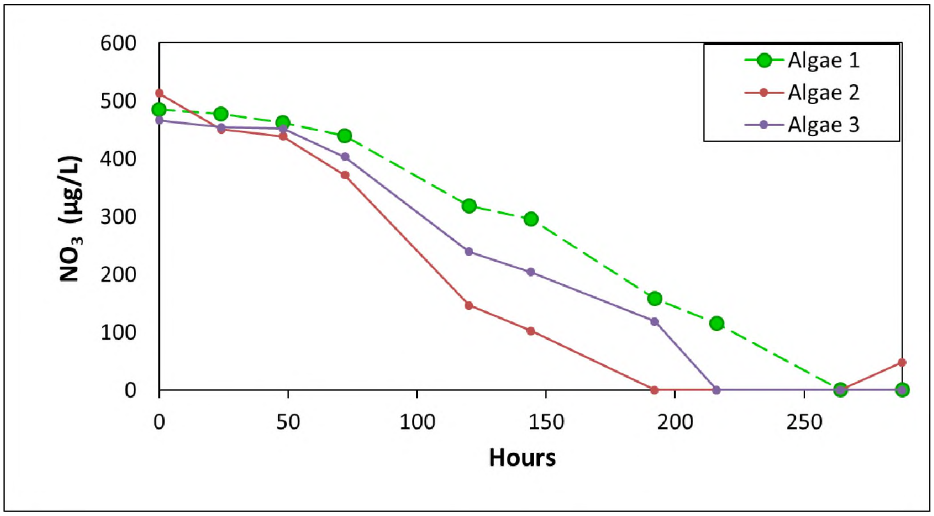

Figure 3.15 NO_3 concentration (µg /L) for July 21st 2020 study.

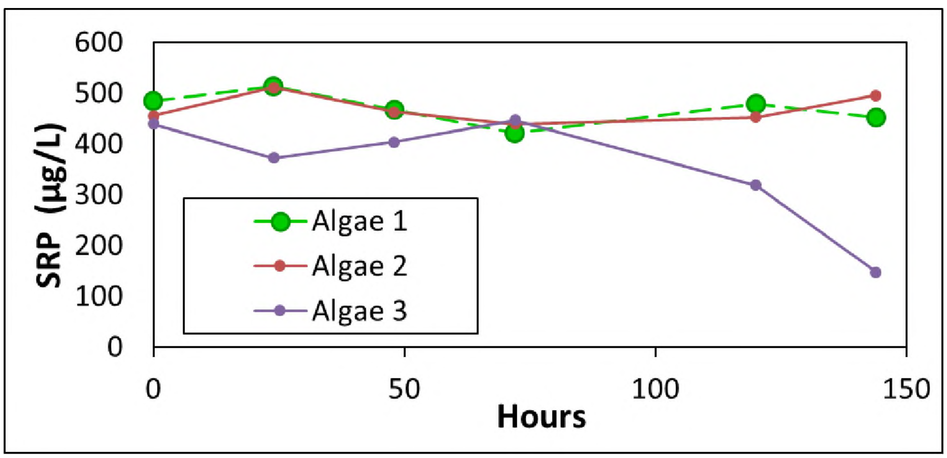

Figure 3.16 SRP concentration (µg/L) for July 21st 2020 study.

3.7. LUDWIGIA DECURRENS

Water primrose, a floating macrophyte, had nutrient uptake rates of -0.362 µg/hr for SRP and 7.85 µg/hr for NO_3-N. The July 21st 2020 study was done in a 20°C temperature controlled chamber. Results for this study are in Figures 3.17 and 3.18.

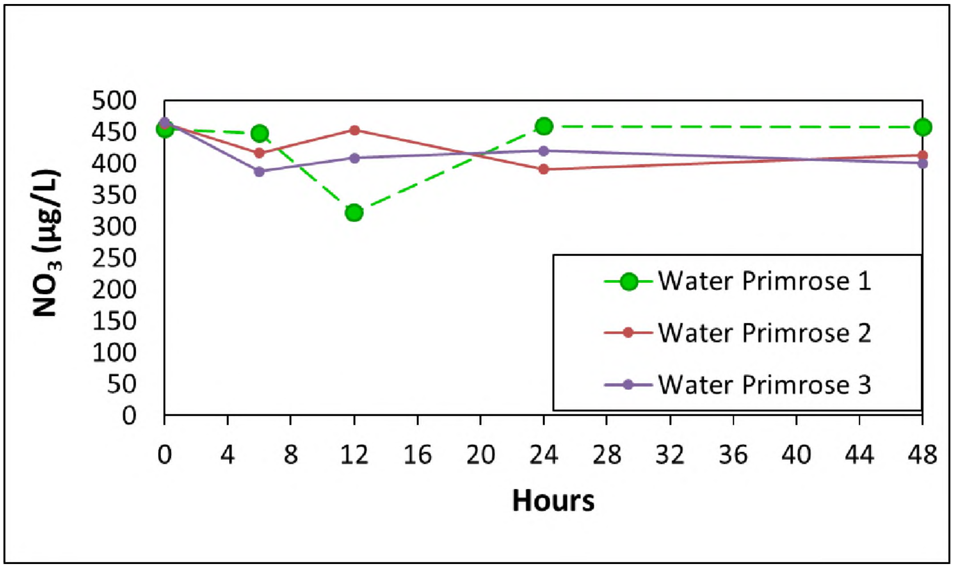

Figure 3.17 NO_3 concentration (µg/L) for July 21st 2020 study.

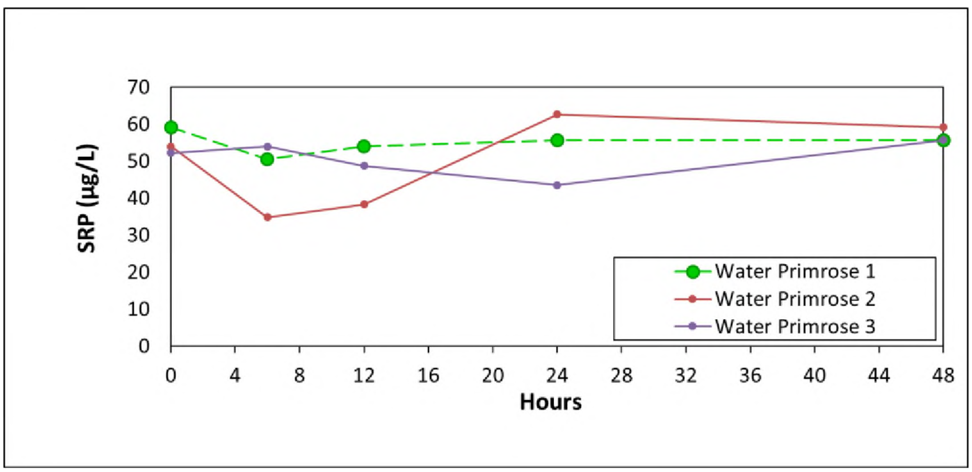

Figure 3.18 SRP concentration (µg/L) for July 21st 2020 study.

3.8. UPTAKE RATES BY PLANT

Nutrient rates are displayed in two different ways: µg/hr and µg/hr/g of plant mass. All replicates from different studies are displayed in Figures 3.19 to 3.22. There was high variability in replicates within each treatment (plant taxon). Algae had the highest nutrient removal rates for SRP. However, water primrose had the highest NO_3 removal rates.

Table 3.1 Treatment types with average nutrient uptake rates for nitrate-N and SRP.

Treatment	Average SRP µg/hr	Average NO3 µg/hr
Cladophora & Spirogyra	33.8	3.55 x 10 ^2
Scirpus atrovirens	6.52	53.7
Ceratophyllum demersum	42.7	7.21
Iris virginica	16.7	46.5
Pontederia cordata	7.97	48.6
Eleocharis compressa	2.51	38.6
Ludwigia decurrens	-0.362*	7.85

* *Ludwigia decurrens* negative SRP rate means that, on average, SRP was being released in this microcosm.

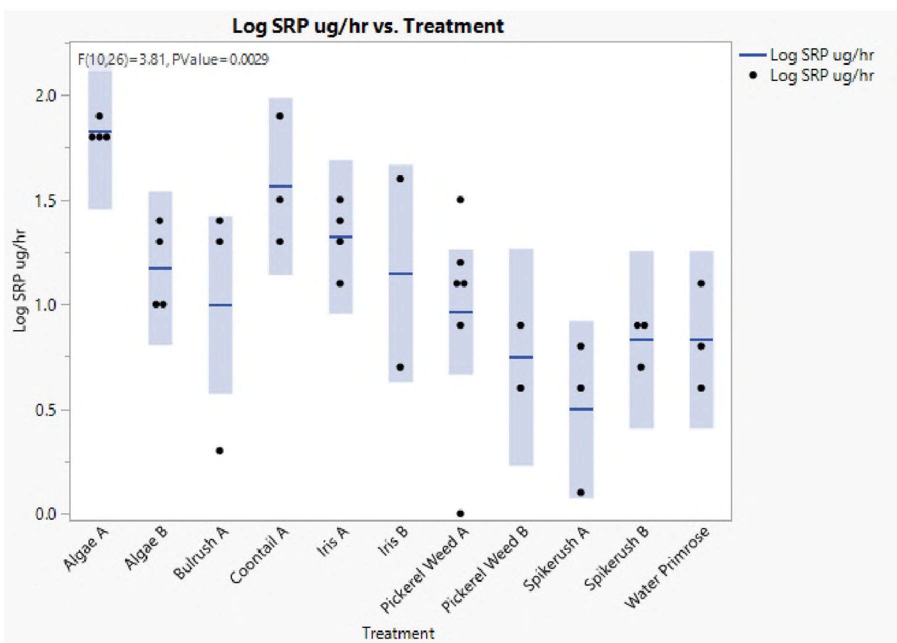

Figure 3.19 SRP uptake rates per microcosm for each treatment (µg/hr).

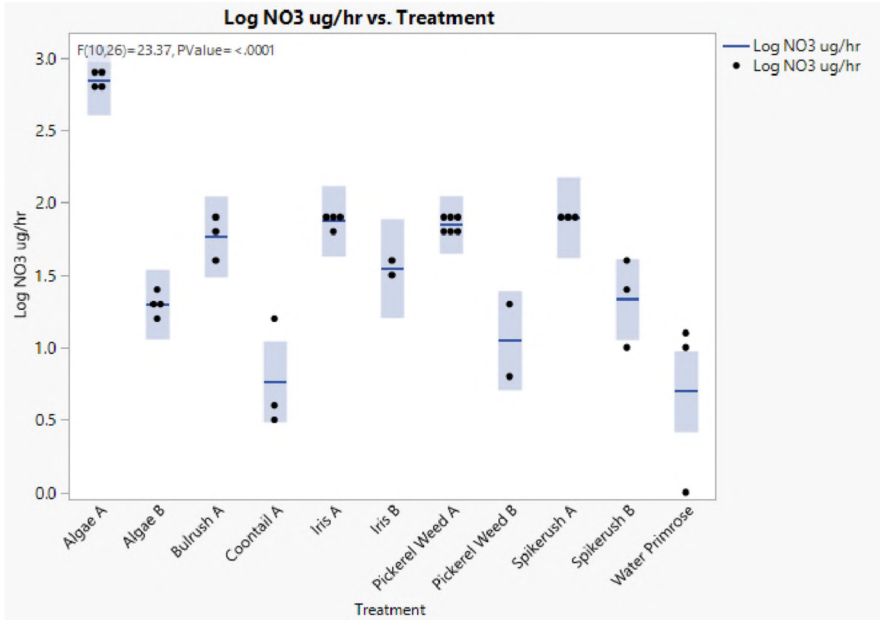

Figure 3.20 NO_3 uptake rates per microcosm for each plant (µg/hr).

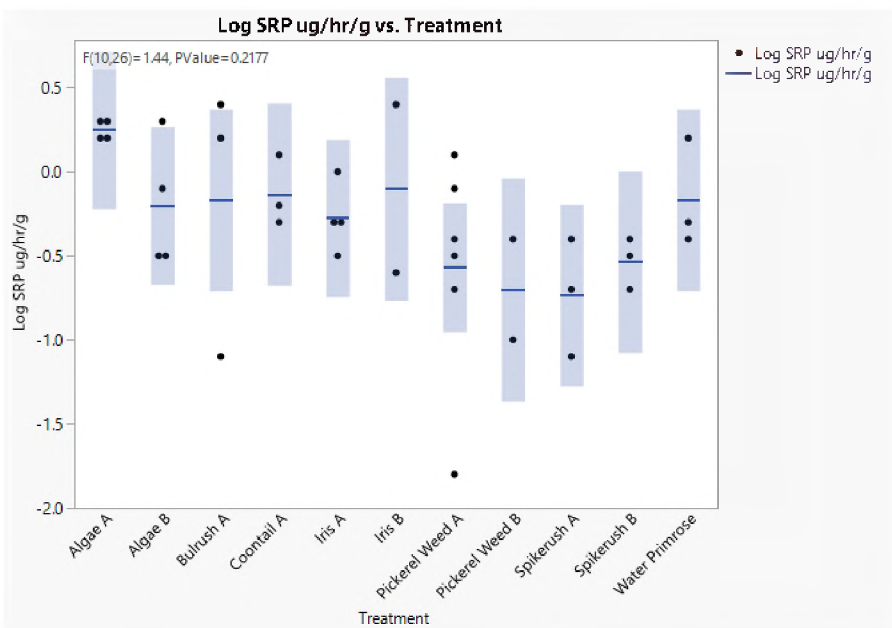

Figure 3.21 SRP uptake rates per plant mass (µg/hr/g plant biomass).

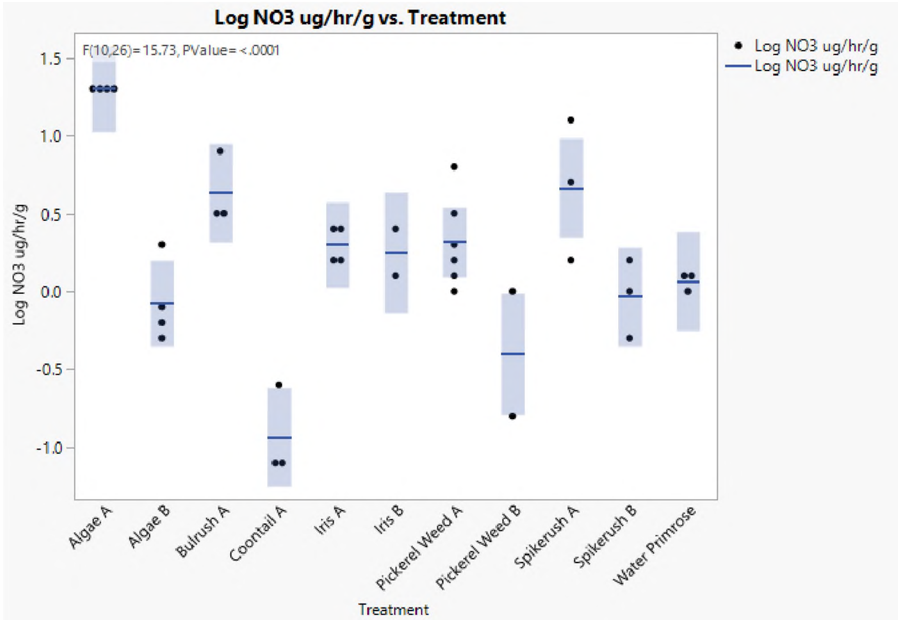

Figure 3.22 NO_3 uptake rates per plant mass (μg/hr/g plant biomass).

Oneway Anova

Summary of Fit

Rsquare	0.594197
Adj Rsquare	0.438118
Root Mean Square Error	0.357654
Mean of Response	1.110811
Observations (or Sum Wgts)	37

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	10	4.8698423	0.486984	3.8070	0.0029*
Error	26	3.3258333	0.127917		
C. Total	36	8.1956757			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Algae A	4	1.82500	0.17883	1.4574	2.1926
Algae B	4	1.17500	0.17883	0.8074	1.5426
Bulrush A	3	1.00000	0.20649	0.5755	1.4245
Coontail A	3	1.56667	0.20649	1.1422	1.9911
Iris A	4	1.32500	0.17883	0.9574	1.6926
Iris B	2	1.15000	0.25290	0.6302	1.6698
Pickerel Weed A	6	0.96667	0.14601	0.6665	1.2668
Pickerel Weed B	2	0.75000	0.25290	0.2302	1.2698
Spikerush A	3	0.50000	0.20649	0.0755	0.9245
Spikerush B	3	0.83333	0.20649	0.4089	1.2578
Water Primrose	3	0.83333	0.20649	0.4089	1.2578

Std Error uses a pooled estimate of error variance

Figure 3.23 Summary of ANOVA results for SRP (μg/hr).

Oneway Anova

Summary of Fit

Rsquare	0.356647
Adj Rsquare	0.109203
Root Mean Square Error	0.455909
Mean of Response	-0.3
Observations (or Sum Wgts)	37

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	10	2.9958333	0.299583	1.4413	0.2177
Error	26	5.4041667	0.207853		
C. Total	36	8.4000000			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Algae A	4	0.25000	0.22795	-0.219	0.7186
Algae B	4	-0.20000	0.22795	-0.669	0.2686
Bulrush A	3	-0.16667	0.26322	-0.708	0.3744
Coontail A	3	-0.13333	0.26322	-0.674	0.4077
Iris A	4	-0.27500	0.22795	-0.744	0.1936
Iris B	2	-0.10000	0.32238	-0.763	0.5627
Pickerel Weed A	6	-0.56667	0.18612	-0.949	-0.1841
Pickerel Weed B	2	-0.70000	0.32238	-1.363	-0.0373
Spikerush A	3	-0.73333	0.26322	-1.274	-0.1923
Spikerush B	3	-0.53333	0.26322	-1.074	0.0077
Water Primrose	3	-0.16667	0.26322	-0.708	0.3744

Std Error uses a pooled estimate of error variance

Figure 3.24 Summary of ANOVA results for SRP (µg/hr/g).

Oneway Anova

Summary of Fit

Rsquare	0.899898
Adj Rsquare	0.861397
Root Mean Square Error	0.235544
Mean of Response	1.616216
Observations (or Sum Wgts)	37

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	10	12.967770	1.29678	23.3735	<.0001*
Error	26	1.442500	0.05548		
C. Total	36	14.410270			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Algae A	4	2.85000	0.11777	2.6079	3.0921
Algae B	4	1.30000	0.11777	1.0579	1.5421
Bulrush A	3	1.76667	0.13599	1.4871	2.0462
Coontail A	3	0.76667	0.13599	0.4871	1.0462
Iris A	4	1.87500	0.11777	1.6329	2.1171
Iris B	2	1.55000	0.16655	1.2076	1.8924
Pickerel Weed A	6	1.85000	0.09616	1.6523	2.0477
Pickerel Weed B	2	1.05000	0.16655	0.7076	1.3924
Spikerush A	3	1.90000	0.13599	1.6205	2.1795
Spikerush B	3	1.33333	0.13599	1.0538	1.6129
Water Primrose	3	0.70000	0.13599	0.4205	0.9795

Std Error uses a pooled estimate of error variance

Figure 3.25 Summary of ANOVA results for NO_3 (µg/hr).

Oneway Anova

Summary of Fit

Rsquare	0.858127
Adj Rsquare	0.80356
Root Mean Square Error	0.267047
Mean of Response	0.240541
Observations (or Sum Wgts)	37

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	10	11.215023	1.12150	15.7262	<.0001*
Error	26	1.854167	0.07131		
C. Total	36	13.069189			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Algae A	4	1.3000	0.13352	1.026	1.574
Algae B	4	-0.0750	0.13352	-0.349	0.199
Bulrush A	3	0.6333	0.15418	0.316	0.950
Coontail A	3	-0.9333	0.15418	-1.250	-0.616
Iris A	4	0.3000	0.13352	0.026	0.574
Iris B	2	0.2500	0.18883	-0.138	0.638
Pickerel Weed A	6	0.3167	0.10902	0.093	0.541
Pickerel Weed B	2	-0.4000	0.18883	-0.788	-0.012
Spikerush A	3	0.6667	0.15418	0.350	0.984
Spikerush B	3	-0.0333	0.15418	-0.350	0.284
Water Primrose	3	0.0667	0.15418	-0.250	0.384

Std Error uses a pooled estimate of error variance

Figure 3.26 Summary of ANOVA results for NO_3 (µg/hr/g).

Based on a one-way ANOVA (Figures 3.23 through 3.26), there was a significant difference among the plant treatments for the rate of uptake for SRP for rates per microcosm ($P = 0.0029$). However, when the rates were accounted for mass of plant used there was no significance ($P = 0.22$). ANOVA did reveal significant differences among plant treatments for uptake rates of N per microcosm ($P < 0.001$) and per biomass of plant used ($P < 0.001$). Algae, water primrose, and bulrush had the highest uptake rates for N in the trials. Since the ANOVA showed statistical differences between treatment for SRP (per microcosm) and NO_3 (per microcosm and plant), a Tukey test was performed to look at the comparison between individual treatments. It was found that algae was different from all other treatment groups when comparing uptake rates NO_3 per microcosm (µg /hr). Iris treatments were also statistically different from water primrose and coontail when comparing uptake rates NO_3 per microcosm (µg/hr). When accounting for biomass algae had statistically different NO_3 nutrient rates (µg/hr/g) when compared to treatments: Pickerel weed, iris, coontail, and water primrose. Spikerush and Iris treatments was statistically different from coontail when comparing biomass accounted NO_3 rates (µg/hr/g). SRP nutrient uptake rates per microcosm (µg/hr) only had statistical differences between spikerush replicate A with coontail and algae.

Controls in this study varied but in most cases each study had at least one positive and negative control. The average change in µg/hr over the course of the trials is displayed in table 3.2.

Table 3.2 Rate of change for control microcosms.

Average Rate of Change Positive Controls	Average rate of change negative	Date of Study
0.449	0	July 21st 2020
-1.07	-0.169	September 4th 2020
9.23	positive only	April 11th 2019
0.612	0.190	July 31st 2019
2.30	**0.00693**	**Average for selected studies**

4. DISCUSSION

4.1. DISCUSSION INTRODUCTION

This study aimed to compare a variety of different aquatic plants that can take up significant amounts of nitrate and phosphate. The successful plants found here are expected to be viable for FTW applications.

4.2. PLANT SELECTION

Native plants attract native life that is important for preserving an ecosystem. Plants also have complex life cycles in which senescence can affect their growth and nutrient removal rate. An undergraduate found while researching for an OURE project in my lab that senesced plants release nutrients as they are decomposing (Mesa, n.d.). Therefore, selecting plants at the beginning of their life cycle or season is beneficial for removing excess nutrients.

4.3. NUTRIENT UPTAKE RATES

Ceratophyllum demersum has been used in submerged aquatic vegetation (SAV) applications in which the floating macrophyte is completely submerged in the water column. *Ceratophyllum demersum* in a SAV application completely reduced SRP in just 3.5 and 7.0 days (Dierberg et al., 2002). Similarly, my study showed that one replicate (coontail #2) of *Ceratophyllum demersum* reduced SRP to low detection levels after 48 hours. *Ceratophyllum demersum* was found to have increasing values for N and P after an initial addition of nutrients (Song et al., 2019) similar to replicates shown in my study.

The spike of P released at the beginning of the study could suggest organic phosphorus from soil leached into the water column.

Iris pseudacorus (similar species to *Iris virgnica*) recovered 31.5% and 26.3% of N and P in a 10-week mesocosm study (Chen et al. 2009). Compared to the other selected plants in the study, it had one of the lowest nutrient recovery rates. In my research, the average nutrient uptake rate for *Iris virginica* was 16.7 and 46.6 µg/hr for P and N, respectively. When compared to other emergent macrophytes in this study, *Iris virginica* had high rates of P uptake but low rates for N.

A mesocosm study found that *Pontederia cordata* (common name) facilitated the highest rates of N (0.31mg/L/day) and P (0.34 mg/L/day) removal (Garcia Chanc et al., 2019a). My research found that N was removed by 11.7 mg/L/day and P 1.91 mg/L/day.

Eleocharis plantaginea (same genus as *Eleocharis compressa*) retained up to 91% of phosphate when combined with other aquatic plants in India (Shardendu et al., 2012). *Eleocharis compressa* has not been studied prior to my research. *Eleocharis compressa* gave promising nutrient uptake results of 2.51 and 38.6 µg/hr for SRP and nitrate respectively. Thus, *Eleocharis compressa* is a promising aquatic plant for FTWs.

Scirpus validus (bulrush) is a commonly studied FTW aquatic plant. In a large scale constructed wetlands study (Rycewicz-Borecki et al., 2017), *Scirpus validus* led to reductions of total dissolved phosphorus (23.1 to 7.8 mg/L) and nitrogen (87.1 to 4.7 mg/L) during the 1-year duration. *Scirpus validus* was found to have the lowest biomass production when compared to other aquatic plants (Rycewicz-Borecki et al., 2017), which may explain the low rates of some replicates. My research found that the average nutrient uptake rate for *Scirpus validus* was 6.52 µg/hr for phosphate and 53.7 µg/hr for nitrate.

4.4. FLOATING TREATMENT WETLAND (FTW) MATERIALS

The materials used in an FTW system are important. The Apache Mills, Inc. Anti-fatigue runner Gray Cast Vinyl Utility Runner from Lowe's Home Improvement in addition to hydroponic pots filled with coconut fiber were used to assist the emergent plants to keep them afloat. Recent work at Missouri University of Science and Technology found that this mat can leach total phosphorus and ammonium (which was nitrified to nitrate) into the water (Campbell, C. unpublished data). Selecting mats where the chemical composition of the materials is known is key to understanding the nutrient cycling. Additionally, if using an FTW in an urban system for a long period of time, it is important to sterilize the mat, hydroponic pots, and coconut fiber. This will reduce the amount of algal growth and keep the FTW in a re-usable condition when removing during the winter season or when harvesting.

4.5. FURTHER RESEARCH

Harvesting strategies are important for recycling biomass and preventing nutrients within the plant from leaching back into the water column. Once plants reached senescence, they no longer took up nutrients. In an FTW application, harvesting should take place just before senescence. Harvesting strategies have the potential of removing nutrients before they are cycled back into the aquatic system. Little research has been done to analyze the success of different harvesting strategies.

Algae is a key concern that is not always addressed in FTW papers. In microcosm and mesocosm studies where there are large pools of nutrient rich waters, algae can quickly establish. This is a problem when algae could be taking up the nutrients rather than the selected aquatic plant being studied. One way to account for algae is to use chl-a measurements, which can be taken through water samples or surface area scrubs to assess how much is present within the volume of water used for the study. Algae had high nutrient uptake rates and might have potential for FTWs. Algae could serve as a main driver or in addition to other aquatic plants in FTW applications. Algae can grow around other plants and could be harvested as well.

4.6. CHALLENGES

Some plants such as pickerel weed and spikerush had a spike in SRP concentrations within the first 24 hours of the experiment. This could be because the root biomass (once planted in the microcosm) was leaching nutrients after being washed. All emergent macrophytes in this study were previously planted in a nutrient-rich soil. This phenomenon could also be explained by the luxury consumption effect where plants take up nutrients in excess rather than maintaining their growth requirements (Chapin et al., 1986). Plants' collection locations and physiology are important for understanding where nutrients are going.

Initially this study aimed to compare temperature to nutrient rates of different aquatic plants. However, at the end of week-long microcosm studies, the plants appeared to be dying and suffering from the new environment. This could be because plants came from nutrient-rich soil and were put into large tubs of cooler water with no substrate (besides the coconut fiber) for the root mass to attach. Additionally, before each plant was placed into a microcosm, they were thoroughly rinsed with tap water which could put them in initial shock. When switching temperatures and environments the plants quickly became stressed which could also explain the initial release of nutrients within the first 24 hours of most experimental runs.

5. CONCLUSION

Floating treatment wetlands are a promising technique for treating nutrient-rich aquatic systems. Native plants should always be considered for selection when implementing an FTW. Native plants have shown to out-perform non-native plants in FTW applications in Italy (de Stefani et al., 2011), similar to the results found in this thesis. The native plants selected in this study showed high nutrient removal rates for both SRP and NO_3.. A One-Way ANOVA showed statistical differences between treatments for NO_3 for both microcosm and plant biomass nutrient rates ($P = 0.0001$). This suggests that the plants take up nutrients at different rates. This is expected since each plant has varying metabolisms, life cycles, and many times came from different areas. Out of all the selected plants, algae took up both N at the highest rate of 355.2 µg/hr and second highest rate for P at 33.81 µg/hr). The highest nutrient uptake rate for SRP was for coontail. Bulrush had the second highest N rate of 53.7 µg/hr.

Based on this data, FTWs should be considered to remove nutrients from nutrient polluted water sources. Additionally, their other benefits such as providing a native plant habitat should be considered. Aquatic plants are the foundation of FTWs and are the working entities for removing the nutrients. More research needs to be done to better understand harvesting strategies, plant to root interactions, and detailed mass-balance models including nutrient pathways.

APPENDIX

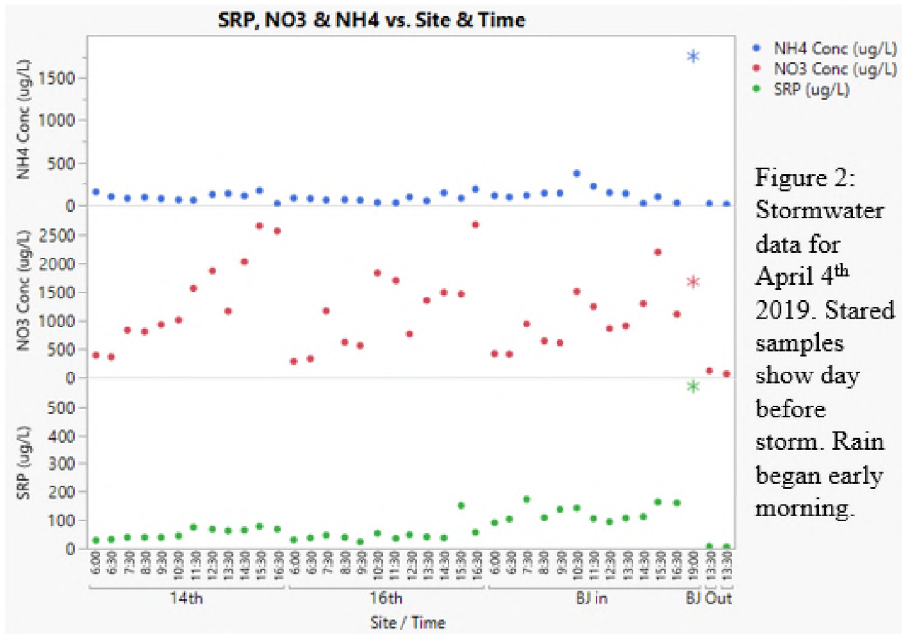

Figure A1. Stormwater graph for April 4th 2019.

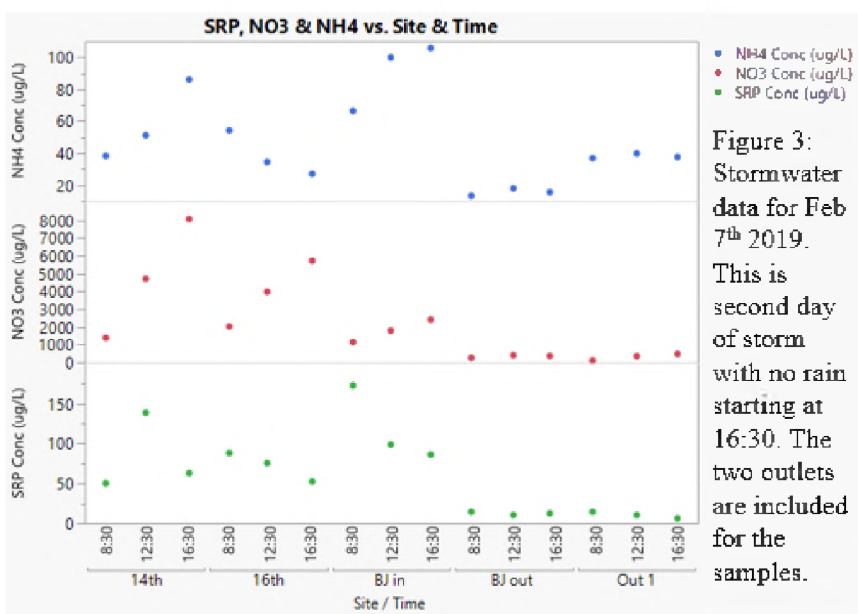

Figure A2. Stormwater graph for February 7th 2019.

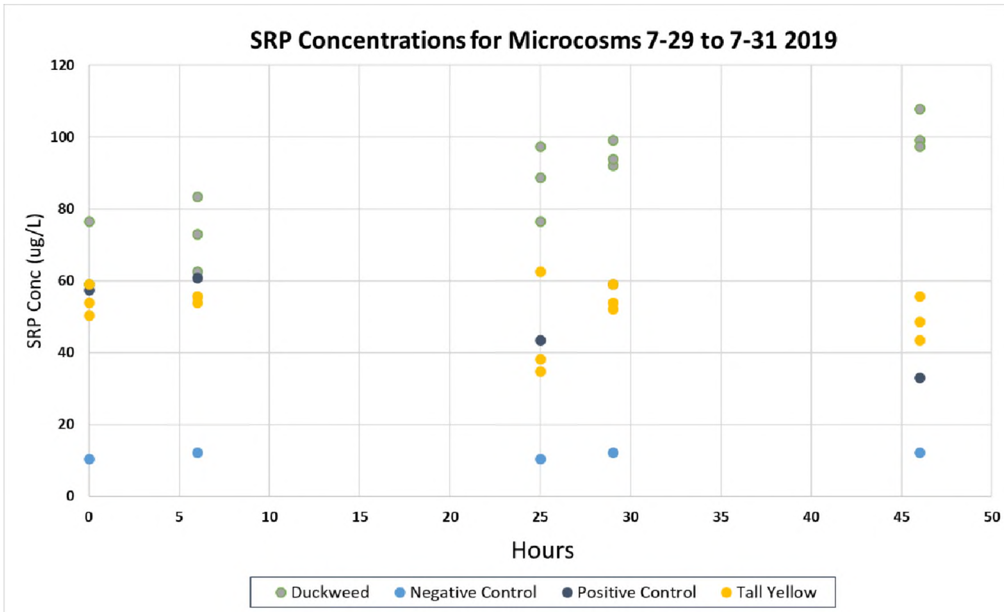

Figure A3. July 29th to 31st 2019. This study looked at Duckweed (*Lemna minor*) and Tall Yellow (Water Primrose). Increase of SRP given by Duckweed could have been explained by nutrient luxury uptake effect. These plants were collected at the inlet of Schuman pond where there are high nutrient concentrations coming into the pond.

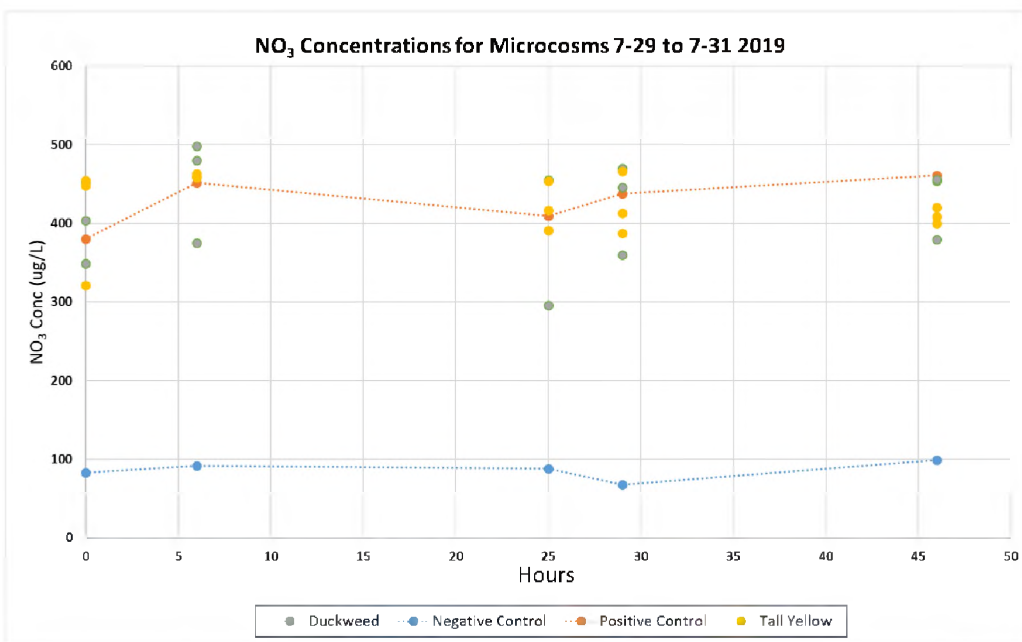

Figure A4. July 29th to 31st 2019. This study looked at Duckweed (*Lemna minor*) and Tall Yellow (Water Primrose). Increase of NO_3 given by duckweed and water primrose could have been explained by nutrient luxury uptake effect. These plants were collected at the inlet of Schuman pond where there are high nutrient concentrations coming in.

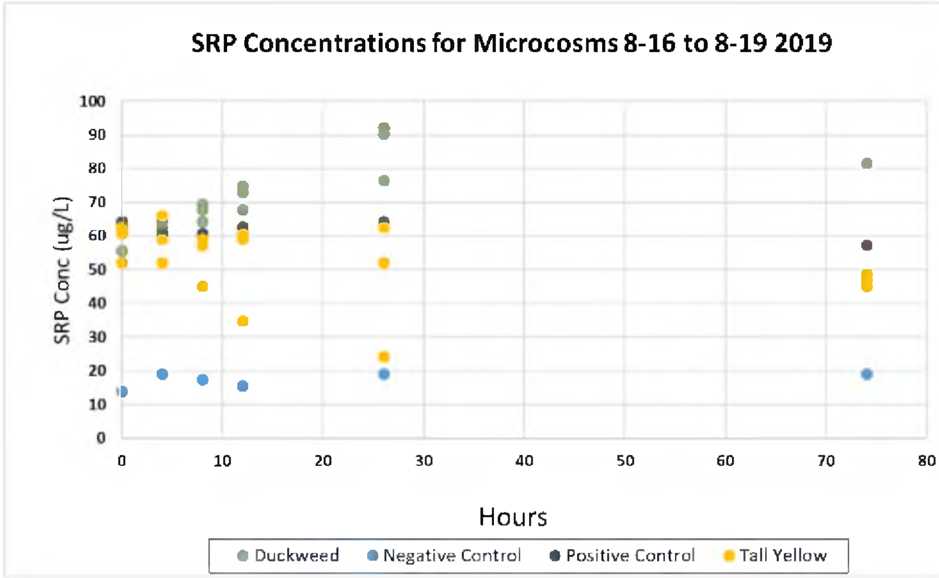

Figure A5. presents data from microcosm study on August 16th to the 19th of 2019. This study was done a lab setting. This study looked at Duckweed (*Lemna minor*) and Tall Yellow (Water Primrose). Increase of SRP given by duckweed and water primrose could have been explained by possible sediment or soluble particulates dissolved from plant matter. These plants were collected at the inlet of Schuman pond where there are high nutrient concentrations coming in.

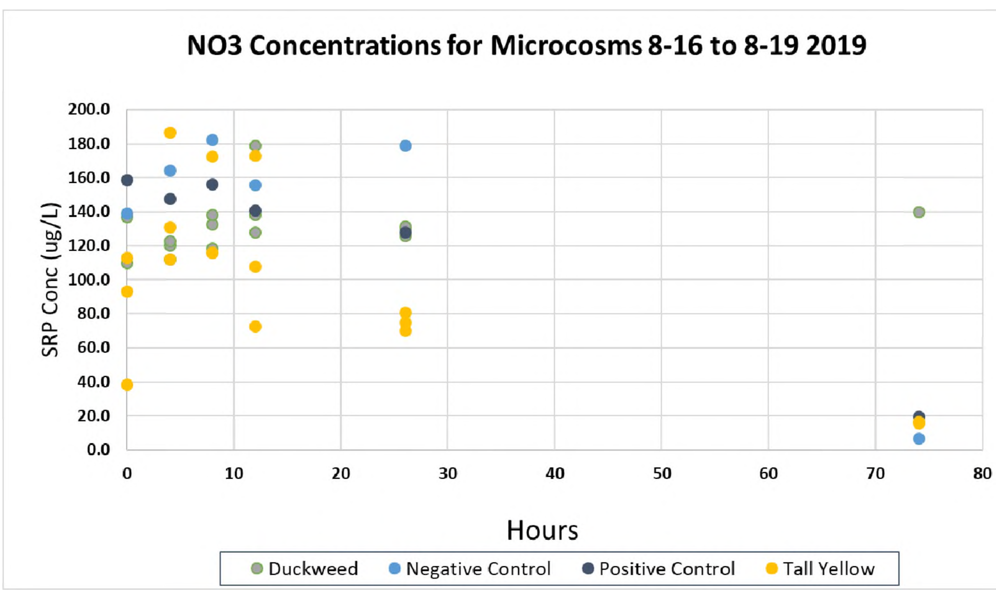

Figure A6. presents data from microcosm study on August 16th to the 19th of 2019. This study was done a lab setting. This study looked at Duckweed (*Lemna minor*) and Tall Yellow (Water Primrose). Negative controls in this study suggest possible contamination between the tubs most likely by human error. Nitrate values for this study are inconsistent, this was around when the IC column was going out.

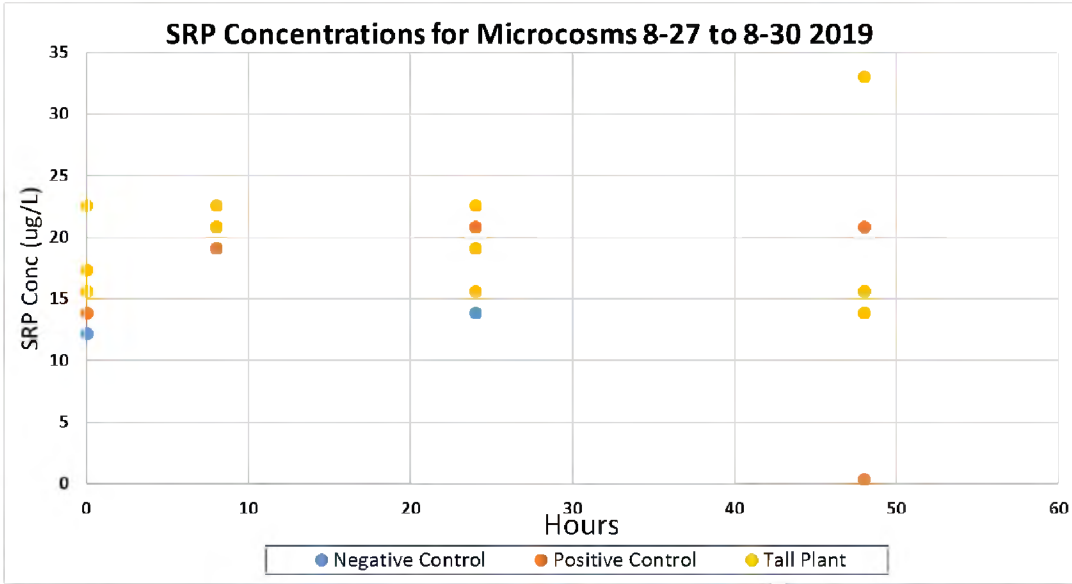

Figure A7. presents data from microcosm study on August 27th to the 30th of 2019. This study was done a lab setting. This study looked at 'tall plant' which was *Sagittaria latifolia* (broadleaf arrowhead). Negative controls in this study suggest possible contamination between the tubs most likely by human error. Micronutrients were used in this study and were found to interfere with results by reacting with PO_4 ions.

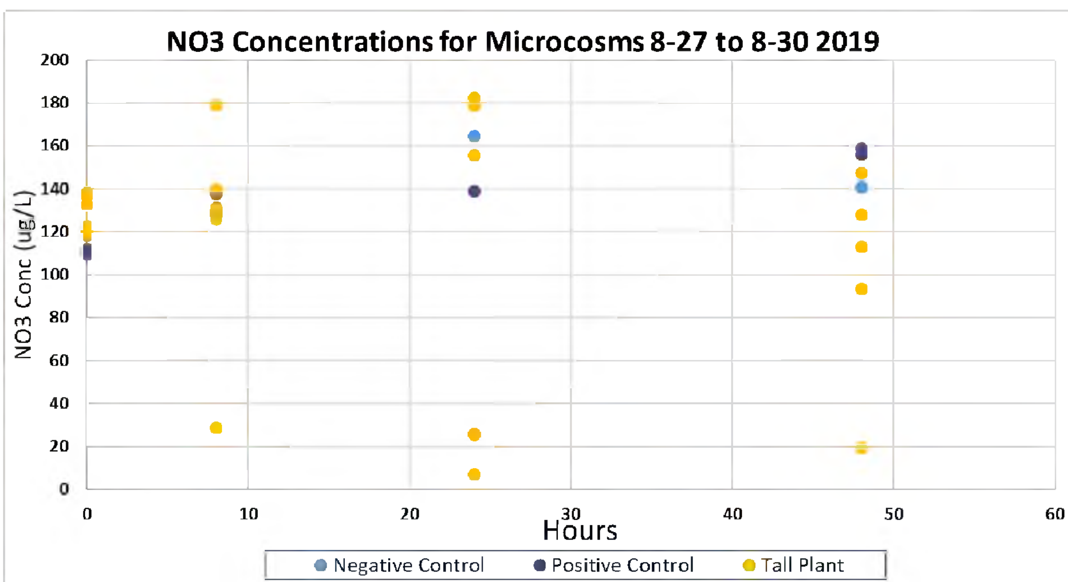

Figure A8. presents data from microcosm study on August 27th to the 30th of 2019. This study was done a lab setting. This study looked at 'tall plant' which was *Sagittaria latifolia* (broadleaf arrowhead). Negative controls in this study suggest possible contamination between the tubs most likely by human error. This experiment was used with micronutrients which interfered with nitrate concentrations.

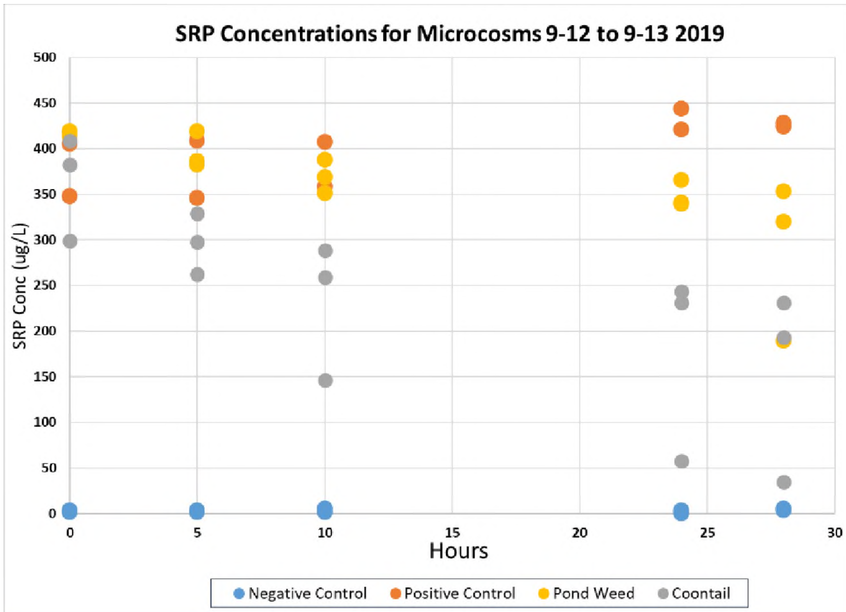

Figure A9. presents data from microcosm study on August 27th to the 30th of 2019. This study was done a lab setting. This study looked at *Ludwigia decurrens* (pondweed) and *Ceratophyllum demersum* (coontail). Coontail and pondweed showed uptake for SRP. However, the positive controls for this study increase which is likely explained by contamination from microcosm to microcosm.

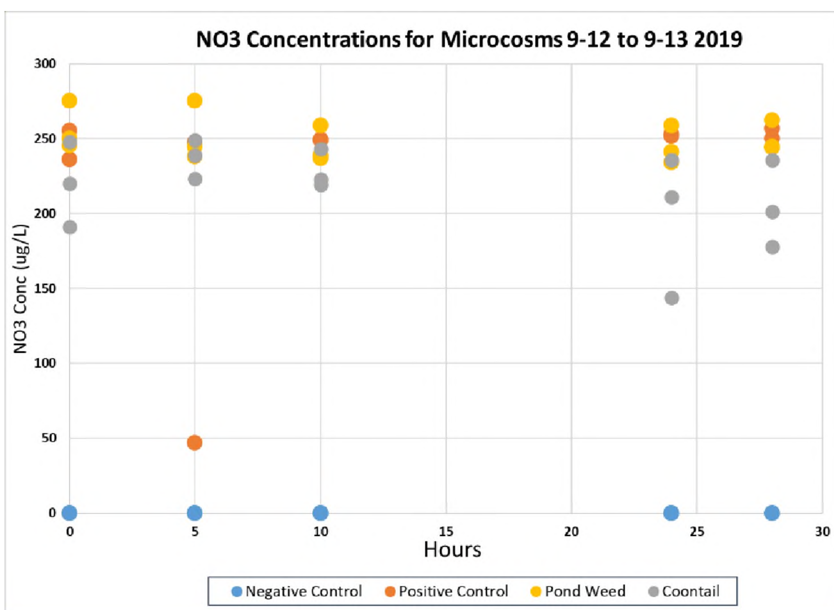

Figure A10. presents data from microcosm study on August 27th to the 30th of 2019. This study was done a lab setting. This study looked at *Ludwigia decurrens* (pondweed) and *Ceratophyllum demersum* (coontail). Coontail and pondweed did not show uptake for nitrate.

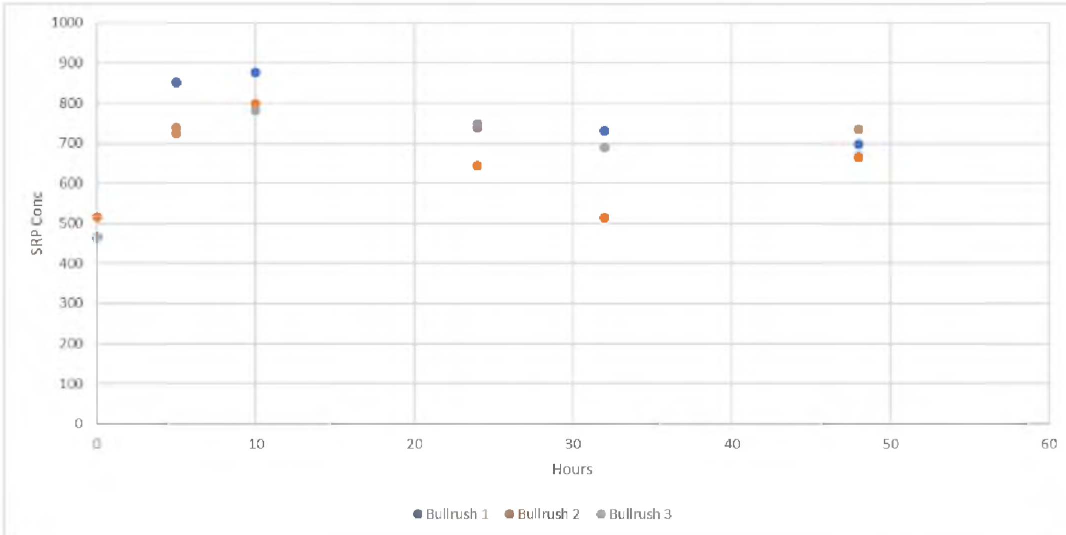

Figure A11. presents data from 25C microcosm study on September 12th to the 15th of 2020. This study was done in temperature-controlled chambers. Bulrush in the first two samples experienced a spike in SRP suggesting that nutrient-rich sediment from root mass was released when completely submerged in the water.

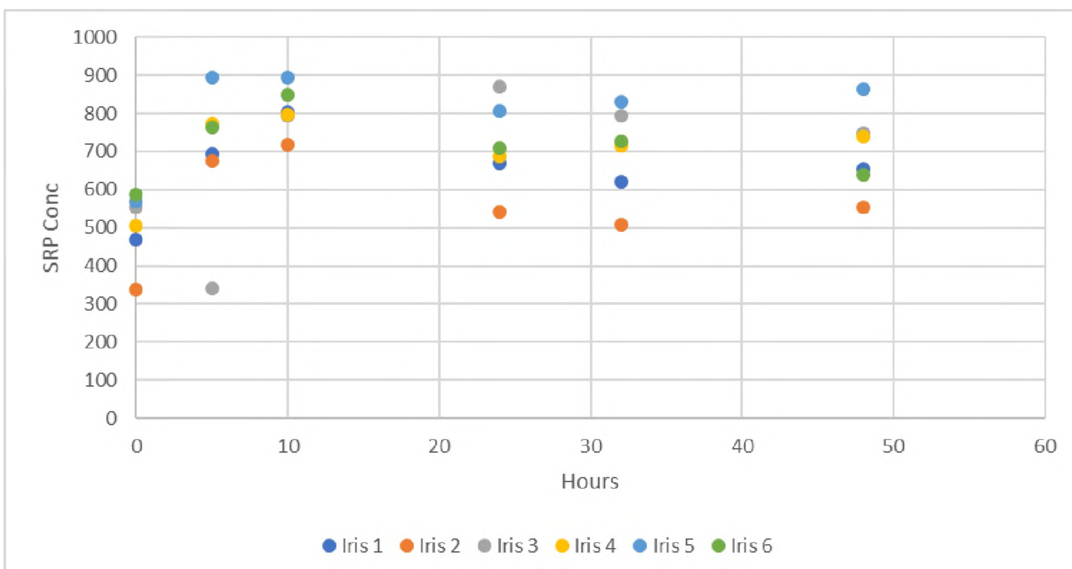

Figure A12. presents data from 25C microcosm study on September 12th to the 15th of 2020. This study was done in temperature-controlled chambers. Iris in the first two samples experienced a spike in SRP suggesting that nutrient-rich sediment from root mass was released when completely submerged in the water.

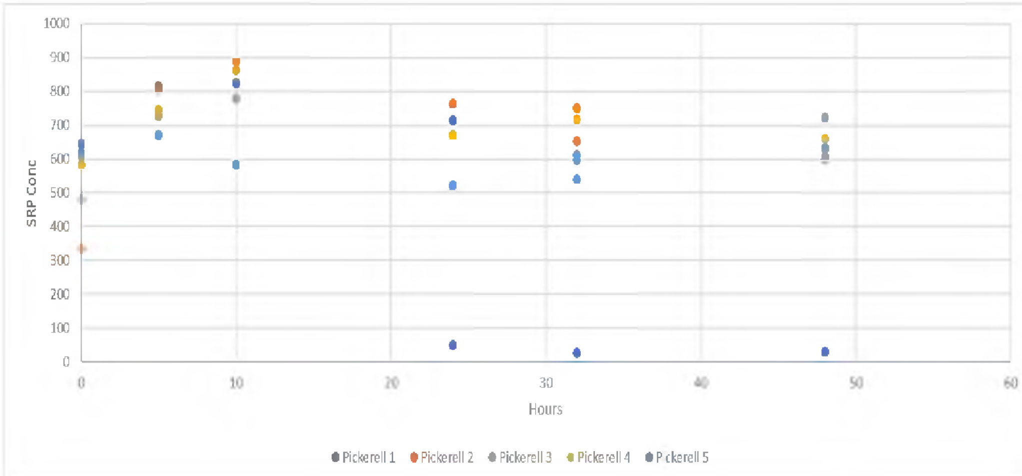

Figure A13. presents data from 25C microcosm study on September 12th to the 15th of 2020. This study was done in temperature-controlled chambers. Pickerel Weed in the first two samples experienced a spike in SRP suggesting that nutrient-rich sediment from root mass was released when completely submerged in the water.

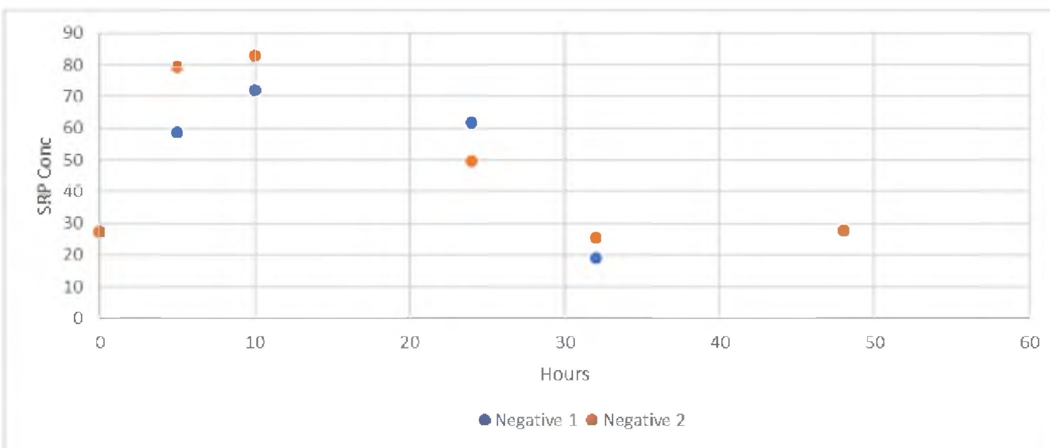

Figure A14. presents data from 25C microcosm study on September 12th to the 15th of 2020. This study was done in temperature-controlled chambers. Negative in the first two samples experienced a spike in SRP suggesting that nutrient-rich sediment from root mass could have contaminated the control tubs or there may have been sampling error.

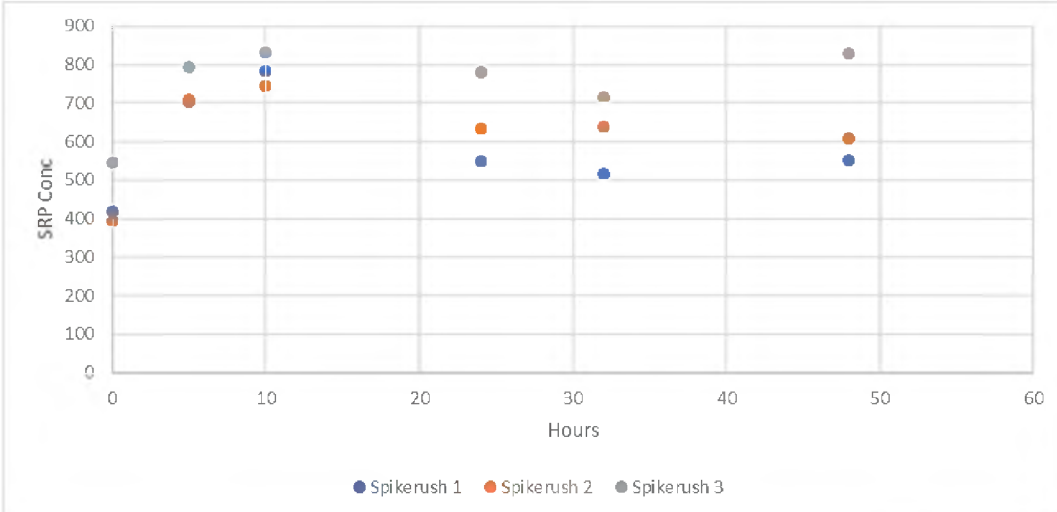

Figure A15. presents data from 25C microcosm study on September 12th to the 15th of 2020. This study was done in temperature-controlled chambers. Spikerush in the first two samples experienced a spike in SRP suggesting that nutrient-rich sediment from root mass could have contaminated the tubs.

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VITA

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