An Assessment of Floating Treatment Wetlands for Reducing Nutrient Loads from Agricultural Runoff in Coastal Virginia

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ABSTRACT

Floating treatment wetlands (FTWs) are an innovative best management practice that can enhance the performance of traditional retention ponds by increasing removal of the nutrients nitrogen (N) and phosphorous (P). FTWs consist of floating rafts on which wetland plants are planted, allowing the roots to be submerged below the water surface while the shoots remain above. A growing body of research has documented FTW performance with regard to urban runoff treatment, however evaluation of FTW effectiveness for treatment of agricultural runoff has received less attention. Due to high fertilization and irrigation rates, commercial nursery runoff contains much higher concentrations of N and P than runoff from urban areas. We conducted this study over two growing seasons (2015 and 2016) to assess the effectiveness of FTWs for use in commercial nursery retention ponds. In the first study we used two different nutrient concentrations, one to simulate nursery runoff (17.1 mg·L^{-1} TN and 2.61 mg·L^{-1} TP) and one to simulate concentrations that fall between urban and nursery runoff (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP). Four treatments were used: 1) *Pontederia cordata* planted in cups supported by a Beemat, 2) *Juncus effusus* planted in cups supported by a Beemat, 3) a Beemat with no plants, and 4) no treatment (open-water). Performance was evaluated based on a 7-day hydraulic retention time (HRT). *Pontederia cordata* removed between 90.3% and 92.4% of total phosphorus (TP) and 84.3% and 88.9% total nitrogen (TN), depending on initial loads. These reductions were significantly more than other treatments at both high and low nutrient loading rates. *Juncus effusus* performed better than the control treatments for TP removal at low nutrient concentrations, but did not perform any better than the control at higher nutrient loads. In the second study, conducted in 2016, we evaluated different plant species over two 8-week trials using simulated nursery runoff. We used five monoculture FTWs with the following species: *Agrostis alba*, *Canna ×generalis*, *Carex stricta*, *Iris ensata*, and *Panicum virgatum*. Additionally, two treatments were created from mixed species plantings and the final treatment consisted of an open water control mesocosm. Nutrient removal performance was evaluated over a 7-day HRT. P removal (phosphate-P) by FTW treatments ranged from 26.1% to 64.7% for trial 1 and 26.8% to 63.2% for trial 2. Trial 1 N removal (sum of ammonium-N, nitrate-N, and nitrite-N) efficiencies ranged from 38.9% to 82.4%, and trial 2 ranged from 12.9% to 59.6%. *Panicum virgatum* removed significantly more N and P than the control and any other FTW treatment in the second study. Both studies indicated, depending upon plant species, that FTWs can effectively remove nitrogen and phosphorous from urban and commercial nursery retention ponds.

An Assessment of Floating Treatment Wetlands for Reducing Nutrient Loads from Agricultural Runoff in Coastal Virginia

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GENERAL AUDIENCE ABSTRACT

Floating treatment wetlands (FTWs) are used to enhance the nutrient removal performance of stormwater retention ponds. FTWs consist of a buoyant raft on which wetland plants are planted, allowing the shoots to extend above the water surface while the roots stay submerged. The purpose of this research was to evaluate FTW nutrient removal performance in a commercial nursery environment where runoff has much higher concentrations of nitrogen and phosphorous than urban stormwater. The study spanned across two growing seasons (2015 and 2016), during which, different plant species and nutrient concentrations where evaluated. The first study evaluated *Pontederia cordata* and *Juncus effuses* as well as two control treatments at a high nutrient concentration and a low nutrient concentration. The *Pontederia cordata* performed better than the other treatments at both the high and low initial nutrient concentrations. In the second study, the following species were evaluated using a combination of mixed and monoculture plantings: *Agrostis alba*, *Canna ×generalis*, *Carex stricta*, *Iris ensata*, and *Panicum virgatum. Panicum virgatum* removed significantly more nitrogen and phosphorous than any other FTW treatment in the second study. Both studies indicated that FTWs can be effective technologies for nutrient removal from urban and commercial nursery retention ponds.

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Chapter 1. Introduction and literature review

1.1 Stormwater pollution

As the world's population has increased, land uses have changed and urbanization has increased. Clean water is important for a variety of reasons, but these land use changes have implications on the natural hydrologic cycle and create a range of water pollution issues. Urban development results in increases in impervious surfaces, which reduce infiltration and result in higher runoff volumes (Dietz and Clausen 2008), increasing erosion (Cianfrani et al. 2006), contaminant loads (Carey et al. 2013), and thermal loads in waterways (Herb et al. 2008). In addition to urbanization, population increases also create more demand for agricultural production. Excess fertilization and sediment loss from agriculture and urban runoff can degrade water quality and can cause eutrophication to surface waters downstream (Carpenter et al. 1998, Qin 2009).

According to the USEPA (2013), agriculture is the leading contributor of impairments to streams and rivers. Agriculture crop production systems and animal grazing can contribute a significant amount of nonpoint source pollution (NPS) through erosion from tillage, application of fertilizers and manures, and poor irrigation practices (Novotny 2003). High concentrations of nutrients, such as nitrogen (N) and phosphorous (P), resulting from chemical and manure fertilizer application can enter nearby water bodies through overland flow or groundwater leaching, thus posing a risk to water quality and aquatic life (Anderson et al. 2002).

Similar to conventional row crop agriculture, nurseries and greenhouses use significant amounts of fertilizer and water to produce "container crops". Due to plant density, substrates, and species diversity, nurseries can use more agrichemicals than row crop operations (White et al. 2010). Majsztrik (2011) reported average yearly container nursery N and P application rates of 680 $kg \cdot ha^{-1}$ and 129 $kg \cdot ha^{-1}$, respectively. Well-drained substrates are typically chosen for container operations to help prevent disease, but the limited amount of water held in those substrates leads to more frequent irrigation (Majsztrik et al. 2011). The combination of well-drained substrates and frequent irrigation can cause water and nutrient leaching. Studies conducted in the southeastern US have shown average concentrations of N and P in nursery runoff to range from 8.27 to 21.7 $mg \cdot L^{-1}$ and 1.41 to 8.27 $mg \cdot L^{-1}$, respectively (Taylor et al. 2006, White et al. 2010, White et al. 2011). This contrasts with concentrations of N and P in urban runoff of 1.19 $mg \cdot L^{-1}$ and 0.15 $mg \cdot L^{-1}$, respectively (Wang and Sample 2014).

1.2 Regulations on nutrient pollutants

To address the environmental damages and human health concerns caused by water pollution, the Clean Water Act (CWA) was created (U.S. Code 1972). Through Section 303d of the CWA, the USEPA requires each state to develop a list of impaired waters, known as the "303d list." The USEPA and states who have been delegated authority under the National Pollutant Discharge Elimination System (NPDES program) are then required to conduct studies to determine the appropriate maximum contaminant loading from each source that the water can receive and still meet its designated use. This program is known as the total maximum daily load, or TMDL program, using "daily" even though average annual loading is normally the criterion used. In the case of N and P, TMDLs need to be met to reduce the rate of eutrophication in lakes, rivers, and estuaries. Perhaps the most well-known example of a nutrient related TMDL is the Chesapeake Bay TMDL.

The Chesapeake Bay TMDL is the largest TMDL imposed by the EPA and is specifically aimed at reducing the N, P, and sediment loads entering the bay through upstream waterways

(U.S. EPA 2010). The Chesapeake Bay TMDL limits yearly N loads by 25% to 84,323 metric tons and P loads by 24% to 5,670 metric tons. After determining what loading can be allowed to the waterway, the next step of a TMDL is to develop a watershed implementation plan (WIP). The WIP outlines the steps and methods to be taken, including identification of load reduction strategies by each source and selection of appropriate best management practices (BMPs) to treat runoff prior to discharge. Since agriculture makes up 24% of the land area in the Chesapeake Bay watershed, identifying suitable BMPs and putting them into place will be necessary to address the Bay TMDL requirements (Majsztrik and Lea-Cox 2013).

1.3 Best management practices and treatment options

In the Commonwealth of Virginia, urban stormwater is managed using the "Runoff Reduction Method" or RRM (Hirschman et al. 2008, Battiata et al. 2010). The RRM uses a variety of post-construction BMPs to reduce, manage and treat runoff prior to its leaving a developed site. RRM favors infiltrative practices such as rooftop disconnection, bioretention, infiltrative basins, but also provides credit for larger treatment BMPs such as wet pond and constructed wetlands. A complete list of BMPs approved for use in Virginia can be found at Virginia Department of Environmental Quality (2012). While some agriculture BMPs focus on some of the same technologies, like riparian buffers and vegetated filter strips, they also include source control efforts such as strip cropping, terracing, conservation tillage, nutrient management planning, and livestock exclusion (Virginia Department of Conservation and Recreation 2016).

Container nursery and greenhouse production systems are agricultural, but operate quite differently than traditional row crop or livestock operations due to the large numbers of plants produced, the increased variety of plant species, and the higher demand for nutrients and irrigation. Yeager et al. (2010) and Majsztrik et al. (2011) identified some BMPs used by the container nursery industry. Most of the BMPs identified focus on water and fertilizer application in efforts to increase the efficiency of the plant production operation while simultaneously improving runoff water quality. Vegetative buffer strips and containment systems, like retention ponds, were identified as BMPs appropriate for nutrient and sediment removal. Retention ponds are commonly used for runoff collection at nurseries, and while adequate for sediment removal, they are inefficient in regards to dissolved pollutant removal (Yeager et al. 2010, Tanner and Headley 2011). To further improve water quality and nutrient removal, a relatively new technology, floating treatment wetlands (FTWs), has emerged that may be suitable for the nursery industry.

With regard to nutrient removal, FTWs are similar in function to constructed wetlands, but float on the surface of the water. This trait makes them independent of the hydrology of the pond; except there must be a minimum water level to keep FTW plants from sinking roots into the pond sediments. Since the surface of the pond is unused and available at no cost, FTWs could be an ideal and cost effective retrofit for conventional retention ponds (Winston et al. 2012, White 2013). Studies on FTWs have demonstrated their ability to remove N and P nutrients from stormwater (Hubbard et al. 2004, Chang et al. 2012, Borne et al. 2013, Borne 2014, Wang and Sample 2014). N and P reduction credits of 12% are allocated for FTWs by the Florida Department of Environmental Protection (Wanielista et al. 2012). The Chesapeake Bay Program Water Quality Goal Implementation Team recently approved FTW removal rates of 0.8% to 4.1% and 1.6% to 8.0% for N and P, respectively, depending on raft coverage as a percentage of the retention pond area (Lane et al. 2016). This latter estimate is intended as a conservative credit based upon the 50^{th} percentile of removal rates from available research on specific FTW trials, and using a first order kinetics in conjunction with a watershed model (Wang and Sample 2013).

1.4 Overview of FTW technology

Floating treatment wetlands are a relatively new technology that can be implemented into existing stormwater retention ponds to aid in pollutant removal (Wang et al. 2014, Borne et al. 2015, Lynch et al. 2015). A FTW consists of a buoyant raft with emergent plants, whereby the raft floats on the surface of the water and the plant roots extend below the surface (Headley and Tanner 2006, White 2013). Unlike a traditional constructed wetland, the floating nature of the design allows FTWs to resist submersion and adapt to water level fluctuations (Headley and Tanner 2007, Lane et al. 2016). Floating rafts and mats are commercially available, but homemade rafts can function adequately as well (Wang and Sample 2014). Representation of a generic FTW setup is shown in Figure 1.1, whereas Figure 1.2 shows implementation of FTWs in an actual pond. Recent research suggests that FTWs provide the following benefits in regards to water quality: nutrient reduction, reduction in metal concentrations, filtration and sedimentation, reduction in shoreline erosion, and provision of wildlife habitat.

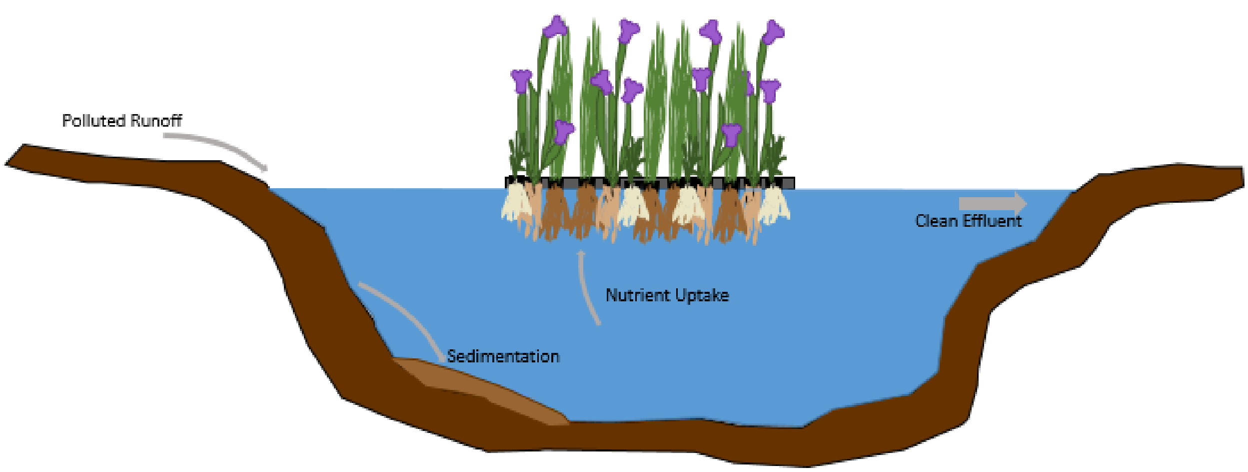

Figure 1.1. Floating treatment wetland cross-section diagram.

Figure 1.2. Newly planted and installed FTWs (image courtesy of Laurie Fox, Virginia Tech).

1.5 FTW benefits

One of the most significant benefits in regards to the application of FTWs is nutrient removal. N and P occur naturally in the environment; however, excess of one or both of these nutrients in surface water or groundwater can have detrimental impacts to human health and aquatic life. While typical stormwater retention ponds are adequate for removing larger particle size sediments from runoff, they are less efficient at removing fine suspended particles and dissolved pollutants (Tanner and Headley 2011). FTW retrofits in existing or newly constructed retention ponds can remove nutrients using mechanisms similar to traditional constructed wetlands (White 2013). Assimilation and denitrification play a major role in N removal (Jayaweera and Kasturiarachchi 2004, Borne et al. 2013). A study in New Zealand by Borne et al. (2013) concluded that denitrification contributed more significantly to N removal than plant uptake, and that sizing a FTW relative to the retention pond surface area is important for sustaining the low dissolved oxygen (DO) environment needed for nutrient removal. Sizing for FTW systems varies based on nutrient loads, pond size, and project budget, but generally, at least 50 m^2 in size or 10% to 50% pond surface area coverage is recommended in order to achieve appropriate anoxic conditions for denitrification (Borne et al. 2015, Lane et al. 2016). According to research by Borne (2014), P removal occurs via sorption, particle entrapment, flocculation and sedimentation.

In addition to removing excess nutrients, FTWs have the ability to remediate waters polluted with metals. Headley and Tanner (2007) found that FTWs were capable of Cu and Zn removal at 3.8-6.4 $mg \cdot m^{-2}\ d^{-1}$ and 25-88 $mg \cdot m^{-2}\ d^{-1}$, respectively. They also concluded that FTWs can reduce turbidity, thus removing fine sediments with greater efficiency than retention ponds alone. Van de Moortel et al. (2010) concluded that Cu and Zn were removed faster with FTWs. In order to capitalize on the accumulated pollutant removal achieved by FTWs, it has been recommended that harvesting be conducted at the end of each growing season by removing either whole plants or the plant shoots (Chang et al. 2012, Wang et al. 2014).

If established appropriately, FTWs can create additional habitat and thriving wetland ecosystems. Wang et al. (2014) observed aquatic macroinvertebrates, birds, and waterfowl utilizing the floating islands for foraging, nesting, or resting spots. Fish seeking cover or spawning areas were found adjacent to the islands as well. Nakamura and Mueller (2008) also mention benefits to fish, birds, and other wildlife. With appropriate plant species selection, pollinators will be attracted to the FTWs (McAndrew et al. 2016).

1.6 Recent FTW research

FTWs have been used and evaluated for treatment of urban stormwater (Tanner and Headley 2011, Borne et al. 2013, White and Cousins 2013, Winston et al. 2013, Wang and Sample 2014, Lynch et al. 2015), combined sewage overflow (Van de Moortel 2008), acid mine drainage (Smith and Kalin 2000), and agricultural runoff and waste water (Hubbard et al. 2004, Stewart et al. 2008, Yang et al. 2008, White and Cousins 2013).

Headley and Tanner (2007) conducted a batch mesocosm study with simulated urban stormwater runoff to evaluate the effects of FTWs on fine particles, copper, and zinc. They used four plant species grown on BioHaven® floating islands. The selection of sedges and rushes included *Carex virgata, Cyperus ustulatus, Juncus edgariae,* and *Schoenoplectus tabernaemontani.* The targeted concentrations for the simulated runoff consisted of 0.3 $mg \cdot L^{-1}$ ammonium-N, 3.0 $mg \cdot L^{-1}$ nitrate-N, and 0.1 $mg \cdot L^{-1}$ total dissolved P. After a 7-day hydraulic retention time (HRT), they observed greater removal of Cu from the planted islands than the unplanted; however, Zn removal was more significant in the unplanted islands. In regards to ammonium N removal, the planted island treatments achieved significant removal, up to 96%, over the course of the 7-day HRT. Reductions between 26 and 51% were observed for dissolved reactive P for the planted islands, which was significantly better performance than observed in the non-planted islands.

Wang and Sample (2014) set up a similar study comparing *Pontederia cordata* and *Schoenoplectus tabernaemontani* performance against control treatments in simulated urban retention pond water. The average initial total nitrogen (TN) and total phosphorous (TP) recorded were 1.19 and 0.15 $mg \cdot L^{-1}$, respectively. Nutrient removal was calculated as a removal efficiency using initial and final nutrient concentrations from water sampling. Both plant species improved nutrient removal in comparison to the control treatment after a 7-day HRT. The *Pontederia cordata* FTW and mesocosm removed significantly more TP than the *Schoenoplectus tabernaemontani,* but showed no statistical difference to *Schoenoplectus* for TN removal.

Lynch et al. (2015) observed similar results to Wang and Sample (2014) in a 7-day HRT batch mesocosm study. Only one plant species, *Juncus effusus,* was observed. Results indicated that TN and TP mesocosm and FTW combined removals of 25% and 4% were achieved, respectively, for BioHaven® floating mats; and 40% and 48%, respectively, for Beemat floating mats. These results indicate that floating island material may affect nutrient removal performance. It is worth noting that the performance numbers mentioned above represent the entire experiment, and the data suggests that after an initiation period, the BioHaven® mats matched the rate of nutrient removal of the Beemats when planted with the same species.

To evaluate nutrient removal from swine lagoon wastewater, Hubbard et al. (2004) set up a FTW study in tanks containing three nutrient concentration treatments. They evaluated three wetland plant species including: *Typha latifolia*, *Juncus effusus, and Panicum hematomon.* A two-week HRT was used, after which half of the tank was drained and then refilled. Initial concentrations for TN were 160, 50, and 53 $mg \cdot L^{-1}$, and TP influent concentrations were 30, 15, and 8 $mg \cdot L^{-1}$. The *Typha latifolia* resulted in the best net nutrient removal performance, which was indicated by plant tissue sampling. Although the *Juncus effusus* removed nutrients early in the trial, ultimately the plants did not grow well and in some cases did not survive at all. In contrast to other studies, this led to the conclusion that *Juncus effusus* was not suitable for FTW applications.

Chang et al. (2012) used *Canna flaccida* and *Juncus effusus* for a FTW mesocosm experiment in Florida. Nutrient input of nitrate was 3 $mg \cdot L^{-1}$ and input of phosphate was 1 $mg \cdot L^{-1}$ to simulate surface water runoff. Over the 3-month study, nitrogen removal was calculated to be 36.39 $mg \cdot m^{-2}\ d^{-1}$ and P uptake was 1.48 $mg \cdot m^{-2}\ d^{-1}$. They concluded that a retention pond with

FTW surface area coverage of 5% was able to remove 61% TN and 53% TP. Plant nutrient content analysis concluded that the *Canna flaccida* performed better than the *Juncus effusus.*

White and Cousins (2013) studied N and P removal from simulated runoff. Their study examined *Canna flaccida* and *Juncus effusus* over two growing seasons. Using troughs and a 3-day HRT, they were able to simulate an average N load of 0.85 mg·L^{-1} during year one and 1.88 mg·L-1 for year two. P influent concentrations were 0.08 mg·$L^{-1}$and 0.22 mg·L^{-1} for years one and two, respectively. The N and P concentrations for year one were reduced by 83.5% and 75%, respectively. The increased inflow concentrations in year two resulted in an N and P concentration reductions of 58% and 45.5%, respectively. These removal efficiencies were determined by measuring inflow and outflow water concentrations. Tissue samples suggest that the *Juncus effusus* removed more nutrients than the *Canna flaccida*, which conflicts with the results of the *Canna flaccida* and *Juncus effusus* experiment by Chang et al. (2012).

Iris pseudacorus and *Typha angustifolia* were used in a study by Keizer-Vlek (2014) and were found to remove more TN and TP than control treatments. The study was conducted over 91 days during which the nutrient levels were maintained at approximately 4 mg·L^{-1} TN and 0.25 mg·L^{-1} TP. The *Iris pseudacorus* treatments had a total removal efficiency of 98% and 92% for TN and TP, respectively while the *Typha angustifolia* resulted in 57% and 23% total removal efficiency for TN and TP, respectively.

Yang et al. (2008) studied FTW systems for purification of nitrate-rich agricultural runoff in Yixing City, China. With initial concentrations of 7.94 mg·$L^{-1}$and 1.54 mg·L-1 TN and TP, respectively, they observed removal of 64% TN and 13% TP by the FTW and mesocosm for a 1-day HRT. Only one species, *Oenanthe javanica,* was used in this study.

In addition to mesocosm or batch tank studies, FTW research has been recently conducted on full-scale retention ponds. Two ponds in Durham, NC, USA were evaluated prior to and after FTW installation (Winston et al. 2013). The ponds had 9% and 18% of surface area covered after FTW installations. They used mixed species plantings consisting of *Juncus effusus, Carex stricta, Andropogon gerardii, Hibiscus moscheutos, and Pontederia cordata.* The nutrient reductions between the pre- and post- retrofits were not significantly different in most cases. The pond with 18% coverage, however, did statistically outperform its pre-retrofit results for TP reduction. Overall, they concluded that the pond with 18% coverage outperformed the pond with only 9% coverage. Borne et al. (2013) evaluated N removal for a pond with FTWs in comparison to a control pond. They concluded that the FTW treatment resulted in higher N removal. Using the same experimental site, TP reductions of 27% were observed (Borne 2014). To realize the full potential of FTWs for TP removal, they concluded that dense root networks needed to be established. Based on extensive full-scale studies, Borne et al. (2015) established a set of design and maintenance guidelines for FTW implementation.

The results presented in the Headley and Tanner (2007) study are encouraging, but nutrient concentrations used in the simulated runoff are much lower than those found in commercial nursery environments. Additionally, three of the four plant species evaluated were not native to the U.S., thus making them a less desirable plant selection for FTWs located in the Chesapeake Bay watershed. The Wang and Sample (2014) and Lynch et al. (2015) studies also used nutrient concentrations typical of urban runoff, not that of the stronger commercial nursery runoff; however, they do identify additional native plant species that show promise for nutrient removal. The swine lagoon wastewater treatment study (Hubbard et al. 2004) used higher nutrient concentrations than those found at commercial nurseries, but the lowest concentration used was close to the typical range observed at nurseries. White and Cousins (2013) reported successful results with *Juncus effusus*, but concluded that more data collection was needed to define nutrient removal rates by plant species at various influent concentrations. These recommendations are further confirmed through the results presented by Chang et al. (2012). Yang et al. (2008) used nutrient concentrations that fall within the typical nursery and greenhouse runoff range, but they only used one plant species, which is non-native to the Chesapeake Bay watershed.

Assigning appropriate credits and removal rates for FTWs is important so that BMP designs function properly and efficiently while meeting regulatory standards. It is clear from reviewing the literature that continued data collection and analysis on FTWs is needed in order to strengthen the reliability of expected FTW performance results. A particular need is to identify suitable plants and methods for nursery runoff applicable to the Chesapeake Bay region.

1.7 Research problem and purpose

Maintaining healthy water quality and quantity is imperative for many aspects of life. As demand for water resources continues to increase, efficient use and treatment of water will also need to increase. This is especially important for the agriculture industry, which includes nurseries and greenhouses. Nationally, approximately 29% of surface water and 65% of fresh groundwater withdrawals in 2010 were used for agricultural crops and other irrigation needs (Maupin et al. 2014). Commercial container nurseries and greenhouses use large quantities of fertilizer and irrigation water that may result in elevated nutrients and sediment in discharges from these sites. Significant effort should be focused on making certain that these pollutants are reduced to the maximum extent feasible to ensure compliance with the Chesapeake Bay TMDL.

The goal of this research was to evaluate the efficacy of FTWs for nutrient remediation at commercial container nursery and greenhouse operations. Current literature and research suggests that FTWs are capable of removing nutrients, but more research is needed to better quantify the performance of different plant species when exposed to varying nutrient concentrations and the overall practicality of each FTW plant species in a commercial environment.

This study evaluated the suitability of different plant species for potential nutrient removal in existing retention ponds with nutrient concentrations representative of commercial nursery runoff and concentrations between urban and nursery runoff. In addition, the study assessed the potential commercial opportunity for growers to harvest FTW plants and sell them for wetland restoration, buffers, and other habitat restoration projects. These objectives were accomplished using mesocosm studies over two separate growing seasons. A mesocosm study was chosen in order to better control the influent nutrient concentrations. This approach facilitates focusing upon key treatment factors (such as species), while also limiting extraneous variables that could be introduced in an actual retention pond setting.

The content in this report is focused on two separate but similar FTW studies. The section titled *Study site and experimental equipment* pertains to both studies. Each study has its own section outlining materials and methods, as well as separate results and conclusions. A comprehensive conclusion section will address the overall key learnings from both studies.

Chapter 2. Study site and experimental equipment

2.1 Study site

The study was conducted in Virginia Beach, VA at the Virginia Tech Hampton Roads Agriculture Research and Extension Center (HRAREC; 36°53′N, 76°10′W). The study site was located on the north central portion of the property and is outlined in red in Figure 2.1. Rainfall and temperature data at the HRAREC for the study periods are shown in Table 2.1.

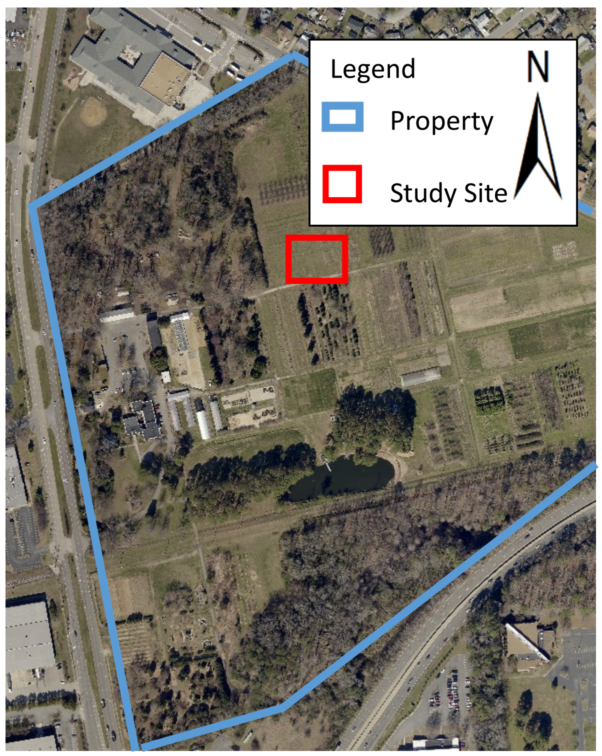

Figure 2.1. Floating treatment wetlands study site at the HRAREC.

Table 2.1. Rainfall and temperature data for the study site.

Study Period Date Range	Total Rainfall (mm)	Mean Air Temperature (°C)	Max Air Temperature (°C)	Min Air Temperature (°C)
June 8, 2015 - October 19, 2015	559	24.7	36.3	7.50
June 1, 2016 - July 27, 2016	255	25.5	36.1	13.7
July 27, 2016 - September 21, 2016	659	26.1	35.9	17.4

2.2 Experimental equipment

The experiments were conducted using a designed and built system of tanks, pumps, and piping. Two 5867 L (1550 gal) plastic storage tanks (Norwesco, St. Bonifacius, MN, USA), also referred to as the mix tanks, were used for the batch process makeup of the water and fertilizer solutions. One tank was capable of supplying water to half of the experimental mesocosms. Each tank drain was connected to the intake of an electric water pump (Model FH40-5500, Little Giant, Oklahoma City, OK, USA). The discharge of each pump consisted of a main supply line and a recirculation line. The recirculation line allowed water to be circulated from the mix tank drains back to the top of the mix tank. This was beneficial for mixing when initially adding the soluble fertilizer to ensure a uniform concentration was delivered.

The main supply line for each mix tank had four headers, one for each of the four rows of mesocosms. The headers were tied into the main supply line on both ends of the piping layout, creating a loop to aid in equalizing the flow in each fill line. Each of those four branches had eight fill lines, two fill lines per mesocosm.

Thirty-two structural foam stock tanks (Rubbermaid Commercial Products, Winchester, VA, USA) were used as mesocosms. Each 378.5 L mesocosm was retrofitted with an overflow hole that allowed a maximum water volume of 302.8 L. Every mesocosm had a drain at the bottom that connected into a main drain system. The reason for creating a drain system was to move the draining water to an appropriate on-site water conveying system and to avoid flooding the experiment area. The main drain line was connected to the intake of a Honda WX15 gas powered water pump (Honda Power Equipment, Alpharetta, GA, USA). The discharge of the drain pump was connected to a series of 15.24 m (50 ft) sections of Goodyear 3.81 cm (1.5 in)

Spiraflex hose. The hose conveyed the water to a ditch on site. The experimental equipment just after construction is shown in Figure 2.2.

Figure 2.2. Constructed system for FTW mesocosm experiments at the HRAREC.

Chapter 3. Effects of nutrient loads on FTW performance for two native species

3.1 Study overview

The experiment for the 2015 growing season was conducted from June 8, 2015 to October 19, 2015 (19 experimental weeks). *Pontederia cordata* (pickerelweed), and *Juncus effusus* (soft rush), two commonly used native plants in floating treatment wetland technology, were evaluated against a set of control studies that consisted of open water or mat-only mesocosms. The plants were planted on commercially available floating treatment wetland systems (Beemats) and implemented in mesocosms used for treatment evaluation. Two nutrient concentrations were used to represent runoff: a low concentration for stormwater between urban and nursery conditions and a higher concentration for commercial nursery runoff. A HRT of 7 days was used for the study. After 7 days, each mesocosm was drained and refilled with new water and fertilizer solution. TN and TP removal were quantified using water sample and plant tissue sample results. The hypotheses of the study were:

1) Nutrient removal is affected by the presence of FTWs in a mesocosm study.

2) Nutrient removal is affected by FTW plant species selection.

3) Nutrient removal is affected by influent concentration.

3.2 Materials and methods

3.2.1 Design and operation of experiment

The experiment was set up in a randomized complete block design that included four replications of eight treatment types. Table 3.1 shows the treatment combinations for the study. For each nutrient concentration, high (17.1 $mg \cdot L^{-1}$ TN and 2.61 $mg \cdot L^{-1}$ TP) and low (5.22 $mg \cdot L^{-1}$

TN and 0.52 mg·L^{-1} TP), there were four replications that included the following: one treatment with *Pontederia cordata*, one treatment with *Juncus effusus*, one treatment with a mat and no plants, and one treatment with no mat and no plants (open water). The mesocosms were blocked by row with the northern most row being block 1 and each subsequent row being blocks 2, 3 and 4. The sole purpose for blocking by row was to aid in organization during sample collection and data measurement. Figure 3.1 below shows the experimental design.

Table 3.1. Treatment combinations for the FTW wetland study.

Treatment	Mat	Plants	Species	Concentration	Reps
1	Yes	Yes	*Pontederia cordata*	Low	4
2	Yes	Yes	*Pontederia cordata*	High	4
3	Yes	Yes	*Juncus effusus*	Low	4
4	Yes	Yes	*Juncus effusus*	High	4
5	Yes	No	n/a	Low	4
6	Yes	No	n/a	High	4
7	No	No	n/a	Low	4
8	No	No	n/a	High	4

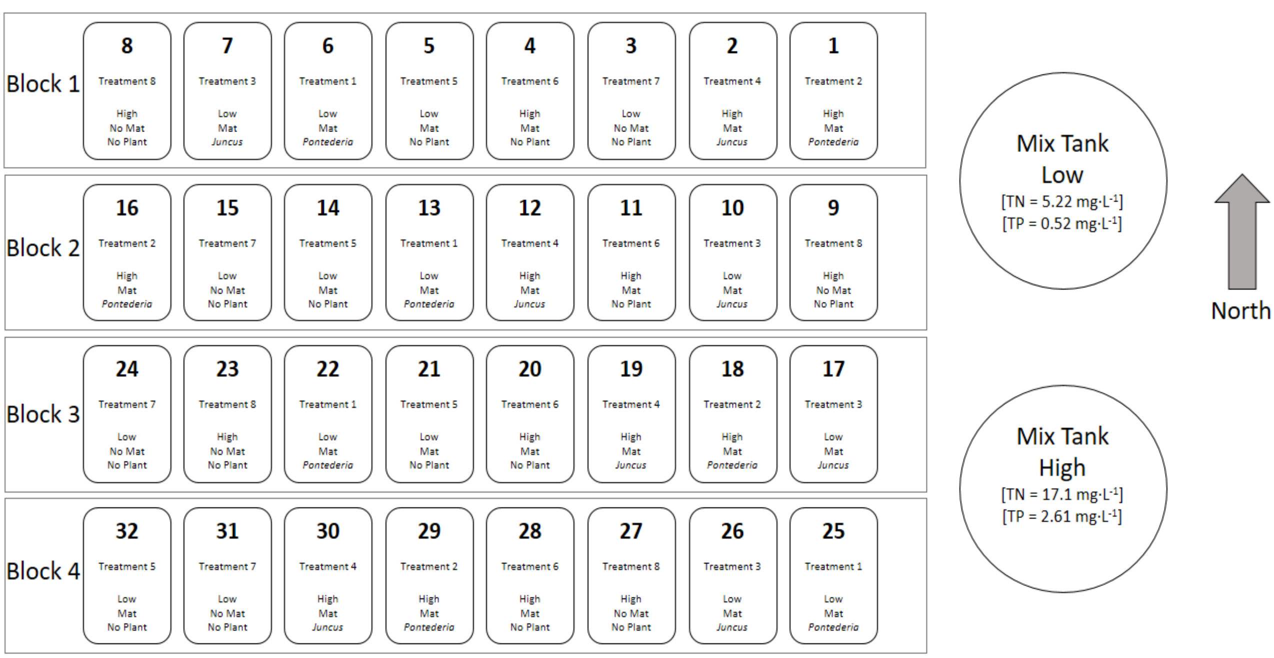

Figure 3.1. Experimental design for the 2015 growing season.

At a depth of 47.3 cm (full), each mesocosm held a volume of 302.8 L, and had a water surface area of 0.79 m^2. The 1.3 cm thick closed cell foam floating rafts used were commercially available FTW systems manufactured by Beemats (Beemats LLC, New Smyrna Beach, FL, USA). The mats came with puzzle-piece edges for custom raft sizing and pre-cut holes that can be used with aerator pots, also distributed by Beemats. The ends of each mat were trimmed to allow for a proper fit in the mesocosms leaving a total mat surface area of 0.64 m^2 (0.55 m^2 if subtracting out the precut holes), as shown in Figure 3.2. At operational depth, the FTW covered 80.3% of the water surface area.

Figure 3.2. Beemat sized to fit mesocosm surface area at operational water levels.

Prior to planting, the roots of each plant were rinsed thoroughly in effort to remove as much of the original planting media as possible. The rinsing process proved effective for the *Pontederia cordata* roots but ineffective for the tightly bound root balls of the *Juncus effusus*. The difference in root balls after rinsing and an example of planting with the aerator pot is shown in Figure 3.3.

Figure 3.3. Roots after rinsing. *Pontederia cordata* (left), *Juncus effusus* (middle), *Juncus effusus* planted in an aerator pot (right).

Immediately after rinsing, the roots were wrapped in fiber coir, placed in the plastic aerator pots, and taken to the study site where they were planted according to the design of experiment. Each mesocosm-fitted mat contained 20 planting holes. During this study, 50 % of the planting holes were used. Five plants were randomly planted on each half of the mat for a total of ten plants per mesocosm (approximate density of 15 plants/m^2). A representation of this planting scheme is shown in Figure 3.4.

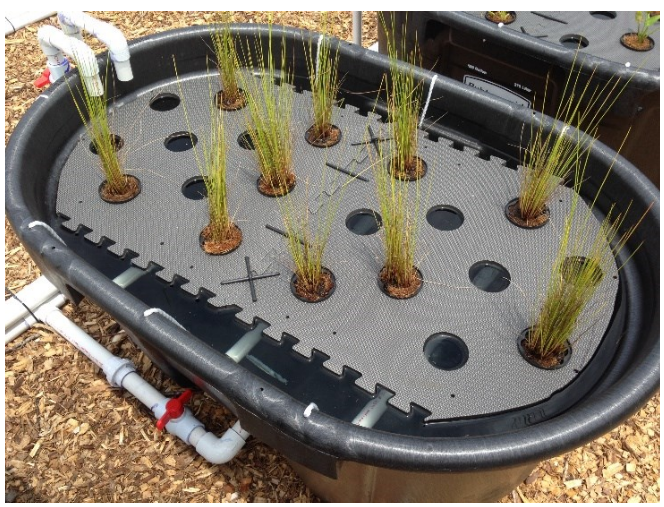

Figure 3.4. Representation of the planting scheme for the 2015 experiment.

After each 7-day HRT, the mesocosms were drained and refilled with a new batch of simulated runoff. The simulated runoff was created by adding water and 24-8-16 soluble fertilizer (Southern Agriculture Insecticides Inc., Hendersonville, NC, USA) to each mix tank. After allowing the solution to recirculate for 1 hour, it was pumped to each mesocosm. To represent the urban stormwater nutrient concentration, 66.24 g of fertilizer was added to the low mix tank which resulted in mean concentrations of 5.22 $mg \cdot L^{-1}$ TN and 0.52 $mg \cdot L^{-1}$ TP. Similarly, the simulated commercial nursery runoff was created by adding 368.03 g of fertilizer to the high mix tank which resulted in mean concentrations of 17.1 $mg \cdot L^{-1}$ TN and 2.61 $mg \cdot L^{-1}$ TP. A lower nutrient concentration was used for the first five weeks of the simulated nursery runoff treatments to help with plant acclimation. During that period, only 220.82 g of fertilizer was added to the high concentration mix tank.

3.2.2 Water sampling and analysis

The start of each experimental week (7-day retention time) was designated as day 0. On day 0, 125 mL wide-mouth Nalgene bottles were used to collect grab samples from each mix tank at a depth of 30.5 cm (12 in) below the water surface after the fertilizer was added and mixed for one hour. The day 0 samples represented the initial nutrient concentration for the 7-day retention period. Water temperature, pH, DO, and EC measurements were taken in situ for both mix tanks using a YSI Professional Plus multi-probe meter (Yellow Springs International Inc. Ohio, USA) at a depth of 60.96 cm (24 in) from the water surface.

The start of each experimental week was also day 7 of the prior 7-day retention time. On day 7, 125 mL grab samples were collected from each of the 32 mesocosms prior to draining. Additionally, water temperature, pH, DO, and EC measurements were taken in situ at a depth of 30.48 cm (12 in). These samples reflected the final (post treatment) nutrient concentration for the retention period. Grab samples and in situ measurements were taken for each mesocosm every other week on days 3 and 5 as well.

After collection, all water samples were kept on ice in a cooler until other necessary fieldwork was complete. Subsamples were acidified to a pH of 2 in preparation for shipping and metals analysis. The remaining samples were frozen until TN and TP analysis could be performed. All collected water samples were analyzed for TN and TP using automated flow injection analysis after persulfate digestion (persulfate digestion methods QuickChem® Method 10-107-04-4B and 10-115-01-4B; Lachat Instruments, Loveland, CO, USA). In addition, water samples were analyzed for metal content using inductively coupled plasma optical emission spectrometry (ICP-OES 7400 Duo, Thermo Fisher Scientific, PA, USA).

One issue with the described water sampling methods is the potential impact of evapotranspiration and precipitation effects on nutrient concentrations. The “open” design of the experiment allows rain to fall directly into the mesocosms, which could potentially dilute nutrient concentrations and overstate nutrient removal performance. In contrast, evapotranspiration effects could result in more nutrient concentrated water samples. The limited project budget did not allow for methods to reduce this error, such as the installation of a rain shelter or more frequent sample collection. However, a simple water balance helped estimate the effects and more accurately quantify the nutrient removal performance. The water balance model was developed to estimate the effects of precipitation and evapotranspiration using weather data collected at the experiment site. This process is described in more detail in the data analysis section.

3.2.3 Plant tissue sampling and analysis

Three plant tissue samples were collected from each mesocosm at the end of the study. Immediately following the harvest, the roots and shoots were separated and stored in paper bags. The roots and shoots from *Pontederia cordata* samples were separated at the crown of the plant as shown in Figure 3.5. The roots and shoots from *Juncus effusus* samples were separated at the bottom of the planting cup and the crown on the plant, respectively as shown in Figure 3.6. The different procedure for *Juncus effusus* was necessary because the portion of roots in the planting cups was too entangled with the planting coir for proper separation.

Figure 3.5. Harvesting *Pontederia cordata* and separating the roots from the shoots.

Figure 3.6. *Juncus effusus* roots separated from the planting cup after harvest.

The samples were dried in a forced air oven at 58 °C until all moisture was released and consistent sample weights were maintained for two consecutive days. Tissue samples were then ground to 0.5 mm particle size using a 3379-K35 Variable Speed Digital ED-5 Wiley Mill set to 900 RPM (Thomas Scientific, Swedesboro, NJ, USA). Due to budget constraints, all three samples of shoots from one mesocosm were ground into one composite sample and mixed thoroughly. The same procedure was used for the roots. Tissue samples were analyzed for TN in a nitrogen combustion analyzer (LECO FP528 Nitrogen Combustion Analyzer, Leco Corp., St. Joseph, MI, USA). Samples were also analyzed for P on an inductively coupled plasma-optical emission spectrometer (Spectro Arcos ICP-OES, Spectro Analytical Instruments-a division of Ametek, Kleve, Germany).

3.2.4 Data analysis

Water sample data was reported with concentration units (mg·L^{-1}) and was converted to mass using mesocosm volume. Since mesocosm evapotranspiration was not measured directly in the field, it was estimated as potential evapotranspiration using the FAO 56 Penman-Monteith reference crop method, shown in equation 1 below:

$$ET_O = \frac{0.408\Delta(R_n - G) + \gamma\frac{900}{T+273}u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad \textbf{(1)}$$

where ET_O is the reference crop evapotranspiration (mm day^{-1}), R_n is the net radiation at the crop surface (MJ m^{-2} day^{-1}), G is the soil heat flux (MJ m^{-2} day^{-1}), T is the air temperature at 2 m height (°C), u_2 is the wind speed at 2 m height (m s^{-1}), e_s is the saturation vapor pressure (kPa), e_a is the actual vapor pressure (kPa), e_s-e_a is the vapor pressure deficit (kPa), Δ is the slope vapor pressure curve (kPa °C^{-1}), and γ is the psychometric constant (kPa °C^{-1}). A water budget model that utilized daily rainfall and potential evapotranspiration was created to estimate water volume at each sampling occurrence. Since the mesocosms had a maximum fixed capacity, spills were taken into account as well. Weekly nutrient removal efficiency was calculated using equation 2 below

$$Removal\ efficiency = \frac{(N_i - N_f)}{N_i} * 100 \tag{2}$$

where N_i is the initial nutrient load and N_f is the nutrient load after a specified retention time. Cumulative removal efficiency was calculated using equation 3:

$$Removal\ efficiency = \frac{(\sum_{w=1}^{n} N_i - \sum_{w=1}^{n} N_f)}{\sum_{w=1}^{n} N_i} * 100 \tag{3}$$

where the sum of N_i was taken across all weeks and the sum of N_f was taken across all weeks.

Weekly curves were fit by nonlinear regression to show removal during the 7-day HRT. The mechanistic growth model shown in equation 4 was used for the fit.

$$Nutrient\ removal = a * (1 - b * e^{-c*days\ after\ load}) \tag{4}$$

where a is the Asymptote in mass units (g or mg), b is the scale, and c is the growth rate in $days^{-1}$.

All values are reported as mean ± the standard error of the mean unless otherwise noted. SAS JMP® Pro 13.0.0 (SAS Institute Inc. Cary, NC, USA) was used to perform statistical analyses. Normality assumptions were tested both visually using the histogram and residuals as well as the Shapiro-Wilk goodness-of-fit test and suggested guidelines for skew and kurtosis. Where appropriate, analysis of variance (ANOVA) was used to identify statistical significance. Student’s t was used for pairwise comparisons and Tukey’s HSD was used for multiple comparisons ($p<0.05$). For non-normal data, Wicoxon/Kruskal-Wallis tests (rank sums) were used for nonparametric each pair comparisons ($p<0.05$).

3.3 Results and discussion

3.3.1 Aqueous nitrogen and phosphorous removal

The average concentration for TP and TN in the high concentration treatments at the start of the 7-day HRT was 2.61±0.04 mg·L^{-1} (0.79 g mass load) and 17.13±0.24 mg·L^{-1} (5.19 g mass load), respectively. The high concentration treatment was meant to simulate nursery runoff; these concentration levels fall within the typical N and P ranges of nursery runoff (Taylor et al. 2006, White et al. 2010, White et al. 2011). Low concentration treatments were intended to simulate a range between nursery and urban runoff. They averaged 0.52±0.01 mg·L^{-1} (0.16 g mass load) and 5.22±0.03 mg·L^{-1} (1.58 g mass load) for TP and TN, respectively, which are both higher than the urban runoff loads observed in a northern Virginia retention pond by Wang and Sample (2014).

Cumulative TN and TP removal for the study are shown in Table 3.2. The mean removal efficiency for TP after 19 weeks ranged from 38.9% to 92.4% for the low concentration nutrient treatments and 15.3% to 90.3% for the high concentration treatments. The removal efficiencies for TP by week over the study period are shown in Figure A.1 and Figure A.2 in Appendix A. *Pontederia cordata* removed an average of 2.77±0.02 g TP over the experimental period from the low nutrient concentration treatment, which was more (p=0.0027) than the *Juncus effusus* (2.20±0.06 g). At low concentration, the FTWs performed better than both the open water control treatment and the mat-only control treatment. The TP removal results suggest there is no advantage to mat coverage without plants in comparison to no coverage at all for the evaluated treatments.

The TN removal efficiency ranged from 34.7% to 88.9% and 25.3% to 84.3% for the low and high concentration treatments, respectively (see Figure A.1 and Figure A.2 in Appendix A). *Pontederia cordata* removed an average of 26.7±0.28 g TN for the low concentration treatment and 83.0±1.90 g TN for the higher concentration, both significantly higher than all other treatments given the same nutrient concentrations. TN removal performance for the low concentration solution by the *Juncus effusus*, 19.9±0.73 g, was no better than the open water control treatment, 18.4±0.71 g. Both the *Juncus effusus* and open water control treatments removed more (p<0.0001, p<0.0001) than the mat-only control treatment at low concentration, which suggests that the addition of a mat without plants adds little value in regards to N removal. At high concentration, the performance of *Juncus effusus* was lower (p=0.0178) on average than the open water control treatment. The high concentration *Juncus effusus* treatments were infected by a rust disease of the Uromyces genus halfway through the study. Uromyces is the most reported disease for *Juncus effusus* in the U.S. (Farr and Rossman 2017). The presence of the rust could be one contributor to the poor nutrient removal performance relative to the control treatments.

Figure 3.7 shows the cumulative TP and TN removal as a function of experimental week for both the high and low concentration treatments. An evident change in slope occurred four weeks after initiation. This increased slope represents a faster nutrient removal rate, and indicates that the FTWs require an establishment phase before they reach their full nutrient removal potential. Lynch et al. (2015) cited similar observations in regards to plant establishment, but selected a period of eight weeks as the establishment phase in contrast to the shorter period observed in this study. The considerable difference in establishment time is likely related to the much higher nutrient loads used in this study, more than 7 times higher for TN and 4 times higher for TP at the low nutrient concentration. Weeks one through four were designated as the establishment phase. Mean removal rate was found using best-fit linear regression for weeks five though nineteen. Table 3.3 shows the removal rates for each treatment combination ($g{\cdot}m^{-2}\ d^{-1}$) and the associated R^2 values. The *Pontederia cordata* had higher removal rates than all other treatments at both high and low concentrations, while the mat-only control performed the worst.

Table 3.2. Mean cumulative removal for TN and TP by nutrient concentration (n = 4 for each treatment) in the FTW experiment conducted from June 2015 – October 2015.

	Cumulative removal after 19 weeks			
Treatment	**TP (g)[1]**	**TP (%)**	**TN (g)[1]**	**TN (%)**
High nutrient concentration				
Control – no cover	3.67b	24.4	49.12b	49.8
Control – mat cover	2.30c	15.3	24.91c	25.3
Juncus effusus	4.08b	27.1	34.96c	35.5
Pontederia cordata	13.56a	90.3	83.03a	84.3
ANOVA F Ratio, p-value	434.95,<0.0001		81.45,<0.0001	
Low nutrient concentration				
Control – no cover	1.52c	50.5	18.37b	61.2
Control – mat cover	1.17c	38.9	10.42c	34.7
Juncus effusus	2.20b	73.4	19.93b	66.3
Pontederia cordata	2.77a	92.4	26.71a	88.9
ANOVA F Ratio, p-value	68.18,<0.0001		97.90,<0.0001	

[1]Means with different letter differ significantly from other means with the same concentration at p<0.05.

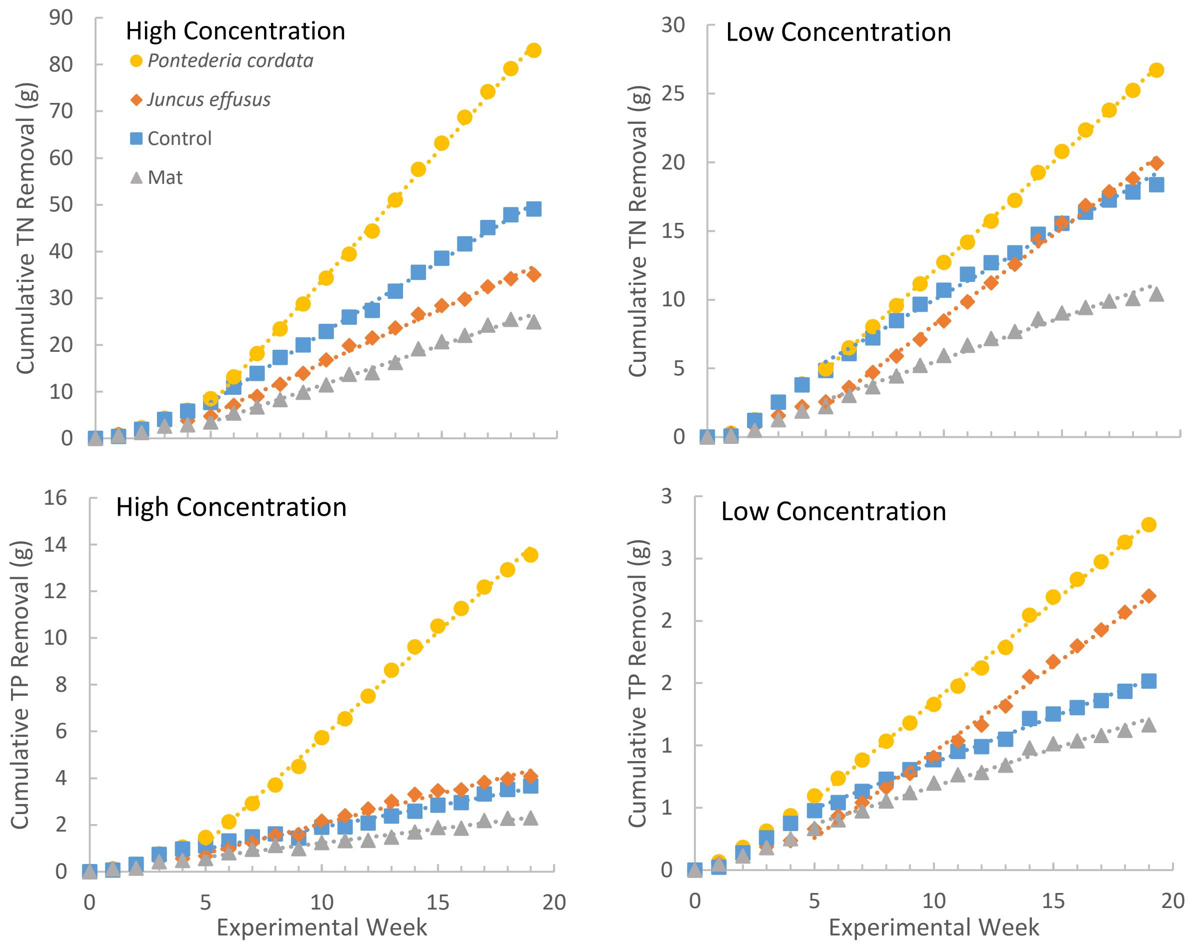

Figure 3.7. Mean cumulative TN and TP removal by treatment type as a function of experimental week for the FTW study conducted from June 2015 – October 2015. Linear regression lines fitted for weeks 5 through 19 represent removal rate after plant establishment phase.

Table 3.3. Mean TN and TP removal rates ($g \cdot m^{-2}\ d^{-1}$) and associated r-square values after plant establishment (weeks 5-19) (n =4) for the FTW study.

Treatment	**TP ($g \cdot m^{-2}\ d^{-1}$)**	**TP (R^2)**	**TN ($g \cdot m^{-2}\ d^{-1}$)**	**TN (R^2)**
High nutrient concentration				
Control – no cover	0.041	0.972	0.680	0.998
Control – mat cover	0.027	0.973	0.370	0.991
Juncus effusus	0.057	0.983	0.505	0.994
Pontederia cordata	0.203	0.998	1.232	0.999
Low nutrient concentration				
Control – no cover	0.016	0.994	0.219	0.992
Control – mat cover	0.014	0.986	0.135	0.983
Juncus effusus	0.031	0.996	0.290	0.998
Pontederia cordata	0.036	0.998	0.351	0.999

Common units for reporting FTW performance are g·m^2 d^{-1}. With regard to mesoscosm studies, these average values assume a linear nutrient removal rate over a study period. Figure 3.8 shows the TN removal rate within the 7-day HRT for the high concentration *Pontederia cordata* treatment for every other week from weeks three to nineteen. The lines were fitted using average removal (n = 4) for 2, 4, and 7 days after initial loading.

Table 3.4 and 3.5 show the summary of fit for the mechanistic growth model as well as the nonlinear regression parameters. While cumulative nutrient removal rates resulted in a linear fit across the growing season, results from sampling throughout the 7-day HRT indicate that nutrient uptake as a function of days after load actually fits an exponential-type (mechanistic growth) model. This is important to note because it suggests that by varying HRT, nutrient removal performance may be affected. More specifically, maximum potential nutrient removal could be reached with shorter HRTs. It is also worth pointing out that week three and five both have a much lower rate than the remainder of the weeks, which could be attributed to plant establishment and total biomass. The uptake rates peaked during week 11 and 13. The figures in Appendix B show the nonlinear regression fits for other treatment and runoff type combinations.

In a FTW mesocosm study using synthetic greywater, Abed et al. (2017) observed no significant differences in ammonium-N between the inflow and outflow for two-day and seven-day contact times, but cited greater removal of nitrate-N by increasing contact time. The study concluded that HRTs should be optimized when using FTWs for pollution removal. Additionally, in a 1:10 scale retention pond replica, Khan et al. (2013) determined that hydraulic performance of the pond depended heavily on the size and position of the FTW in regards to the pond surface and inlet.

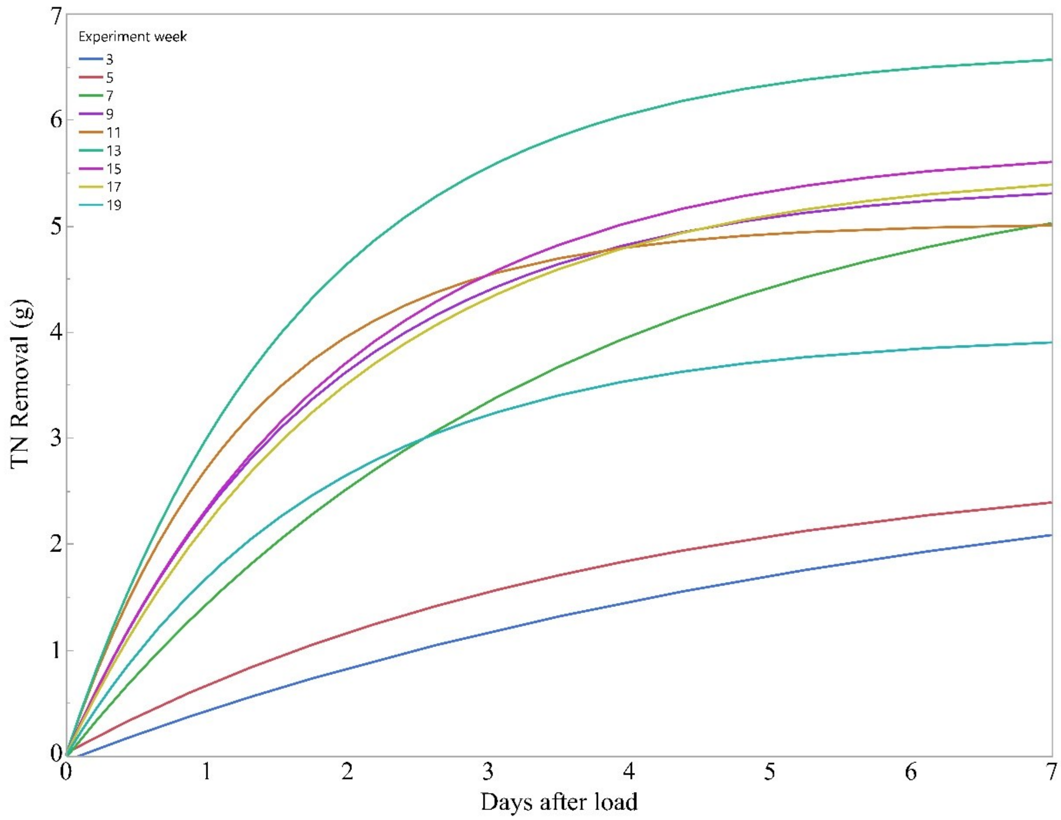

Figure 3.8. Weekly fitted TN removal curves for high concentration *Pontederia cordata* treatments during the FTW study conducted from June 2015 – October 2015.

Table 3.4. Summary of fit for the FTW study TN removal by day for *Pontederia cordata* high concentration treatments.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	211.308	23.647	0.250	0.028	0.167	0.998

Table 3.5. Nonlinear regression parameters for the FTW study TN removal by *Pontederia cordata* high concentration treatments.

Experiment Week	Asymptote	Scale	Growth Rate
3	3.074	1.015	0.164
5	2.866	0.991	0.255
7	5.807	1.003	0.286
9	5.416	0.999	0.554
11	5.025	0.998	0.775
13	6.667	0.999	0.596
15	5.753	0.998	0.519
17	5.554	0.999	0.500
19	3.984	1.000	0.548

3.3.2 Plant growth

Figure 3.9 visually displays the root and shoot growth throughout the study for two low concentration treatments. Root and shoot dry weights for harvested plants are shown in Figure 3.10. *Pontederia cordata* shoots from high concentration treatments had a mean dry weight of 260±28 g, which was larger than *Pontederia cordata* low concentration shoots (177±14 g, p=0.0166), *Juncus effusus* high concentration shoots (94.9±8.8 g, p<0.0001), and *Juncus effusus* low concentration shoots (79.0±5.8 g, p<0.0001). The *Juncus effusus* shoot dry weights did not differ for high and low concentrations (p=0.0885). The root dry weight of *Pontederia cordata* high and low concentration treatments did not differ from each other (p=0.8389) but were larger than both *Juncus effusus* treatments. The low concentration *Juncus effusus* roots had a mean dry weight of 14.2±1.9 g, which is larger (p=0.001) than the high concentration *Juncus effusus* treatment. This result could be attributed to root morphological changes under conditions with lower P availability (Mengel et al. 2001). Since nutrient availability is low, the roots should grow longer in search of more nutrients. Initial nutrient load influenced the plant shoot biomass of *Pontederia cordata*, where higher initial concentrations enabled larger biomass production, but this was not observed for *Juncus effusus*. The opposite is true for plant root biomass, where low initial nutrient loads increased root growth in *Juncus effusus*, but not *Pontederia cordata*.

06/29/15 07/27/15 08/24/15 09/21/15

Low concentration *Pontederia cordata*

06/29/15 07/27/15 08/24/15 09/21/15

Low concentration *Juncus effusus*

Figure 3.9. Plant growth over time for *Pontederia cordata* (top) and *Juncus effusus* (bottom) during the FTW study conducted from June 2015 – October 2015.

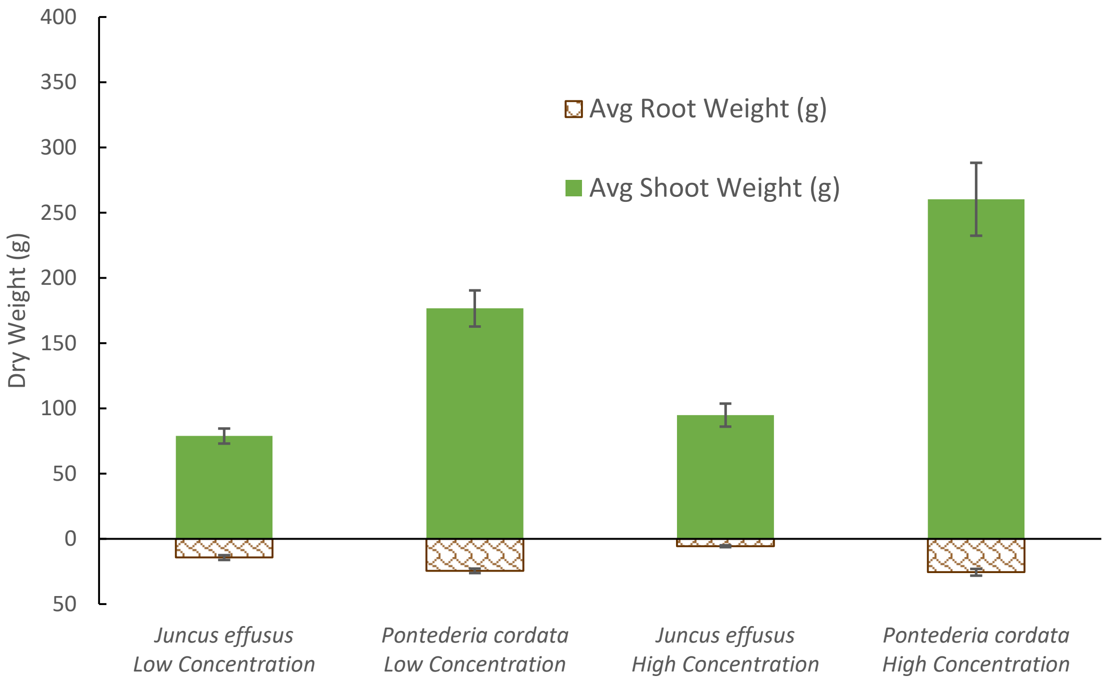

Figure 3.10. Root and shoot dry weights (mean ± standard error) by nutrient treatment and species for the FTW study conducted from June 2015 – October 2015. n=12.

Figure 3.11 shows the N and P for each treatment combination accumulation after the 19-week study. *Pontederia cordata* high concentration shoots accumulated 4.87±0.49 g of N, more than all other combinations of species and nutrient treatments (*p*=0.0304). The low concentration *Pontederia cordata* shoots (1.84±0.05 g) and the high concentration *Juncus effusus* shoots (2.05±0.07 g), both accumulated more N (*p*=0.0304) than the low concentration *Juncus effusus* shoots (1.37±0.04 g). *Pontederia cordata* roots at the high concentrations accumulated 0.42±0.06 g, more than the other three treatment combinations. *Pontederia cordata* (0.26±0.02 g) and *Juncus effusus* (0.18±0.03 g) roots absorbed more N (p=0.0304) than the high concentration *Juncus effusus* roots (0.11±0.01 g). Chang et al. (2012) found that *Juncus effusus* collected 0.036 $g{\cdot}m^{-2}\ d^{-1}$ TN after an operational period of 3 months with influent nutrient concentrations of approximately 4 $mg{\cdot}L^{-1}$. Chang's results are about 5 times lower than the TN uptake (0.182 $g{\cdot}m^{-2}$

d^{-1}) observed by *Juncus effusus* given low concentration treatments during the FTW study conducted from June 2015 – October 2015. One reason for the additional nutrient accumulation is the extended time covered by this study. Additionally, surface area coverage and planting density could be contributing factors.

The P sequestered in the shoots of each treatment combination differed (p=0.0027). The shoots of *Pontederia cordata* exposed to the high treatment accumulated 0.90±0.06 g of P. The shoots of *Juncus effusus* exposed to the high treatment accumulated only 0.27±0.01 g. The shoots of *Pontederia cordata* exposed to the low treatment accumulated 0.19±0.01 g. The shoots of *Juncus effusus* exposed to the low treatment accumulated 0.14±0.01 g. Root accumulation of P was greater for *Pontederia cordata* at the high treatment (p=0.0304), but there was no difference among all other treatment combinations. The roots of *Pontederia cordata* in the high treatment absorbed 0.04±0.01 g P, while in the low treatment, only 0.02±0.01 g P was absorbed. The roots of *Juncus effusus* absorbed 0.02±0.00 g P at the high treatment, and the same amount of P was absorbed by the roots in the low treatment. These results contrast those reported by Chang et al. (2012) where there was not a determinable conclusion for which species (*Pontederia cordata* or *Juncus effusus*) performed better for nutrient removal.

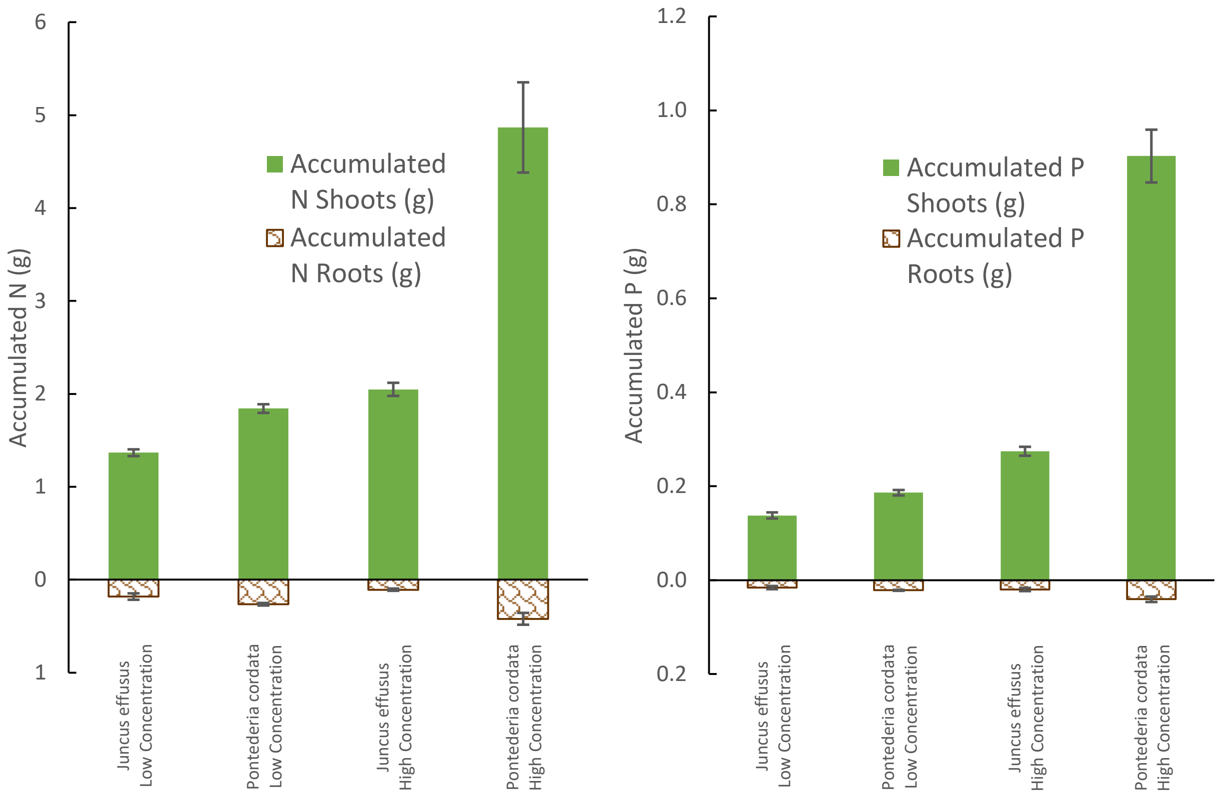

Figure 3.11. The N and P (mean ± standard error) accumulated in roots and shoots of *Juncus effusus* and *Pontederia cordata* after 19 weeks of exposure to 2 nutrient levels (high = 17.1 mg·L^{-1} TN and 2.61 mg·L^{-1} TP; low = 5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) in a FTW study.

The mass balance for TP and TN, shown in Table 3.6, indicates that plant uptake of nutrients does not account for all nutrient removal. The ratio of plant uptake to total nutrient load reduction was similar for both plant species at a given concentration. The ratio of uptake to total load reduction for TN was higher for *Pontederia cordata* (p=0.0284) and *Juncus effusus* (p=0.021) at low concentration in comparison to the same plant species at high concentration. If DO levels are low, unaccounted for removal of N likely results from denitrification processes. Unaccounted for P removal can be attributed to sediment binding and settling. Since grab samples were taken in the middle of the water column, P representation in the samples could be low. White and Cousins (2013) reported similar mass balance results, which confirmed that additional removal processes occurred. For example, in a mixed planting using *Juncus effusus* they found that 32.9% of P was removed by processes other than plant uptake, which compares with unaccounted for P removal of 30.0% in the low concentration *Juncus effusus* treatment.

Table 3.6. Mass balance after the 19 week FTW study (n = 4 for each treatment).

	High Nutrient Concentration		**Low Nutrient Concentration**	
	TP (g)	**TN (g)**	**TP (g)**	**TN (g)**
Total initial load[1]	15.02	98.55	3.00	30.04
<u>Pontederia cordata</u>				
Total load after 7-day HRT	1.46	15.5	0.23	3.33
Load reduction	13.6	83.0	2.77	26.7
Plant uptake[2]	9.43 (69.5)	52.9 (63.7)	2.08 (75.1)	21.1 (78.8)
Other removal processes	4.13	30.1	0.69	5.65
<u>Juncus effusus</u>				
Total load after 7-day HRT	10.9	63.6	0.80	10.1
Load reduction	4.08	35.0	2.20	19.9
Plant uptake[2]	2.94 (72.1)	21.6 (61.7)	1.54 (70.0)	15.5 (77.7)
Other removal processes	1.14	13.4	0.66	4.44

[1]n = 1 for initial load data. [2]Mean uptake (% of total load reduction)

3.3.3 Physicochemical responses

Table 3.7 shows the mean physicochemical properties after 7-day HRTs for the entire study. The FTWs had significantly lower DO in comparison to the treatments without plants. Tanner and Headley (2011) observed similar results where treatments containing higher biomass experienced lower DO levels. The shade produced by FTWs can reduce photosynthetic activity, thus reducing DO production (Borne et al. 2015). If DO is lowered enough to create anoxic conditions, N removal will occur through denitrification in addition to plant uptake. This concept supports the results shown in Table 3.6 where plant uptake is not solely responsible for TN reduction. So, the surface coverage area and DO relationship is an important factor to consider when implementing FTWs as BMPs. In addition to DO, pH was significantly lower for the FTWs in comparison to the control treatments. Wang and Sample (2014) also reported significantly lower pH levels for FTWs planted with *Pontederia cordata*, but their results were still closer to neutral than those observed in this study. In a pond scale study, Borne et al. (2014) observed lower pH for FTWs in comparison to the control, but they concluded that the water treated with FTWs had a near neutral pH. Lower pH could occur because plant roots have the potential to release acidic exudates (Marschner 1995, Coleman et al. 2001). Diurnal fluctuations in DO and pH can be attributed to photosynthesis and respiration processes (Reeder 2011). Since physicochemical properties were consistently recorded during morning hours, respiration effects from the previous night could be the reason for lower pH and DO in the FTW treatments. There was no difference between treatments for water temperature on day 7.

Table 3.7. Mean physicochemical properties after 7-day HRT for the 19 week FTW study (n = 76).

Treatment	DO ($mg \cdot L^{-1}$)[1]	pH[1]	Temperature (°C)[2]	EC ($\mu S \cdot cm^{-1}$)[1]
Low, Control – no cover	11.18a	9.05a	23.2	205c
High, Control – no cover	7.66b	7.29b	23.3	253a
Low, Control – mat cover	8.75b	7.19b	24.5	201c
High, Control – mat cover	5.74c	6.02c	24.3	268a
Low, *Juncus effusus*	4.24d	5.91c	24.3	194cd
High, *Juncus effusus*	3.35de	4.90d	24.1	264a
Low, *Pontederia cordata*	2.61e	4.71d	23.9	183d
High, *Pontederia cordata*	2.40e	4.16e	23.8	225b
ANOVA F Ratio, *p*-value	15.74, <0.0001	8.64, <0.0001	10.89, 0.1437	6.18, 0.0004

[1]Means with different letter differ significantly from other means at $p<0.05$.
[2]Data did not meet normality assumption. Wilcoxon tests used. Reported statistical values Chi Square, *p*-value.

3.4 Conclusion

This mesocosm study evaluated the nutrient removal performance of FTWs at two nutrient loading rates, one similar to nursery runoff, and the other to urban runoff. Nutrient load and species selection both had an effect on nutrient removal over the growing period. An establishment phase of 4 weeks was observed, after which the removal rates increased. Those rates should be considered when estimating the operational performance of the particular species at a given nutrient loading rate. *Pontederia cordata* removed 90.3 to 92.4% of TP and 84.3 to

88.9% of TN at high and low nutrient loading rates, respectively. Those cumulative removal results were significantly higher than the observations for *Juncus effusus*, making it the preferable plant choice for urban or nursery treatment applications. *Juncus effusus* is a suitable species if the goal is to remove TP from urban runoff, but the results suggest that it performs no better than control treatments with respect to TN removal. More research is needed to determine the suitability of *Juncus effusus* at the higher loading rates introduced in commercial nursery and other agriculture environments.

While species selection had an impact on overall nutrient removal, plant uptake was not the only contributor to that removal. Other removal processes accounted for up to 30% and 38% removal for TP and TN, respectively. Although FTWs with higher loading rates removed more nutrients overall, plant uptake at lower loading rates accounted for a larger percentage of the overall nutrient removal. Denitrification likely played a role in removing the unaccounted for N in the mass balance and settling could explain the unaccounted for P removal.

This study used a large surface area coverage ratio (80.3%) which could be cost prohibitive for implementation in a retention pond. The scaling up of these results for field applications could over estimate performance if close attention is not given to surface area coverage and planting density. Additionally, the nutrient removal within the 7-HRT was shown to be nonlinear, which should be considered when selecting species for given loading rates and HRTs. The growing volume of research generally agrees that FTW performance can have significant benefits at lower nutrient loading rates. However, further research is recommended to identify additional plant species that can handle the high nutrient concentrations presented in nursery environments as well as the scalability of these systems.

Chapter 4. FTW nutrient removal effectiveness for simulated nursery runoff

4.1 Overview of study

The experiment for the 2016 growing season consisted of two trials. The first trial took place from June 1, 2016 to July 27, 2016 (8 weeks) and the second trial took place from July 27, 2016 to September 21, 2016 (8 weeks). The methods used to conduct each trial were identical. Each trial evaluated *Agrostis alba* (redtop), *Carex stricta* (tussock sedge), *Panicum virgatum* (switchgrass), *Iris ensata* (iris), and *Canna ×generalis* 'Firebird' (firebird canna) against mixed species plantings and a control study that consisted of an open water mesocosm. The plants were installed on a commercially available floating raft system and a HRT of 7 days was used to evaluate FTW performance, after which the mesocosms were drained and refilled with new simulated runoff. N and P removal were quantified using water sample and plant tissue sample results. The hypothesis for this experiment is that FTWs exposed to high nutrient loads will increase N and P removal in comparison to the control treatments. After each trial, plants were transplanted into containers and into a field setting to evaluate the survivability when being moved from a FTW environment to a soil media planting.

4.2 Materials and methods

4.2.1 Design and operation of experiment

The experimental design was set up in a randomized complete block design that included four replications of eight treatment types. Table 4.1 shows the treatment combination used in this study. The treatments were as follows: one treatment with *Agrostis alba*, one treatment with *Carex stricta*, one treatment with *Panicum virgatum*, one treatment with *Iris ensata*, one treatment with *Canna ×generalis*, one treatment with a uniform mixture of the first three treatments listed (grass and sedge mixture), one treatment with a uniform mixture of the first five treatments listed, and a control treatment with no plants. The control in this study did not include a mat. The mesocosms were blocked by row with the northern most row being block 1 and each subsequent row being blocks 2, 3 and 4. Figure 4.1 below shows the proposed experimental design. A block design was implemented for organizational purposes when conducting fieldwork. This entire study was replicated.

Table 4.1. Treatment combinations for the FTW study conducted from June 2016 – September 2016.

Treatment	Mat	Plants	Species	Reps
1	Yes	Yes	*Agrostis alba*	4
2	Yes	Yes	*Canna*	4
3	Yes	Yes	*Carex stricta*	4
4	No	No	n/a	4
5	Yes	Yes	*Iris ensata*	4
6	Yes	Yes	Mixed all	4
7	Yes	Yes	Mixed partial	4
8	Yes	Yes	*Panicum virgatum*	4

Figure 4.1. Experimental design for the FTW study conducted from June 2016 – September 2016.

Each mesocosm had a volume of 302.8 L and water surface area of 0.79 m^2 at operational depth of 47.3 cm. Beemats (Beemats LLC, New Smyrna Beach, FL, USA) were used as floating rafts, and biodegradable cup distributed by Beemats were inserted into the pre-cut holes in the mats shown in Figure 4.2. The ends of each mat were trimmed to allow for proper fit in the mesocosms, leaving a total mat surface area of 0.64 m^2, which covered 80.3% of the water surface area. Before planting, the root balls of each plant were rinsed to remove as much of the original planting media as possible. For this study, all planting holes were used resulting in 20 plants per mesocosm, or 31 plants/m^2. Figure 4.3 shows a newly planted FTW at the beginning of the study.

After each 7-day HRT, the mesocosms were drained and refilled with a new batch of simulated runoff. The simulated runoff was created by adding water and 24-8-16 soluble fertilizer (Southern Agriculture Insecticides Inc., Hendersonville, NC, USA) to each mix tank. After allowing the solution to recirculate for one hour, it was pumped to each mesocosm. To represent the simulated commercial nursery runoff, 368.03 g of fertilizer was added to each mix tank.

Figure 4.2. Plant installed in a biodegradable Beemat cup for the FTW study.

Figure 4.3. Twenty *Carex stricta* plants in a mesocosm at the beginning of the 2016 FTW study.

4.2.2 Water sampling and analysis

The start of each experimental week was designated as Day 0. Day 0 occurred on each Wednesday of every experimental week. On Day 0, samples were collected from each mix tank representing the initial nutrient concentration for the 7-day retention period. Each sample was collected in a 125 mL wide mouth Nalgene bottle. Water temperature, pH, DO, and EC measurements were taken in situ for both mix tanks using a YSI Professional Plus multi-probe meter (Yellow Springs International Inc. Ohio, USA) at a depth of 60.96 cm (24 in) from the water surface.

Wednesday of each experimental week was also referred to as Day 7. On Day 7 of the experimental week, 125 mL grab samples were collected from each of the 32 mesocosms, and water temperature, pH, DO, and EC measurements were taken in situ at a depth of 30.48 cm (12 in). These samples reflect the post treatment nutrient concentration for the retention period. The depth from the water surface to the bottom of the mesocosms was recorded to estimate evapotranspiration and convert concentration data to mass.

After collection, all water samples were kept on ice in a cooler until other necessary fieldwork was complete. Each water sample was filtered through a 0.2-micrometer Thermo Scientific™ Target2 30 mm PVDF Syringe Filter into a Thermo Scientific™ Dionex AS-AP Auto Sampler Vial. The samples were frozen until they were ready for analysis using Ion Chromatography (Thermo Scientific(TM) Dionex(TM) ICS2100 for anion and ICS1600 for cation; Waltham, MA, USA) for nitrate, nitrite, ammonium, and phosphate.

4.2.3 Plant tissue sampling and analysis

Three plant tissue samples were collected at the end of each trial study from each monoculture mesocosm, and three plant tissue samples were collected for each species in each mixed planting mesocosm. The plants were kept in a walk-in cooler at 8.9°C until initial measurements and processing were complete. Each plant was rinsed with deionized water prior to measuring the root and shoot length. After measurements were taken, the roots and shoots were separated at the crown as shown in Figure 4.4.

Figure 4.4. Processing of plant tissue samples included detaching the roots from the shoots.

The samples were dried in a forced air oven at 58 °C until all moisture was released and consistent sample weights were maintained. Tissue samples were then ground to 0.5 mm particle size using a 3379-K35 Variable Speed Digital ED-5 Wiley Mill set to 900 RPM (Thomas Scientific, Swedesboro, NJ, USA). For the monoculture mesocosms, each of the three root and shoot tissue samples were processed individually. For the mixed planting mesocosms, the three shoots samples from each species were ground into a composite sample and the same process was used for the roots. In this case, the use of composite samples was due to project budget. Tissue samples were analyzed for N content by dynamic flash combustion (Thermo Scientific FLASH 2000 Elemental Analyzer; Waltham, MA, USA). P was analyzed using ICP-OES (Thermo Scientific iCAP 6500 Duo view ICP-OES; Waltham, MA, USA).

In addition to harvesting plants for tissue analysis, six plants from each monoculture mesocosm were harvested after each trial to study the effects of transplanting floating wetland plants into an environment in which the roots are in a soil media. Three of the six plants were transplanted into 11.4 L high-density polyethylene containers (Nursery Supplies Inc. ®, Chambersburg, PA, USA) with Sun Gro Fafard 52 soil (Sun Gro Horticulture, Agawam, MA, USA). The remaining three samples from each monoculture mesocosm were planted in a 5.2 m by 7.6 m plot at the HRAREC. The field was dressed with mulch for weed control. Both the container and field transplant studies were set up as serial repeated measures designs (RMD). The biodegradable Beemat cups were left intact on the plant roots during planting. Transplants in the containers received daily irrigation, whereas water for the field plantings was limited to plant available water in the soil. Plant health was subjectively rated on a scale of 1-5 (1 being poor and 5 being excellent) once per week for a total of 4 weeks after transplanting.

4.2.4 Data analysis

Nitrogen values are reported as the sum of ammonium-N, nitrate-N, and nitrite-N. P values are phosphate-P values recorded during sample analysis. Water sample results were converted from units of concentration to mass using depth measurements and a correlation between volume and depth for the mesocosms. All values are reported as mean ± standard error of the mean unless otherwise noted. SAS JMP® Pro 13.0.0 (SAS Institute Inc. Cary, NC, USA) was used to perform statistical analyses. Normality assumptions were tested both visually using the histogram and residuals as well as the Shapiro-Wilk goodness-of-fit test, and suggested guidelines for skew and kurtosis. Where appropriate, analysis of variance (ANOVA) was used to identify statistical significance. The reported F-ratio is the ANOVA F-ratio for data with equal variance and Welch's ANOVA F-ratio for data with unequal variance. Student's t was used for pairwise comparisons and Tukey's HSD was used for multiple comparisons (p<0.05). For non-normal data, Wicoxon/Kruskal-Wallis tests (rank sums) were used for nonparametric each pair comparisons (p<0.05).

4.3 Results and discussion

4.3.1 Aqueous nitrogen and phosphorous removal

The mean N load at the start of each 7-day HRT for trial 1 and trial 2 was 10.42 ± 0.15 $mg \cdot L^{-1}$ (3.28 g mass load) and the mean P load was 2.96 ± 0.10 $mg \cdot L^{-1}$ (0.93 g mass load). It is important to reiterate the fact that N values reported for water samples for the 2016 study are not TN. Rather, they are the sum of nitrate-N, nitrite-N, and ammonium-N. The P values reported reflect phosphate-P not TP. These concentrations fall within the range used by Polomski et al.

(2007) in a study aimed at remediating runoff from nurseries using subsurface constructed wetlands.

The mean cumulative N and P removal for trial 1 and trial 2 by species are presented in Table 4.2. During trial 1, removal efficiencies ranged from 21.9% to 64.7% and 4.8% to 82.4% for P and N, respectively. *Panicum virgatum* removed more N and P than all other treatments, with a cumulative mean removal of 21.62±0.42 g N and 4.88±0.21 g P (p<0.0001). The mixed plantings removed more nutrients than *Agrostis alba*, *Canna*, *Carex stricta*, and the control treatment. It is possible that the higher performance of the mixed plantings was due to the *Panicum virgatum* plants, as they comprised 20% and 30% of the complete mix and partial mix plantings, respectively. Sometimes mixed plantings consisting of flowering plants are desired for diversity and aesthetic reasons (Headley and Tanner 2012). If that is the case, choosing the proper ratio of high nutrient removal species to other species should be considered to maximize effectiveness. The control treatment performed the worst in regards to N removal at 1.21±1.27 g. It removed 1.59±0.25 g of P, which was lower than all FTWs, but not low enough to be considered significantly different from *Agrostis alba*, *Canna*, *Carex stricta*.

Trial 2 removal efficiencies ranged from 12.9% to 59.6% and 26.8% to 63.2% for N and P, respectively. Similar to trial 1, trial 2 resulted in higher N and P removal by *Panicum virgatum* in comparison to all other treatments. No differences were detected for N and P removal between the remaining treatments. Collectively, the trial 1 plants removed more (p<0.0003) N than the trial 2 plants, but cumulative P removal was similar between the two trials. One explanation for the variation in the results could be the product of initial plant uniformity in regards to size and quality. Figure 4.5 shows *Iris ensata* and *Canna* plants at the time of planting. The *Iris ensata* image shows variation in plant size within the trial, and the *Canna* image shows variation of initial plant size between trial 1 and trial 2. These effects should be considered when estimating performance of FTWs.

Table 4.2. Mean cumulative N and P removal (g and %) for trials 1 and 2 by species for the FTW study conducted from June 2016 – September 2016. (n = 4).

	Cumulative removal after 8 weeks			
Treatment	**P (g)[1]**	**P (%)**	**N (g)[1]**	**N (%)**
Trial 1				
Agrostis alba	2.18cd	29.8	10.57d	41.5
Canna	1.92d	26.1	11.17d	43.7
Carex stricta	2.09d	28.3	10.04d	38.9
Control	1.59d	21.9	1.21e	4.8
Iris ensata	3.57b	48.6	12.91cd	50.4
Mixed all	3.28bc	44.2	16.34bc	63.4
Mixed partial	3.27bc	44.2	17.44b	67.7
Panicum virgatum	4.88a	64.7	21.62a	82.4
ANOVA F ratio, p value	20.46, <0.0001		47.55, <0.0001	
Trial 2				
Agrostis alba	2.00b	26.8	3.44b	12.9
Canna	2.75b	37.1	7.74b	29.2
Carex stricta	2.24b	29.6	7.09b	26.2
Control	2.53b	34.6	3.77b	14.4
Iris ensata	3.19b	42.9	5.84b	22.0
Mixed all	2.50b	33.3	7.01b	26.1
Mixed partial	2.65b	35.1	9.64b	35.8
Panicum virgatum	4.86a	63.2	16.34a	59.6
ANOVA F ratio, p value	11.70, <0.0001		8.84, <0.0001	

[1]Means with different letter differ significantly from other means with for the same trial at $p<0.05$.

Figure 4.5. Left: *Iris ensata* plants. Right: *Canna* plants. Both pictures taken at the time of planting.

Sun et al. (2009) used *Canna* in a floating bed study to remove N pollution. They observed a 44% removal rate of N (ammonium-N, nitrate-N, and nitrite-N), which is similar to the removal rate observed for the Canna treatment in Trial 1 of this study, 43.7%. However, this study used a mean initial concentration that was 4.45 $mg \cdot L^{-1}$ higher, and a HRT that was 2 days longer than that used by Sun.

Similar to the 2015 study, there appeared to be an inflection in the slopes of the cumulative nutrient removal data. The figures in Appendix D show the cumulative removal for each treatment and each trial, and a slope change is evident at week four. Therefore, the time from initiation until week four was defined as the plant establishment phase, after which the plants reach their optimal nutrient removal potential. Figure 4.6 shows the cumulative removal for the operational phase. The linear fits through the mean cumulative removal represent the removal rate in $g \cdot week^{-1}$ for each treatment. The rates were converted to $g \cdot m^{-2}\ d^{-1}$ by dividing the weekly rates by the mat area and 7 days. Table 4.3 shows the mean N and P removal rates for each treatment and the associated R^2 value. The *Panicum virgatum* treatment had the highest N removal rates at 0.738 $g \cdot m^{-2}\ d^{-1}$ and 0.656 $g \cdot m^{-2}\ d^{-1}$ for trials 1 and 2, respectively. *Panicum virgatum* also had the highest P removal rates at 0.200 $g \cdot m^{-2}\ d^{-1}$ and 0.194 $g \cdot m^{-2}\ d^{-1}$ for trials 1 and 2, respectively. Tanner and Headley (2011) used the sedge species *Carex virgata* and recorded P removal rates of 0.027 $mg \cdot L^{-1}$, about three times lower than the *Carex stricta* rates observed in this study. The higher P removal rates by the sedge in this study could be attributed to the higher initial P loading or the fact that the Tanner and Headley (2011) study recorded removal rates after 3-days.

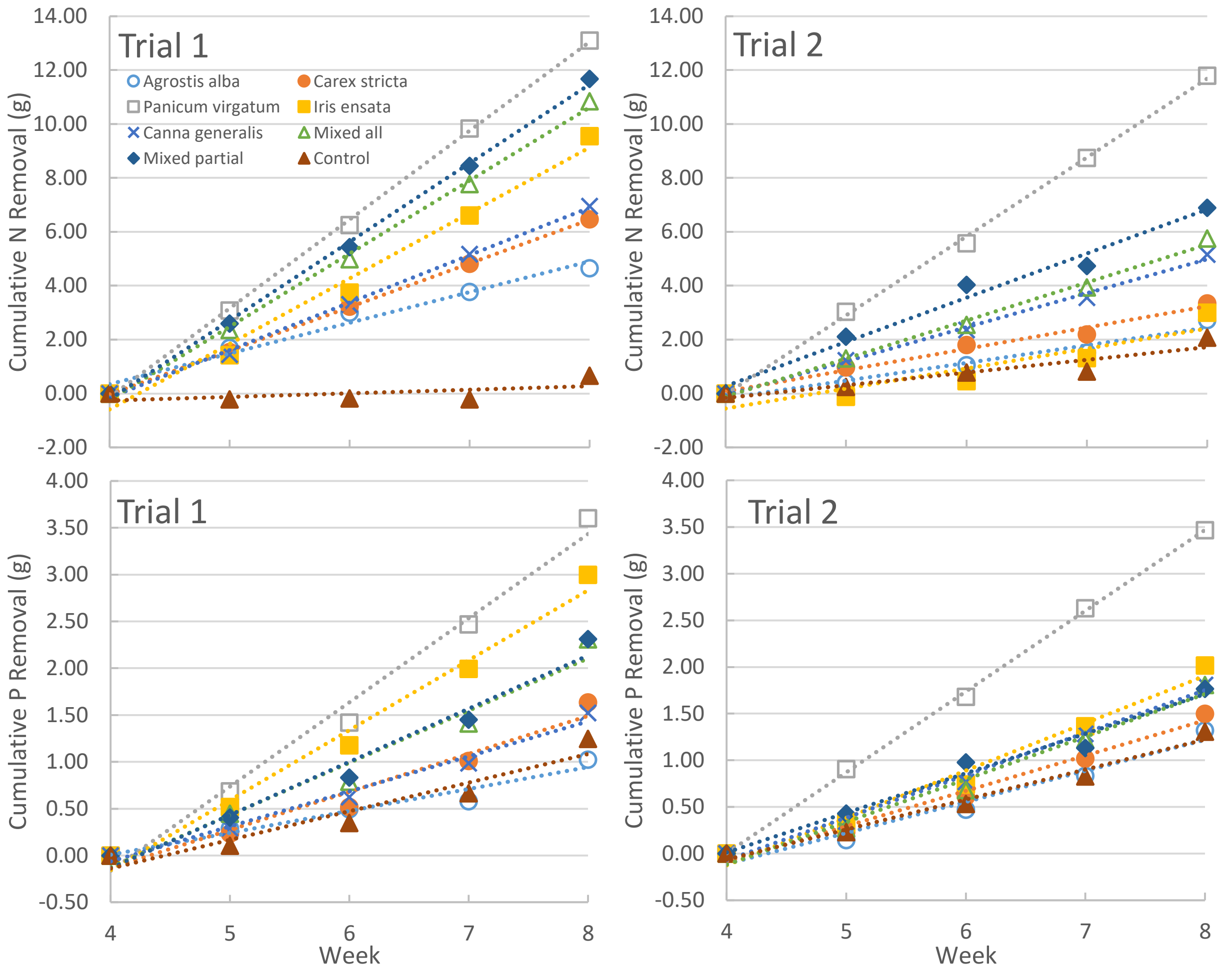

Figure 4.6. Mean cumulative N and P removal (g) by treatment for the FTW study conducted from June 2016 – September 2016. Cumulative removal starts from week four. Fitted linear regression lines represent operational rates after 4-week plant establishment phase.

Table 4.3. Mean N and P removal rates ($g \cdot m^{-2} d^{-1}$) and associated R^2 values after plant establishment (weeks 5-8) during the FTW study (n =4).

Treatment	P ($g \cdot m^{-2} d^{-1}$)	P (R^2)	N ($g \cdot m^{-2} d^{-1}$)	N (R^2)
Trial 1				
Agrostis alba	0.052	0.926	0.255	0.973
Canna	0.084	0.985	0.394	0.998
Carex stricta	0.091	0.958	0.362	0.999
Control	0.068	0.923	0.029	0.307
Iris ensata	0.167	0.984	0.544	0.984
Mixed all	0.125	0.964	0.607	0.997
Mixed partial	0.127	0.972	0.653	0.998
Panicum virgatum	0.200	0.987	0.738	0.999
Trial 2				
Agrostis alba	0.074	0.966	0.147	0.956
Canna	0.102	0.992	0.283	0.995
Carex stricta	0.085	0.986	0.177	0.983
Control	0.072	0.981	0.105	0.868
Iris ensata	0.113	0.979	0.166	0.834
Mixed all	0.102	0.980	0.316	0.994
Mixed partial	0.095	0.978	0.367	0.980
Panicum virgatum	0.194	0.999	0.656	0.999

4.3.2 Plant growth

Plant nutrient content followed a similar pattern to cumulative nutrient removal. Table 4.4 shows the mean N and P content per plant by species and trial. For trial 1, *Panicum virgatum* removed more N than *Agrostis alba*, *Canna*, and *Iris ensata*, but not *Carex stricta.* The *Panicum virgatum* in the complete mix treatment removed significantly more P than all other treatments not containing *Panicum.* For trial 2, *Panicum virgatum* removed more N and P than all other treatments. Keizer-Vlek et al. (2014) reported 18.6 $g \cdot m^{-2}$ N removal and 0.51 $g \cdot m^{-2}$ P removal after a 91 day study using *Iris*. These N removal results fall within the range of N removed by *Iris ensata* in this study, which were 24.8 $g \cdot m^{-2}$ and 13.6 $g \cdot m^{-2}$ for trial 1 and 2, respectively. However, the P removal from plant uptake is approximately four to ten times lower than the P removal in this study, which ranged from 2.09 $g \cdot m^{-2}$ to 5.03 $g \cdot m^{-2}$. One difference between the two studies that would contribute to such a large difference in total removed nutrients is the initial nutrient load. This study had an initial N concentration 2.5 times higher and an initial P concentration 12 times higher than the Keizer-Vlek et al. (2014) study.

Table 4.4. Mean N and P content (roots plus shoots) per plant by treatment and trial for the FTW study conducted from June 2016 through September 2016.

	Trial 1		Trial 2	
Treatment	**P (g)[1]**	**N (g)[1]**	**P (g)[1]**	**N (g)[1]**
Agrostis alba[2]	0.068e	0.533cde	0.020b	0.128c
Agrostis alba, mixed all[3]	0.095cde	0.646cde	0.001b	0.049bc
Agrostis alba, mixed partial[3]	0.068de	0.491cde	0.003b	0.046bc
Canna[2]	0.097de	0.520cde	0.063b	0.294bc
Canna, mixed all[3]	0.038e	0.205e	0.018b	0.077bc
Carex stricta[2]	0.088e	0.820bc	0.048b	0.397bc
Carex stricta, mixed all[3]	0.082de	0.772bcde	0.041b	0.416bc
Carex stricta, mixed partial[3]	0.089cde	0.840bcde	0.052b	0.440bc
Iris ensata[2]	0.161bcd	0.791cd	0.067b	0.435b
Iris ensata, mixed all[3]	0.048e	0.246de	0.041b	0.251bc
Panicum virgatum[2]	0.220ab	1.483ab	0.196a	1.115a
Panicum virgatum, mixed all[3]	0.313a	1.825a	0.177a	1.031a
Panicum virgatum, mixed partial[3]	0.210abc	1.347a	0.259a	1.288a
Welch's ANOVA F ratio, *p*-value	9.54, <0.0001	14.41, <0.0001	27.56, <0.0001	18.06, <0.0001

[1]For each column, means not connected by the same letter differ significantly at $p<0.05$.
[2] n = 12, [3] n = 4

Figure 4.7 and Figure 4.8 show the plant dry weights (mean ± standard error) and the root and shoot length (mean ± standard error) for the monoculture treatments. Total plant weights differed ($p<0.0001$) by plant species for both trial 1 and trial 2. *Panicum virgatum* had the largest mean total dry weights at 92.1 ± 9.01 g (69.2 g for shoots and 22.9 g for roots) for trial 1 and 64.4 ± 6.89 g (43.9 g for shoots and 20.5 g for roots) for trial 2. The root weights of *Panicum virgatum*, *Carex stricta*, and *Agrostis alba* did not differ for trial 1. For trial 2, *Panicum virgatum* and *Carex stricta* had similar root weights, but shoot mass differed ($p=0.0006$). No differences in total weight, root weight, or shoot weight were detected between *Iris ensata* and *Canna* for trial 1 or trial 2.

For Trial 1,the mean root length of *Iris ensata* (24.1 ± 0.6 cm) was longer than all other monoculture species. The *Panicum virgatum* and *Carex stricta* had consistently taller shoots than the other species for both trial 1 and trial 2. This is partly due to the growth characteristics of those two plant species. For trial 2, *Agrostis alba* had significantly lower root lengths and shoot lengths than all other species, which was not the case for trial 1. The *Agrostis alba* did not acclimate well during the trial 2 study, and nearly 50% of the plants died, which likely contributed to the slower growth observed.

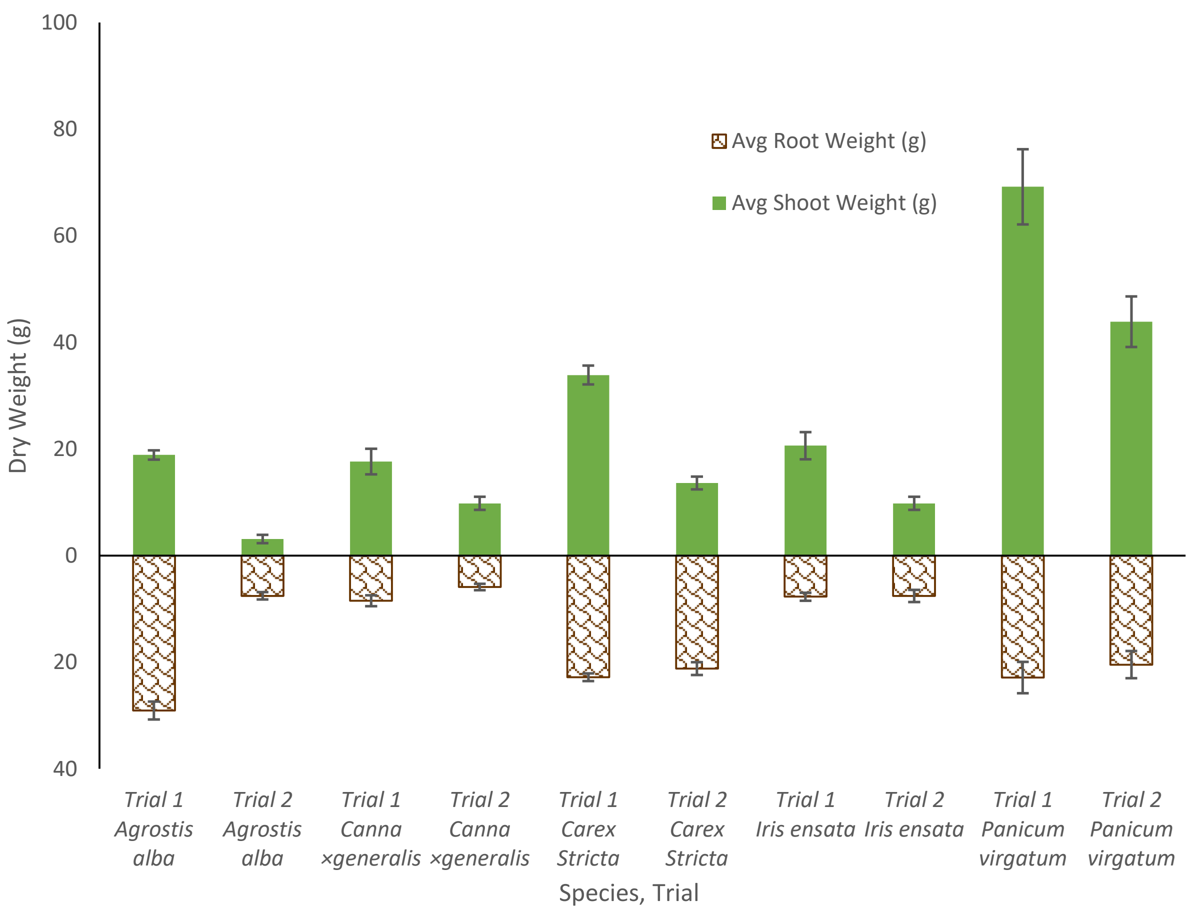

Figure 4.7. Root and shoot dry weight (mean ± standard error) by trial for monoculture mesocosms after 8 weeks of growth in the FTW study conducted during the 2016 growing season. n=12.

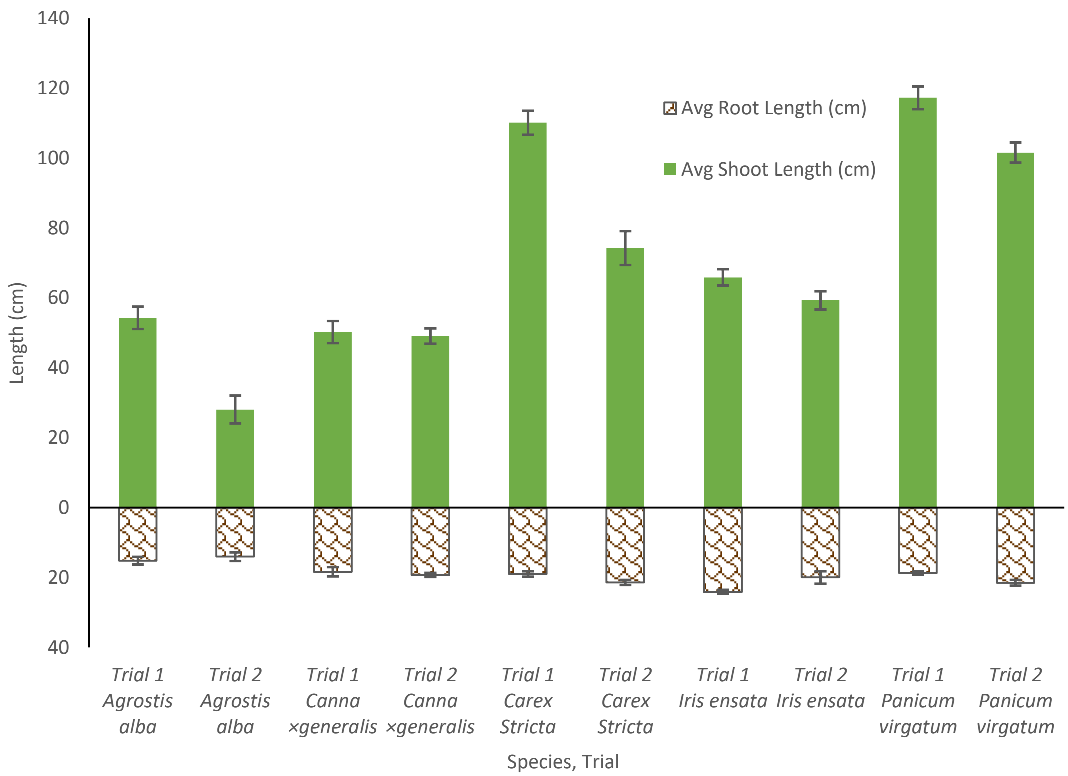

Figure 4.8. Root and shoot length (mean ± standard error) by trial for monoculture mesocosms after 8 weeks of growth in the FTW study conducted during the 2016 growing season. n=12.

Correlations between the mean dry weight of the plant tissue (g·plant^{-1}) and the cumulative N and P removed (g) after 8 weeks are shown in Figure 4.9 and Figure 4.10. Cumulative N removed was correlated to plant dry (p<0.0001) with a linear fit R^2 value of 0.64. The P removal after 8 weeks also correlated with plant dry weight (p<0.0001) with a linear fit R^2 value of 0.34. Wang et al. (2014) reported that plants with higher biomass accumulated more N and P in the plant tissue in a microcosm study. These correlations suggest similar results; however, they show total nutrient removal from the FTW treatment as opposed to just the plant uptake portion of removal. Therefore, by correlating the dry mass to the total FTW removal, unaccounted for nutrient removal can be estimated.

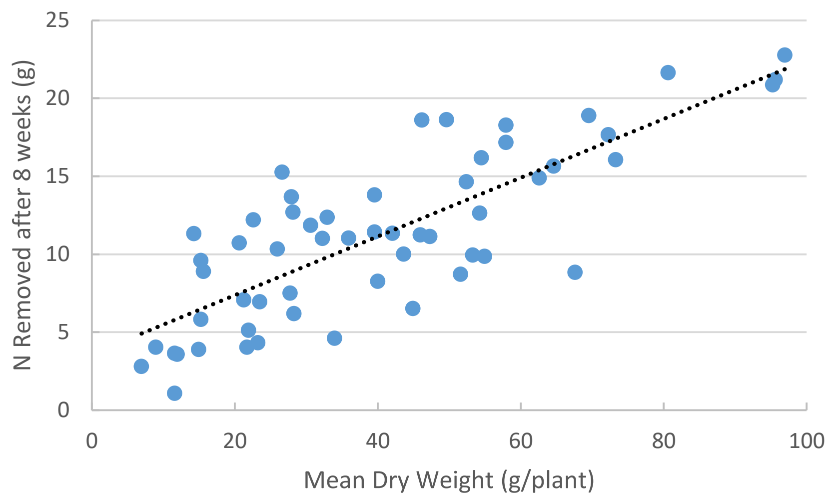

Figure 4.9. Cumulative N removed from water after 8 weeks (Trial 1 and 2) as a function of mean dry weight per plant (n=8). $R^2 = 0.64$, F-ratio = 97.85, p<0.0001.

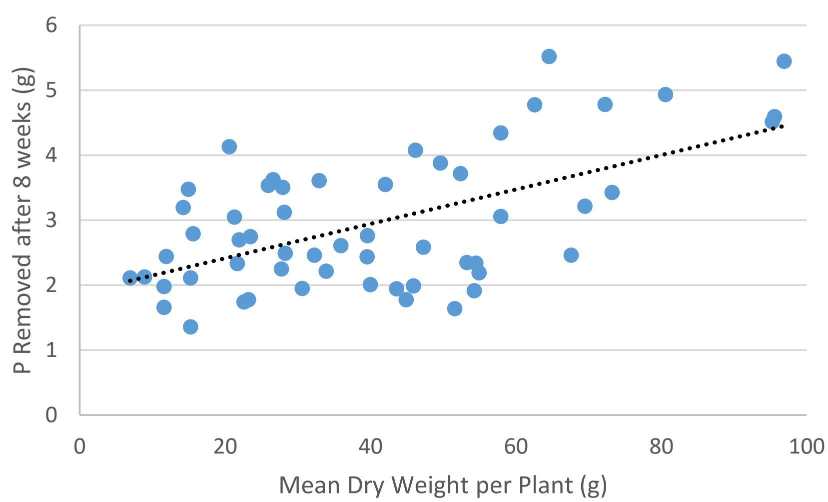

Figure 4.10. Cumulative P removed from water after 8 weeks (Trial 1 and 2) as a function of mean dry weight per plant (n=8). $R^2 = 0.34$, F-Ratio = 27.78, p <0.0001.

4.3.3 Physicochemical properties

Table 4.5 shows the mean DO (mg·L^{-1}), pH, Temperature (°C), and EC (µS·cm^{-1}) for trials 1 and 2. The mean DO for the control treatment during trial 1 was 8.31 ± 0.32 mg·L^{-1}, higher than all other treatments which ranged from 0.39 mg·L^{-1} to 0.99 mg·L^{-1}. Trial 2 showed similar results where the control treatment had a mean DO level of 8.28 ± 0.23 $mg \cdot L^{-1}$ and all other treatments ranged from 1.20 $mg \cdot L^{-1}$ to 1.62 $mg \cdot L^{-1}$. The lower DO levels of the FTW treatments increased the likelihood of creating anoxic conditions. If the primary goal of the FTW is to remove nutrients, then low DO levels could positively contribute by encouraging denitrification processes. However, if the goal of the FTW is nutrient removal as well as creating wildlife habitat, then precaution should be taken when sizing FTWs as to not create toxic conditions for freshwater organisms (Borne et al. 2015). The pH for the control treatments in trials 1 and 2 was 8.07 ± 0.12 and 7.37 ± 0.85, respectively. All FTW treatments had lower mean pH levels than the control. Similar observations were reported in the study conducted from June 2015 through October 2015. The lower pH could be attributed to the release of acidic substances from the roots around the rhizosphere (Marschner 1995, Blossfeld et al. 2011). Temperature did not differ between the treatments in trials 1 or 2. The EC was lowest for treatments that performed the highest in regards to nutrient removal.

Table 4.5. Mean physicochemical responses for floating treatment wetlands after a 7-day HRT for a study conducted from June 2016 through September 2016 (n = 32).

Treatment	DO ($mg \cdot L^{-1}$)	pH	Temperature (°C)	EC ($\mu S \cdot cm^{-1}$)
Trial 1				
Agrostis alba	0.44[1]	6.10[1]	27.1	273.9
Canna	0.39[1]	6.05[1]	26.8	256.9
Carex stricta	0.99[1]	5.36[1]	27.4[1]	258.4
Control	8.31	8.07	25.9	281.4
Iris ensata	0.87[1]	5.46[1]	26.9	240.3[1]
Mixed all	0.61[1]	5.64[1]	26.9	238.4[1]
Mixed partial	0.70[1]	5.68[1]	26.9	236.0[1]
Panicum virgatum	0.63[1]	5.29[1]	26.7	210.5[1]
Chi Square, *p*-value	109.25, <0.0001	151.97,<0.0001	8.71,0.271	97.48,<0.0001
Trial 2				
Agrostis alba	1.20[1]	5.68[1]	25.9	250.1
Canna	1.27[1]	5.63[1]	25.8	234.6
Carex stricta	1.54[1]	5.26[1]	26.2[1]	246.0
Control	8.28	7.37	24.8	247.2
Iris ensata	1.62[1]	5.20[1]	25.9	240.3
Mixed all	1.29[1]	5.48[1]	26.0	234.4
Mixed partial	1.20[1]	5.27[1]	26.0	228.9
Panicum virgatum	1.31[1]	4.97[1]	26.0	207.5[1]
Chi Square, *p*-value	89.94,<0.0001	97.91,<0.0001	9.51,0.218	23.36,0.0015

[1] Means differ significantly from the control using Dunn's Method for Joint Ranking at p<0.05.

4.3.4 Transplant effects

After each trial, three plants from each mesocosm were planted in a field and three were planted in containers in order to evaluate transplant survivability. The study period and ambient weather conditions during the evaluation are shown in Table 4.6. The plants were observed weekly and given a score a score from 1 to 5 based on visual health. Figure 4.11 gives an example of how each plant was scored based on visual appearance.

Table 4.6. Rainfall and temperature data for the transplant observation periods.

Study Period Date Range	Total Rainfall (mm)	Mean Air Temperature (°C)	Max Air Temperature (°C)	Min Air Temperature (°C)
July 27, 2016 - August 24, 2016	265	27.1	35.9	17.5
September 21, 2016 - October 19, 2016	424	20.8	30.3	10.4

Figure 4.11. Example of the rating scale used for the evaluation of plants during two 4-week FTW transplant studies. From left to right the ratings are 5, 3, and 1.

For the field plantings, trial 2 had better (*p*<0.0001) survivability ratings than trial 1. This difference can be explained by ambient conditions during transplant. The temperature reached 36.7°C on the day of the trial 1 transplant, and the evaluation period had a higher mean temperature and less total rainfall, thus inducing higher stress on the plants during the acclimation phase. Differences between plant species were also notable (*p*<0.0001), and plant health decreased between the time of transplant and the fourth week of evaluation. Mean ratings by week for each trial and species are shown in Figure 4.12 The field transplants for the trial 1 plants after 4 weeks are shown in Figure 4.13.

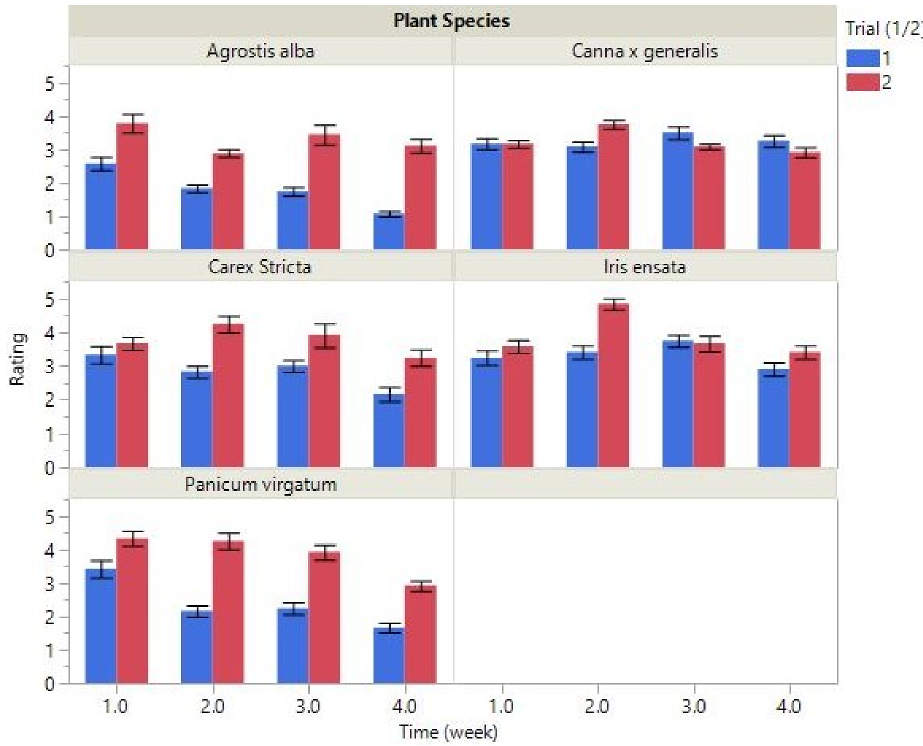

Figure 4.12. Field transplant ratings (mean ± standard error) by week for trial and plant species during a FTW transplant and survivability study.

Figure 4.13. Trial 1 plants four weeks after being transplanted in the field.

Agrostis alba was not included in the container transplant analysis because of plant availability at the end of trial 2. Figure 4.14 shows the mean ratings by week for trial 1 and 2 container transplants. For the container transplants, overall plant aesthetic ratings were better in trial 1 than trial 2 ($p<0.0001$), but how the plant species responded were similar in trial 1 and 2 (no interaction). Plant species response differed over the four week evaluation ($p<0.0001$) differences occurred for the interaction between plant species and time. For *Carex stricta* and *Panicum virgatum* week four ratings were lower than the week one ratings, but the *Canna* and *Iris ensata* showed improved ratings in all cases except the trial 2 *Canna* plants. The mean rating for the container transplants was 3.63 in comparison to the 3.16 mean rating of the field transplants. More research is needed to understand whether harvesting FTW plants for the purpose of resale is a viable option for nurseries. The results from this study suggest that transplanting into containers to be the better option as opposed to direct field transplants. However, plant size, ambient weather conditions during transplant, and soil and hydrologic conditions of the transplant area could all affect the outcome of plant survivability.

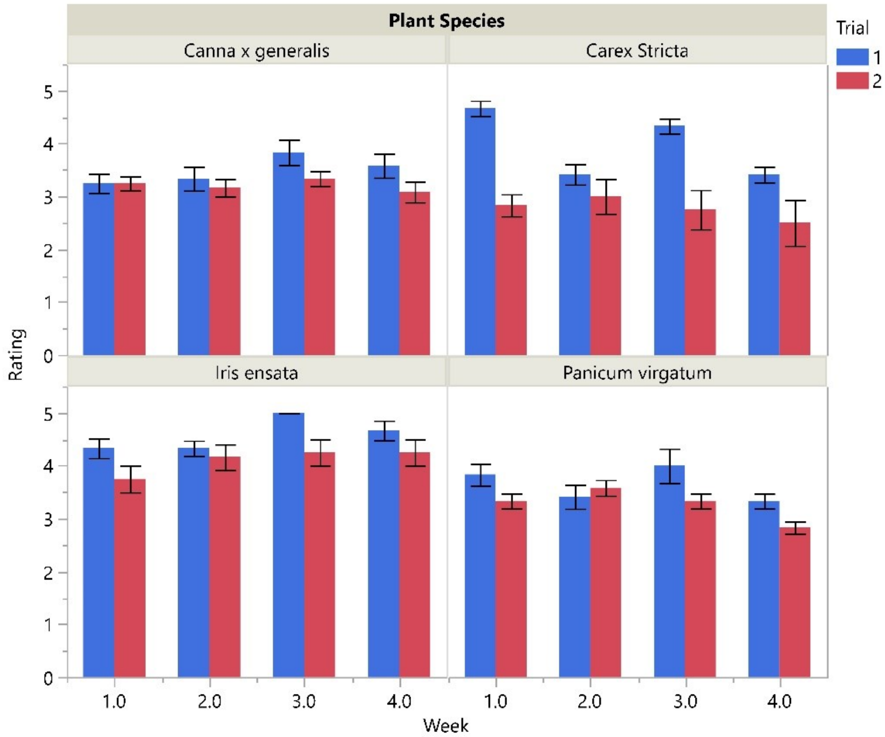

Figure 4.14. Container transplant ratings (mean ± standard error) by week for trial and plant species during a FTW transplant evaluation.

4.4 Conclusion

The 2016 mesocosm study evaluated FTW performance with simulated nursery runoff over two 8-week trials. Nutrient removal performance was dependent on plant species. *Panicum virgatum* performed the best in regards to cumulative N and P removal, removing up to 64.7% P and up to 82.4% N. *Panicum virgatum* would be the recommended species from the 2016 study for use in nursery retention ponds to control nutrients. Mixed species plantings also performed well. If mixed plantings are required for species diversity or aesthetic reasons, a higher performing species should be included to maximize nutrient removal. After the four week establishment phase, N removal from FTW treatments ranged from 0.255 $g \cdot m^{-2}\ d^{-1}$ to 0.738 $g \cdot m^{-2}\ d^{-1}$ and 0.147 $g \cdot m^{-2}\ d^{-1}$ to 0.656 $g \cdot m^{-2}\ d^{-1}$ for trial 1 and 2, respectively. P removal ranged from 0.052 $g \cdot m^{-2}\ d^{-1}$ to 0.128 $g \cdot m^{-2}\ d^{-1}$ for trial 1 and 0.074 $g \cdot m^{-2}\ d^{-1}$ to 0.194 $g \cdot m^{-2}\ d^{-1}$ for trial 2.

Biomass per plant correlated with overall nutrient removal by FTW treatments, which suggests that biomass could be used to estimate removal performance including plant uptake as well as other removal processes.

Transplant effects on plant health were monitored over four weeks after each trial. Overall, plants transplanted into containers had higher survival rates than those transplanted into the field. The better health of container transplants was likely due to the daily irrigation.

This research suggests that nursery runoff can be effectively processed by FTWs, but more research is needed to evaluate these plant species and quantify the removal rates in full-scale nursery retention ponds. The nutrient removal results can easily be swayed by a number of factors including weather, initial plant size, initial overall plant health and uniformity. Using harvested FTW plants for resale into ornamental or restoration markets is a possibility; however further testing is needed to determine the survivability when transplanted into environments containing no irrigation.

Chapter 5. Conclusion

Over the course of two growing seasons, FTW nutrient removal performance was evaluated for simulated urban and nursery runoff using a mesocosm study. A variety of general conclusions from the two-year study can be made. These include:

1. Results indicate that, depending upon the species, FTWs can reduce N and P loads from urban and nursery runoff.
2. Plant species has a significant effect on nutrient removal performance, so species selection is an important factor when designing a FTW.
3. Results from the study conducted from June 2015 through October 2015 indicated *Pontederia cordata* is better suited for urban and nursery environments than *Juncus effusus*. *Juncus* did provide P removal better than the control (no mat); however, it did not for N removal.
4. Results from the study conducted from June 2016 through September 2016 showed that *Panicum virgatum* removed significantly more N and P than the control treatment as well as other FTW treatments, thus making it a preferred species for commercial nursery retention ponds.
5. *Pontederia cordata* plants accumulated (by plant uptake) 0.62 $g·m^{-2}$ d^{-1} and 0.11 $g·m^{-2}$ d^{-1} of N and P, respectively. Similarly, *Panicum virgatum* accumulated between 0.62 $g·m^{-2}$ d^{-1} and 0.83 $g·m^{-2}$ d^{-1} N and 0.12 $g·m^{-2}$ d^{-1}, but the *Panicum virgatum* FTWs had twice the planting density in comparison to the *Pontederia cordata*.

It is important to mention that neither of these studies were field-scale studies, so extrapolating these results to estimate large-scale performance may be unwise. More research is needed to evaluate FTW performance in full-scale nursery retention ponds to completely assess performance of this technology and particular plant species, provide a better understanding of physicochemical treatment processes, and potentially provide better design guidance and limitations.

Chapter 6. References

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Appendix A. Mean removal efficiency by week

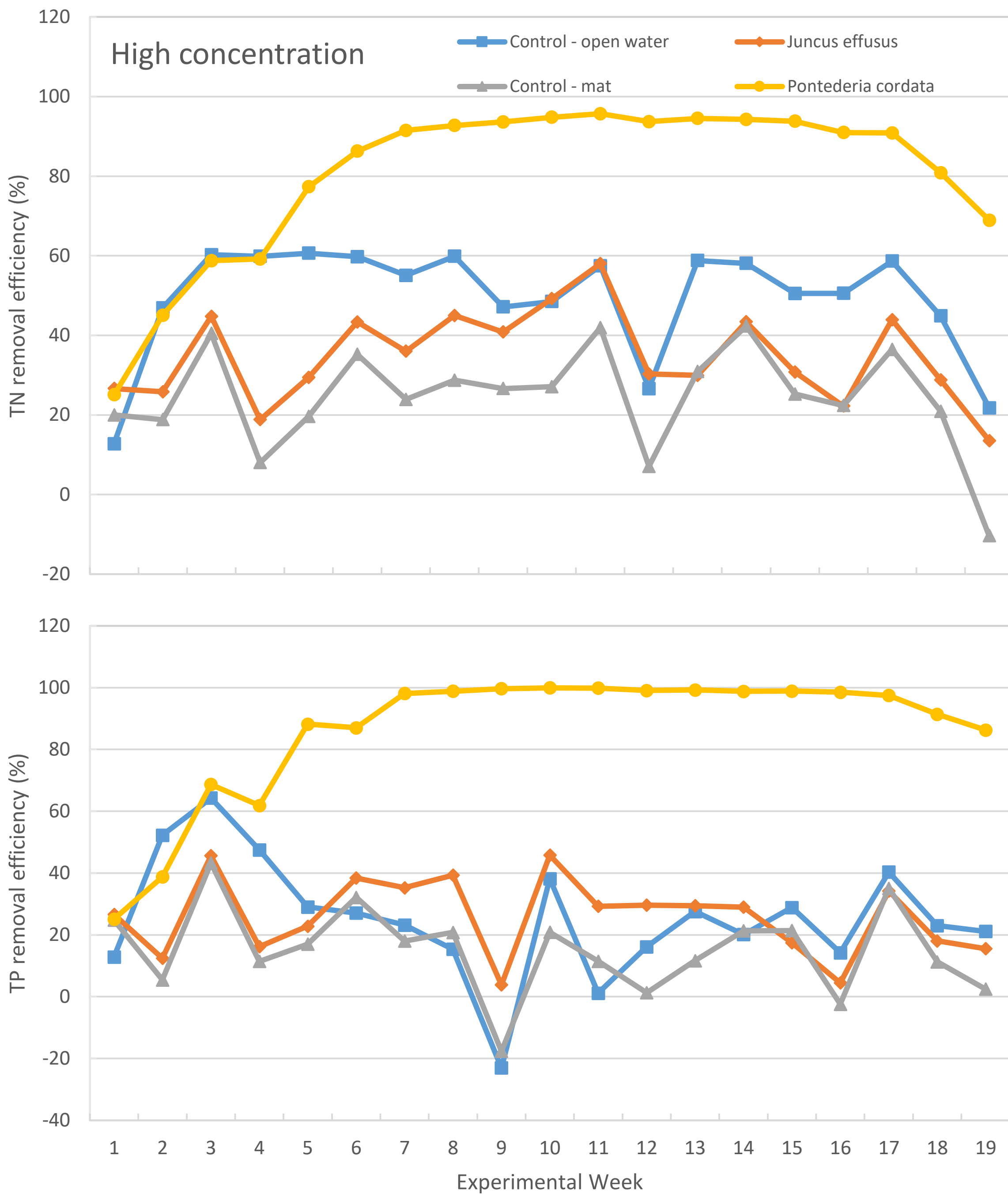

Figure A.1. Mean TP and TN removal efficiency for high concentration treatments (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) by week in a floating treatment wetland study conducted from June 2015 through October 2015.

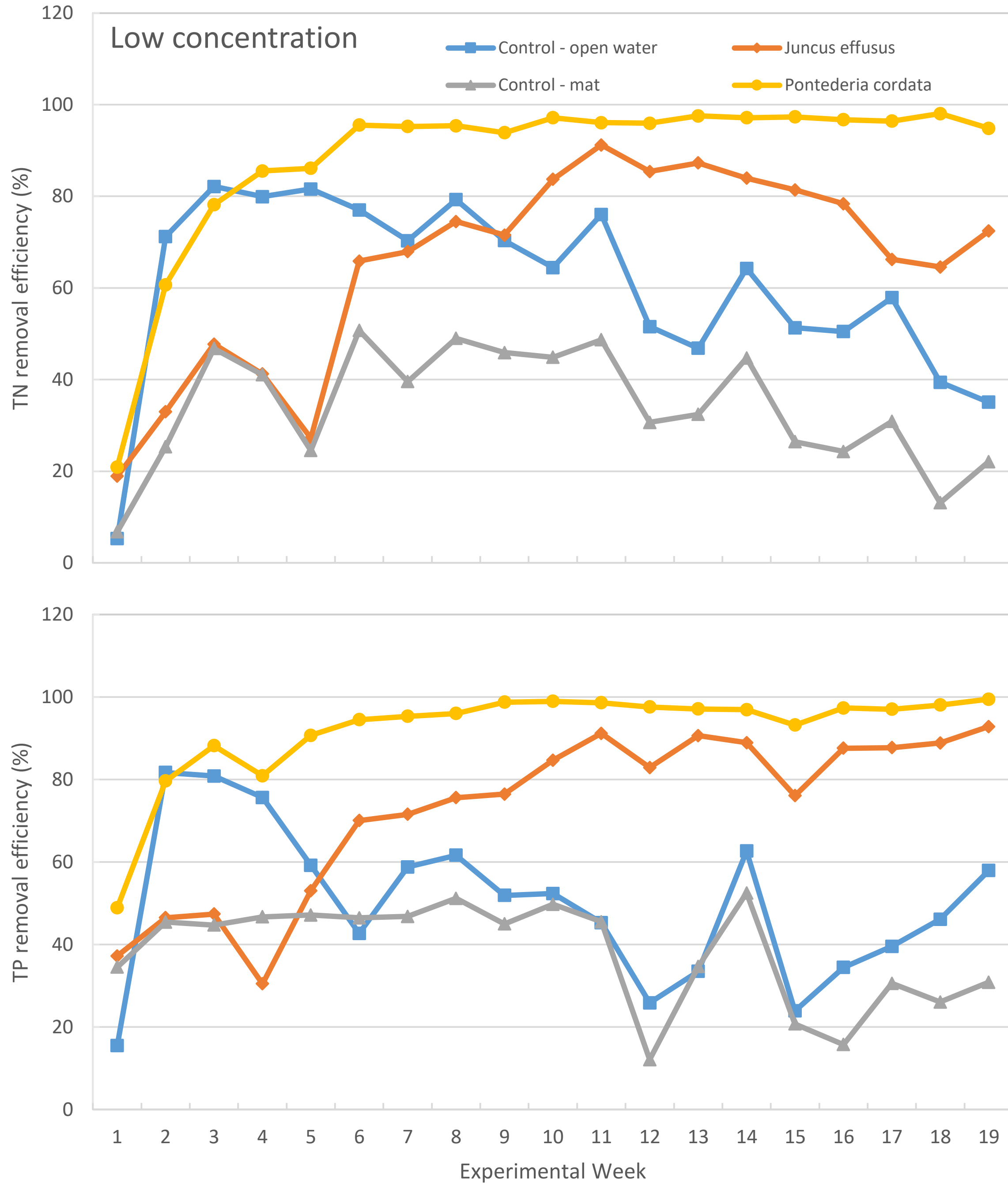

Figure A.2. Mean TP and TN removal efficiency for low concentration treatments (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) by week for a floating treatment wetland study conducted from June 2015 through October 2015.

Appendix B. Nonlinear fit nutrient removal curves for 7-day HRT

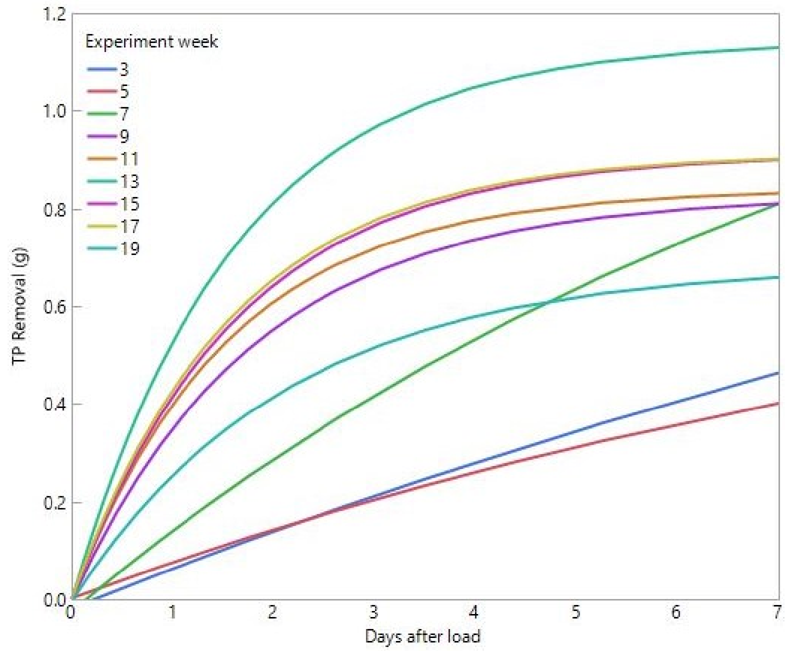

Figure B.1. Weekly fitted TP removal curves for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.1. Summary of fit for TP removal curves by day for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	133.837	-53.825	0.029	0.003	0.057	0.994

Table B.2. Nonlinear regression parameters for TP removal by day for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	1.471	1.012	0.056
5	0.857	0.997	0.090
7	1.467	1.017	0.117
9	0.828	1.004	0.553
11	0.841	1.002	0.645
13	1.145	1.002	0.620
15	0.913	1.002	0.610
17	0.913	0.999	0.633
19	0.685	1.006	0.469

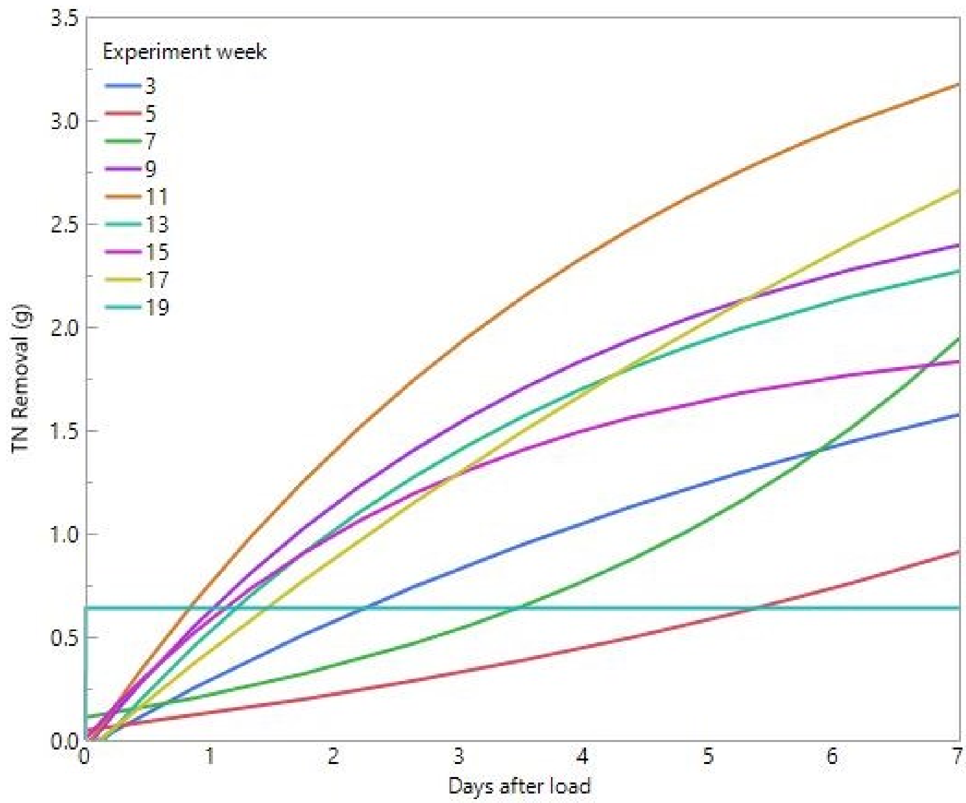

Figure B.2. Weekly fitted TN removal curves for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.3. Summary of fit for TN removal by day for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	261.767	74.106	1.017	0.113	0.336	0.965

Table B.4. Nonlinear regression parameters for TN removal for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	2.70	1.01	0.13
5	-0.56	1.09	-0.13
7	-0.27	1.41	-0.25
9	2.86	1.01	0.26
11	4.10	1.01	0.21
13	2.78	1.04	0.25
15	2.02	1.00	0.34
17	6.25	1.01	0.08
19	0.64	1.00	1087079.85

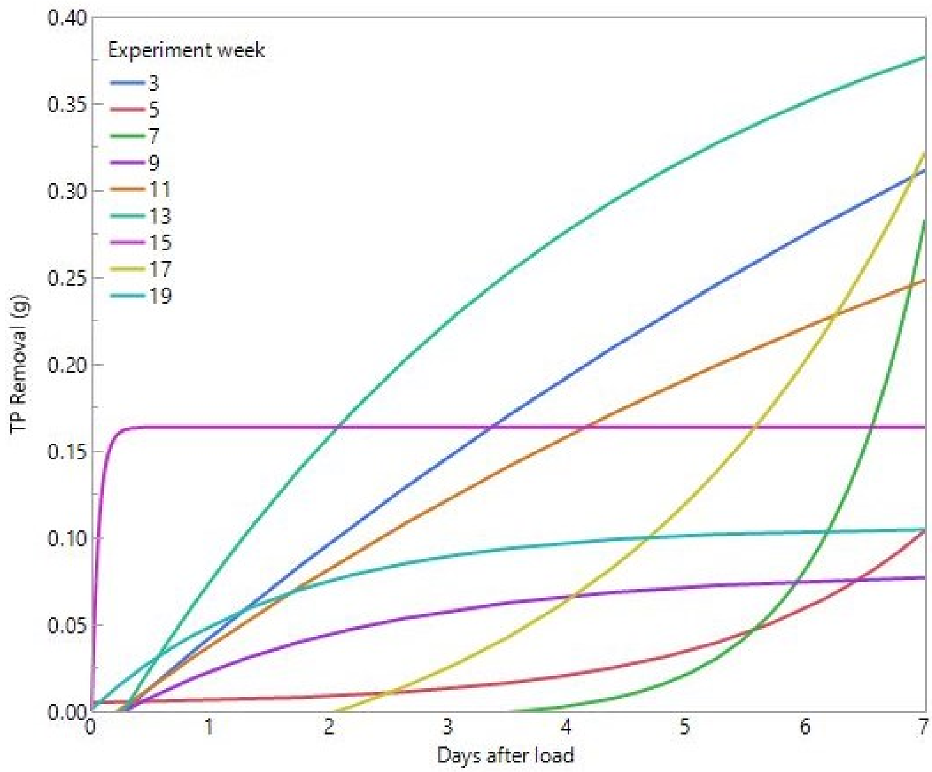

Figure B.3. Weekly fitted TP removal curves for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.5. Summary of fit for TP removal by day for high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	162.874	-24.787	0.065	0.007	0.085	0.873

Table B.6. Nonlinear regression parameters for TP removal by high initial concentration (17.13±0.24 mg·L^{-1} TN and 2.61±0.04 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	0.760	1.020	0.078
5	0.003	-0.500	-0.585
7	-0.005	0.013	-1.192
9	0.081	1.131	0.462
11	0.507	1.021	0.099
13	0.474	1.065	0.236
15	0.164	1.000	17.015
17	-0.060	0.462	-0.375
19	0.106	0.988	0.611

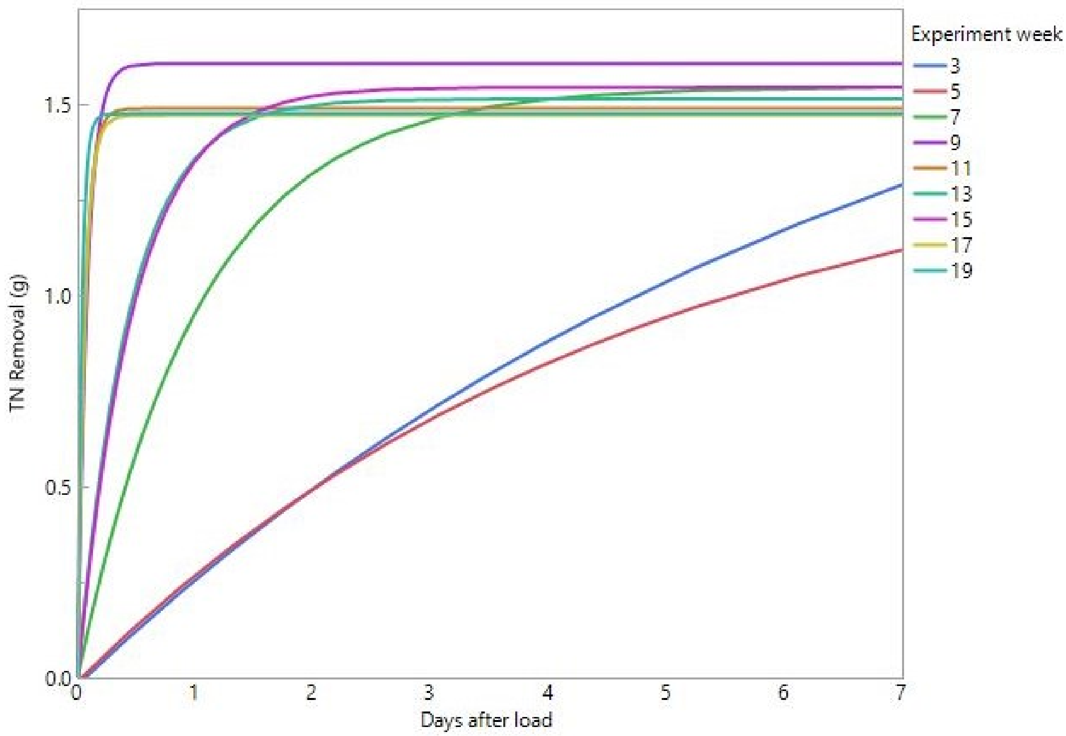

Figure B.4. Weekly fitted TN removal curves for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.7. Summary of fit for TN removal by day for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	109.549	-78.112	0.015	0.002	0.041	0.999

Table B.8. Nonlinear regression parameters for TN removal by low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	2.058	1.009	0.142
5	1.453	1.007	0.211
7	1.547	1.000	0.963
9	1.607	1.000	12.307
11	1.490	1.000	16.374
13	1.514	1.000	2.281
15	1.545	1.000	2.083
17	1.471	1.000	16.746
19	1.476	1.000	28.571

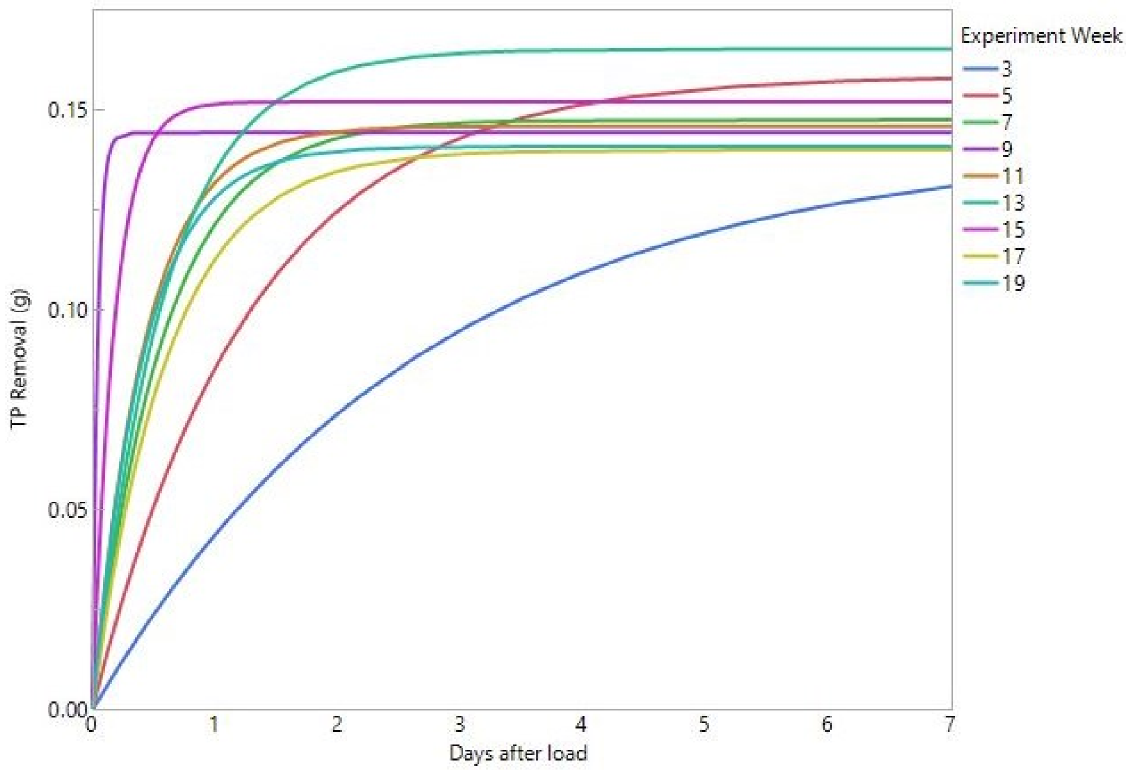

Figure B.5. Weekly fitted TP removal curves for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.9. Summary of fit for TP removal by day for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	-80.391	-268.053	0.000	0.000	0.003	0.999

Table B.10. Nonlinear regression parameters for TP removal by low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Pontederia cordata* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	0.141	1.002	0.371
5	0.158	1.000	0.772
7	0.147	1.000	1.733
9	0.144	1.000	24.062
11	0.146	1.000	2.339
13	0.165	1.000	1.693
15	0.152	1.000	5.617
17	0.140	1.000	1.634
19	0.141	1.000	2.397

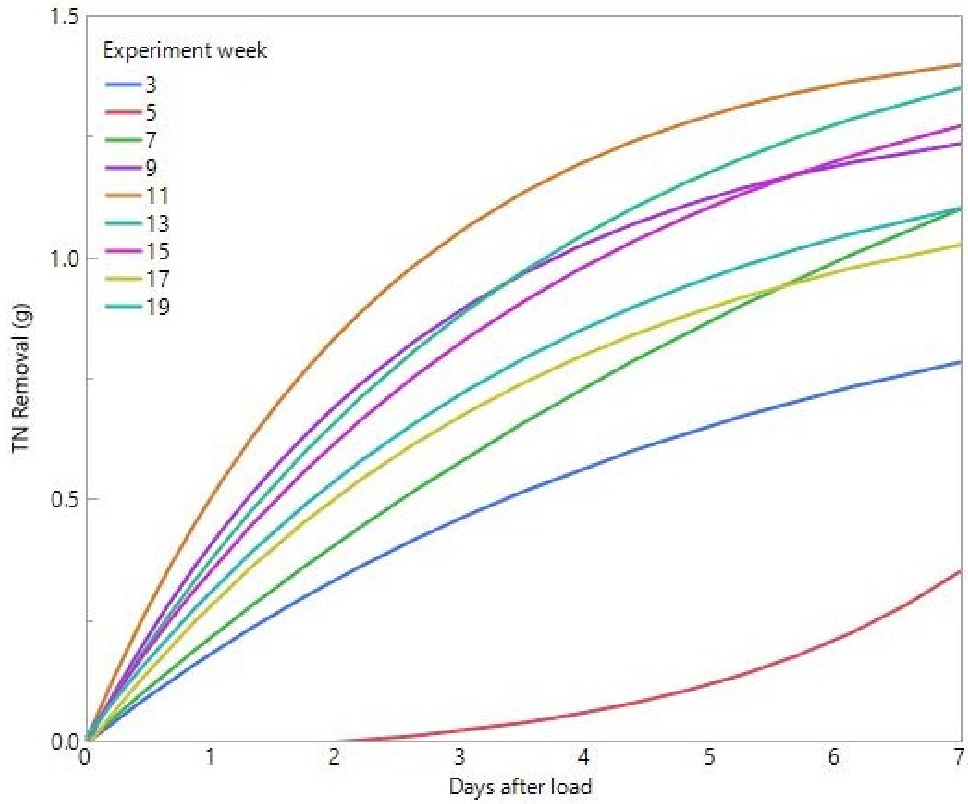

Figure B.6. Weekly fitted TN removal curves for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.11. Summary of fit for TN removal by day for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	127.754	-59.908	0.025	0.003	0.052	0.997

Table B.12. Nonlinear regression parameters for TN removal by low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	1.065	1.005	0.190
5	-0.048	0.381	-0.441
7	1.999	1.001	0.114
9	1.336	1.007	0.369
11	1.479	1.000	0.416
13	1.593	1.001	0.269
15	1.517	0.995	0.259
17	1.200	1.011	0.277
19	1.308	0.994	0.263

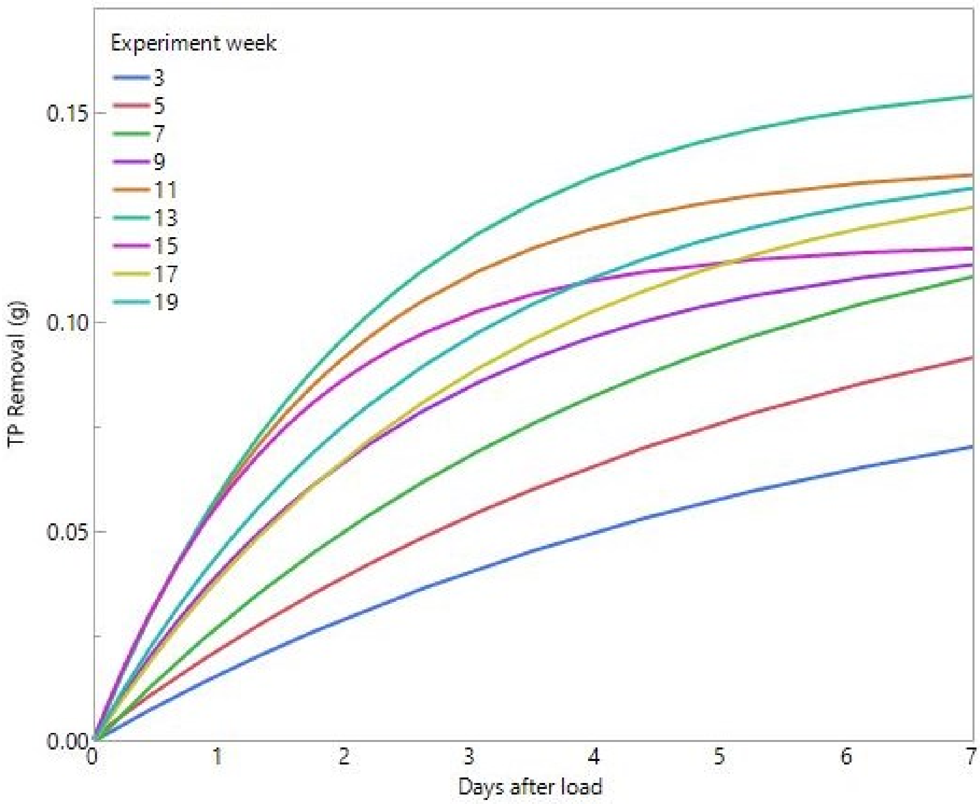

Figure B.7. Weekly fitted TP removal curves for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Table B.13. Summary of fit for TP removal by day for low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Mechanistic Growth	-75.334	-262.995	0.000	0.000	0.003	0.999

Table B.14. Nonlinear regression parameters for TP removal by low initial concentration (5.22 mg·L^{-1} TN and 0.52 mg·L^{-1} TP) *Juncus effusus* treatments in a floating wetland study conducted from June 2015 through October 2015.

Experiment Week	Asymptote	Scale	Growth Rate
3	0.100	1.002	0.173
5	0.127	0.993	0.180
7	0.141	1.005	0.222
9	0.121	0.999	0.401
11	0.138	1.001	0.546
13	0.160	1.000	0.459
15	0.119	0.998	0.650
17	0.143	1.003	0.319
19	0.142	1.001	0.380

Appendix C. Mean day-7 physicochemical properties by week

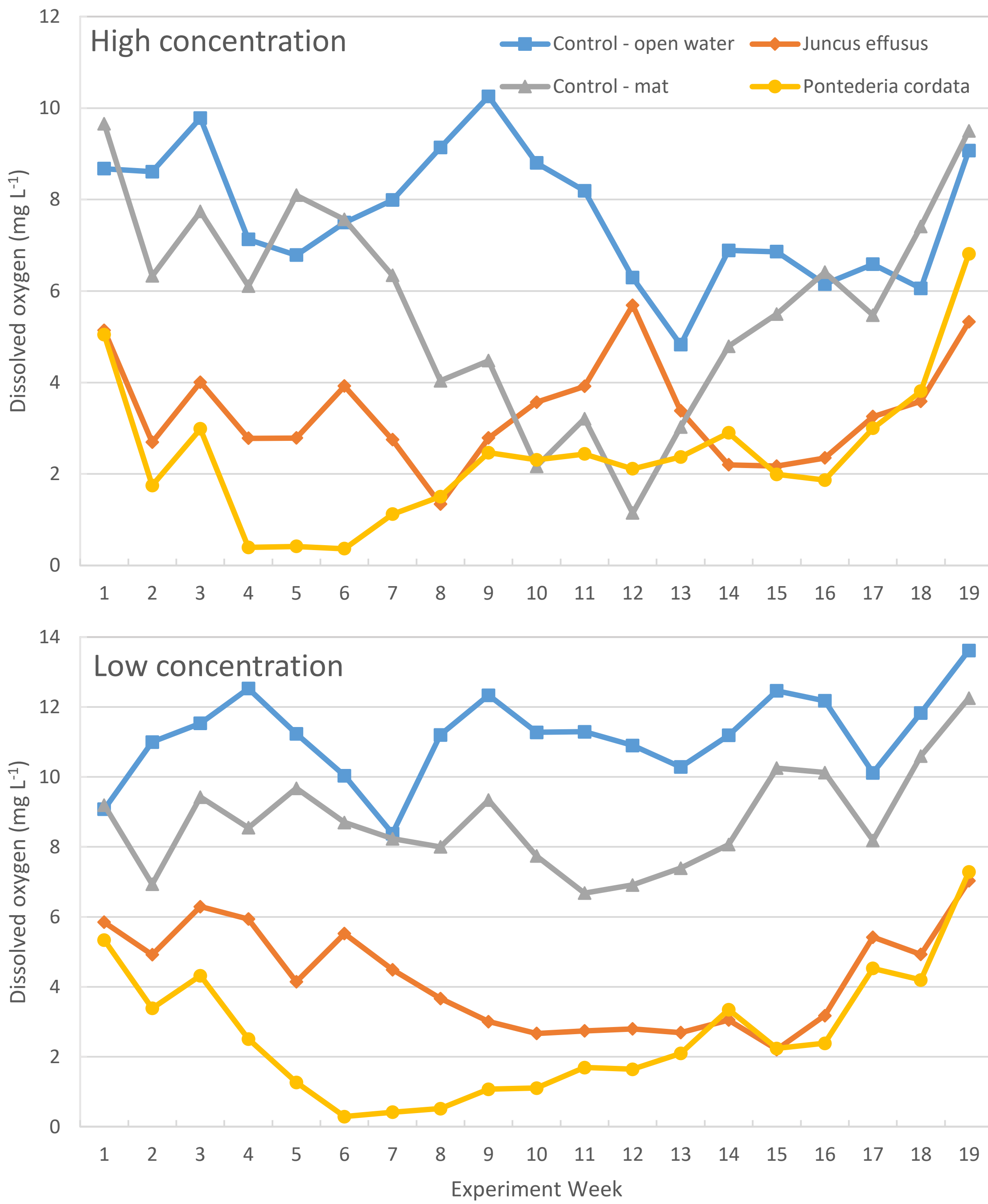

Figure C.1. Mean DO (mg L^{-1}) after a 7-day hydraulic retention time for each treatment combination in a floating treatment wetland study conducted from June 2015 through October 2015 in Virginia Beach, VA.

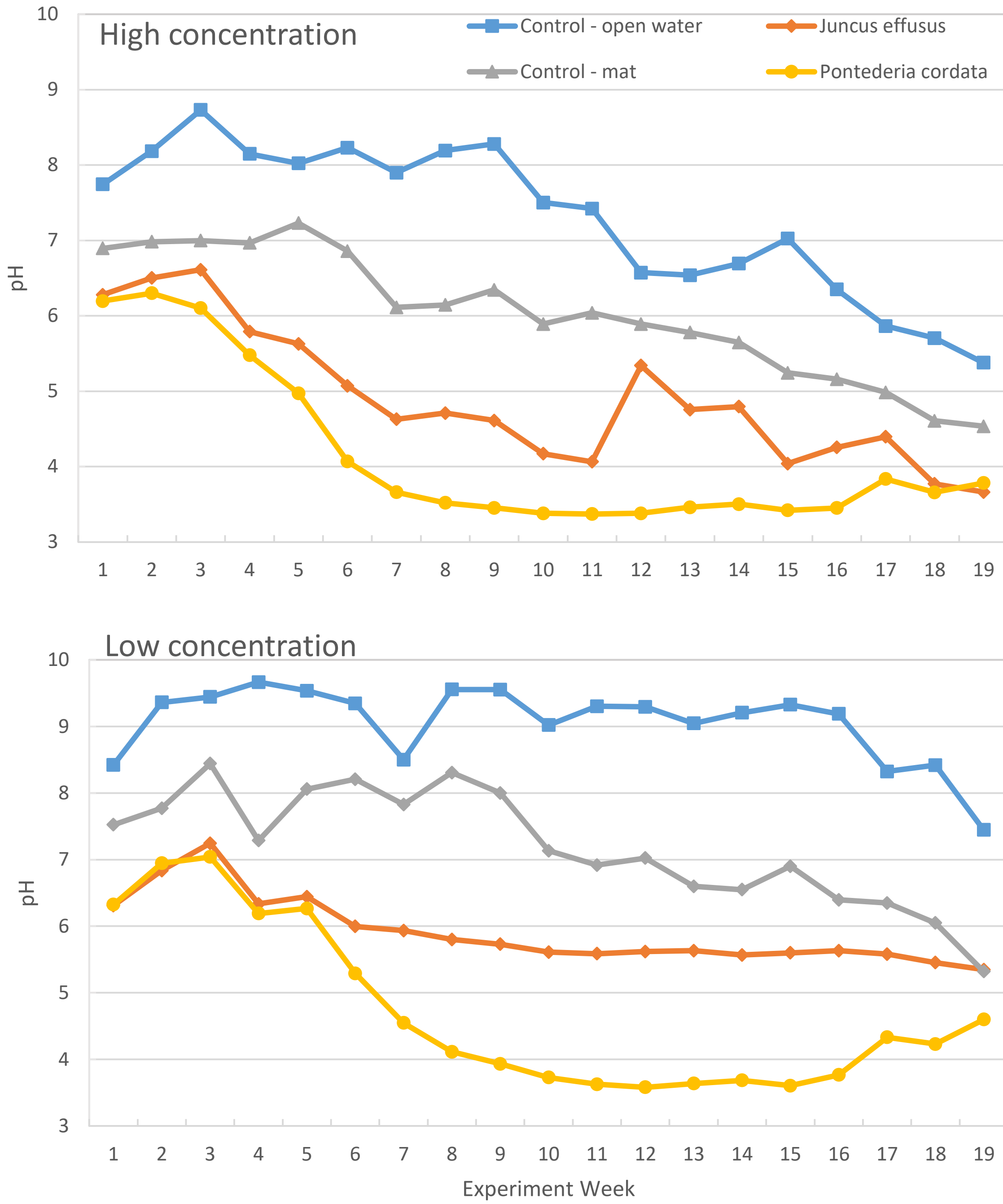

Figure C.2. Mean pH after a 7-day hydraulic retention time for each treatment combination in a floating treatment wetland study conducted from June 2015 through October 2015 in Virginia Beach, VA.

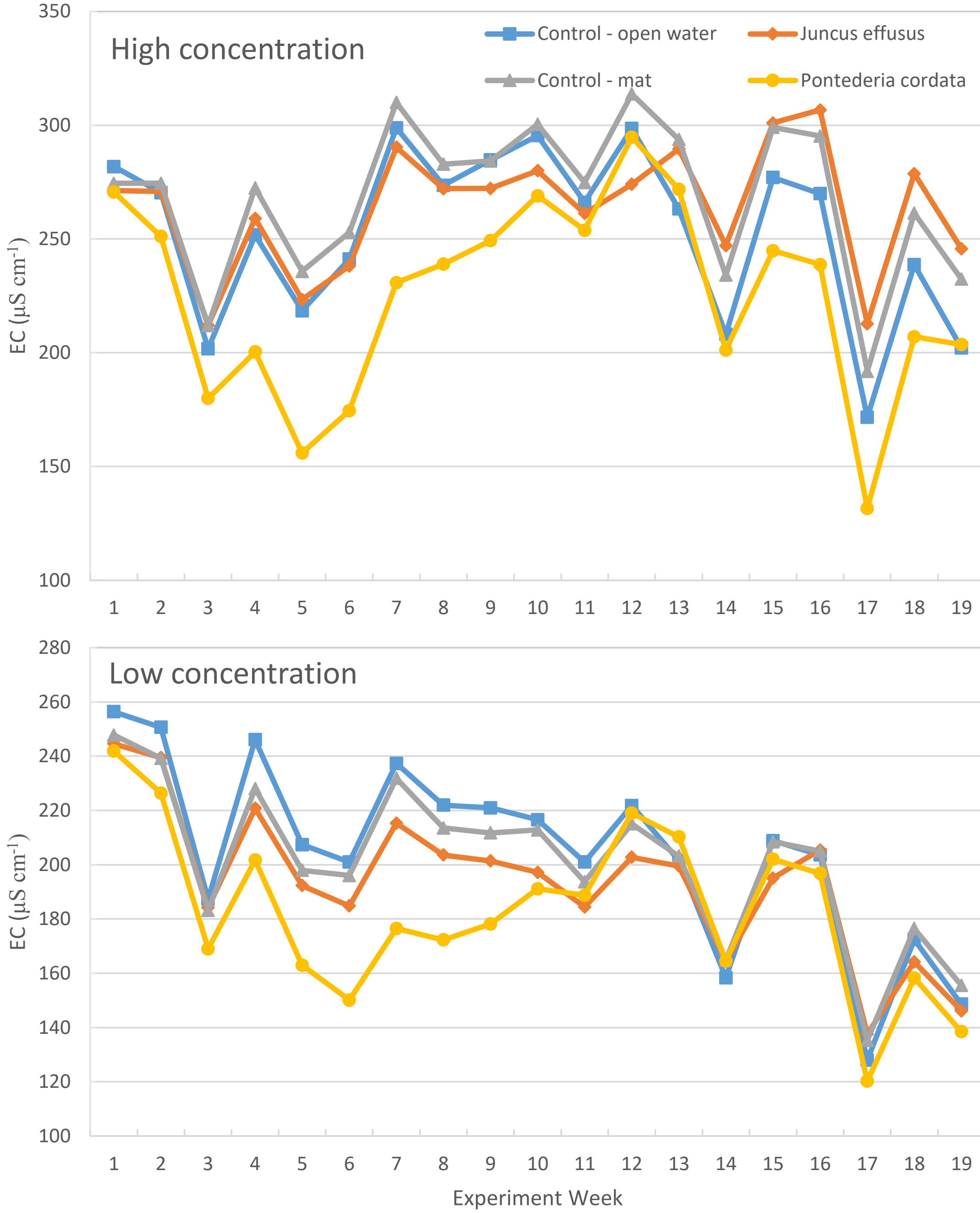

Figure C.3. Mean EC (µS cm^{-1}) after a 7-day hydraulic retention time for each treatment combination in a floating treatment wetland study conducted from June 2015 through October 2015 in Virginia Beach, VA.

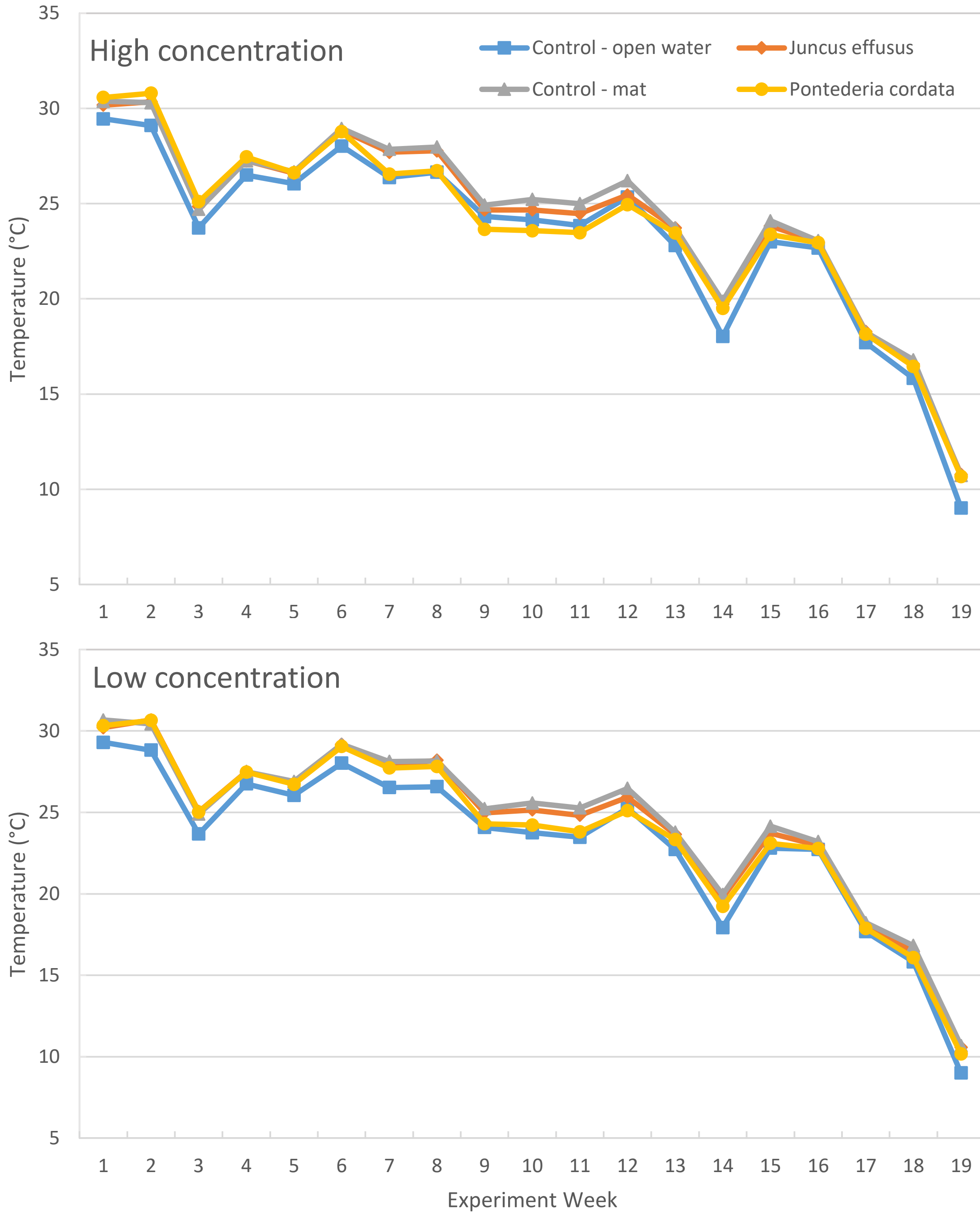

Figure C.4. Mean temperature (°C) after a 7-day hydraulic retention time for each treatment combination in a floating treatment wetland study conducted from June 2015 through October 2015 in Virginia Beach, VA.

Appendix D. Cumulative N and P removal by week

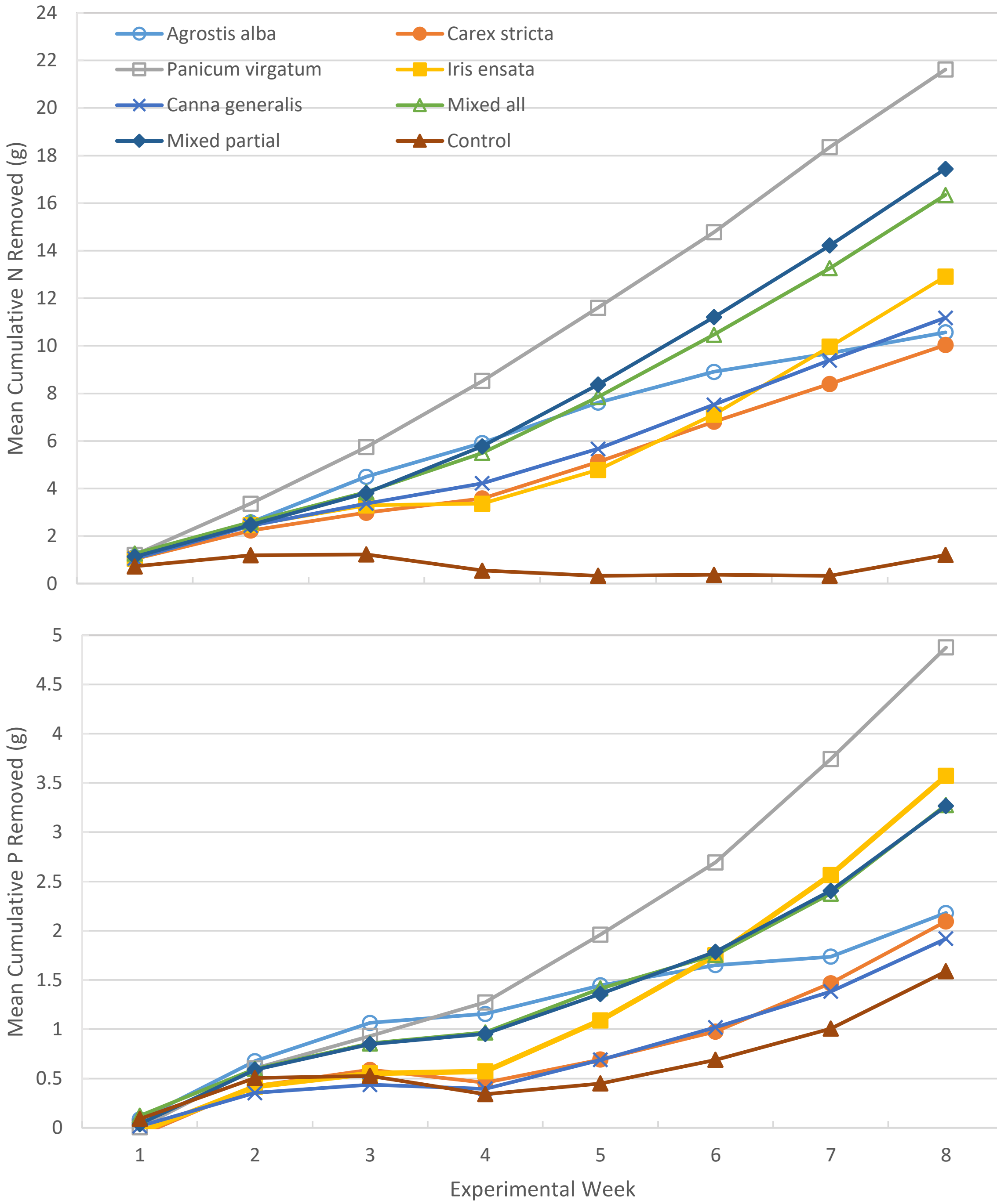

Figure D.1. Mean cumulative nitrogen (N) and phosphorous (P) removal (g) by week for Trial 1 of a floating treatment wetland study conducted from June 2016 through July 2016 (n = 4). N is the sum of ammonium-N, nitrate-N, and nitrite-N. P is phosphate-P.

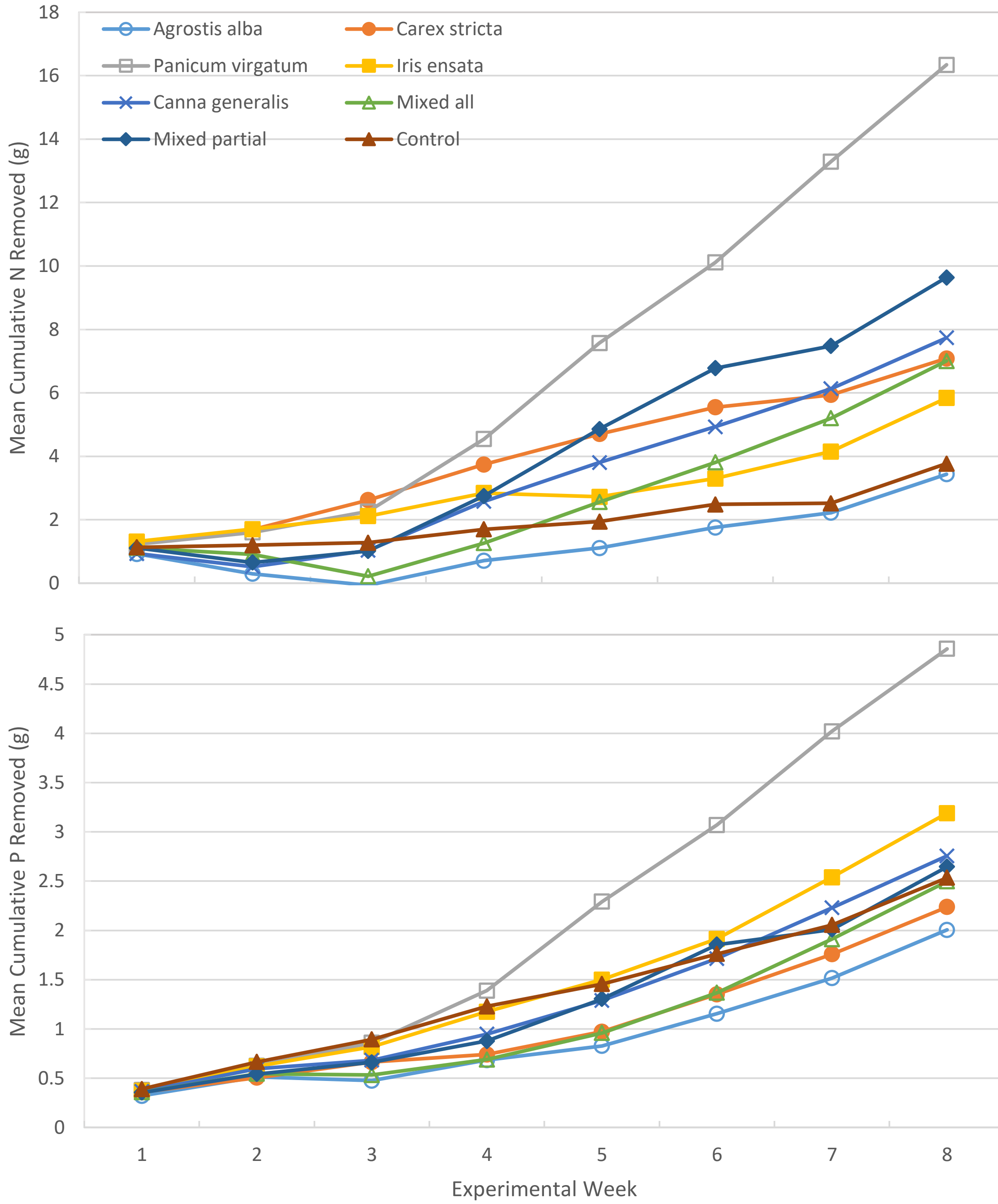

Figure D.2. Mean cumulative nitrogen (N) and phosphorous (P) removal (g) by week for Trial 2 of a floating treatment wetland study conducted from July 2016 through September 2016 (n = 4). N is the sum of ammonium-N, nitrate-N, and nitrite-N. P is phosphate-P.

Appendix E. Plant growth pictures June 2016 – July 2016

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.1. *Agrostis alba* growth over time during Trial 1 of a floating wetland mesocosm study conducted from June 2016 through July 2016 in Virginia Beach, VA.

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.2. *Carex stricta* growth over time during Trial 1 of a floating wetland mesocosm study conducted from June 2016 through July 2016 in Virginia Beach, VA.

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.3. Growth over time for a three species mixed planting floating wetland mesocosm. The study was conducted from June 2016 through July 2016 in Virginia Beach, VA.

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.4. *Panicum virgatum* growth over time during Trial 1 of a floating wetland mesocosm study conducted from June 2016 through July 2016 in Virginia Beach, VA.

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.5. *Iris ensata* growth over time during Trial 1 of a floating wetland mesocosm study conducted from June 2016 through July 2016 in Virginia Beach, VA.

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.6. *Canna* x *generalis* growth over time during Trial 1 of a floating wetland mesocosm study conducted from June 2016 through July 2016 in Virginia Beach, VA.

06/11/16 06/29/16 07/13/16 07/27/16

Figure E.7. Growth over time for a five species mixed floating wetland mesocosm. The study was conducted from June 2016 through July 2016 in Virginia Beach, VA.