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Evaluation of Seasonal Reservoir Water Treatment Processes in Southwest Florida: Protection of the Caloosahatchee River Estuary

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Abstract: In southwest Florida, the Caloosahatchee River flows from Lake Okeechobee into a biologically productive tidal estuarine system. A combination of excess water during the wet season, insufficient fresh water in the dry season, and poor quality of the river water are damaging the estuarine ecosystem. To better control the quality and quantity of the water entering the estuary, reservoirs are being constructed to store excess, poor quality water during the wet season and return it to the river for discharge into the estuary at an appropriate time. This stored water is enriched in nutrients and organic carbon. Because of the subtropical nature of the climate in southwest Florida and potential increases in temperature in the future, the return flow of water from the reservoirs must be treated before it can be returned to the river. Hence, an experimental water treatment system was developed and operated to compare biological treatment processes consisting of solely wetland plants versus adding some engineered processes, including slow sand filtration and a combination of slow sand filtration and ultraviolet (UV) treatment. These three treatment trains were operated and monitored through a seasonal cycle in 2021–2022. All three treatment methods significantly reduced the concentrations of nutrients and total organic carbon. While the enhanced engineered wetlands' treatment trains did slightly outperform the wetland train, a comparison of the three process trains showed no statistically significant difference. It was concluded that upscaling of the slow sand filtration and UV process could improve the treatment efficiency, but this change would have to be evaluated within a framework of long-term economic benefits. It was also concluded that the Caloosahatchee River water quality is quite enriched in nutrients so that reservoir storage would increase the organic carbon concentrations, making it imperative that it be treated before being returned to the river. It was also discovered that the green alga *Cladophora* sp. grew rapidly in the biological treatment tubs and will present a significant challenge for the treatment of the reservoir discharge water using the currently proposed alum treatment.

Keywords: Caloosahatchee River; Lake Okeechobee; eutrophication; nutrient loading; biological water treatment; engineered water treatment; slow sand filtration

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1. Introduction

The Caloosahatchee River of southwestern Florida currently begins at Lake Okeechobee and drains west into a large, biologically productive tidal estuary. This river was originally a natural system with headwaters in Lake Hikpochee prior to the excavation of a shallow connection to Lake Okeechobee by native Indians in the early 1800s. Later, in the 1880s, a deeper connection was hand dug by fisherman and a land development company. In 1886, a schooner containing an exploration team succeeded in traversing Lake Hikpochee and entering Lake Okeechobee via the shallow canal [1]. Before modification of the river by the U. S. Army Corps of Engineers into a transportation canal in 1937, it contained meanders and water runoff from an essentially natural landscape composed of various wetland and pine flatwood environments, producing a high-quality water discharge into the estuary [2].

The river was later channelized, and a series of locks were installed beginning in the 1950s and were completed in the 1960s. Today, the Caloosahatchee River is greatly affected by the water quality in Lake Okeechobee and the drainage basin upstream of the W. P. Franklin Dam (S-79; Figure 1). Lake Okeechobee is in a eutrophic condition, adversely affected by excessive nutrient concentrations, and periodically undergoes harmful algal blooms that pass into the river during high water periods [3–5]. In addition, the river basin contains ranch lands and various other types of agriculture, particularly near the cities of Clewiston and LaBelle, which all contribute to nutrient loading of the Caloosahatchee River.

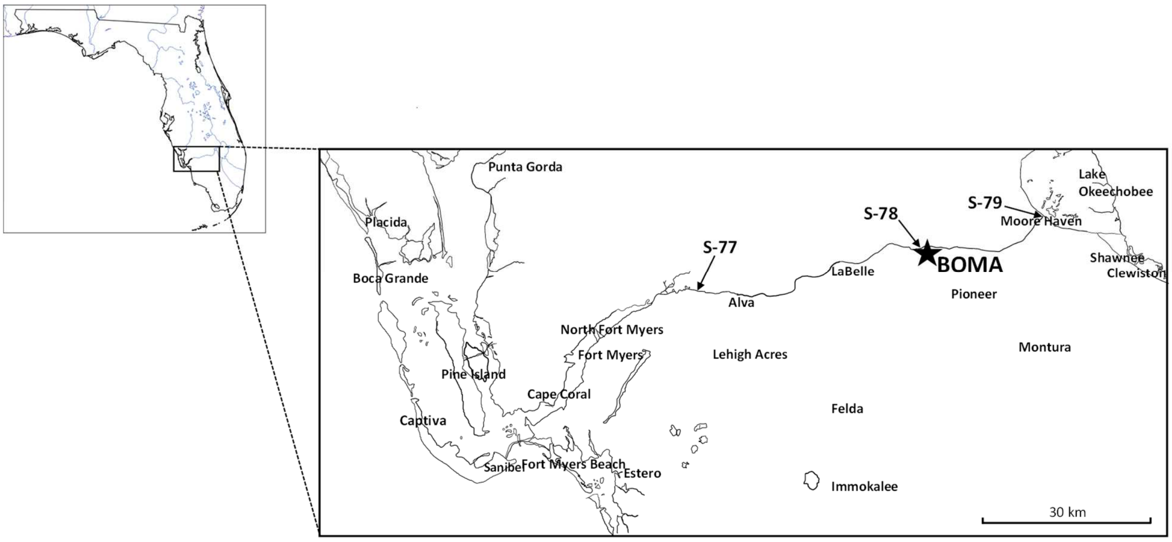

Figure 1. Location map showing the Caloosahatchee River channel, the drainage basin, the location of the research site (BOMA), and S-79, which is the location of river water entering the estuary.

Documented impacts of excessive discharges of freshwater and poor water quality entering the estuarine system from the Caloosahatchee River beginning at structure 79 created the necessity to develop remedial measures to control both problems [6–26]. To control the freshwater discharges from Lake Okeechobee into the river, the Hoover Dike that surrounds the lake was strengthened to allow it to peak at a maximum stage of 4.88 m NGVD and a series of large reservoirs were to be constructed along the river upstream from structure 79 with a goal of moderating freshwater discharges entering the estuary during excessively wet periods and increasing discharges during excessively dry periods [27].

While the concept of creating large storage reservoirs to control the water budget of the Caloosahatchee River Estuarine system has considerable merit, the quality of water in the Caloosahatchee River during the highest discharge periods of the summer months is commonly laden with excess concentrations of nutrients, total organic carbon, turbidity, color, and associated harmful algal blooms. Once the river water is pumped into the reservoirs, the high nutrient content along with high water temperature and lentic hydrology will exacerbate the issue of algal blooms in the reservoir along with other aquatic plant growth [28–32]. Therefore, treatment of the reservoir water will be required before it can be discharged back into the river to meet the goals of the estuary freshwater management plan.

Several methods have been applied to shallow lakes for the control of algal blooms, aquatic vegetation growth, and high organic carbon concentrations [33]. Chemical methods have been applied using copper-based algaecides [34] or herbicides including glyphosate or 3-(3,4-dichlorophenyl)-1,1dimethylurea (Diuron) [35,36]. However, residues of these compounds could be discharged into the river during water recovery cycles, thereby exacerbating downstream impacts. The addition of a coagulant (alum) plus a ballast compound has been suggested to remove algae and other organic compounds from the water column [37]. Some successful combinations of adding a natural clay flocculant to the water stored in the reservoirs could bind the organic material, causing it to flocculate and sink to the bottom of the water body [38]. If the clay is bentonite, which is chemically inert, the combination of the clay and the organic material tends to harden on the bottom and cannot be re-suspended by wind mixing. In this case, the bottom can be periodically dredged. This technique has been used in southwest Florida in lakes to manage plant growth in real estate lakes.

Another means of water treatment is to use vegetation to uptake the nutrients and filter the turbidity and particulate organic carbon, like the stormwater treatment areas in the Everglades [39–42]. To access the potential for development of a "natural" stormwater treatment strategy, the South Florida Water Management District (SFWMD) developed a research site in Hendry County, located upstream of the S-78 water management structure on the south side of the Caloosahatchee River (Figure 1). The C-43 mesocosms site has 12 tanks with dimensions of about 7 m $\times$ 3.5 m $\times$ 1.5 m containing wetland vegetation. The tank vegetation system was used to conduct a water quality assessment of nutrient removal from Caloosahatchee River water that was allowed to flow through the wetland cells [43]. There were three key findings of the research: (1) no single plant community appears to control nitrogen removal (denitrification), (2) the sediments in the tanks represent a net sink for nitrogen and phosphorus, and (3) the average denitrification was 14.4 $\pm$ 23.0 mg N/m^2/day with the highest rate occurring in June at 24.3 $\pm$ 29.7 mg N/m^2/day and the lowest rate occurring in December at 10.9 $\pm$ 11.4 mg N/m^2/day. They concluded that denitrification was significant in the mesocosms.

It was concluded that upscaling of this type of biologic nutrient removal system was both impractical and ineffective at removing organic nitrogen. The consulting engineer suggested that the reservoir cells could be successfully treated with alum to control algal blooms and aquatic weed growth during storage. Unfortunately, alum may be only partially effective at removing organic materials and does not tend to fully harden on the lake bottom, thus making it unfeasible to dredge. It could easily be remobilized during strong winds, thereby adding turbidity to the reservoir water and delaying discharge back into the river. In addition, disposal of residual alum combined with organic material is difficult, very expensive, and laden with several additional potential environmental issues.

It is the purpose of this research to evaluate an alternative to treatment of the water stored in the reservoir prior to its discharge. This alternative includes biological treatment processes using plants with enhanced nutrient removal facilitated by engineered water treatment. The goal would be to convert the nitrogen in the surface water from nitrate/nitrite to ammonium and to reduce the concentration of organic nitrogen. The conversion would facilitate the uptake of nitrogen by the plant communities, particularly algae. An investigation of this process was conducted at the C-43 mesocosm site using 6 of the 12 tanks from past research. The primary objective of the research is to ascertain if well-known engineering processes, that have high potential for upscaling, can be used to facilitate the plant uptake of nutrients. If this process is feasible, it could be developed on the sites of the reservoirs and leave less residuals for disposal and perhaps operate at a lesser cost. The novelty of the investigation was the advanced control and monitoring systems' design to allow for remote operation to ensure operational stability and constant water flow rates.

2. Methodology

2.1. Experimental Design

The original design of the project included three water treatment trains. Engineered water treatment was proposed for the feed water of two of the three trains, and one train was to be used as a control. Each train consisted of two tanks containing different wetland communities, with the first tank being emergent plants (primarily *Typha* sp., cattail) and the second tank containing submergent vegetation (primarily *Vallisneria* sp. tape grass) (Figure 2). Entry of river water into the first train was taken directly from the supply tank without treatment and was fed by gravity into the two wetland tanks. The second train was designed to be treated by slow sand filtration of the river water before entering the two wetland tanks. The third train water treatment scheme included slow sand filtration of the river water followed by UV treatment and then wetland treatment in the two tanks (Figure 2).

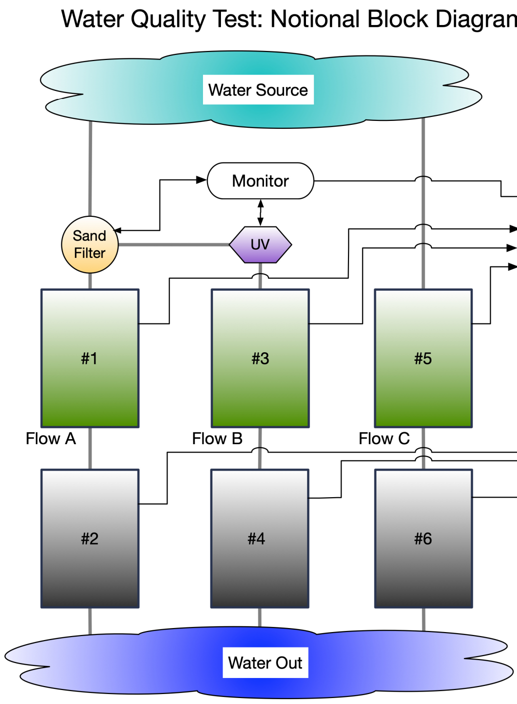

Figure 2. Schematic diagram of the initial water treatment test design.

The reasoning behind the design was that the slow sand filtration would remove a large part of the particulate organic carbon and algae and create anoxia in the tank to help convert nitrate to ammonium. In addition, the UV following slow sand filtration would help break down some of the organic nitrogen to smaller size molecules that could be taken up by the aquatic plants in the tanks and kill bacteria, therefore enhancing the nutrient removal.

The duration of the experimental treatment processes was designed for a period not to exceed 12 months after the installation and pretesting for a period of two months.

2.2. Final Experimental Design with a Description of the Power System, Controls, and Monitoring

Site conditions and a series of health events triggered some design changes to the project. The COVID-19 pandemic caused major cost increases in construction materials and sharp price increases in pumps and electronic components. Thus, a more efficient design was developed and constructed to be close to the original budget. A primary system was designed to control the movement of water through the various process trains. A subsystem provided a means to remotely control the pumps (flow regulation), processes, and track the movement of the Caloosahatchee River source water.

Water from the Caloosahatchee River was pumped via a pipe to a main supply tank at the Boma test facility (Figure S1, note that all figures containing an "S" designation are in the Supplementary Materials). The original Boma facility consisted of the river water pump, the main supply tank, two holding tanks, and twelve test tubs. The experiment used the existing main supply tank, six tubs, and added a new sand filter tank and a filtered water holding tank. Three parallel water paths were created, referenced, filtered, and UV-treated to compare water quality treatments. Two tubs were assigned to each path, the first with emergent plants and the second with submergent plants (Figures 2 and 3).

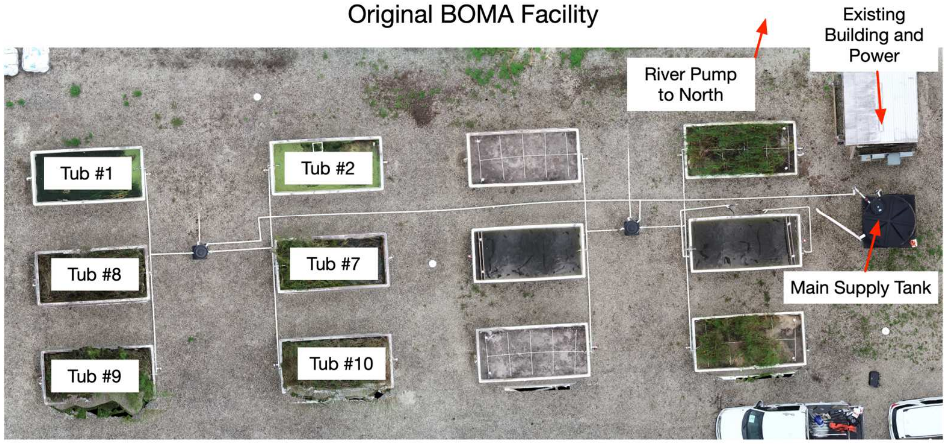

Figure 3. The layout of the C-43 mesocosms site including the main supply tank, the storage building with the power supply, and the tubs containing the vegetation. Note that the mesocosms will be referred to as tubs in the text.

The three experimental paths utilized the following tanks and tubs: the reference path used gravity feed from the main supply tank to Tub 8 containing emergent plants. Pump T1 then pumped water from Tub 8 to the submergent plant Tub 1. Pumps from the emergent plant tubs to the submergent tubs were required because the water level in the submergent tubs was higher than in the emergent tubs. Both the filtered and UV paths utilized river water filtered by the new slow sand filter tank. Pump SF moved river water from the main supply tank to the top of the slow sand filter tank. Since the sand filter tank did not have enough gravity head, pump HT moved water from the base of the sand into the filtered water holding tank.

The filtered water tank used gravity to feed water directly to Tub 10 for the filtered water flow. For the UV path, gravity fed the water through a UV light and then to Tub 9. The UV light consists of a 12 VDC powered Blackcomb LB5-06 rated for 23 L per minute with a 22-watt bulb. The UV light was set to run continuously. Two pumps moved the water from Tub 10 to pump T7 to Tub 7 to for the filtered flow, and from Tub 9 to pump T2 to Tub 2 for the UV flow (Figure S2). The three experimental paths utilized the following tanks and tubs: the reference path used gravity fed from the main supply tank to Tub 8 containing emergent plants. Pump T1 then pumped water from Tub 8 to the submergent plant Tub 1. Pumps from the emergent plant tubs to the submergent tubs were required because the water level in the submergent tubs was higher than in the emergent tubs. Both the filtered and UV paths utilized river water filtered by the new slow sand filter tank. Pump SF moved river water from the main supply tank to the top of the slow sand filter tank. Since the sand filter tank did not have enough gravity head, pump HT moved water from the base of the sand into the filtered water holding tank.

Under normal conditions, the system operated as follows: the main river water pump ran continuously and filled the main holding tank. Any excess water was drained out to an adjacent canal. The reference path used gravity to feed water into Tub 8. Pump T1, running continuously, moved water from Tub 8 to Tub 1. Any excess water was drained to the adjacent canal. Pump SF moved water from the main holding tank to the top of the sand filter tank. The river water percolated through the sand filter and then was moved by pump HT to the top of the filtered water holding tank. Gravity fed water from this tank directly to Tub 10 and through the UV light to Tub 9. Pumps T7 and T2 moved water from Tub 10 to Tub 7 and from Tub 9 to Tub 2 (Figure S3). Any excess water was drained to the adjacent canal. The monitoring system continuously checked that this operation was functioning nominally and reported any voltage, current, and water level problems.

To enable a safe and cost-effective system, pumps operating on 12 VDC provided the required pump and UV light power. The field site contained only the power wiring, control switches, pumps, and UV light. The supply power and power monitoring systems were installed in an existing onsite building. Figure S4 shows the tanks, two field control boxes, and the UV light system. The power control system provided a method to check the power availability, control pump, and UV light. All control systems were installed in water-resistant enclosures, with cable penetration protected by gland fittings. The UV control module and one of the control boxes are shown in Figure S5.

The monitoring system provided the status of the available power and water levels. Sensors included source AC power, 12 VDC power supply voltages, current flow to the pumps and UV light, and float switches to indicate the water level in the tanks and tubs. Since the water supply contained high concentrations of particulate matter and the water flow was small (≈3.79 L/m from emergent to submergent tubs), standard flow meters would not work. Instead of expensive flow meters, low-cost float level sensors provided the continuous monitoring of the water status. An example of the float switch and mounting system is shown in Figure S6. A summary of the monitoring system is given in Table 1.

Table 1. Summary of monitoring indicators.

Monitored Data	Type	Comment	Failure Indication
Binary Inputs			
Main Supply Tank	Float Switch	River water available	Loss of pumping from the river
Sand Filter Tank	Float Switch	Sand filter feed water status	Pump SF failure
Filtered Water Tank	Float Switch	Filtered water status	Pump HT failure, clogged Sand Filter
Tub #1	Float Switch	Low water level in tub	Pump for T1
Tub #2	Float Switch	Low water level in tub	Pump for T2
Tub #7	Float Switch	Low water level in tub	Pump for T7
Tub #8	Float Switch	Low water level in tub	Main holding tank level
Tub #9	Float Switch	Low water level in tub	Low filtered water tank level
Tub #10	Float Switch	Low water level in tub	Low filtered water tank level
Input AC Power	Relay	AC input power	Loss of AC power to the facility
Analog Inputs			
Power Supply #1	Voltage	Status of 12VDC power	Power supply failure
Power Supply #2	Voltage	Status of 12VDC power	Power supply failure
Power Supply #3	Voltage	Status of 12VDC power	Power supply failure
Power Supply #4	Voltage	Status of 12VDC power	Power supply failure
UV Light	Current	System operational	UV light bulb failure
Current to SF pump	Current	System operational	Pump failure
Current HT pump	Current	System operational	Pump failure
Current to pump T1	Current	System operational	Pump failure
Current to pump T2	Current	System operational	Pump failure
Current to pump T7	Current	System operational	Pump failure

An Arduino microcontroller collected and digitized the analog inputs. A Raspberry Pi computer was used to collect digital status information and analog data transferred from the Arduino. Once the data were assembled, a daily status email was sent to enable remote monitoring of the Boma system. The email consisted of three general parts. The first is a header stating the project name and ending in the day of week, month, day, the time, and the year. The second part is the daily summary, with an easy-to-identify green check for OK and a red X for a problem. The third part listed the server data acquisition system status including temperature, uptime, and other technical performance information. An internet connection, using a cell modem, was installed at the site to allow for the reporting of the system to the project team. If there was an AC power failure, an immediate email was sent. Since the monitoring system was powered by an uninterruptable power supply (UPS), the monitoring system continued to operate even with a relatively short power failure. Two example emails are shown in Figures S7 and S8 that represent a full report and a partial report. The first shows a good status, the second one with problems.

An example of an email showing the failure of the main river water pump is shown in Figure S9. With no input water, the main tank water ran low. With no water available, the follow-on system also ran low. The email notifications were very helpful in identifying issues and enabling quicker repairs, especially the indications of the sand filter system clogging. With a clogged sand filter, the filtered water holding tank did not fill correctly and was reported as low. Since Tubs 9 and 10 were gravity-fed from the sand filter, they were also reported as low. In addition, when the slow sand filter clogged, an alert was transmitted as also shown in Figure S9. With an understanding of the water flow architecture, trouble-shooting issues could be conducted remotely from the status report. The clogging was then repaired by scrapping the top of the sand filter.

The monitoring and power systems were installed in the existing building. The system was connected to existing AC power and provided the 12 VDC required for the field devices and a collection point for status monitoring. An image of the panel and labels for the major components are shown in Figure S10. The entire control system allowed for the efficient operation of the experimental apparatus.

2.3. Design of the Slow Sand Filter

The original design contained two small slow sand filters. This design was found to be economically inefficient and was replaced by a single unit with a substantially larger volume of graded sand. The dimensions of the 9500 L slow sand filter tank were approximately 260 cm in diameter and 201 cm in height. The tank was modified by adding a 76 cm diameter hole in the center of the top and a discharge hole 5 cm in diameter at the base. Approximately 10,545 kg of sand were placed into the tank in graded layers (Figure S11). As shown in Figure S4, it was necessary to stabilize the tank by installing a series of 9 × 9 cm posts that were cemented into the ground. Straps were placed around the structure to prevent the tank walls from splitting. The tops of the posts were interconnected to allow for the ease of entry of a person into the tank during cleaning.

The slow sand filter was constructed based on the standard design used in potable water treatment facilities [44,45]. The basal layer of gravel was 30.5 cm thick and consisted of 3.175 mm × 6.35 mm gravel. The gravel base was constructed by the placement of an initial 7.6 cm. Then, a network of 5.1 cm diameter, schedule 40, machine slotted PVC pipes were placed atop the gravel layer. The ends of the screen were capped, and the screen extended into a 5.1 cm diameter, schedule 40 PVC outflow pipe. A special fitting was used to seal the discharge line from the tank to prevent leakage. An additional 22.9 cm of gravel was placed above the collection screen. The approximate flow rate through the slow sand was about 11.4 L/min to produce a contact time of about five hours. A spillover at the top of the filter maintained 30.5 cm of driving head.

A series of four sand layers were placed above the gravel with sufficient grading to prevent the layers of sand from plugging the intake screen. The first layer was 1.19–2.38 mm sand with a thickness of 15.2 cm. The next layer upward was a 15.2 cm layer of sand with a size range of 0.85–1.68 mm. This layer was followed by 30.4 cm of sand with a size range of 0.59–1.19 mm. The top and primary sand filtration layer was 61 cm thick and had a size range from 0.42 to 0.50 mm. The space between the top of the sand filter and the inflow pipe provided the driving head to operate the filter by gravity. The inflow pipe contained several "spokes" containing holes to allow for the water to flow into the filter evenly during startup. A spillover pipe was also installed to maintain the head in the tank at a constant number. The hole in the top of the tank was sufficiently large to allow for manual cleaning of the tank when the control system provided an alert that the sand filter was clogged.

An organic and particulate layer formed at the water sand interface, which is termed the "schmutzdecke". A considerable amount of biochemical water treatment occurs in this layer, which tends to become a few centimeters thick. When the rate of water flow through the schmutzdecke became too slow, the filter had to be cleaned by removing this organic layer and replacing it with clean sand of the same size. The automated telemetry system provided an indication of when cleaning was necessary (see control section). The duration of operation before cleaning was dependent on the quality of the source water being filtered. In major water treatment plants using rivers or reservoirs, the cleaning time typically ranges from 1 to 3 months. The Caloosahatchee River water quality contains an extremely large quantity of organic material and turbidity, which caused cleaning in the early operational stages (test stage) every 20 to 23 days. After a month and a half of operation, it was necessary to clean the filter every 12 to 15 days.

2.4. Water Quality Sample Collection

A water quality sampling scheme was developed to assess the veracity of the water treatment technologies employed in comparison to the baseline system. Within the operating system, 16 locations were established to adequately monitor water quality to allow full technology evaluation. The sample locations are given in Table 2. There was some purposeful redundancy in the sampling because some organic material can accumulate within the plumbing system and could cause some variation in both the inflow water and in the transport of water between the wetland treatment tubs.

Table 2. Locations of the water quality samples collected with the used quality control assurance and tracking.

Station No.	Description	Sample No.	Sample Date	Sample Time
1	Control inflow to *Typha* tub			
2	Control outflow from *Typha* tub			
3	Control inflow to *Vallisneria* tub			
4	Control outflow from the *Vallisneria* tub			
5	Slow sand filter inflow			
6	Slow sand filter outflow			
7	Slow sand filter secondary holding tank			
8	Slow sand filter inflow to *Typha* tub			
9	Slow sand filter outflow from *Typha* tub			
10	Slow sand filter inflow to *Vallisneria* tub			
11	Slow sand filter outflow from *Vallisneria* tub			
12	Slow sand filter + UV treatment discharge			
13	Slow sand filter + UV treatment inflow to *Typha* tub			
14	Slow sand filter + UV treatment outflow from *Typha* tub			
15	Slow sand filter + UV treatment inflow to *Vallisneria* tub			
16	Slow sand filter + UV treatment outflow from *Vallisneria*			

The water quality of the inflow water from the Caloosahatchee River was measured at two locations, which are stations 1 and 5. Effects of the vegetation treatment on the Caloosahatchee River water as a control can be evaluated by a comparison of the data from stations 1 and 4. The treatment provided in the control train solely for the *Typha* tub were evaluated by comparing data from stations 1 and 2 and the tape grass tub by comparing data from stations 3 and 4. Variation in the water quality caused by growth in the pipe between the two vegetation tubs can be observed by comparing data from stations 3 and 4.

The impact on water quality from slow sand filtration can be assessed by comparing the data from stations 5 and 6. The full impact of slow sand filtration and vegetation treatment can be compared by assessing water quality changes between stations 5 and 11. Any water quality changes occurring in the holding tank (used for hydraulic flow balance) can be evaluated by comparing data from stations 6 and 7. Note that the holding tank was painted black to inhibit aquatic plant and biofilm growth. Any water quality changes caused by pipe transport between the holding tank and the slow sand filter *Typha* tub can be evaluated by assessing changes between stations 7 and 8. The effectiveness of water treatment by the slow sand filter *Typha* tub was evaluated by assessing changes between stations 8 and 9. Any impacts of water quality of the pipe connecting the slow sand filter water between the *Typha* and *Vallisneria* tub were evaluated by comparing data from stations 9 and 10. The treatment effects of the slow sand *Vallisneria* tub were evaluated by comparing data from stations 10 and 11.

The combined slow sand filtration and UV treatment with vegetation treatment were evaluated by comparing data from stations 5 and 16. The impacts of any connection pipe organic shedding between the combined slow sand filter and UV discharge and the *Typha* tub were evaluated by comparing the data from stations 12 and 13. The impacts of the *Typha* tub treatment for the slow sand filter and UV treatment were evaluated using a comparison between stations 13 and 14. Any pipe impacts to water quality between the wetland plant tubs for slow sand filter and UV-treated water were evaluated by comparing the data from stations 14 and 15. The treatment provided for the slow sand filter and UV treatment by the *Vallisneria* tub were evaluated by comparing the data from stations 5 and 15.

2.5. Water Quality Measurements and Laboratory Methods

A series of chemical parameters were measured in the field using meters during each of the sampling events, while water samples were collected for transportation to the laboratory for chemical analyses. The sampling methods followed a filed Quality Assurance Project Plan (QAPP) as approved by Lee County and the Florida Department of Environmental Protection (FDEP). The FDEP required that the laboratory conducting the primary analytical work on the samples was NELAC certified. Therefore, the samples were analyzed by Sanders Laboratories and their subcontractor Pace Analytical. These laboratories are certified and approved by the FDEP and have filed QAPP documents with the department. They follow the Standard Operating Procedures (SOPs) required by the FDEP.

The only analytical procedure performed at the Florida Gulf Coast University Emergent Technologies Institute laboratory was the quantification of the bacteria in the water using a flow cytometer. The SOP and description of the analytical methods used is described based on the research work of Harvey et al. [46]. While these samples were analyzed for seawater, the procedure was the same. The detailed methodology is given in the Supplementary Materials.

2.6. Pre-Sampling Planting and Establishment of the Mesocosm Vegetation

When the project was started, the vegetation in the three tubs that utilized emergent vegetation (i.e., tubs 8, 9, and 10) already contained *Typha domingensis* (cattail) as well as some *Schoenoplectus californicus* (giant bullrush) and some other sedges and grasses such as the invasive plant *Panicum repens* (torpedo grass, Figure S12). All tubs also contained a significant amount of phytodetritus, some of which had turned into a dark organic sediment overlying a layer about 30.5 cm thick of sand sourced from the property.

From 12 July 2021 to 15 July 2021, the tanks were cleaned of their vegetation and phytodetritus and sediment were removed (Figure S12). This was accomplished by first unrooting the vegetation by hand and hand tools while taking care of preserving the roots of *Typha* sp. and *S. californicus*. The least severely damaged individuals of those two species were kept in a horse trough filled with water until they could be replanted (Figure S13). The heads of *T. domingensis* were removed with a machete while special attention was taken to limit the damage to the rhizomes (Figure S13). The removal of the phytodetritus and the sediment was conducted after the tanks were drained overnight. These materials were allowed to dewater and cake on top of the sand, so that they could be removed from each tub. On average, about 6 cart loads (approximately 1.7 m^3) were removed from each tub (about 18 to 20 cm of sediment + detritus accumulation in each tub). The planting then occurred from 16 July 2021 to 17 July 2021 (Figure S13) by splitting the amount of *T. domingensis* (about 195 individuals with about 65 transplants per tub) and *S. californicus* (about 300 individuals with 100 transplants per tub) amongst the three tubs. Plants were thus about 25 to 30.5 cm from one another to achieve a plant density of about one plant per 929 cm^2. The water level in these tubs was then set at about 30.5 cm above the surface of the sand substrate and the plants were randomly planted to occupy the entire surface of each tub. The plants were then allowed to grow for approximately two months using untreated river water flowing through the tubs in and out.

The three tubs selected for the submerged vegetation (tubs 1, 2, and 7) contained either emergent rooted vegetation as aforementioned in tubs 8, 9, and 10 (albeit with more undesirable vegetation) or a mixture of mostly macroalgae (*Chara* sp., muskgrass or stonewort) with some invasive *Hydrilla verticillata* (waterthyme) as well as a mixture of filamentous green algae with the dominant alga being *Cladophora* sp. These tanks were cleaned similarly to the other tanks, and the planting of *Vallisneria americana* (tape grass or eel grass) was accomplished between 16 July 2021 and 17 July 2021. These shoots originated from a donor detention pond in Cape Coral, Florida, and they were left acclimating in tanks under an 80% canopy at the FGCU Buckingham property. Plants were planted by hand in the sandy substratum at every 13 to 15 cm (about 4 plants per 0.093 m^2) so that each tub was planted with about 400 plants. The water level was set in the submergent tubs at about 91.4 cm above the soil. The tape grass was fed with river water for about four months before treatment was initiated.

For all the tubs, before the experimental treatment system was turned on, all tanks were inspected and almost all undesirable algae and vegetation were removed. The environmental conditions in the tanks were in very good condition at the start of the experiment (Figure S13).

2.7. Monitoring of the Mesocosm Vegetation

The vegetation was monitored during each sampling of water quality and during cleaning of the slow sand filter. Floating vegetation (i.e., *Lemna minor* (duckweed) and the fern *Azolla* sp.) was netted out of all tubs after the first event only, and it was conducted in all submerged vegetation tubs for all other events. The tubs with submerged vegetation were also cleaned from encroaching green filamentous algae (mainly *Cladophora* sp.) using nets and by gentle raking. This green alga grew in abundance as metaphyton (a floating mat) as well as epiphyton (attached on the *V. americana*) within all tanks and especially in the control tank. It interfered with the light source in the submergent vegetation tanks. During the months of March through the end of the experiment, the tubs were covered with a tan shade cloth (light blocking of approximately 50%) to limit the growth of the green filamentous algae. Additionally, some sparse stands of *H. verticillata* appeared, but those were left in place as submergent plants.

Drone surveys using a DJI™ phantom 4 Pro were conducted during all water sampling events, and photographs of each tub were taken over time to show the condition of the vegetation. To cut through the glare of the water surface in the tubs, the camera of the drone was covered with a polarized lens. These photographs were taken after the water was sampled and were ideally taken when the sun was at its zenithal position. However, for some events, these photographs were taken in the middle of the afternoon with less ideal lighting. Special care was taken to have the drone positioned on the apex of each tub with the drone stationary at about 3.1 m above it. At the office, each photograph was rotated to orient it horizontally, and it was cropped to show the border of each tub only. Attempts to enumerate *V. americana* were in vain as the water contained tannic acid (tea color) and only the plants close to the surface could be accounted for. Passing the hand above the bottom of each tank confirmed that many *V. americana* could not be accounted for using the drone. This issue was, however, not a real problem for accounting the emergent vegetations, which could be well accounted for at the beginning of the experiment as well as at the end especially in the absence of wind. For this enumeration, photographs were contrasted in PowerPoint and then marked with a digital pen. For *T. domingensis*, a shoot with several leaves would count as an individual, whilst for *S. californicus*, each stem was counted if they were not visually too clustered spatially. This would not replace an actual count in situ, but this surrogate method gave a fair and pretty consistent assessment of the plant expansion (Figures S14 and S15). Notes accounting for the extent of floating vegetation and algae as well as the number inflorescences for *T. domingensis* were also recorded.

2.8. Statistical Methods

It is essential to perform a statistical analysis at a meaningful abstraction level to find interesting patterns and to determine whether the results of the dataset comparisons were statistically significant [47]. Three treatment trains that were considered in this study include (1) Treatment Train A (TTA)—Control: Raw Water/Vegetation Tank 1 out (Typha)/Vegetation Tank 2 Out (*Vallisneria*), (2) Treatment Train B (TTB): Raw Water/Sand Filter In/Sand Filter Out/Vegetation Tank 1 Out (Typha)/Vegetation Tank 2 Out (*Vallisneria*), and (3) Treatment Train C (TTC): Raw Water/After Sand Filtration/After UV/Vegetation Tank 1 out (Typha)/Vegetation Tank 2 Out (Vallisneria). Raw water quality parameters were measured for each treatment train.

The inflow to TTA was directly connected to the control *Typha* tub, while the inflow for the TTB and TTC was connected to the slow sand filtration system. The Shapiro–Wilk test was performed on the data to ascertain normality. The results showed that data were normally distributed ($p > 0.05$), so parametric statistical analyses, such as *t*-tests and ANOVA, are required. If the data were non-normal, a non-parametric test, such as Kruskal–Wallis, would be required.

A two-sample *t*-test was performed to compare water quality parameters in the inflow to TTA and TTB/C. In addition, a two-sample *t*-test was performed to compare water quality parameters within each treatment train. The two-sample *t*-test is used to determine if the means of two groups are equal. A one-way ANOVA was performed to compare three treatment trains for 12 key water quality parameters. A one-way ANOVA compares the means of two or more independent groups to determine whether there is statistical evidence that the associated group means are significantly different.

3. Results

An extremely large quantity of data were collected during this investigation. Hence, only plots of critical data associated with the research questions being investigated are included in the paper text. However, plots of all remaining data are included in the Supplementary Materials and the figures therein are designated with the "S" prefix.

3.1. Sand Filter Treatment Effectiveness Independent of the Vegetation Tubs

Data were collected from stations 5 and 6 to evaluate the treatment provided by the slow sand filter. Station 5 was the raw river water from the main storage tank onsite. The samples were collected from the inflow to the sand filter at the top. Station 6 was located at the sand filter outflow at the outflow valve.

In most cases, the total nitrogen was lower after sand filtration, and there was also a reduction in the organic nitrogen (Figure S16). The box plots in Figure 4 show the same trend with a slight lowering of total nitrogen and organic nitrogen provided by sand filtration. It should be noted that some of the outlier measurements do impact the box plot full ranges in concentrations.

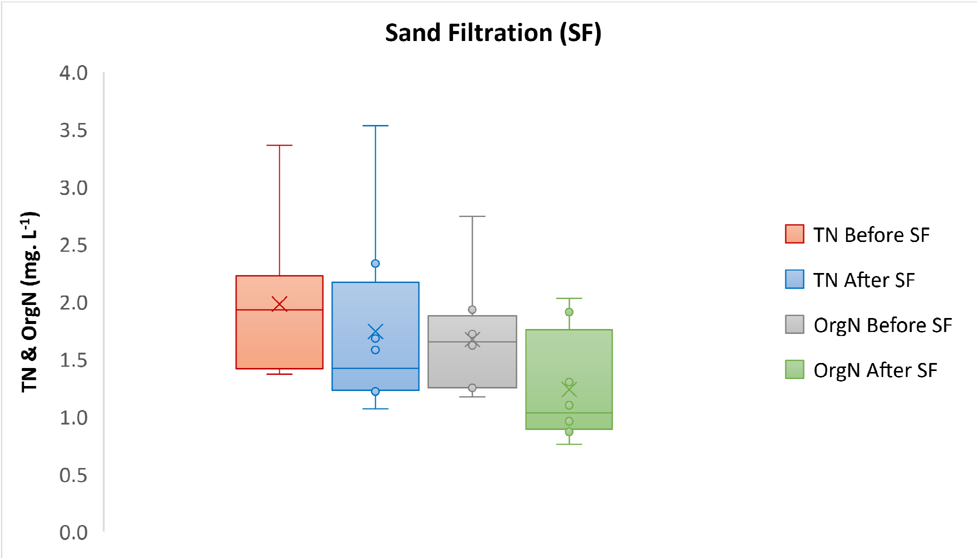

Figure 4. Box plots of total (TN) and organic nitrogen (OrgN) before and after sand filtration (SF).

Nitrate and nitrite concentrations increased during sand filtration, but ammonia decreased (Figures 5 and S17). There is considerable scatter in the data obtained. Based on the reductions in total and organic nitrogen, it appears that the increase in nitrate and nitrite concentration occurred based on the breakdown of ammonium, suggesting that there may have been nitrification occurring within the sand matrix.

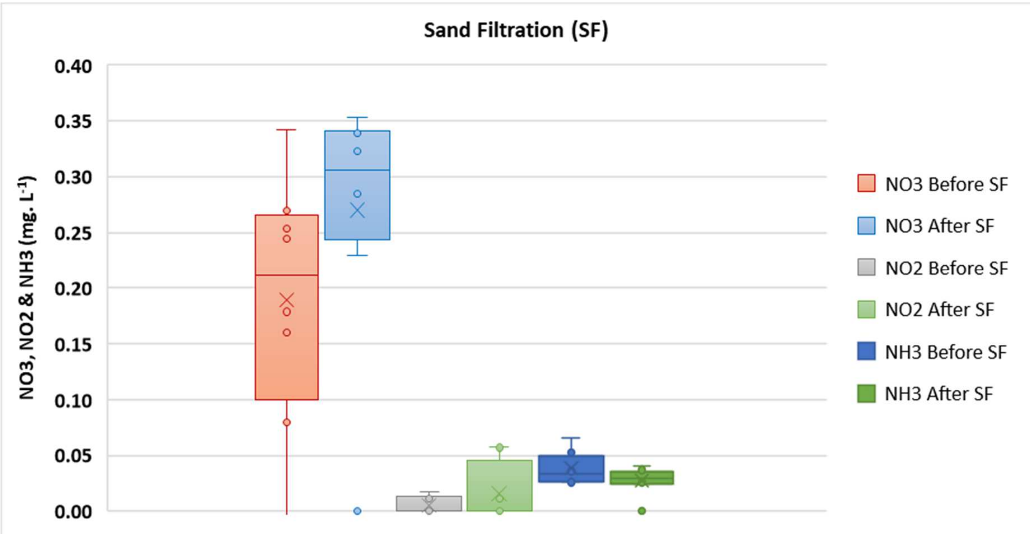

Figure 5. Box plots of nitrate (NO_3), nitrite (NO_2), and ammonia (NH_3) before and after sand filtration (SF).

Changes in the concentrations of total phosphorus and orthophosphate are shown as a temporal variation plot in Figure S18 and as a box plot in Figure 6. In the temporal plot, there is considerable scatter in the data. The box plot shows an increase in the concentrations of both total phosphorus and orthophosphate during sand filtration. There were outlier measurements that somewhat impacted the analysis.

Both total and dissolved organic carbon concentrations decreased during slow sand filtration (Figures S19 and S20). The scale of the temporal changes does not clearly show the reductions, but the box plots clearly show them.

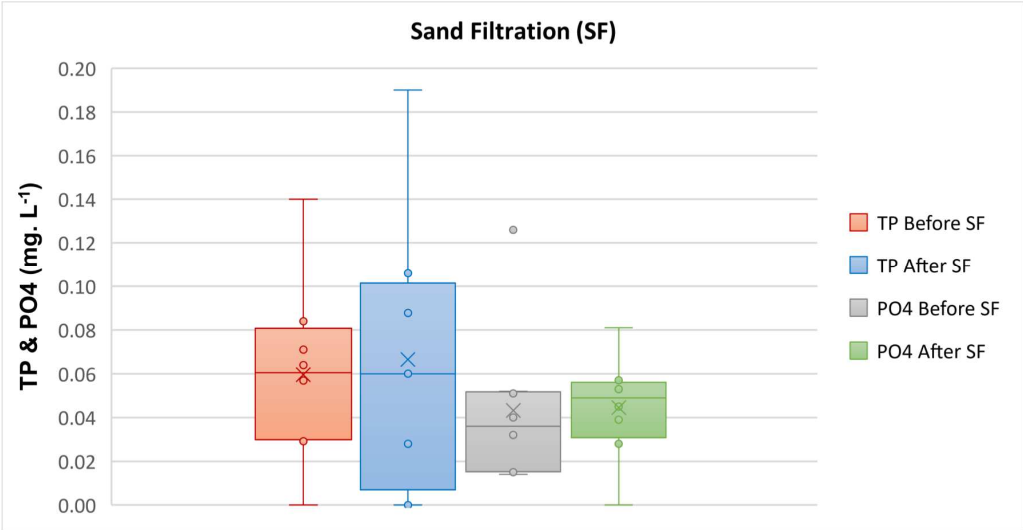

Figure 6. Box plots of total phosphorus (TP) and orthophosphate (PO_4) before and after slow sand filtration (SF).

The concentration changes in chlorophyll A before and after slow sand filtration are shown in Figures S21 and S22. In this case, both laboratory and field measurements were made and showed differing results. The field instrument data showed a slight decrease in chlorophyll A concentration, while the laboratory data exhibited a major reduction. The laboratory data are supported by observations during operation where the top of the filter required the removal of an organic crust every 13 to 23 days. Much of this material was organic debris dominated by living algal and bacterial material.

Both the temporal and box plot data show substantial reductions in the concentration of total bacteria, algae, and cyanobacteria during slow sand filtration (Figures S23 and S24). It is particularly interesting that some concentrations of total bacteria, algae, and cyanobacteria did manage to pass through the sand filtration. The percentage of breakthrough was total bacteria > algae > cyanobacteria.

Temporal plots of actual conductivity, specific conductivity, total dissolved solids (TDS), and turbidity are shown in Figure S25. Box plots of actual and specific conductivity are shown in Figure S26 with box plots of TDS and turbidity shown in Figure S27. The temporal data and box plots of the conductivity and the TDS have some temporal scatter, but the box plots show that expectedly little variation occurred through the sand filter. Turbidity was greatly reduced by the sand filtration, which was supported by the required number of cleanings of the filter (Figure S27).

Temporal and box plot variations in the real oxygen concentration and real oxygen saturation showed a reduction across the sand filter (Figures S28 and S29). Reduction in dissolved oxygen was expected based on the very high concentration of biochemically active organic matter in the water. The dissolved oxygen changed from 60% at the influx to 50% during the sampling period.

Water temperature was nearly constant across the sand filter (Figures S30 and S31). Temporal variations in oxidation–reduction potential (ORP) and pH measured before and after sand filtration show minimal variation in most of the measurements, but some outlier values were obtained (Figure S32). The box plot for these data shows minimal variation across the sand filter (Figure S33).

3.2. Treatment of Organic Nitrogen Using UV Treatment Independent of Slow Sand Filtration and the Vegetation Tubs

Temporal variation in the total and organic nitrogen before and after UV treatment shows considerable scatter, and in many cases little variation (Figure S34). Box plots for the total nitrogen and organic nitrogen before and after UV treatment confirm that the UV process is not effectively breaking down the organic nitrogen (Figure 7).

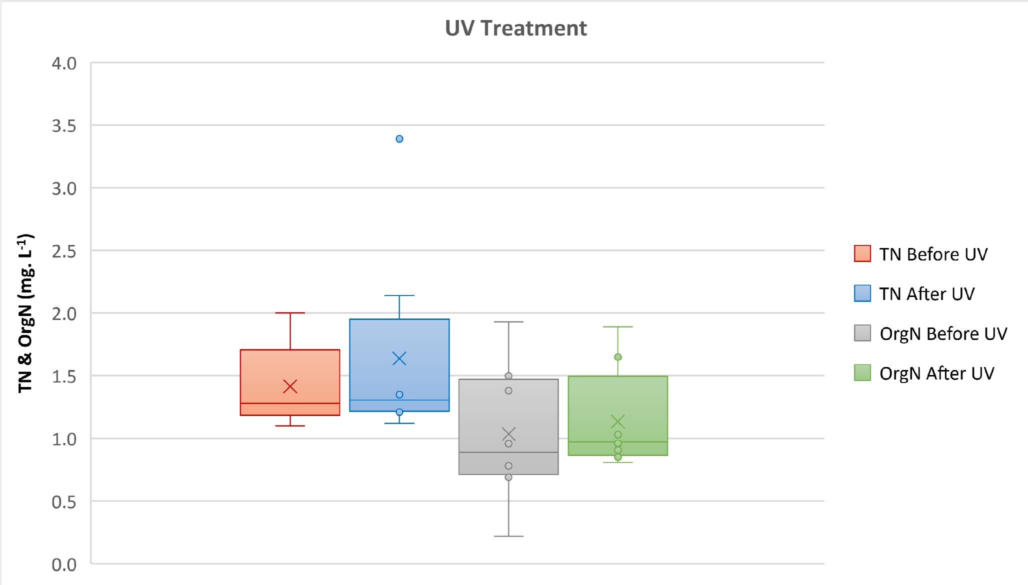

Figure 7. Box plot for variation in total (TN) and organic nitrogen (OrgN) before and after UV treatment.

UV treatment had a minimal impact on the other parameters measured during the investigation. Plots of all parameters are given in Figures S35–S53. One surprise was that the UV had little impact on the concentrations of total bacteria, algae, and cyanobacteria. A stronger UV light combined with a longer contact time could have led to a different outcome.

3.3. Effectiveness of Water Treatment of the Emergent and Submergent Vegetation in Train A (Control Train)

The changes in water quality and other parameters were measured to assess the water treatment effectiveness of the aggregated emergent (*Typha*) and submergent (*Vallisneria*) vegetation. This was achieved by comparing the data between stations 1 and 4, which is the base case condition or control. The raw water from the river enters the first tank at station 1 (*Typha*) and leaves the last tank (*Vallisneria* sp., tape grass) at station 4.

The temporal and box plot data for total and organic nitrogen show major reductions in concentrations from the vegetation (Figures 8 and S54). There is some scatter in the data, but the overall pattern is distinctive.

Temporal and box plot concentrations of nitrate and ammonia show major reductions between the river water at station 1 and the discharge from tank 4 (Figures S55 and S56). The concentration of nitrite was so small that any real change in concentration was not significant. In many sample events, nitrite concentrations were below detection limits.

Like the nitrate and ammonia removal, the vegetation treatment in the control train showed a high removal of both total phosphorus and orthophosphate (Figures S57 and S58). If the single outlier concentration was removed from the phosphate data, the percentage of removal would be even higher.

Measured concentrations of TOC and DOC before and after vegetation treatment show some reductions in each case (Figures S59 and S60). However, the statistical significance of these changes is reported in a later part of the report. There are some significant outliers in the data, which may influence the changes in concentration (Figure S59).

The vegetation treatment produced significant reductions in chlorophyll A in both the field measurements (meter) and in the laboratory measurements (Figures S61 and S62).

Some scatter in the temporal data can be observed (Figure S61), but the box plot clearly illustrates the reduction (Figure S62).

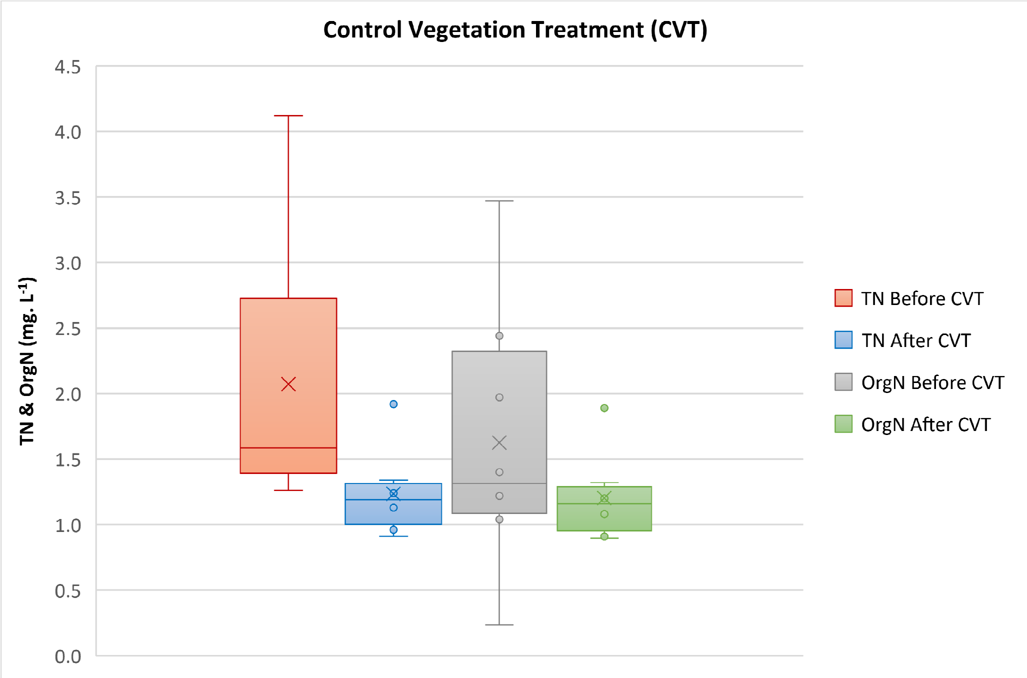

Figure 8. Box plots of total and organic nitrogen concentrations before and after passage through both vegetation tanks in the control train.

The temporal and box plots of the total bacteria, algae, and cyanobacteria before and after the vegetation treatment show reductions in all three parameters (Figures S63 and S64). There are some outliers in the data as clearly shown in the box plots (Figure S64).

The evaluation of changes in conductivity, TDS, and turbidity in the control vegetation train before and after treatment showed that actual and specific conductivity and TDS did not change significantly (Figures S65 and S66). The scatter in the meter data did produce some variation based on observations of the box plot. The turbidity was reduced significantly based on the box plot of before and after vegetation treatment (Figure S67).

Based on the meter data collected in the field, the dissolved oxygen concentration and saturation increased during vegetation treatment (Figures S68 and S69). The saturation changes were dramatic from about 59% to 99% (Figure S69). Note that the measurements were made during daylight hours and no data showing nychthermal variations are available.

A minor water temperature reduction was observed before and after vegetation treatment in the control chain (Figures S70 and S71). The change was a few tenths of a degree Celsius, which is not believed to have significance.

Oxidation–reduction potential declined during the vegetation treatment in the control chain (Figures S72 and S73). In contrast, the pH significantly increased during the vegetation treatment, which is best illustrated in the box plot (Figure S73).

3.4. Full Treatment Train A Analysis (Control): Raw Water/Vegetation Tank 1 Out (Typha)/Vegetation Tank 2 Out (Vallisneria sp., Tape Grass)

The control case is based on the influx of raw water with only vegetative treatment. Box plots were used to assess the changes in various parameters during treatment. These analyses were used in comparison to trains B and C to assess the overall effectiveness of the engineered solution versus solely vegetation.

Impacts of the vegetation treatment types on concentrations of total and organic nitrogen based on the median values were reduced by both the *Typha* and *Vallisneria* tubs

(Figure S74). Organic nitrogen was reduced during both vegetation treatment processes with an overall reduction of about 25% and the total organic nitrogen was reduced by about 7.5%.

The reduction in nitrate, nitrite, and ammonia concentrations was greater compared to organic and total nitrogen (Figure S75). The mean discharge of nitrate was reduced from 0.23 mg.L^{-1} to near zero. Nitrite was low at the beginning and was close to zero at the discharge. Ammonia was reduced by 37.5%.

Reduction in total phosphorus and phosphate exhibited a similar pattern to the nitrogen nutrients. However, the tub containing *Typha* exhibited a greater reduction than the reduction occurring in the *Vallisneria* tub. The overall reduction in concentrations of P and PO4 from the river water to the discharge were 82.6 and about 90%, respectively (Figure S76).

Total and dissolved organic carbon showed some net reduction between the inflow water and discharge water, which occurred primarily in the *Vallisneria* tub (Figure S77). The reductions were $\leq$1% and 3.7%, respectively.

Based on the laboratory measures of chlorophyll A, both vegetation tubs produced significant reduction. The overall reduction between the inflow and outflow water was about 90%.

Total bacteria, algae, and cyanobacteria were reduced in both vegetation tanks. A few outlier values measured for total bacteria in the inflow water masked the effects of the *Typha* tub reduction. The overall reductions measured between the inflow and outflow waters using the mean values were about 50, 90, and 90% (Figure S79).

The vegetation in the tanks did not significantly impact the actual conductivity, specific conductance, and the TDS concentrations (Figures S80 and S81). A slight lowering of the conductivity values was likely caused by rainfall events during the year. Turbidity showed a large reduction of about 50% (Figure S81).

Dissolved oxygen concentration and saturation showed an interesting relation in treatment train A. The mean dissolved oxygen concentration and saturation values were similar in the raw water and the discharge water but increased by 40% within the *Vallisneria* tub (Figure S82). Water temperature showed a slight decline between the inflow water and as it passed through the two tubs (Figure S83).

The oxidation–reduction potential (ORP) was lower in the vegetation treatment tubs compared to the raw water (Figure S84). The change between the raw water and the discharge of the *Typha* tub was small but was greater between the *Typha* and *Vallisneria* tubs.

Variation in pH from the raw river water to the discharge of the *Typha* tank showed minimal variation, but a significant rise in the mean occurred between the Typha tank and the discharge of the *Vallisnera* tank (Figure S84). The mean pH rose from 7.4 to 8.3.

3.5. Full Treatment Train B Analyses: Raw Water/Sand Filter in/Sand Filter Out/Vegetation Tank 1 Out (Typha)/Vegetation Tank 2 Out (Vallisneria)

Train B contained the engineered enhancement of the vegetative treatment of the river water, which was slow sand filtration. The approximate flow rate through the slow sand filter was about 11.4 L/min to produce a contact time of about five hours. A spillover at the top of the filter maintained 30.5 cm of driving head. Box plots were used to evaluate the induced changes in water quality.

Total nitrogen concentration was significantly reduced by slow sand filtration (Figure S85). No change occurred in the *Typha* tub and a small decrease was observed in the *Vallisneria* tub. Based on a comparison of the mean values, the sand filter reduced the organic nitrogen concentration by about 40%. The two vegetation tubs added some organic nitrogen back into the water, but overall, the exit concentration was lower than the inflow concentration.

Nitrate and ammonia concentrations followed a similar pattern, with an increase from the raw water to the sand filter discharge to some reduction in the *Typha* tub to a very strong reduction in the *Vallisneria* tub (Figure S86). The mean values of nitrate and ammonia were

0 and 0.2 mg·L^{-1} discharge at the final discharge. The nitrite values are not meaningful based on their very low concentrations with many values falling below detection limits.

Slow sand filtration had no impact on total phosphorus concentration, but the vegetation treatment was effective at removing it (Figure S87). Total phosphorus removed by the *Typha* and *Vallisneria* tubs was about equal, resulting in the discharge concentration of <0.1 mg·L^{-1}. The pattern of changes in orthophosphate concentrations was different. The sand filter discharge produced a higher concentration compared to the raw water, while the *Typha* tub produced a reduction of about 28% comparing the inflow to the outflow and the *Vallisneria* tub lowered the concentration by about 80%.

Both TOC and DOC were reduced to a degree by slow sand filtration but only between 1 and 2 mg·L^{-1}, which is a small part of the river water concentration which had a mean value of about 15.4 mg.L^{-1} (Figure S78). The two vegetation treatment tubs had little impact on the concentration.

Chlorophyll A was effectively removed by slow sand filtration as demonstrated in both the field meter and laboratory-analyzed measurements (Figure S88). However, some chlorophyll A was added back into the water by the *Typha* and *Vallisneria* vegetation tubs. The laboratory-analyzed chlorophyll A values (Figure S89) appear to add back rather large amounts, but a close look at the mean values shows that the outlier values greatly impact the box size.

Total bacteria, algae, and cyanobacteria were effectively removed by slow sand filtration (Figure S90). A slight increase in all parameters occurred in the *Typha* tub and a slight reduction followed in the *Vallisneria* tub. A comparison of the inflow to outflow shows that the overall treatment for all three was effective with reductions ranging from 93 to 98%.

The processes in treatment train B did not affect the conductivity values nor the TDS concentrations (Figures S91 and S92). The variations observed were likely caused by instrument drift and small changes in temperature. Turbidity removal was quite effective in the sand filter, but some turbidity was added in the *Typha* tub discharge. Turbidity values were reduced in the discharge of the *Vallisneria* tub. By comparison of the mean values, inflow turbidity was reduced by about 80% in the treatment train.

Dissolved oxygen concentrations and saturation decreased in the sand filter (Figure S93). Then, both the concentration of the oxygen and the saturation percentage increased in both vegetation tubs. The most extreme increase occurred in the *Vallisneria* tub where the mean value was very close to saturation and many of the temporal values were above saturation.

Changes in water temperature in treatment train B were only a few tenths of a degree Celsius (Figure S94). It cooled slightly in the sand filter and both the vegetation tubs which were shaded to a degree.

The median of the oxidation–reduction potential (ORP) increased slightly through the slow sand filter and subsequently decreased slightly in the *Typha* tub (Figure S95). It decreased further in the *Vallisneria* tub to a greater degree compared to the lowering in the *Typha* tub.

Field measurements of pH show that sand filtration did not change it very much, and the pH stayed near 7.5 or slightly alkaline (Figure S96). There was a slight lowering of the mean in the *Typha* tub to about 7.4, and then a substantial increase in the *Vallisneria* tub to a mean near 8.7.

3.6. Full Treatment Train C Analyses: Raw Water/after Sand Filtration/after UV/Vegetation Tank 1 Out (Typha)/Vegetation Tank 2 Out (Vallisneria)

In train C, the sequence of the full process is raw water/after slow sand filtration/after UV treatment/after emergent vegetation tub (*Typha*) and at the discharge of the submergent vegetation tub (*Vallisneria*). One of the primary objectives of the study was to evaluate whether the full process train would reduce organic nitrogen concentrations, particularly the UV exposure which had the potential of breaking down the organic nitrogen molecules. Figure 9 shows total and organic nitrogen through the entire process. Slow sand filtration reduced the total and organic nitrogen by about 25 and 40%, respectively. The UV process, comparing inflow to outflow, reduced the concentrations of total and organic nitrogen by about 7 and 9%, respectively. The mean after the *Typha* tub appeared to have risen slightly, which may be a function of a single outlier point. The largest reduction in organic nitrogen was achieved in the sand filtration process with minor changes in the UV and vegetation treatment processes.

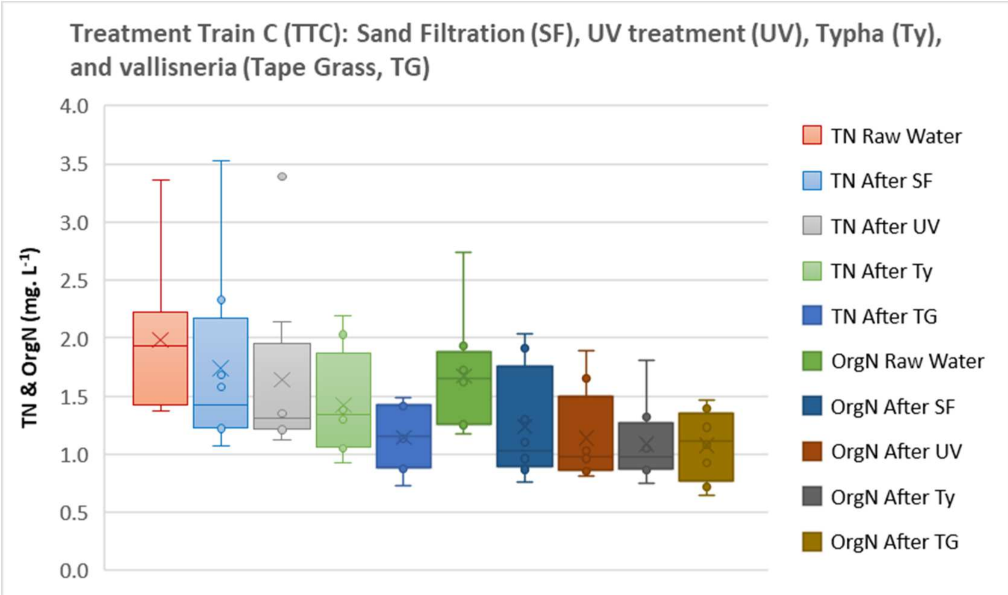

Figure 9. Comparison of effectiveness of the full process train C on concentrations of total and organic nitrogen.

Process train C had mixed results on nitrate, nitrite, and ammonia reduction (Figure S97). Total nitrogen concentration during the slow sand filtration process increased above the raw water concentration. The UV process had no impact on concentration. The two vegetation treatment tanks showed a significant uptake of nitrate with the last tank (*Vallisneria* tub) being the most significant with an 89% reduction. Nitrite is not a significant parameter, occurs at low concentrations, and was not included in Figure S97. The ammonia concentrations showed a reduction during sand filtration and UV, with a slight increase after the *Typha* tub and a final lowering in the *Vallisneria* tank.

Removal of total phosphorus and orthophosphate showed a similar pattern (Figure S98). Total phosphorus concentration remained rather constant through the first four processes and showed a significant drop in the last process, which was the *Vallisneria* tub. Orthophosphate increased slightly during sand filtration, stayed constant through UV treatment, declined slightly in the *Typha* tub, and declined most significantly in the *Vallisneria* tub. A comparison of the initial raw water concentration means of total phosphorus and orthophosphate are at about 0.06 and 0.02 mg·L^{-1}, respectively, and the final means of about 0.02 and 0.015 mg·L^{-1} show reductions of 67 and 25%.

Based on the box plot comparisons of TOC and DOC, concentrations through treatment train C show a narrow range of mean values indicative of minimal treatment (Figure S99). The slow sand filtration did remove some TOC and DOC, but the UV and vegetation treatment processes did not greatly change the concentrations, and the vegetation treatment tanks contributed slightly increased concentrations.

Changes in the field meter and laboratory-analyzed values for chlorophyll A show a dramatic reduction of about 93% from the raw water through the sand filtration process (Figure S100). The UV process showed a minor reduction. The chlorophyll A values increased in the vegetation treatment processes. However, the reduction of the means in the laboratory chlorophyll A data from above 21 RFU to near 2 RFU is a significant reduction.

Concentrations of total bacteria, algae, and cyanobacteria in treatment train C showed that the sand filtration was very effective at the removal of all microbes at 87, 95, and

98% respectively (Figure S101). Additional concentration reduction does occur in the UV process to bring the values down to near zero. The two vegetation tubs did not significantly change the counts but did add some microbes.

As expected, the treatment occurring in train C did not have significant impacts on the conductivity values and TDS concentrations (Figures S102 and S103). The turbidity showed some rather odd trends in that the slow sand filtration process removed most of the turbidity, but in each subsequent process, it increased until at the final discharge it was above the raw water (Figure S103). This was likely caused by the buildup of organic material in the tank discharge pipes and is not a true analysis of the turbidity removal. The median value after treatment is still below the raw water mean and 25th percentile, indicating an upward skew from outliers.

Field measurements of the dissolved oxygen concentration and percentage of saturation show that the slow sand filter lowered the values as expected (Figure S104). The UV did not have any significant effect, but each vegetation treatment tub added oxygen, with the *Vallisneria* tub increasing it to above saturation.

Water temperature varied little (<1 °C) throughout treatment train C (Figure S105). Comparison of the mean values shows some cooling in the vegetation tanks but not of significance.

The oxidation–reduction potential (ORP) varied through treatment train C (Figure S106). The raw water and sand filter discharge were nearly equal followed by a minor reduction after UV treatment. It increased in the *Typha* tub and then reduced by about 15% in the *Vallisneria* tub based on the difference between the inflow and outflow.

The pH stayed in a very narrow range centered near 7.5 from the raw water through sand filtration and UV treatment (Figure S107). It increased slightly in the *Typha* tub and then increased greatly in the *Vallisneria* tub due to photosynthesis.

3.7. UV Treatment Impacts on Water Quality Parameters Other Than Total and Organic Nitrogen

The UV treatment process had significant effects on only one other parameter other than organic and total nitrogen concentration (Figures S108–S126). After the removal of most of the algae, total bacteria, and cyanobacteria by the slow sand filtration process, treatment with UV removed most of the remaining concentrations. There was some variation in other parameters measured but these are believed to be associated with outlier measurements.

3.8. Impacts of the Holding Tank on Water Quality

It was necessary to install a holding tank after the slow sand filter treatment to provide a gravity flow balance in part of the system. Although the tank was painted black to reduce biochemical activity within the temporarily stored water, inevitably, some water quality impacts had to occur. A detailed series of graphics are given in the Supplementary Materials to show the details of the changes in water quality observed (Figures S127–S147). Since the tank is not a significant part of the water treatment system, it is not discussed in detail. Based on the sampling of the before and after water quality data collected, the holding tank had the following impacts on water quality: (1) slight decreases in total and organic nitrogen concentration occurred, (2) no significant changes occurred to nitrate, nitrite, and ammonia concentrations, (3) total phosphorus concentration increased slightly and orthophosphate concentration stayed the same, (4) TOC and DOC concentrations showed no change, (5) measurements of chlorophyll A by field instrument and laboratory analysis showed no significant variation, (6) total bacteria concentration declined slightly and algae and cyanobacteria concentration showed little change, (7) conductivity values and TDS concentrations were unchanged, (8) turbidity showed a very minor increase, (9) the dissolved oxygen concentration declined slightly and the saturation increased from about 5 to 45%, (10) water temperature remained constant, (11) the mean oxidation–reduction potential declined from about 250 to 220 mV, and the pH mean declined from about 7.48 to 7.38.

3.9. Impacts on Water Quality Caused by the Piping System

The piping that connects the various processes on the site is schedule 40-, one- and two-inch diameter, white-colored PVC pipe. It was observed during sampling that if the connecting piping was stepped on or jarred, the water would become turbid at the entry point into a treatment process. There are four possible explanations for possible pipe impacts which are as follows: (1) organic matter formed a biofilm on the inside of the pipe based on the high organic composition of the water, (2) the possible charge of the pipe may impact the deposition of organic material, (3) the low flow rate prevents organic buildup scouring, and (4) there is some light penetration through the pipe that promotes initial organic biofilm growth with enhancement from the raw river water. The biofilm formation may be caused by all four issues. It should be noted that the high concentrations of total bacteria, algae, and cyanobacteria in the water make it likely that transparent exopolymer particles (TEP) are also abundant in the raw water. TEP is composed of acidic polysaccharides, which are gels and quite sticky. This substance could form the base of the biofilm to promote growth in thickness and at the same time provide food for some of the living bacteria. Periodic vapor locks in the system also tended to mobilize some of the biofilm by sluff off.

The section of pipe chosen for analysis connected the holding tank to the *Typha* tub or connected sampling stations 7 and 8. To assess the impact of the biofilm within the transmission pipe, several affected parameters were measured to evaluate the impact on water quality.

Data collected show that there are small increases in the total and organic nitrogen added in the pipeline between the two points (Figures S148 and S149). The total nitrogen mean is affected by an outlier (high concentration), so the change is probably less than indicated by comparison of the means.

Changes in nitrate, nitrite, and ammonia concentrations in the pipeline were minimal (Figures S150 and S151). A small reduction in nitrate mean can be observed in Figure S151.

The concentration of the total phosphorus was lowered to a significant degree in this segment of the pipeline (Figure S152). The orthophosphate concentration showed no significant changes.

There were no significant changes in the concentrations of total and dissolved carbon and the graphics for this comparison are in Figures S153 and S154. Water transport in the pipeline between stations 7 and 8 also significantly affected the chlorophyll A values and total bacteria, algae, and cyanobacteria concentrations based on comparisons of the mean values (Figures S155–S158).

Transport of the water through the pipeline did not affect the real conductivity and specific conductivity values and the TDS concentrations (Figures S159–S161). However, there was a slight decrease in the turbidity (Figure S161).

The dissolved oxygen concentration and percentage of saturation increased in the pipeline between stations 7 and 8 (Figures S162 and S163). The water temperature did not change significantly between stations 7 and 8 (Figures S164 and S165). The oxidation–reduction potential rose significantly, and the pH rose slightly through the pipeline connecting stations 7 and 8 (Figures S166 and S167).

3.10. Biomass Removed during Cleaning of the Schmutzdecke on the Surface of the Slow Sand Filter

Based on observations made in the field while cleaning the surface of the slow sand filter, it is estimated that between 8 and 12 kg of organic matter were removed during each cleaning. The amount of organic carbon removed was based on the time between cleaning and the TOC concentration in the river water. Approximately 15 cleaning events occurred during the project duration, thereby removing between 120 and 180 kg of organic carbon. The flow rate through the slow sand filter was about 11.4 L/min. This illustrates how poor the quality of the river water is in terms of treatment difficulty.

3.11. Change in the Timing of Sampling Events and Loss of the Last Two Events

The original experimental design included 12 monthly sampling events after the system was installed and tested. Two issues impacted the ability to complete the experimental work as described in Section 2. First, the heavy load of organic carbon from the Caloosahatchee River necessitated more frequent cleaning of the slow sand filter than anticipated, so the monthly duration between samplings was decreased to every two weeks toward the end of the experiment. The last two sampling events were lost when lightning struck the transformer and destroyed the pump and connecting electric line. There was no budget to replace the feed pump.

3.12. Statistical Analysis of the Data

The raw water quality parameters were measured for each of the treatment trains. The inflow to TTA is directly connected to the control *Typha* tub while the inflow for the TTB and TTC is connected to slow sand filtration system. A two-sample *t*-test was performed to compare raw water quality parameters in the inflow to TTA and TTB/C. The results in Table 3 show there was not a significant difference in raw water quality between inflow to TTA and TTB/C. In addition, a two-sample *t*-test was performed to compare water quality parameters within each treatment train. The result shows there was a significant improvement in water quality; however, there was small improvement shown in NO2, NH3, and OrgN. A one-way ANOVA was performed to investigate the difference in treatment trains for 12 key water quality parameters. The difference between the outflow and inflow water quality is used to analyze the statistical difference between the three treatment trains. The one-way ANOVA revealed that the differences between the means of the treatment trains for most of the water quality parameters are not statistically significant (Table 3).

Table 3. ANOVA and *t*-test results for comparison of measurement parameters.

Parameter	*t*-Test Result for the Comparison of Raw Water to TTA and TTB/C	*t*-Test Result for the Comparison of Raw Water and Treated Water			ANOVA Test Result for Comparison of TTA, TTB, and TTC	
		TTA	TTB	TTC		
	p-Value	*p*-Value	*p*-Value	*p*-Value	F	*p*-Value
Turbidity	0.679	0.020 **	0.002 **	0.978	1.908	0.173
TN	0.829	0.039 **	0.098*	0.005 **	0.555	0.0582
NO_2	0.921	0.101	0.320	0.631	0.179	0.837
NO_3	0.833	0.001 **	<0.001 **	0.004 **	0.092	0.913
NH_3	0.360	0.252	0.954	0.840	1.219	0.316
OrgN	0.909	0.268	0.136	0.012 **	0.328	0.724
TP	0.056 *	0.055	0.018 **	0.170	2.598	0.098
PO_4	0.421	0.019 **	0.039 **	0.355	0.986	0.390
Chl A (Field)	0.904	0.017 **	0.075 *	0.006 **	0.369	0.696
Chl A (Lab)	<0.001 **	0.031 **	<0.001 **	<0.001 **	0.006	0.994
Total Bacteria	0.528	0.530	0.027	0.018	1.835	0.185
Algae	0.602	0.299	0.044	0.030	0.913	0.418
Cyanobacteria	0.679	0.343	0.067	0.048	0.471	0.631

Notes: * $p < 0.1$, ** $p < 0.05$.

4. Discussion

4.1. Raw Water Quality from the Caloosahatchee River

The water quality is quite poor in terms of TOC and DOC load. This conclusion is based on the high concentrations found in the raw water, the 8 to 12 kg of organic debris removed during each cleaning of the slow sand filter, and the presence of cyanobacteria, green algae, fungi, and diatoms in the water. A sample of the schmutzdecke (organic detritus) on 17 November 2021, showed the presence of microbes (Figures S168–S171). Nematodes and fungi were also found in the debris along with amorphous organic material (Figure S172).

Samples of the raw water during the project were collected from two locations on the site, including at the entrance to train A (control) and at the inflow to the slow sand filter. The reason for the duplicate sampling was to ascertain if any differences in water quality occur based on the highly heterogeneous nature of the raw water and the interior biofilm coating of the piping system. The statistical analysis between the raw water samples showed that it was not significant, but there were some observed differences in some parameters.

4.2. Changes in the Vegetation with Time and Treatment Activities

Even though great care was taken to evenly plant the emergent vegetation, after four months of acclimation and growth, each tank started with a slightly different number of plants ranging from 61 (tub 9, UV treatment) to 104 (tub 8, reference treatment, Figure 10). This equates to a loss of plants ranging from 50% to about 15%. Emergent plants overall grew slightly in number (1.8 times greater than average) during this experiment, growing from their rhizomes (asexual vegetative multiplication). This growth seems to have slowed down at the end of the experiment. Not accounted for numerically, *T. domingensis* also grew taller and expanded laterally as its foliage grew. The foliage looked browner and less expansive as it was in the middle of the dry season. Although it was not possible to determine from the photographs when *S. californicus* started to reproduce sexually, it was found that *T. domingensis* began reproducing as early as 21 February 2022 (event 3) starting in tub 10 (filter treatment) and was present in all tubs at the next event (23 March 2022) with tub 8 (reference) having the most inflorescences (seven in total). In comparison, tubs 9 (UV treatment) and 10 (filter treatment) reached similar values a month later (18 April 2022). The number of inflorescences in all tubs declined rapidly thereafter (Figure 10). The amount of floating vegetation in the tubs (mixture of *L. minor* and the fern *Azolla* sp. predominantly) started in tub 9 (UV treatment) but then receded quickly whilst it grew very thick in the two other tubs and persisted until the end of the experiment. It is not known why this floating vegetation disappeared in tub 9. Because *S. californicus* was harder to decipher in the photographs, less assertions can be made for that species. This plant followed the same growth dynamics as *T. domingensis,* but the results for this species have to be taken with caution at the end of the experiment as the stems of *S. californicus* were hard to distinguish from those of *T. domingensis,* which gained in height and foliage.

V. americana growth dynamics were difficult to track using the monitoring method chosen. However, the raking in tub 1 (reference treatment) was incommensurably more intense, which lacked floating vegetation but had thick metaphyton and epiphyton (both mainly from the alga *Cladophora* sp.) on the leaves of *V. americana*. This severely pulled the plant up as well as blocked its photosynthesis, so that tub 1 lost tremendous amounts of plants by the end of the experiment. Tubs 2 (UV treatment) and 7 (filter treatment) were more successful at growing healthy stands of *V. americana,* but those were also covered with epiphytes and a thick blanket of both floating plants and filamentous algae. At the end of the experiment, *V. americana* was still present but was visually less abundant than at the beginning of the experiment.

Overall, the sand filter water in conjunction or not with the UV treatment lowered the ability of *T. domingensis* to grow and especially reproduce, also in the reference treatment. This effect was even more pronounced for *V. americana*, which is severely impacted by the growth of *Cladophora* sp., which grew at a faster rate. However, even with filtered water, *V. americana* would still likely lose the competition against this filamentous green alga. The nutrient levels in the filtered water are high enough to promote the growth of microphytes (here *Cladophora* sp.) compared to macrophytes.

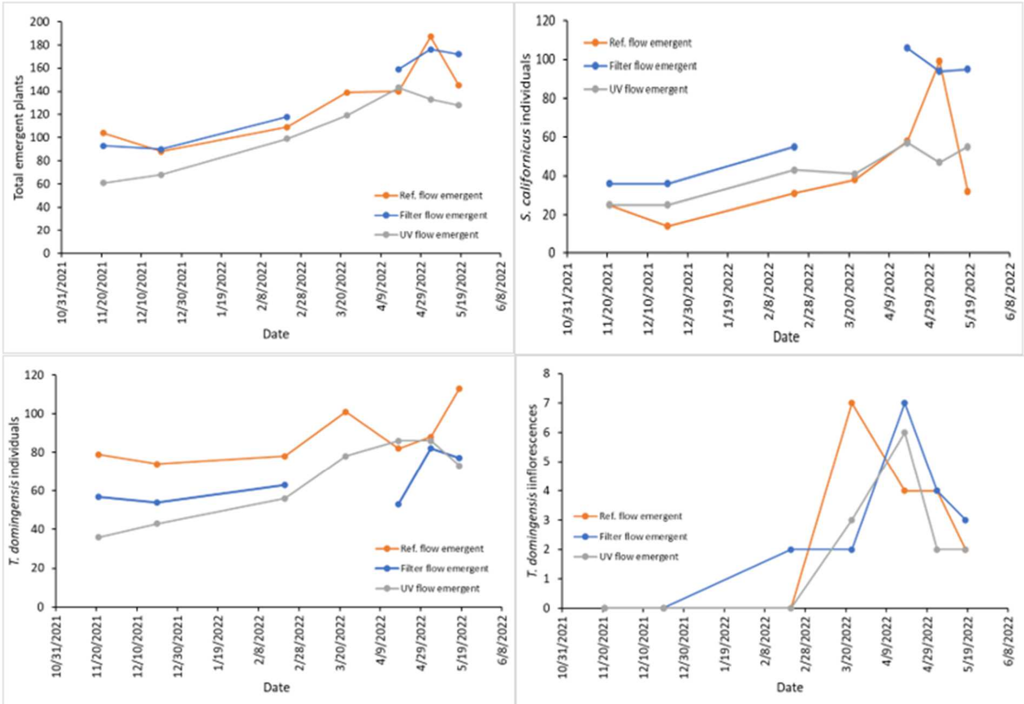

Figure 10. Growth dynamics of emergent plants in tubs #8 (reference treatment), #9 (UV treatment), and #10 (filter treatment). (**Top left**): change in total number of plants; (**top right**): change in *S. californicus*; (**bottom left**): change in *T. domingensis*; and (**bottom left**): change in *T domingensis* inflorescences. Note: the missing datum for tub 10 is due to a corrupted photograph. This missing datum is not present for the bottom right graph since inflorescences were visible by zooming on the photograph encapsulating all six tubs.

4.3. Overall Assessment of the Effectiveness of the Two Treatment Technologies (Trains B and C) versus Only Vegetative Treatment (Control Train A)

As shown in Table 3, the statistical analysis showed that a comparison of the raw water that is coming out of each treatment train produced no statistical difference. In each case, total nitrogen, organic nitrogen, nitrate, nitrite, total phosphorus, and orthophosphate were reduced during treatment (Figures 11–13). There were, however, some differences and special circumstances that need to be discussed based on how the systems operated (Figures 14–16).

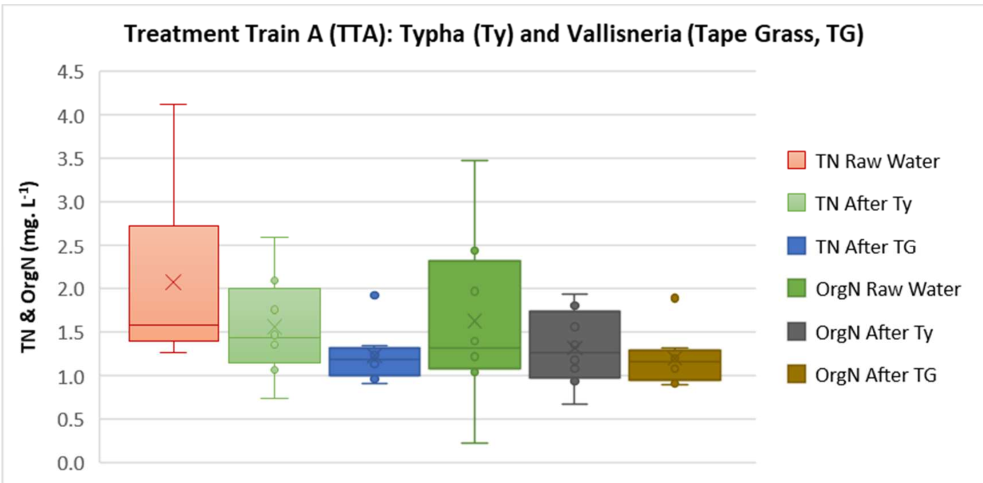

Figure 11. Box diagram of the changes in the concentrations of total and organic nitrogen in train A (vegetation only, control).

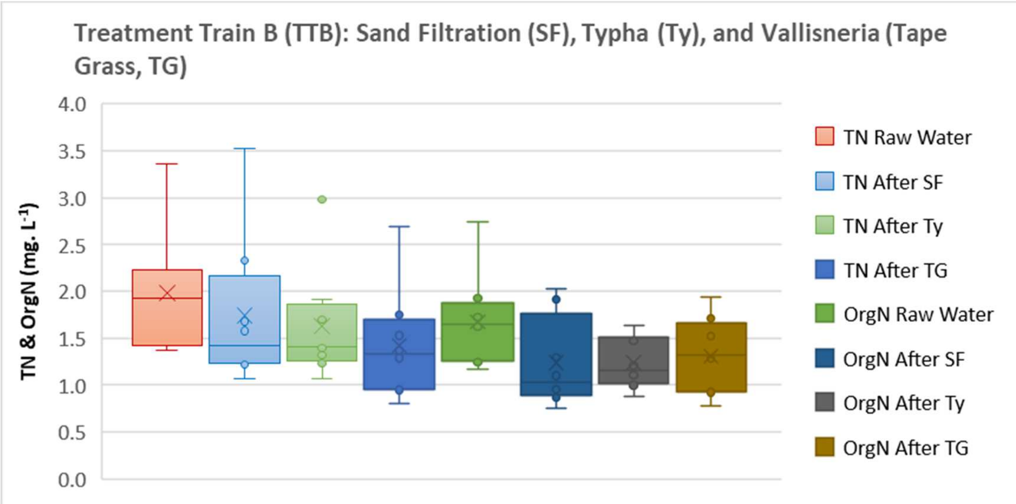

Figure 12. Box diagram of the changes in the concentrations of total and organic nitrogen in train B (slow sand filtration + vegetation).

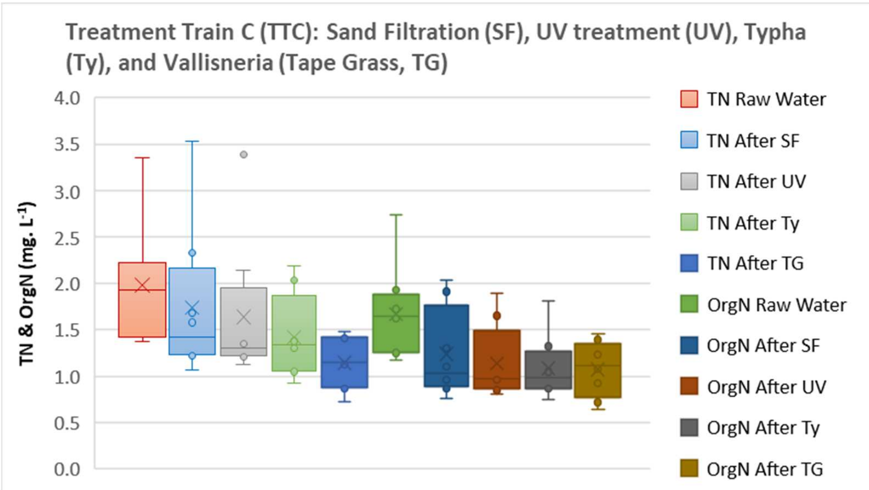

Figure 13. Box diagram of the changes in the concentration of total and organic nitrogen in train C (slow sand filtration + UV + vegetation).

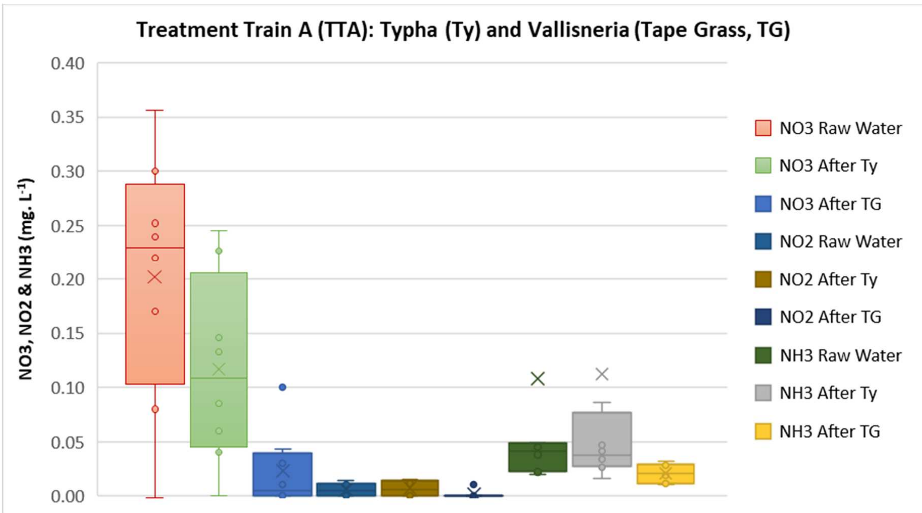

Figure 14. Box plot of the variation in the concentration of nitrate, nitrite, and ammonia in treatment train A (vegetation only, control).

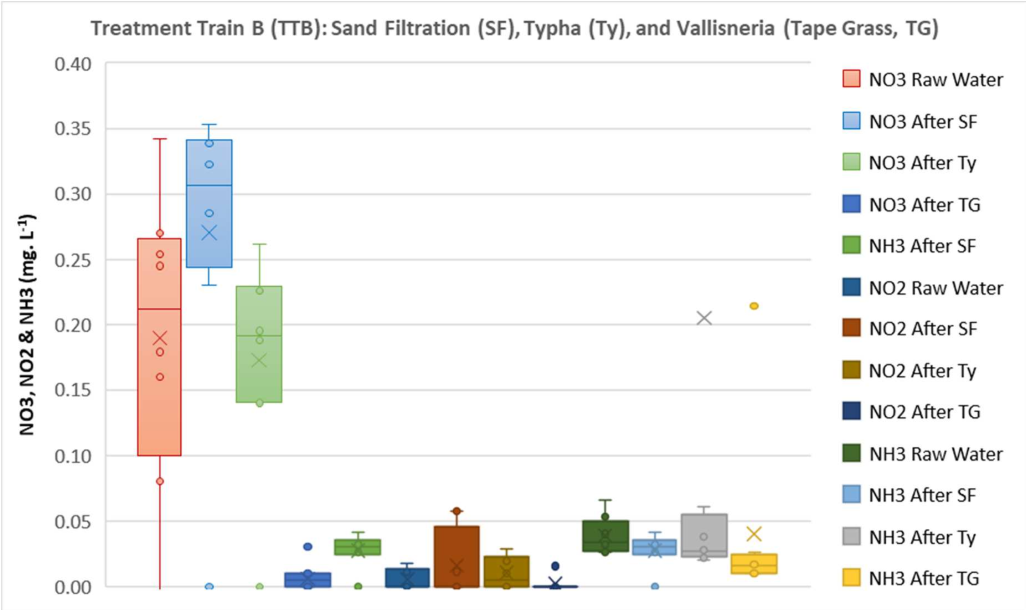

Figure 15. Box plot of the variation in the concentration of nitrate, nitrite, and ammonia in treatment train A (slow sand filtration + vegetation).

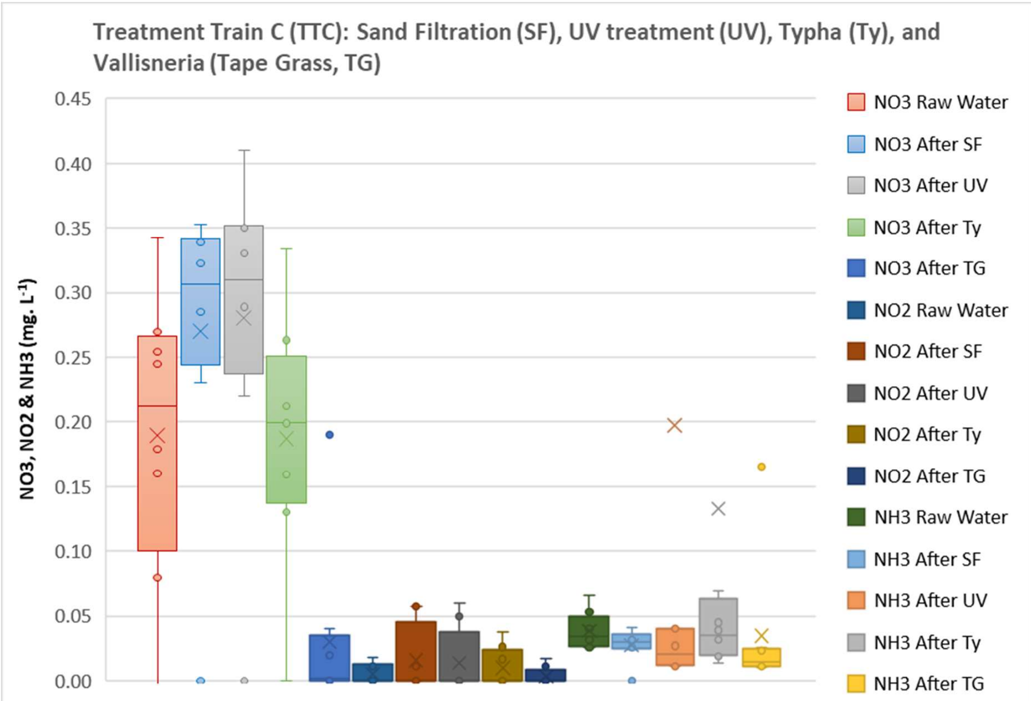

Figure 16. Box plot of the variation in the concentration of nitrate, nitrite, and ammonia in treatment train c (slow sand filtration + UV + vegetation).

Based on a comparison of the three treatment trains, the slow sand filter and UV both removed some organic nitrogen (Figures 15 and 16). It was postulated that the slow sand filter would be somewhat effective in creating reducing conditions at its base, which would convert some of the nitrogen to ammonia. This was not as effective as possible based on the rather low retention time in the filter. The high turbidity and color of the water also impacted the effectiveness of the UV in breaking down some of the organic nitrogen.

The removal of nitrate in all three trains was most effective in the *Vallisneria* tub or the last treatment process. In the vegetation-only treatment train A, this last tank contained a variety of vegetative types, not just tape grass. Other fast-growing vegetation was recruited from the river water and aided the removal of the nitrogen nutrients. However, it was necessary to harvest a large algae species (e.g., *Cladophora* sp.) to maintain the tape grass in a living state. The fast-growing algae species also had to be cleaned to a lesser degree in the tape grass tanks of trains B and C.

All three treatment trains were effective at reducing concentrations of total phosphorus and orthophosphate (based on mean values) (Figures 17–19). With regard to total phosphorus, train B was most effective with a concentration reduction to less than 0.10 mg·L^{-1}, while train C lowered the concentration to below 0.20 mg·L^{-1} and train A to below 0.25 mg·L^{-1}. Orthophosphate was also effectively lowered, but train A was most effective with a reduction to below 0.01 mg·L^{-1}, while trains B and C lowered it to less than 0.02 mg·L^{-1}. Despite the statistical analyses, there are some differences in how the trains were effective for the removal of specific analytes.

The slow sand filtration process effectively removed particulate biomass including algae, bacteria, turbidity, and reduced chlorophyll A. The UV also reduced the total bacteria, algae, and cyanobacteria concentrations.

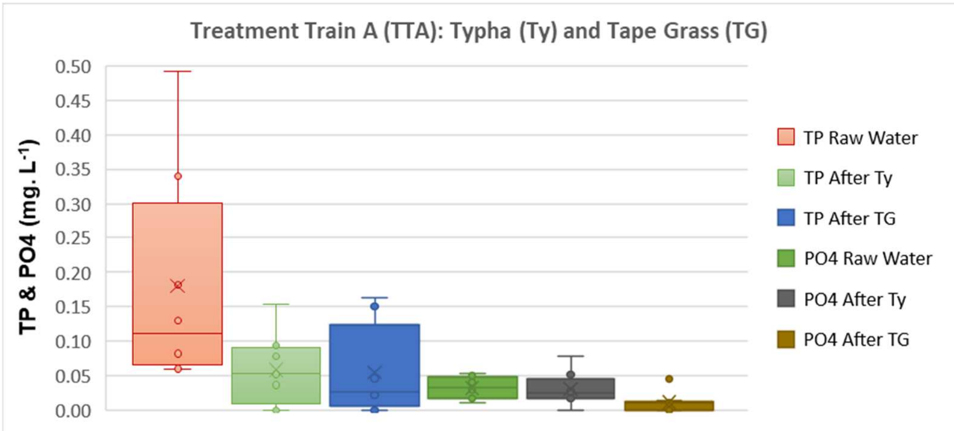

Figure 17. Box plot showing the changes in total phosphorus and phosphate in treatment train A (vegetation only, control).

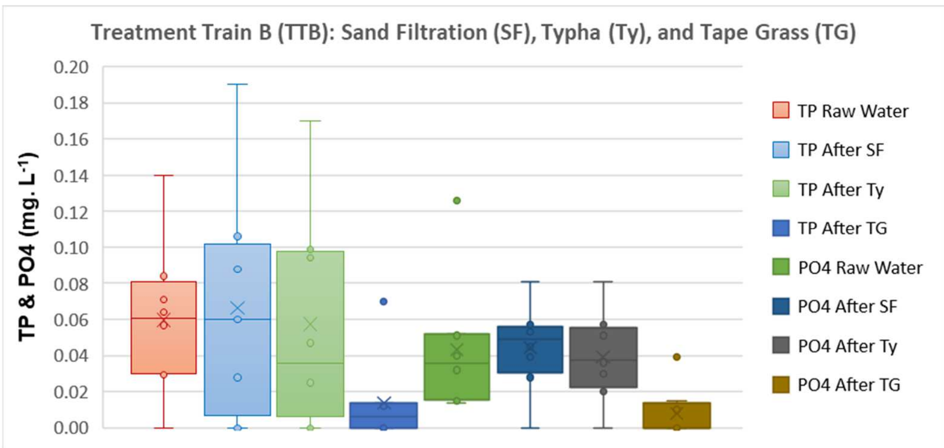

Figure 18. Box plot showing the changes in total phosphorus and phosphate in treatment train B (slow sand filtration + vegetation).

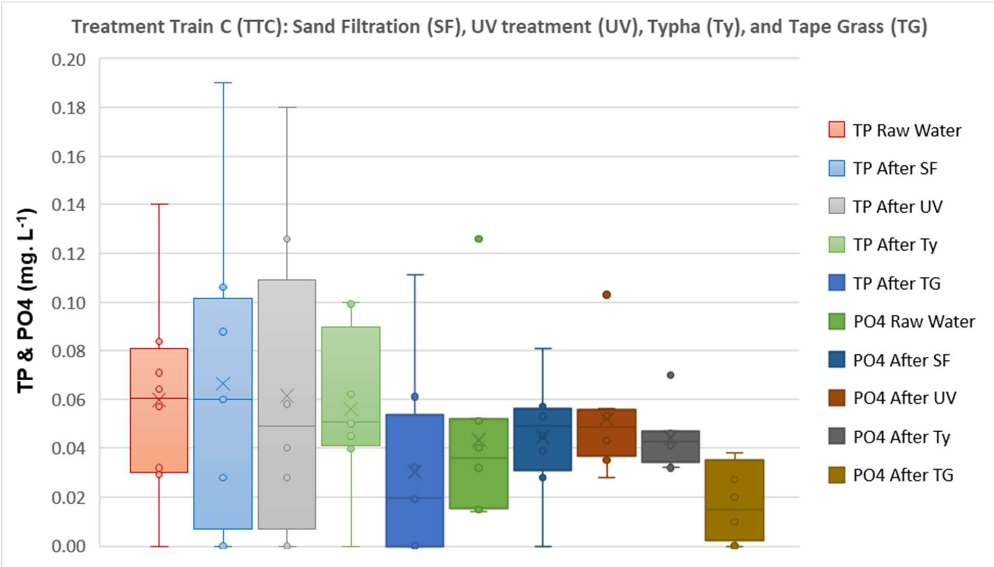

Figure 19. Box plot showing the changes in total phosphorus and phosphate in treatment train C (slow sand filtration + UV + vegetation).

4.4. Effect of UV Treatment in Reducing the Concentration of Organic Nitrogen

UV treatment did lower the concentration to a very limited degree but was not effective because of the high color and turbidity of the raw water, the flow rate, and the limited power of the UV lamp. Upscaling the process could help it to be more effective by increasing the UV power, reducing the flow rate, and using a tray exposure geometry.

4.5. Water Treatment and Impacts on Vegetation Growth in the Mesocosms

Based on the mass of floating organic material, mostly the algae *Cladophora* sp., the engineered treatment did reduce the amount of growth in trains B and C *Vallisneria* tubs compared to the train A *Vallisneria* tub. It should be noted that the mass of *Cladophora* sp. was mostly floating. If harvested, this material could be composted or used as a natural fiber.

4.6. Lessons Learned: What Experimental Design Changes Could Be Used to Improve the Engineered Treatment?

If the processes were upscaled to provide a greater degree of treatment at very high volumes, the slow sand filtration process would need to have a thicker media bed, perhaps six to eight feet, and the flow rate would need to be sufficiently low to increase the retention time to eight hours or longer. This design would aid in creating reducing conditions within the filter and would encourage the conversion of more organic nitrogen into soluble nutrients, such as ammonia and orthophosphate, which are taken up rapidly in the vegetation treatment tanks. In addition, the cleaning of the large-scale sand filter tanks would need to be accomplished using an automated process, such as those used in many existing slow sand filter, potable water treatment facilities.

If a UV process were to be implemented as part of an engineered process train, the flow hydraulics would need to provide a longer contact time with the raw water (tray design) and would have to be coordinated with the slow sand filtration retention time to provide water with a lower turbidity. In addition, the power of the UV light source would need to be increased.

4.7. Could the Engineered System Function to Lessen Algal Blooms in the Storage Reservoirs or Any Stormwater Storage Facilities Occurring in the Caloosahatchee River Basin over Critical Times?

Perhaps the treatment process could be implemented in any stormwater facility before the water is returned to the Caloosahatchee River rather than when the raw water is pumped into the reservoir. This would provide the river with better water quality. In addition, algal blooms could be allowed to occur in the reservoir to allow the floating algae to aid in the treatment process. The algae could then be harvested as part of the treatment process.

4.8. Is There Some Commercial Value for Harvesting Cellulose or Fiber from the Green Algae Cladophora sp. to Offset Tater Quality Treatment Costs in the Reservoir Lakes?

One of the important observations made during this research was the incredible growth rate of *Cladophora* sp. in the submergent vegetation tanks, particularly in train A (control). After this plant was harvested to maintain the health of the *Vallisneria*, it was found that if left in the sunlight for several weeks to dry, it produced a fiber similar to hemp. The fiber appears to be strong and could be harvested for commercial use. This issue has been explored by Mihranyan [48]. Extraction of the cellulose fiber appears to be easier than the hemp extraction process. This could have commercial value that could be used to offset the treatment of the reservoir water.

4.9. Does the Prolific Growth of the Green Algae Cladophora sp. Impact the Proposed Use of Alum to Sequester Organic Material in the Reservoirs for Treatment?

The rapid growth of *Cladophora* sp. would impact the use of alum for treatment of the reservoir water. This green alga tends to float and the application of an alum slurry or even a bentonite slurry will not make it sink. Therefore, it provides a serious challenge to the use of alum treatment.

5. Conclusions

The research objectives of the Boma project were achieved despite the challenging times (COVID-19 pandemic) causing supply chain disruptions, cost increases, and pump system failures caused by lightning damage and part failures. Despite the reduced number of samples collected, the sampling events were representative of all seasonal climatic conditions and did allow for a detailed analysis of the three treatment trains originally suggested for evaluation.

It was found that all three treatment trains were effective at the removal of nutrients and organic biomass from poor water quality in the Caloosahatchee River water. There were no statistical differences among the three treatment process trains, which were as follows: A. emergent vegetation (Typha) with submergent vegetation (*Vallisneria*), B. low sand filtration with emergent (Typha) and submergent (*Vallisneria*) vegetation, and C. slow sand filtration, UV, and emergent (Typha) and submergent (*Vallisneria*) vegetation.

The detailed data collected allowed for a more thorough understanding of how these treatment processes work in the field under pilot-scale operation. The recruitment of the filamentous algae *Cladophora* sp. from the river water was an important observation because not only did it aid in the treatment performance of the submergent vegetation tub, but it also provided insight into difficulties for future operation of the reservoirs and other stormwater retention areas in the Caloosahatchee River Basin. The rapidly growing and floating algae will provide a serious challenge to future reservoir water quality management that will make coagulation with alum unlikely as a successful method to reduce nutrient concentration and biomass. The presence of the *Cladophora* sp. may also provide an opportunity for the harvest of the plant for use as a commercial source of cellulose fiber.

This research also suggests that new approaches need to be evaluated in the large-scale management of reservoir water quality. A combination of using vegetation for water quality treatment with some engineered enhancements still needs to be assessed and investigated with some design improvements. This research should include a detailed economic analysis of the treatment alternatives.

Supplementary Materials: The following support information can be downloaded at https://www.mdpi.com/article/10.3390/w16152145/s1. There are 172 figures included in the supplementary materials and they include all data collected during the investigation.

Author Contributions: T.M.M. wrote the grant proposal, wrote the first draft of the report and paper, worked on the construction of the test site, helped clean the slow sand filter, and helped collect and analyze the water samples. S.T. (Seneshaw Tsegaye) helped design the experiment, helped construct the infrastructure, collected water quality samples, cleaned the slow sand filter, drafted the figures, performed the statistical analyses on the water quality data, and helped revise the paper text. S.T. (Serge Thomas) designed the vegetation used in the test tubs, installed the vegetation, helped construct the site infrastructure, monitored the changes in the vegetation, cleaned the nuisance vegetation, collected water samples, measured field parameters, and wrote several sections of the original report and paper. A.D.-T. was the project manager and worked on the QAQC documents required by the grant agency, helped arrange student participation, and managed the project finances. P.R.M. designed the electrical system and controls, helped construct the infrastructure, obtained water quality samples, helped clean the slow sand filter, monitored the operation of the experiment, and wrote the sections of the paper on the controls. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data collected during this investigation are contained either in the text of the paper or are contained in the Supplementary Materials.

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