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Nitrate Removal by Floating Treatment Wetlands under Aerated and Unaerated Conditions: Field and Laboratory Results

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Abstract: Urban and storm water retention ponds eventually become eutrophic after years of receiving runoff water. In 2020, a novel biological and chemical treatment was initiated to remove accumulated nutrients from an urban retention pond that had severe algae and weed growth. Our approach installed two 6.1 m × 6.1 m floating treatment wetlands (FTWs) and two airlift pumps that contained slow-release lanthanum composites, which facilitated phosphate precipitation. Four years of treatment (2020–2023) resulted in median nitrate-N concentrations decreasing from 23 μg L^{-1} in 2020 to 1.3 μg L^{-1} in 2023, while PO_4-P decreased from 42 μg L^{-1} to 19 μg L^{-1}. The removal of N and P from the water column coincided with less algae, weeds, and pond muck (sediment), and greater dissolved oxygen (DO) concentrations and water clarity. To quantify the sustainability of this bio-chemical approach, we focused on quantifying nitrate removal rates beneath FTWs. By enclosing quarter sections (3.05 × 3.05 m) of the field-scale FTWs inside vinyl pool liners, nitrate removal rates were measured by spiking nitrate into the enclosed root zone. The first field experiment showed that DO concentrations inside the pool liners were well below the ambient values of the pond (<0.5 mg/L) and nitrate was quickly removed. The second field experiment quantified nitrate loss under a greater range of DO values (<0.5–7 mg/L) by including aeration as a treatment. Nitrate removal beneath FTWs was roughly one-third less when aerated versus unaerated. Extrapolating experimental removal rates to two full-sized FTWs installed in the pond, we estimate between 0.64 to 3.73 kg of nitrate-N could be removed over a growing season (May–September). Complementary laboratory mesocosm experiments using similar treatments to field experiments also exhibited varying nitrate removal rates that were dependent on DO concentrations. Using an average annual removal rate of 1.8 kg nitrate-N, we estimate the two full-size FTWs could counter 14 to 56% of the annual incoming nitrate load from the contributing watershed.

Keywords: floating treatment wetland (FTW); water quality; nitrate-N; phosphate-P; dissolved oxygen concentrations

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1. Introduction

Storm water retention ponds strategically placed in urban areas as flood control structures receive a variety of pollutants—from sediments bearing heavy metals to organic contaminants and soluble nutrients. While retention ponds can physically remove coarse sediments by slowing down water flow, they are limited in their ability to remove nutrients and will eventually become eutrophic. Adding a constructed wetlands to a storm water retention pond can remove nutrients and contaminants by utilizing plants rooted in soil, with water flowing either above or below the soil surface. One potential disadvantage to constructed wetlands is that high water depths following rains can lead to water levels intolerable for conventional wetlands [1]. Moreover, additional space beyond the border of the pond and landscaping may be needed for the constructed wetlands. An alternative to a constructed wetland is a floating treatment wetland.

Floating treatment wetlands (FTWs) are buoyant systems where plant roots hang into the water and plant growth removes nutrients directly from the water column [1]. The buoyant mats are adept at handling water depth fluctuations and the flexibility in size and shape of FTWs make them easy to retrofit into existing storm water retention ponds.

Numerous researchers have used floating treatment wetlands (FTWs) to improve the water quality of waste waters and eutrophic ponds [1–12]. In 2020, McKercher et al. [2] initiated a novel approach of combining FTWs with airlift pumps to treat a storm water retention pond (Figure 1). Results from this initial two-year field-scale trial showed a dramatic reduction in algae and weeds, increased dissolved oxygen (DO) concentrations, and improved water clarity.

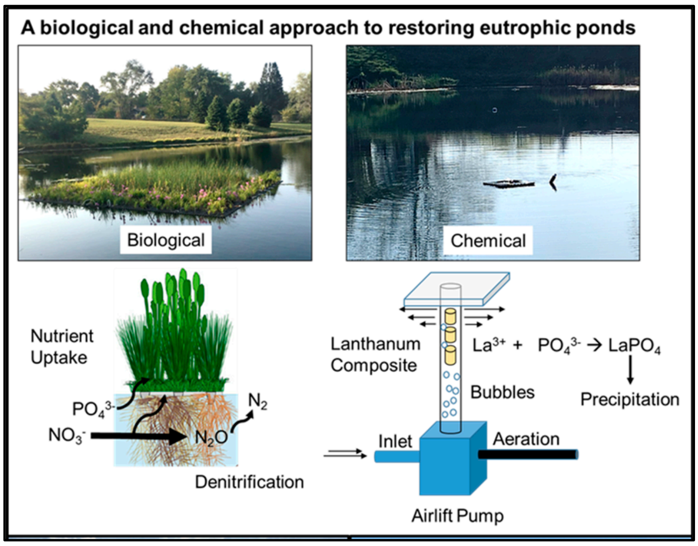

Figure 1. Photos and schematics of biological and chemical approach used by McKercher et al. [2] to restore eutrophic ponds. Photos are of Densmore Pond (Lincoln, NE, USA).

The ability of FTWs to remove nitrogen and phosphorus has been well documented [1–11]. While nitrogen removal is an established function of FTWs, delineating the relative contributions of plant uptake versus denitrification is still being investigated. Another potential knowledge gap is quantifying just how much nitrogen is actually removed by full-scale FTWs when placed in a storm water retention pond.

Critical reviews on FTWs have documented differences in the mass of nitrogen that is removed via uptake versus denitrification. Messer et al. [13] reported microbial denitrification was a key removal process in mesocosm-scale wetlands, accounting for approximately 9 to 32% of the $^{15}NO_3$-N (^{15}nitrate-N) removed. Keizer-Vlek et al. [14] reported 74% of the total nitrogen removed was attributed to plant uptake in mesocosms planted with *Iris pseudacorus*. Given that plant uptake, denitrification, and nitrification can all influence nitrate concentrations beneath FTWs, understanding conditions that favor one process over others is critical.

Although the exact mechanisms involving denitrification are complex, the environmental factors affecting denitrification are reasonably well known. Few factors, however, influence denitrification independently [15] and, therefore, multiple mechanistic processes must be considered to understand denitrification in soil–water systems.

Provided temperature and pH are favorable for microbial growth, denitrification generally occurs when three conditions are satisfied: (i) nitrate is present, (ii) oxygen concentrations are reduced, and (iii) electron donors (i.e., carbon substrates) are available. One factor preventing these conditions from occurring simultaneously is that the production of nitrate requires oxygen (O_2), while denitrification requires the absence of oxygen [15].

This dichotomy dictates that denitrification occurs at oxic/suboxic interfaces, with this interface being a separation in space, time, or both [14,16]. Garcia Chance and White [17] added aeration as an experimental variable in their FTW mesocosm experiments and found that as aeration increased DO concentrations, N removal from the water column decreased. Adding oxygen would logically hinder denitrification and explain the lower N removal. However, of the N that was removed, plants in aerated mesocosms took up more N than nonaerated plants by as much as 55% [17]. Given nitrate is the preferred N form taken up by plants [18], the added aeration may have increased the NO_3 available via nitrification.

Previous researchers have reported a large range of DO concentrations beneath FTWs [19–21]. Plants can release oxygen through their roots during daylight via photosynthesis. This released oxygen directly affects the redox potential in the water column and creates oxic/suboxic interfaces that can influence nitrogen transformations. As pointed out by Tanner and Headly [20], low DO concentrations in the presence of wetland plants are perhaps surprising given the ability of wetland plants to transport atmospheric oxygen into the rhizosphere. This added oxygen, however, is outweighed by the additional respiratory oxygen demand fueled by the release of plant root exudates [22] and the physical barrier created by the FTW to shield waters below from atmospheric gas exchange. This shielding may also influence daytime pH by curbing algae and submerged macrophyte photosynthesis [23]. Enclosing an FTW's root zone for experimental purposes could similarly disrupt natural DO concentrations by confining the carbon released from the plant roots into a smaller zone.

If the goal for FTWs is to remove as much nitrogen as possible via denitrification, then there appears to be some advantage to having both oxic and suboxic regions beneath FTWs. Using the diffusion-dominated denitrification scenario described by Seitzinger et al. [15], oxic regions near the air/water interface and perimeter of the FTW would be beneficial for the oxidation of total nitrogen and ammonium, producing nitrate, while suboxic regions within the central root architecture beneath the FTWs would allow the permanent removal of nitrate via denitrification.

We report herein on a series of field and laboratory experiments designed to quantify nitrate removal by floating treatment wetlands. Measuring nitrate removal by FTWs requires an enclosed root zone. Creating an enclosed root zone that is representative of natural conditions, however, can be challenging. For FTWs placed in an open pond, plant roots are in contact with the bulk water whereas once enclosed, the carbon released by the roots can facilitate heterotrophic respiration and decrease DO concentrations. In an effort to create DO concentrations more representative of an open (i.e., unenclosed), full-scale FTW, we included an aeration treatment in our experiments.

2. Materials and Methods

Field and laboratory experiments were performed to quantify nitrate removal by FTWs. Field experiments were first conducted, followed by laboratory experiments. Laboratory experiments were conducted to ensure reproducibility of results under controlled conditions. Additionally, the smaller experimental units made investigating routes of nitrogen removal with ^{15}N feasible.

2.1. Field Site: Densmore Pond

Field experiments were conducted at Densmore Pond (Lincoln, NE, USA). Densmore Pond (0.5 ha) was constructed in 2002 to attenuate runoff from a 55.4 ha urban watershed. Densmore Pond was designed to handle 100-year storms and drains to a 0.9 m diameter, 48 m length culvert located in the northwest corner (Figure S1).

In 2020, McKercher et al. [2] began a chemical-biological treatment to remove N and P from the water column (Figure 1) and reported the result from this treatment during the years 2020 and 2021. This study continued with the treatments started by McKercher et al. [1] and collected data in 2022 through 2023. The experimental setup used at Densmore Pond is detailed in McKercher et al., [2] but in brief, Densmore Pond was equipped with two 6.1 m × 6.1 m FTWs and two airlift pumps that contained slow-release lanthanum composites (Figure S1; [2]).

The sampling design changed slightly from 2021 to 2023. Water samples collected after 1 August 2022, were analyzed for total nitrogen (TN) and total phosphorus (TP) in addition to nitrate-N (NO_3-N) and phosphate-P (PO_4-P). The final sampling change occurred in 2022, when one of the FTWs was moved from east of GPS 3 to its current position (Figure S1). There were no changes to the sampling locations in 2023.

Analytical Methods

Discrete water samples were collected using 125 mL HDPE sample bottles at a depth of 0.15 m after rinsing three times. All water samples collected were cooled to 4 °C and analyzed for NO_3-N and PO_4-P within 48 h. Analysis for TN and TP was completed within 28 d of collection. Samples run for NO_3-N and PO_4-P were filtered using a 0.45 µm filter paper and analyzed using an AQ300 discrete autoanalyzer (Seal Analytical, Mequon, WI, USA) using SEAL methods EPA 127-D Rev 2A and EPA 145-D Rev 1. To analyze for TN and TP, samples were digested with a persulfate digestion reagent for one hour in a Tuttnauer 1730M Manual Valueklave (Breda, The Netherlands) at 121 °C and 17 psi. Samples were then analyzed using an AQ300 using method EPA-126-D Rev 1 for TN and EPA-134-D Rev 2A for TP.

All data generated using the AQ300 discrete analyzer utilized the following quality control (QC) requirements: a calibration coefficient (R^2) greater than 0.995 and passing continuing calibration validation (CCV) and blank (CCB) samples. The CCV and CCB samples were run every ten samples with limits of CCV concentration ±10% of true value and CCV concentration <0.05 mg NO_3-N/L. If any QC failed, analysis was stopped, the problem identified and corrected, and any affected sample(s) re-analyzed.

2.2. Field Mesocosm Experiments

A field-scale experiment was conducted to quantify nitrate removal rates beneath the FTWs in the Densmore Pond. To accomplish this, we separated one of the two 6.1 m × 6.1 m FTWs into quarters (3.05 m × 3.05 m) and placed three of these sections inside 4.57 m diameter, 1.2 m deep Easy Set pool liners (Intex, Long Beach, CA, USA) that were filled with water from the pond (Figure 2). Prefabricated holes in the liners used for pump hoses were plugged with rubber stoppers. Each floating wetland section (3.05 m × 3.05 m) consisted of 250 mature plants, composed of 64 softstem bulrush (*Schoenoplectus tabernaemontani*) and 186 sedges (*Carex comosa*, *Carex scoparia*, and *Carex frankii*). Each FTW section covered approximately 90% of the surface area inside the pool liner. Buoyancy for each wetland was provided by foam mats (Beemats LLC, New Smyna Beach, FL, USA).

2.2.1. Field Experiment 1

For Experiment 1, there were three experimental units (i.e., pool liners): two contained FTWs and one control, which consisted of a floating mat with a tarp cover to replicate the blockage of light without plants and reduce atmospheric gas exchanges (Figure 2). This specific control was chosen to isolate the effects of plants versus no plants. Each mesocosm was spiked with 70 g of $NaNO_3$ delivered as an aqueous solution prepared using ultrapure water. The mass of nitrate added was chosen to produce an initial concentration of ~1.0–1.5 mg NO_3-N/L inside the pool liner, which is easy to detect analytically, but not unrealistically high.

Water quality grab samples and water quality parameters were collected daily for 14 d, then every other business day until NO_3-N concentrations were below detection. Daily NO_3-N loss rates were calculated using the difference between water sample results and dividing by the number of days between samples. Water quality parameters were recorded daily using a YSI Pro Plus Multiparameter water quality meter (YSI Inc., Yellow Springs, OH, USA) at a depth of 0.15 m. Water quality parameters recorded included temperature (°C), pH, DO concentration (mg/L), specific conductivity (µS/cm), and oxidation-reduction potential (mV). To record water quality parameters beneath the center portions of FTW, the YSI water quality probe was attached to a 3.6 m telescoping Swing Sampler (Nasco Sampling, Fort Atkinson, WI, USA). Precipitation was recorded using rainfall collected from the Lincoln Municipal Airport. Graphical figures of NO_3-N concentrations and water quality parameters were created using SigmaPlot (Systat Software Inc., San Jose, CA, USA).

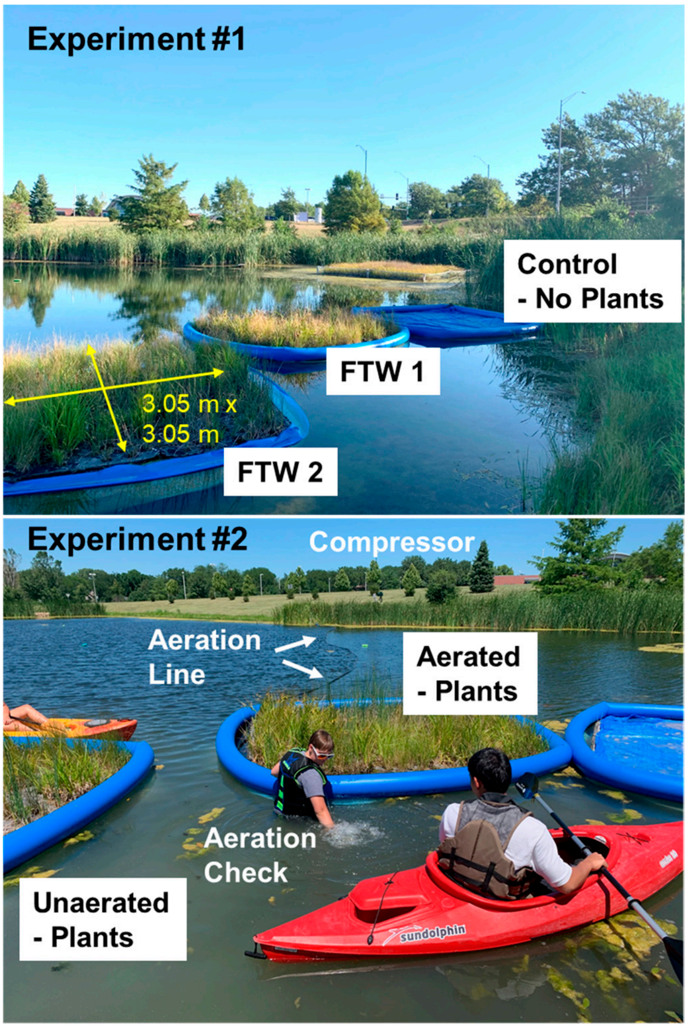

Figure 2. Photographs of field experimental units (pools) used for Experiment 1 and Experiment 2.

2.2.2. Field Experiment 2

To achieve a greater range of DO concentrations beneath the FTW than those observed in Experiment 1, we used the same experimental units but added aeration as a treatment variable. This gave us three treatments: (i) aerated (with plants), (ii) unaerated (with plants), and (iii) control (unaerated, no plants). Aeration was supplied by a rotary vane compressor that was connected to a plastic hose and commercial diffuser (Figure 2). Each mesocosm was spiked with 80 g of $NaNO_3$, prepared and delivered as described in Experiment 1. We used a slightly higher mass (70 g vs. 80 g) to increase the initial nitrate concentration. Likewise, water grab samples and water quality parameters were measured as described in Experiment 1, with the experiment extending for 26 d.

2.3. In Situ Dissolved Oxygen Measurements

The root system beneath the full-scale FTWs was photographed to visualize the root architecture and a Hydrolab MS5 DO probe (Hach, Loveland, CO, USA) was then installed into a cluster of roots 15 cm below the surface to record DO measurements every 15 min over several days. Following the DO measurements beneath the FTW, a similar set of measurements were then taken in parallel outside the perimeter of the FTW at a similar depth. These two measurements allowed the comparison of diurnal DO concentrations beneath, and adjacent to, the field-scale FTW.

2.4. Complementary Laboratory Experiments

To provide insight into the results obtained from the field experiments, similar experimental treatments to those used in the field were imposed on laboratory mesocosm units under more controlled conditions (i.e., temperature and light). Here, the experimental units were 85 L plastic tubs filled with 70 L of H_2O. Each unit contained four clusters of plants that covered approximately 80% of the surface area (Figure 3). Like the field experiments, plant species used in the laboratory experiments included softstem bulrush (*Schoenoplectus tabernaemontani*) and sedges (*Carex comosa* and *Carex crinita*). Small sections of foam mats (Beemats LLC., New Smyna Beach, FL, USA) were also used to support the plants. Illumination was provided daily for 12 h by a Sunsystem HPS 150 grow lamp (Sunlight Supply, Inc., Vancouver, WA, USA) suitable for full spectrum plant growth.

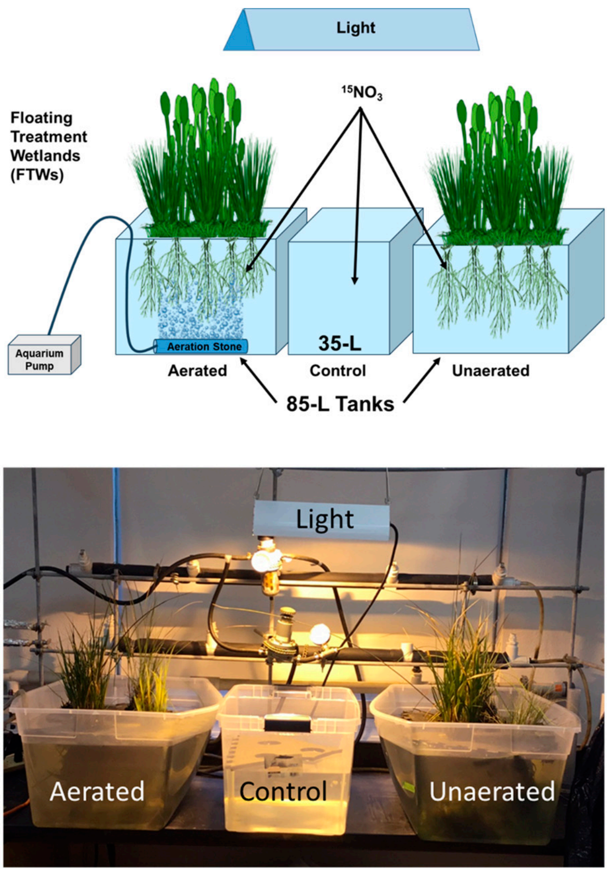

Figure 3. Schematic and photograph of lab-scale mesocosms.

2.4.1. Laboratory Experiment 1

Treatment variables in Laboratory Experiment 1 included aerated and unaerated mesocosms. Aeration was supplied using an aquarium compressor (Whisper AP 150, Tetra, Blacksburg, VA, USA) capable of delivering 2.5 L/min. The air line from the compressor was connected to a diffuser. Water was spiked with nitrate ($NaNO_3$) to a concentration similar to the field experiments (~1.5 mg NO_3-N/L). Immediately before starting the experiment, the inside of each mesocosm tank was cleaned with an abrasive brush to remove built-up algae and filled with fresh tap water.

Dissolved oxygen was measured in two ways during Experiment 1. The first method used the same YSI Pro Plus Multimeter used in the field with the DO probe calibrated daily before use. The second method used a prototype version of an optic fiber technology from Intelligent Optical Systems, Inc. The commercial version of the system we used (ISO Apollo) is available from ISO (https://intopsys.com/ios-apollo/, accessed on 18 September 2024).

As performed in the field experiments, water quality grab samples and water quality parameters were collected daily for 17 d, until NO_3-N concentrations were below detection. The YSI sensors and sensor guard were rinsed twice with nanopure water prior to sampling each treatment.

2.4.2. Laboratory Experiment 2 ($^{15}NO_3$)

Laboratory Experiment 2 used a similar setup to Experiment 1 but expanded upon treatments by including a control (unaerated, no plants, Figure 3) and using a nitrate spike enriched with $^{15}NO_3$-N. Mesocosm cleaning and water replacement were completed 10 days prior to starting the experiment to limit elevated DO. Each treatment received 0.45 g of $NaNO_3$ and 0.47 g of KNO_3 composed of 60.7% ^{15}N from separate spike solutions prepared with ultrapure water. In total, the nitrate spikes added 140 mg N with a ^{15}N enrichment of 46% for a concentration of ~2 mg NO_3-N/L in mesocosm tanks. Prior to the spike additions, a root and shoot sample was collected from each plant.

Water grab samples were taken daily for 14 d, then every other weekday along with DO concentrations using the YSI Pro Plus Multimeter. Once NO_3-N concentrations were undetectable in the aerated and unaerated treatments, sampling ceased, and plants were harvested for analysis. Each plant species was removed and isolated and allowed to air dry for 24 h before processing. Detailed procedures used to analyze plant tissue for ^{15}N enrichment and percent N and C are provided in the Supplementary Materials.

2.5. Calculations of Nitrate Loads

To determine the sustainability of the FTWs to remove NO_3-N, we used two methods to estimate the mass of nitrate entering Densmore Pond. The first method used the SCS Curve Number method to estimate incoming runoff in 2020, as described in McKercher et al. [2]. Curve numbers were based on land use classification and hydrologic soil group for each contributing section of the Densmore Pond watershed under wet and dry conditions. This estimate did not include runoff entering Densmore Pond from a sub-catchment upstream of Densmore Pond. The runoff volumes were then multiplied by the 50% exceedance probabilities for NO_3-N, itself calculated using runoff NO_3-N concentrations.

The second method utilized the Storm Water Management Model (SWMM). SWMM is an open-source hydraulic and hydrologic water modeling software developed by the US Environmental Protection Agency and CDM Smith, Inc. (Boston, MA, USA) [24]. Key factors making SWMM the modeling software of choice included its ability to model street drainage and weirs, infiltration into soil layers, and dry-weather pollutant build-up over different land uses [23]. The SWMM accounted for the same contributing sections of the Densmore Pond watershed, and additionally included spillover from the upstream sub-catchment when rainfall was sufficient for water to discharge into Densmore Pond.

The model was calibrated and validated using 15 min interval precipitation and runoff NO_3-N and PO_4-P concentrations collected from rain events in 2021 by an ISCO 6712 autosampler (Teledyne, Inc., Lincoln, NE, USA). Following calibration and validation, daily rainfall data were obtained from the High Plains Regional Climate Center ACIS-CLIMOD database (Lincoln, NE, USA) from 2013 through 2022. SWMM used precipitation inputs to calculate NO_3-N loads to Densmore Pond.

2.6. Statistical Analysis

The normality of nutrient concentration and water quality parameters was tested using the Anderson–Darling test ($p > 0.05$). As normality was not present, a nonparametric Kruskal–Wallis test ($p > 0.05$) determined differences using the median rank of the data. All statistical analysis was computed using MATLAB ver. 9.10 software with the statistics toolbox (MathWorks, Natick, MA, USA).

3. Results and Discussion

By maintaining the biological-chemical treatment started in 2020, photographs from a pole-mounted, time-lapse camera clearly showed improvement in water quality of the Densmore Pond. In July 2020, a thick mat of *Cladophora*, a lime green filamentous algae species, covered most of the pond surface, but by 2023, the pond was largely devoid of algae (Figure 4). The lack of algae did not occur all at once but rather the frequency of algae observations diminished with time. During the initial years of treatment (2020–2022), some algae would form on occasion. No algae have been observed in 2024.

Figure 4. Photographs of Densmore Pond from 2020 to 2023.

Consistent with the improved aesthetics of the pond, UNL's biological and chemical treatment decreased PO_4-P and NO_3-N concentrations while DO concentrations increased (Figure 5). Four seasons of treatment (2020–2023) resulted in decreased median NO_3-N concentrations from 23 μg/L in 2020 to 1.3 μg/L in 2023, while PO_4-P decreased from 42 μg/L to 19 μg/L. It was also noted that the variability in PO_4-P and NO_3-N values observed in 2020 and 2021 diminished as concentrations decreased (Figure 5). Water nutrient content and water quality parameters for each year are summarized in Table S1, with more specific details and statistics on yearly changes in (i) NO_3-N and PO_4-P, (ii) TN and TP (Figure S2), (iii) DO, and (iv) specific conductivity provided in the Supplementary Materials.

Given that nitrate decreased in the Densmore Pond over the four seasons following initiation of the bio-chemical treatment (56 μg/L in 2020 to 1.3 μg/L in 2023, Figure 5), the questions we wanted to answer were: Were the two FTWs partially responsible for this decrease? And if so, what contribution did they play? To answer these questions, we split one of the full-scale FTWs into smaller units where nitrate removal rates could be quantified (Field Experiments 1 and 2).

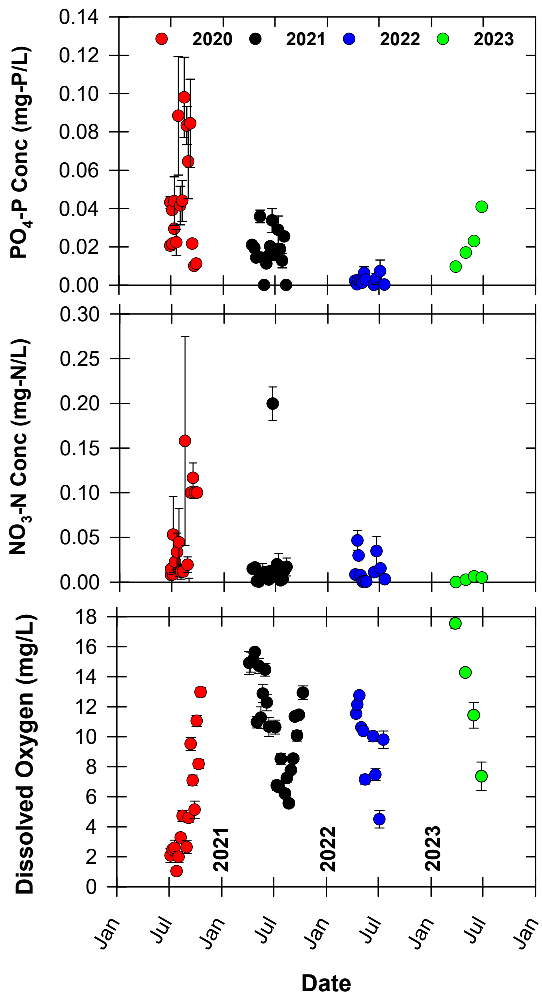

Figure 5. Temporal changes in PO_4-P, NO_3-N, and dissolved oxygen concentrations in Densmore Pond from 2020 through 2023. Error bars on symbols represent standard errors; where absent, bars fall within symbols.

3.1. Field Experiments

3.1.1. Field Experiment 1

The results from the FTWs (3.05 × 3.05 m) placed inside pool liners showed NO_3-N concentrations decreased at a zero-order rate (0.1975–0.200 mg NO_3-N/d) (Figure 6), with rates relatively consistent between pools (i.e., replicates). Dilution from rainfall was considered insignificant as 0.127 cm of rain was only received on Day 0, when the pool liners were spiked. Using the initial concentrations (0.76–0.92 mg NO_3-N/L) and the volume of water in each pool (~13,000 L), the mass of NO_3-N removed ranged from 9800 to 11,700 mg NO_3-N. Each FTW removed NO_3-N at a rate of ~1950 mg NO_3-N/d. In both unaerated FTWs, median DO concentrations were lower than 1.0 mg/L, likely leading to rapid denitrification. Median DO concentrations were not different between FTW1 (0.25 mg/L) and FTW2 (0.32 mg/L). The control pool, which had no plants, had significantly higher ($p < 0.001$) median DO concentrations (1.19 mg/L). While NO_3-N concentrations declined immediately in FTW1 and FTW2, NO_3-N concentrations remained constant in the control until Day 5, after which, a slight decline was observed, which we attribute to algal growth (Figure 6). Given DO concentrations and the pH of water outside the pool liners (DO = 11.36 mg/L, pH = 8.47) were significantly higher (both $p < 0.001$) than inside the pool liners (DO, Control = 1.19 mg/L, FTW1 = 0.25 mg/L, FTW2 = 0.32 mg/L; pH: Control = 7.51, FTW1 = 7.20, FTW2 = 7.04), we acknowledge that the vinyl pool enclosure minimized air exchange with the atmosphere and extraneous carbon released from the wetland plant roots likely facilitated low DO values. Thus, the results from Field Experiment 1 provided an example of how quickly NO_3-N can be removed beneath FTWs under low DO concentrations.

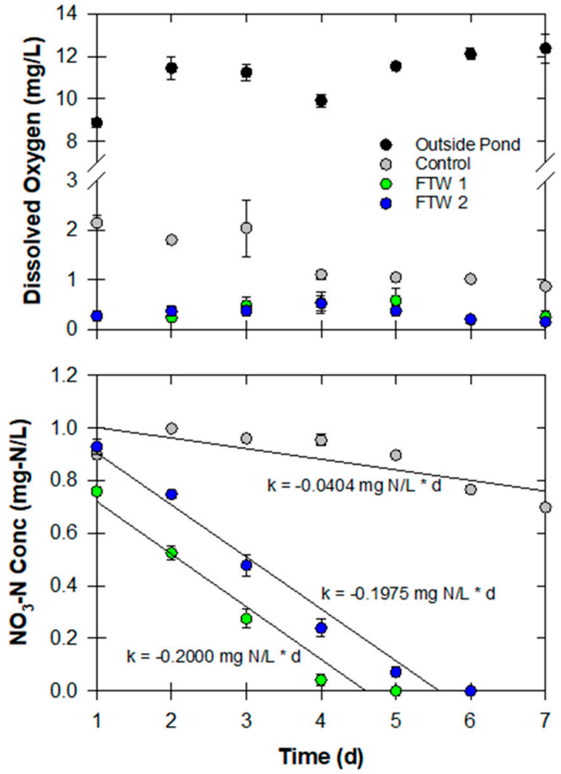

Figure 6. Field Experiment 1. Top: Temporal changes in dissolved oxygen concentrations inside treatment pools and outside (ambient). Bottom: Temporal changes in NO_3-N concentrations in aerated, unaerated, and control pools. Error bars on symbols represent standard errors; where absent, bars fall within symbols.

3.1.2. Field Experiment 2

To achieve a greater range of DO concentrations beneath the FTW than those observed in Experiment 1, we used the same experimental units, but added aeration as a treatment variable. Using three treatments: aerated (with plants), unaerated (with plants), and control (no plants), we spiked each pool to ~1.6 mg NO_3-N/L and measured removal rates. A check of dissolved organic carbon (DOC) concentrations in the three treatments showed that pond water in the control pool liner was 6.59 mg C/L; 7.25 mg C/L in the non-aerated plant treatment and 7.59 mg C/L in the aerated plant. Rainfall dilution was again insignificant, with a total of 0.43 cm received over the course of the experiment. Dissolved oxygen was increased in the aerated pool by pumping air into the water via an aeration line from a rotary vane compressor (Figure 2). By creating two FTWs with divergent DO concentrations, the unaerated pool removed the NO_3-N significantly faster than the aerated pool (p = 0.048) (9 d vs. 23 d; Figure 7).

Over the course of 26 d, NO_3-N concentrations decreased in the treatment pools until concentrations fell below detectable limits. The unaerated pool had the most significant NO_3-N loss, taking only 9 d to fall below detectable concentrations. The aerated and control mesocosms also had linear decreases in NO_3-N concentration, with a higher rate of loss in the aerated (plants) treatment mesocosm than the control (Figure 7). NO_3-N loss in the control mesocosm was attributed to the growth of filamentous algae along the edges of the wetland mat. Only the median concentration of the control (1.202 mg/L) was significantly different (p = 0.002) than the unaerated (0.755 mg/L) and aerated (0.385 mg/L) treatments. However, the median daily change in NO_3-N concentrations was significantly

(p = 0.048) higher for the unaerated mesocosm (0.146 mg/L d^{-1}), while the loss rates of the control (0.052 mg/L d^{-1}) and unaerated (0.057 mg/L d^{-1}) mesocosms were similar and not significantly different.

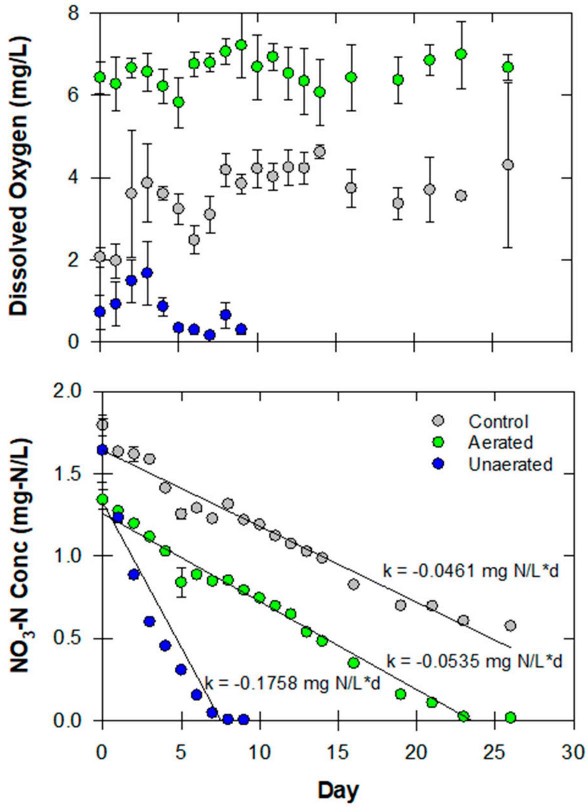

Figure 7. Mesocosm Experiment 2. Top: Temporal changes in dissolved oxygen concentrations inside treatment pools and outside (ambient). Bottom: Temporal changes in NO_3-N concentrations in aerated, unaerated, and control pools. Error bars on symbols represent standard errors; where absent, bars fall within symbols.

Using pool volumes (L) and initial NO_3-N concentrations (mg NO_3-N/L), the mass of NO_3-N in each pool (treatment) was calculated (Table 1). Likewise, the rate of NO_3-N removal (mg NO_3-N/d) for each treatment was estimated by two methods. Option 1 divided the initial mass of NO_3-N in the pool liner by the number of days needed to remove the NO_3-N. Option 2 used the fitted zero-order rates shown in Figures 6 and 7.

To estimate the total mass of NO_3-N removed by the two field-scale FTWs in the Densmore Pond, we extrapolated results obtained from the quarter-sized FTWs in the pool experiments to two full-sized FTWs (a scale-up factor of 8) and then estimated potential removal over a spring–fall season (i.e., May–September, 153 d). Details of this scale-up calculation are provided in the Supplementary Materials. While the method used to calculate NO_3-N removal rates (i.e., Option 1, 2) slightly influenced the overall estimates (Table 1), the mass of NO_3-N removed was much more contingent on whether the pools were aerated or unaerated. We estimate the mass of NO_3-N removed by Densmore Pond's two FTWs could vary between 0.63 kg NO_3-N and 3.73 kg (Table 1). This range, however, represents extremes in DO concentrations. The lowest estimate (0.63 kg) was from the mesocosm that was constantly aerated and had an average DO of 6.58 mg/kg. This high DO likely severely limited denitrification. Thus, the NO_3-N removal observed from this treatment was mainly attributed to plant uptake. The high estimate of NO_3-N removal (3.73 kg) was from the field experiment that had an average DO of 0.35 mg/L, where denitrification would be more optimal (Table 1). The NO_3-N removed from the unaerated treatment in Field Experiment 2 (1.82 kg, DO = 0.74 mg/L. Table 1) fell in between the observed range, and may be closer to denitrification losses under ambient conditions.

Table 1. Nitrate-N removal rates from in situ pool experiments extrapolated to two full-sized floating treatment wetlands.

Variable	Treatment								
	Field Experiment 1			Field Experiment 2			Laboratory 2		
	U 1	U 2	C	U	A	C	U	A	C
Pool volume (L)	12,619.5	15,226.6	13,184.3	8138.9	9991.3	7628.3	67.4	63.7	30.3
Pre-experiment NO_3-N conc (mg/L)	0.017	0.002	0.026	0.020	0.019	0.063	0.150	1.170	0.440
Starting NO_3-N conc (mg/L)	0.93	0.76	0.90	1.64	1.34	1.79	2.22	3.37	5.07
Mass of NO_3-N (mg)	11,736	11,572	11,869	13,369	13,398	13,688	150.4	214.2	153.5
Days to remove (d)	6	5	32	9	26	39	10	23	30
Average DO conc (mg/L)	0.35	0.35	1.50	0.74	6.58	3.60	5.97	6.48	8.07
NO_3-N removal rate (mg/d)	1956.0	2314.4	370.8	1485.4	515.3	351.0	15.04	9.31	5.12
Zero-order rate; k in figures. (mg/L*d)	0.1975	0.2000	0.0404	0.1758	0.0535	0.0461	0.2626	0.1686	0.1831
OPTION 1 Calc estimated kg of NO_3-N removed from 2 FTWs over 153 d	2.394	2.833	0.454	1.818	0.631	0.430	1.151	0.712	0.392
OPTION 2 Calc estimated kg of NO_3-N removed from 2 FTWs over 153 d	3.051	3.727	0.652	1.751	0.654	0.430	1.354	0.820	0.424

U = Unaerated, A = Aerated, and C = Control treatment.

3.1.3. Root Architecture and DO Concentrations Beneath Full-Scale FTW

The results from Field Experiment 1 and 2 clearly demonstrated the added aeration affected DO concentrations beneath the FTWs and that DO significantly influenced NO_3-N removal rates. Experiment 1 demonstrated that when DO concentrations are below 1.0 mg/L, nitrate has the potential to be removed within a few days. Measurements outside of the pool liners, however, indicated DO measurements inside the pool liners were not representative of the DO outside the pools (Figure 6). The results from Field Experiment 1 demonstrated the challenge of creating an enclosed root zone beneath an FTW that is representative of natural conditions.

The added aeration in Experiment 2 increased DO and slowed NO_3-N removal significantly. Although the field pool mesocosms represented large experimental units (9.3 m^2), the lack of constant exchange of water between the pool and the outside pond water likely kept DO concentrations lower than if the pool liners were not present. Therefore, to get a better estimate of how much NO_3-N was being removed by the two full-sized FTWs, we needed to know the range in DO concentrations beneath the full-sized FTWs.

Therefore, the root system beneath the FTWs was photographed (Figure 8) and the DO was monitored. A Hydrolab MS5 DO probe (Hach, Loveland, CO, USA) was installed into a cluster of roots and allowed to record temperature and DO measurements every 15 min over several days. The results from the MS5 DO probe demonstrated diurnal fluctuations in DO, likely caused by changes in temperature, sunlight (photosynthesis), atmospheric gas exchange, and heterotrophic consumption of plant carbon (Figure 9). Median DO concentrations were not significantly different (p = 0.23) for the under (8.44 mg/L) and adjacent (8.74) probe deployments. Despite no statistically significant differences in median DO concentrations, there were notable differences when comparing the timeseries of each deployment (Figure 9).

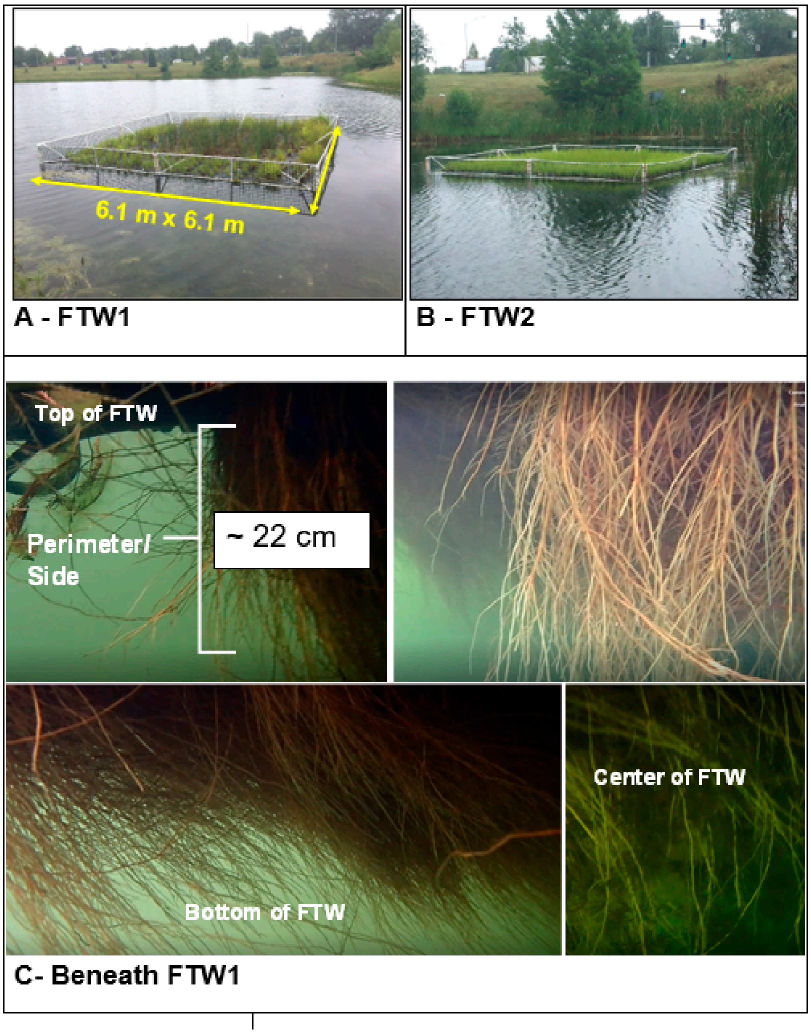

Figure 8. (**A**,**B**) Photographs of FTW1 and FTW2 (above water). (**C**) Underwater photographs of rooting system of FTW1.

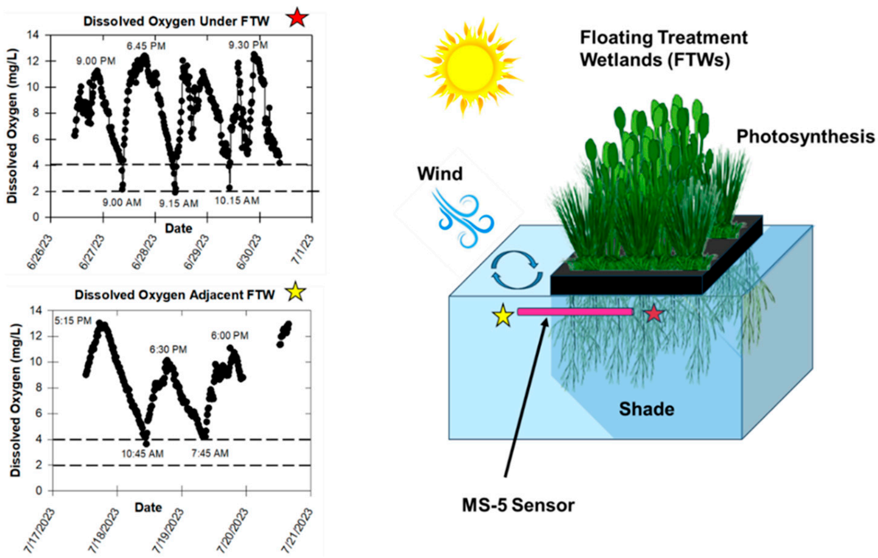

Figure 9. (**Top Left**): Diurnal fluctuations in dissolved oxygen concentrations beneath FTW 1 (red star). (**Bottom Left**): Diurnal fluctuations in dissolved oxygen concentrations outside of FTW1 (yellow star). (**Right**): Schematic of MS-5 Sensor deployments.

Outside the FTW, the DO fluctuated between highs of 10 to 13 mg/L to lows of 4 mg/L (Figure 9). Beneath the FTW, similar fluctuations occurred, but timings of peaks and minimums were slightly different; specifically, the highest DO readings were later in the evening and the lowest DO measurements were later in the morning (Figure 9). Also, the DO concentrations beneath the FTW varied from high values of 10 to 13 mg/L to lows around 2 mg/L (Figure 9), which were roughly 2 mg/L lower than lows recorded outside the FTW (~4 mg/L). Reddy and DeLaune [25] reported that anoxic and anaerobic micro-zones are likely to occur beneath floating mats, in the soil media, and in biofilms, with the potential to induce microbial denitrification. Anoxic and anaerobic micro-zones can increase at nighttime, when photosynthesis is not operative, and respiration could lead to decreased oxygen concentrations.

While use of the MS5 DO probe identified different diurnal fluctuations of DO concentrations when placed beneath or outside of the FTW (Figure 9), we acknowledge the spatial resolution of the probe was in centimeters (cm) and not fine enough to measure DO concentrations in microsites (mm) or in biofilms surrounding the roots. Nonetheless, the 2 mg/L lower DO readings we observed beneath the FTW, compared to outside of the FTW (Figure 9) indicate even lower DO concentrations are likely prevalent within microsites and biofilms in the FTW's rhizosphere. Further, evidence that denitrifying conditions can form under the FTW was reported by Borne et al. [26] who demonstrated the size of FTWs can influence DO concentrations. A 23 m^2 FTW in North Carolina (USA) maintained DO concentrations below 0.5 mg/L for 5 to 7% of the time, while a 50 m^2 FTW in New Zealand induced denitrification conditions (DO < 0.5 mg/L) during most of the summer months. Given the size of the FTW can impact DO concentrations, we acknowledge that our nitrate removal estimates may have been higher had we been able to encompass the entire 6.1 m $\times$ 6.1 m FTW inside a pool liner rather than a quarter section (3.05 m $\times$ 3.05 m).

3.2. Laboratory Microcosms

3.2.1. Laboratory Experiment 1

Laboratory Experiment 1 compared NO_3-N removal rates between an aerated and unaerated mesocosm. The unaerated microcosm removed NO_3-N at a faster rate but there was a lag, or slower rate of decline, during the first 8 d, followed by a more rapid rate of decline during the last 8 d (Figure 10). This lag was most likely due to a limited amount of carbon released from the smaller root system (i.e., lower root density compared to field), combined with chlorine present in tap water temporarily shocking microbes in the treatment tank. In stark contrast, the aerated microcosm did not exhibit a decrease in NO_3-N over the entire experiment (17 d). This may be due in part to the experiment taking place in winter (9–26 January), when plant uptake of NO_3-N is at its minimum [7]. The DO concentrations in both treatments were also significantly different for measurements taken continuously and discretely ($p < 0.001$). Discrete DO concentrations in the aerated tank ranged between 6 and 8 mg/L with a median of 6.89 mg/L, while DO concentrations in the unaerated tank were between 3 and 4 mg/L. Median continuous DO concentrations were 7.02 mg/L for the aerated tank and 3.57 mg/L for the unaerated tank (Figure 10). The fiber-optic measurements matched the YSI DO measurements in the aerated tank, but in the unaerated tank, diurnal DO fluctuations, like those observed in the field (Figure 9), were evident. These diurnal fluctuations in DO, however, were not manifested in observed NO_3-N removal rates, which were generally constant or zero-order (e.g., Figures 6, 7 and 9). This means denitrification was occurring within biofilms or microsites where DO concentrations remained below the threshold needed for constant NO_3-N removal.

The results from Laboratory Experiment 1 confirm NO_3-N removal rates were affected by DO concentrations, with no significant NO_3-N loss occurring when DO values were above 6 mg/L. By contrast, NO_3-N removal was observed when DO values were ~4 mg/L or lower (Figure 10).

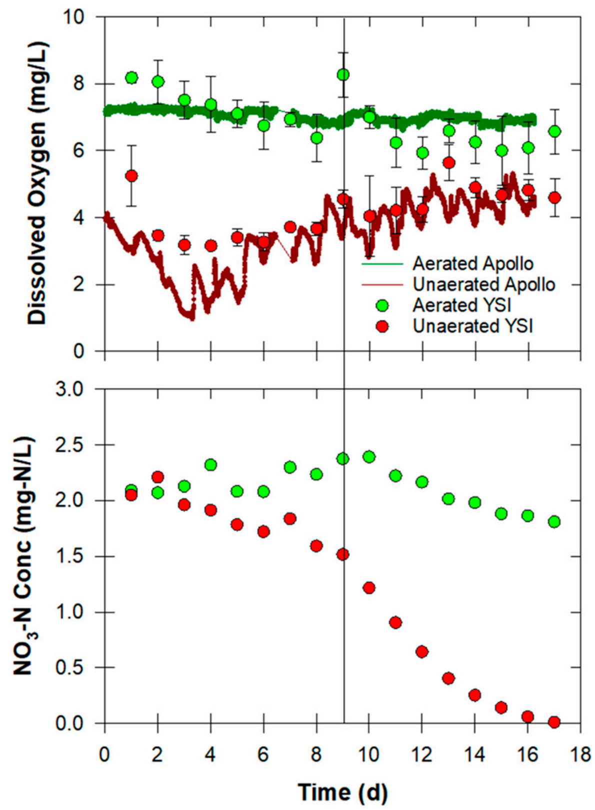

Figure 10. Laboratory Experiment 1. Top: Temporal changes in dissolved oxygen concentrations in aerated and unaerated mesocosms. Bottom: Temporal changes in NO_3-N concentrations in aerated, unaerated mesocosms. Error bars on symbols represent standard errors; where absent, bars fall within symbols.

3.2.2. Laboratory Experiment 2 (^{15}N-NO_3)

Plants used in Laboratory Experiment 1 were utilized, with the experiment occurring from 2 to 25 August 2023. Although artificial light was supplied for both experiments, annual growth cycles may not have been in sync between experiments, with plants being more active in the summer [8]. Nitrate removal rates differed between the control (0.191 mg/L*d), aerated (0.205 mg/L*d), and unaerated tanks (0.280 mg/L*d) (Figure 11). The unaerated tank removed NO_3-N within 9 days, while the aerated tank took over 20 d. The observed NO_3-N removal rates in Laboratory Experiment 2 (Figure 11) resembled the results obtained from Field Experiment 2 (Figure 7).

We similarly extrapolated NO_3-N removal rates from Laboratory Experiment 2 to the field (two 6.1 by 6.1 m FTWs over 153 d), as performed for the field mesocosm experiments (see Supplementary Materials for detailed calculations). Despite the large difference in scale-up factors between the laboratory experiments and the field experiments (see Supplementary Materials), the overall NO_3-N removal (kg NO_3-N) estimates were reasonably close: Unaerated 1.35–1.51 kg (Lab Exp 2) versus 1.82–1.75 kg (Field Exp 2); Aerated 0.71–0.82 kg (lab) versus 0.63–0.65 kg (field) (Table 1). The fact that the estimated mass of nitrate removed by two full-scale FTWs was similar when using input data collected in Field Experiment 2 versus Laboratory Experiment 2 (Table 1), lends credence to the experimental approach. It is also noteworthy that the DO values of the laboratory experiments were higher than the field. This difference may simply be due to the ratio of plant roots to water volume in the enclosures. High root densities would favor more carbon release and lower DO concentrations.

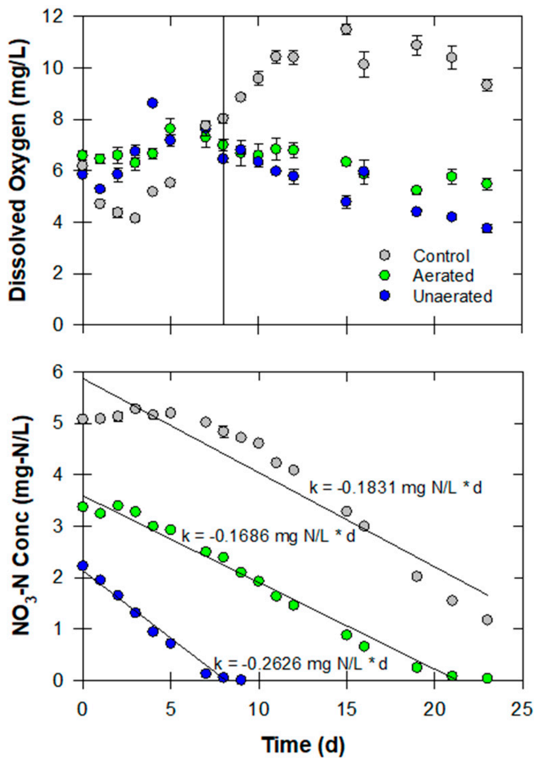

Figure 11. Laboratory Experiment 2. Top: Temporal changes in dissolved oxygen concentrations in aerated and unaerated mesocosms. Bottom: Temporal changes in NO_3-N concentrations in aerated and unaerated mesocosms. Error bars on symbols represent standard errors; where absent, bars fall within symbols.

Dissolved oxygen concentrations among treatments in Laboratory Experiment 2 were also similar to Laboratory Experiment 1, where the aerated tank had a median DO of 6.45 mg/L; the control had a median DO of 4.65 mg/L but later increased to over 10 mg/L (Figure 11). The unaerated tank remained above 4 mg/L, with a median of 6.09 mg/L. Only the unaerated and control tanks had significantly different DO (p = 0.039) over the course of the experiment. For the control treatment (Figure 11), the growth and photosynthesis of *Aphanizomenon* cyanobacteria on all sides of the tank were clearly responsible for the increase in DO and NO_3-N removal.

The DO of the unaerated tank was not consistent during the first 8 d (4–6 mg/L) and then linearly declined (Figure 11). Despite the higher DO concentrations (4–6 mg/L), NO_3-N in the unaerated treatment decreased rapidly during the first 8 d. The removal of NO_3-N in the laboratory experiments under higher DO concentrations than those observed in the field experiments indicate that either the YSI measurements were not adequately reflecting DO concentrations near the plant roots, or more likely, other processes were operative. The first major difference between the field and laboratory experiments was that the rooting density beneath the field FTW was much greater, meaning it was easier to place the YSI probe into the roots zone (Figure 8). Secondly, the laboratory experiments had significant growth of *Aphanizomenon* cyanobacteria, forming films on roots and attached to the sides of the treatment tank. Similar growth in the field experiments was not observed due to the opaque pool liners, whereas laboratory tanks were translucent.

Plant analyses exhibited no significant difference in per-species tissue TN or ^{15}TN content. Aerated biomass (which included the cyanobacteria) had slightly higher TN content (2.66%) than the unaerated treatment (2.38%) (Table 2). Conversely, the aerated biomass had lower ^{15}TN% (2.11%) than the unaerated treatment (2.40%). The *Aphanizomenon* cyanobacteria had the highest TN content and ^{15}TN content for both the unaerated and aerated microcosms.

Table 2. Results of ^{15}N% and N% from Laboratory Experiment 2.

Treatment	Species	Sample Type	15 N	N
			%	%
Aerated	*C. crinita*	Shoot	0.56	2.54
		Root	1.52	1.35
	C. comosa	Shoot	0.53	2.77
		Root	3.08	1.15
	S. tabernaemontani	Shoot	0.75	1.68
		Root	0.77	2.60
	Aphanizomenon	-	7.55	6.55
		Average	2.11	2.66
Unaerated	*C. crinita*	Shoot	0.45	2.61
		Root	1.33	1.47
	C. comosa	Shoot	0.70	1.95
		Root	4.16	1.27
	S. tabernaemontani	Shoot	0.70	1.95
		Root	1.94	2.41
	Aphanizomenon	-	7.55	5.02
		Average	2.40	2.38

Due to the relatively high ^{15}TN content of the photosynthetic cyanobacteria, ^{15}N enrichment in tank waters was perhaps less than what normally would be observed during denitrification, where $^{14}NO_3$ is known to react faster than $^{15}NO_3$ [27]. While the ^{15}N enrichment in biomass was higher in the unaerated treatment than the aerated treatment, these values were not significantly different (Table 2). Future research should consider analyzing $^{15}N/^{14}N$ ratios in water and plants; shielding light from the root zone to limit photosynthetic cyanobacteria from growing would also be beneficial.

3.3. Nitrate Load and Sustainability of FTWs

Estimates from this research showed for a 153 d treatment season (May–September), between 0.63 and 3.73 kg NO_3-N could be removed per year by two mature 6.1 by 6.1 m FTWs (Table 1). At the start of this project in 2020, average NO_3-N concentration in the water column was 0.056 mg/L but decreased to 0.0013 mg/L in 2023. Given the average volume of the Densmore Pond (4,046,850 L), the initial mass of NO_3-N in the water column was 0.23 kg NO_3-N. Our calculated estimates of NO_3-N removed by the two FTWs (0.63–3.73 kg NO_3-N) combined with the decline in NO_3-N concentrations throughout this four-year study (Figure 5) supports that the FTWs contributed to the removal of NO_3-N from the Densmore Pond.

The sustainability of the FTWs to continue to remove incoming NO_3-N was also considered. McKercher et al. [2] calculated a total of 0.83 kg NO_3-N could enter the Densmore Pond in 2020 from the surrounding watershed using the SCS Curve number method. We expanded on this estimate by utilizing the Storm Water Management Model. In 2020, SWMM predicted four times as much NO_3-N entering Densmore Pond (3.21 kg) than the SCS Curve Number method (0.83 kg). The NO_3-N load from 2020 to 2022 was similar between years (3.21–4.86 kg per year), but the higher precipitation years preceding resulted in much higher variability (6.15 to 14.37 kg per year) (Table 3). As previously mentioned, the range of NO_3-N removed by the FTWs (0.63–3.73 kg NO_3-N) represents two extremes in DO concentrations beneath the FTWs. Given diurnal and seasonal fluctuations in DO and temperature beneath FTWs (Figure 9), the true mass of NO_3-N removed likely lies between these maximum and minimum values. Using an average removal rate of 1.8 kg NO_3-N, and predicted SWMM nitrate loads (Table 3), we estimate the Densmore Pond two FTWs could remove 14 to 56% of the annual incoming nitrate despite only covering ~2% of pond surface. The remaining NO_3-N could be removed by existing coontail (*Ceratophyllum demersum*) and American pondweed (*Potamogeton nodosus*) submerged in the pond, and cattails on the pond's edge, which are a hybrid of narrowleaf cattail (*Typha angustifolia*) and broadleaf cattail (*Typha latifolia*).

Table 3. Predicted nitrate loads to Densmore Pond using Storm Water Management Model (SWMM) from 2013 to 2022.

Year	Precipitation (cm)	SWMM Load (kg NO_3-N)
2013	64.26	6.15
2014	76.99	11.53
2015	89.69	14.37
2016	71.76	9.28
2017	82.17	12.35
2018	72.77	9.43
2019	66.55	6.16
2020	46.20	3.20
2021	45.49	4.86
2022	45.90	4.72

In summary, the results from our field and laboratory experiments showed NO_3-N removal beneath FTWs can be rapid when DO concentrations are low, confirming our initial hypothesis. Given our biological-chemical treatment increased the overall DO concentrations throughout the Densmore Pond over four seasons (Figure 5), using larger FTWs that favor denitrifying conditions is recommended. Spatially separating the FTWs from the airlift pumps (or pond aerators) to avoid aerating the root zone is also recommended. While our estimates of nitrate removal by two FTWs are specific to a midwestern retention pond in Lincoln, NE (USA), the range in nitrate removals presented (0.63–3.73 kg NO_3-N) should provide guidance in determining if FTWs are a logical choice for other retention ponds, based on local watershed nitrate loads.

Future research on quantifying nitrogen removal by FTWs should include analyzing a greater suite of nitrogen compounds (nitrite, nitrate, ammonium, and nitrous oxide), as well as dissolved oxygen and soluble carbon. Recognizing that enclosing a root zone for experimentation can shift the dynamics of available carbon, dissolved oxygen, and nitrogen species, more detailed and temporal in situ measurements of these parameters within the root zones of FTWs could aid in determining if experimental units (i.e., enclosed root zone) are adequately mimicking ambient field conditions and providing realistic estimates of nitrogen removal by floating treatment wetlands.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nitrogen5040053/s1. Figure S1. Aerial view of Densmore Pond, annotated with sampling points, FTWs and airlift pumps. Figure S2. Temporal changes in total nitrogen and total phosphorus concentrations in Densmore Pond from 2021 through 2023. Figure S3. Temporal changes in NO_3-N concentrations in aerated, unaerated and control pools. Figure S4. Photographs showing extrapolation of field experiments to two full-scale floating treatment wetlands. Table S1. Statistical analysis results of median water quality parameters at Densmore Pond. Significantly different with respect to [a] 2020, [b] each other (α = 0.05).

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