

# Research Paper Floating Wetlands beyond Retention Ponds: Estimating Nitrogen Cycling and Removal in Tidal Waters

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Efforts to meet nutrient reduction goals in the Chesapeake Bay watershed involve a variety of practices that remove nutrients before they are delivered to estuarine systems. Floating treatment wetlands (FTWs) have been a certified best management practice (BMP) implemented for decades in retention ponds and wastewater treatment plants but have only recently been placed in tidal waters to remove nutrients directly. Given the paucity of data on nitrogen (N) cycling within tidal FTWs, we measured N transformation and removal within FTWs deployed in estuarine-like mesocosms during a 12-week experiment in summer 2019 and a 10-week experiment in spring 2021. Results indicate a comparable, net removal of total N (TN) for both summer 2019 and spring 2021, but substantial transformations of nitrogen within the FTW. Nitrate + nitrite (NO<sub>2+2</sub>) was generated while ammonium (NH<sub>4</sub><sup>+</sup>) and particulate N (PN) were removed from the mesocosm. Nitrogen concentrations measured in different parts of the mesocosms and wetland media also indicate signs of transformation, where  $NO_{2+3}$  concentrations were 0.2 mg L<sup>-1</sup> higher in the media porewater than the inflowing water for both control and experimental mesocosms. This suggests relatively high rates of nitrification within the media. This nitrification could support measured denitrification rates (2.4 mg  $N_2$ -N m<sup>2</sup>h<sup>-1</sup> – 10.9 mg  $N_2$ -N m<sup>2</sup>h<sup>-1</sup>), which were at least 4 times higher than in oligohaline marshes and almost half the rates reported in restored oyster reefs that are also considered a BMP. This research has shown that floating wetlands have the capability to transform and remove N in estuarine-like environments, potentially expanding the areas in which floating wetlands can be deployed. Furthermore, this study provides measurements of these transformation and removal rates to inform future estimates of impact and remediation following FTW use in estuarine environments.

# **Keywords** Constructed floating wetland, Nitrogen removal, Estuaries, Ecological engineering

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# 1. Introduction

Nutrient pollution and eutrophication remains a problem globally, degrading coastal ecosystems through excess phytoplankton biomass and associated depletion of dissolved oxygen (DO) and food web disruption. Nutrients are essential for plants and animals to grow, but they may constrain coastal productivity in pristine environments where nitrogen (N) and phosphorus (P) are often limiting. However, when nutrient concentrations increase, associated increases in productivity drive elevated algal biomass (Nixon 1995), low oxygen (O<sub>2</sub>) zones (Diaz and Rosenberg 2008), and the degradation of coastal systems (Deegan et al. 2012). High rates of productivity and organic matter degradation also stimulate microbially mediated nutrient regeneration and transformation, including O<sub>2</sub>-sensitive processes like nitrification and denitrification (Kemp et al. 1990; Cornwell et al. 1999). This nutrient-enrichment driven eutrophication significantly degrades water quality, which can negatively affect coastal organisms and hence recreational and economic benefits of coastal systems. To try to reverse this pollution problem, many socio-economic commitments to reduce watershed nutrient loading have been put into place worldwide (e.g., NRC 2009, Backer et al. 2010).

Efforts to meet watershed nutrient reduction goals in the Chesapeake Bay watershed (i.e., total maximum daily loads, or TMDLs) involve a variety of practices. Known as best management practices (BMPs), these range from cover crops and conservation tillage for managing diffuse pollution sources from agricultural lands, to biological N reduction technologies implemented for point sources from single wastewater treatment facilities. Improvements to wastewater treatment plants have allowed for a considerable reduction in N load to the Chesapeake Bay since 1985 (Clune et al. 2021). However, watershed model estimates suggest that there are still 42 million lb/y in N point sources and 209 million lb/y of non-point sources of N (CBP 2017) still entering the Chesapeake Bay. In the coming decades, additional nutrient load reductions will have to be made from these diffuse sources, which range from agricultural fields to urban stormwater. Many of these reductions will need to come from technologies involving land management practices, approved by the Environmental Protection Agency (EPA), that mitigate the polluted water from nearby surfaces and groundwater (Choi et al. 2020). However, issues of cost and limited adoption of these BMPs present a challenge to reaching land-based nutrient removal goals.

Alternatives to land based BMPs in the Chesapeake Bay TMDL framework include floating treatment wetlands (FTWs), which are a certified BMP technology (evaluated and approved by the EPA, academic researchers, engineers, and implementers) in which macrophytes are grown on a floating raft and/or media within a retention pond. The roots are in contact with the water column and they remove nutrients and other pollutants via several physicochemical and biological processes (Sharma et al. 2021). The implementation of FTWs has occurred primarily in retention ponds as a way to increase their efficiency at treating runoff and to overcome operational challenges and flaws, such as inconsistent hydrologic loads of nutrients and other pollutants. The buoyant nature of FTWs allows for the constant removal of nutrients and pollutants regardless of the water level in the retention pond. Previous research exploring the effectiveness of FTWs in retention ponds has employed the use of in-situ measurements (Nahlik and Mitsch 2006) and mesocosm experiments (Tanner and Headley 2011). While the relatively simple design of retention ponds allows for the quantification of N removal via input-output analysis (i.e., differences between inflow and outflow concentrations), this approach limits deeper understanding of N transformations and removal mechanisms that drive these reductions. FTW technology and research have evolved and expanded to engineered FTWs to treat other polluted waters, including urban and agricultural runoff (Stewart et al. 2008; Spangler 2017), secondary effluent (Gao et al. 2018), greywater (Faulwetter et al. 2011), mine tailings water (Gupta et al. 2020), and industrial wastewater (Knight et al. 1999).

In contrast to the common implementation of FTWs in retention ponds, implementation of FTWs in estuarine systems has not been widely employed or studied. In those places that have deployed FTWs, the same technology developed for retention ponds has been applied but utilizing coastal wetland plants like Phragmites australis (Sanicola et al. 2019). Microcosms installed in the floating wetlands deployed in the Inner Harbor of Baltimore, Maryland, United States, in 2009 provided evidence of nutrient transformation and potential removal, but extrapolation of these impacts, accounting for the ultimate fate of the nutrients, or the details of their transformation was not clear (Streb 2013). Research performed in FTWs deployed in the Southern Baltic Sea's tidal lagoons indicates that FTWs serve as protection for shorelines, as well as nursery habitats for shrimp and eels (Karstens et al. 2021). However, measurements of nutrients around those FTWs did not indicate any nutrient reductions in the surrounding waters (Karstens et al. 2021). Other research performed by Sanicola et al. (2019) in Queensland, Australia, explored shoot and root growth of suitable FTW plant species under different salinity environments. Research identified 2 plant

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species, *Isolepis nodosa* and *Baumea juncea*, with the potential to significantly reduce P as long as the plant is harvested (Sanicola et al. 2019). Thus, despite recent efforts to understand the role of the FTW as a habitat provider for local wildlife, a detailed understanding of the biogeochemical transformations in these wetlands is lacking.

To increase our understanding of the mechanisms of N removal and transformation in estuarine FTW, we explored the potential of FTWs to remove N from estuarine waters by measuring a comprehensive suite of N removal processes using mesocosm experiments under brackish water conditions. One experiment focused on measuring N removal and transformations in summer 2019 while the second experiment focused on measuring N removal and transformations in spring 2021. The experiments included triplicate mesocosms with vegetated FTWs and control tanks that included only the floating wetland media. The mesocosms allowed for a high degree of control, daily sampling frequency, and ease of measuring process rates (denitrification, plant uptake) that allowed us to assess N transformations and associated N losses. We hypothesized that treatments with vegetated media would remove a higher fraction of the N inputs than the control treatments with only the wetland media, as a result of enhanced nutrient removal through plant uptake and plant-associated denitrification.

# 2. Materials and Methods

# 2.1 Experimental Design

We designed and executed 2 mesocosm experiments to measure N removal and transformation during the spring and summer. The first experiment was performed from 2019 Jun 21 to 2019 Sep 9. The second experiment was performed from 2021 Apr 16 to 2021 Jul 22. These experiments were carried out in different years due to COVID-19 interruptions as well as logistics. The mesocosm design consisted of a flow-through system with 3 control tanks and 3 experimental tanks. Control tanks consisted of unplanted floating wetland media, while the experimental tanks consisted of the floating media planted with Spartina patens. To measure the effects of the floating wetland N removal, we measured N accumulation in plant material (roots, shoots, flowers, and seeds) and periphyton and detritus within the mesocosm tanks, as well as rates of N removal (denitrification) within the floating wetland matrix. Dissolved and particulate N (PN) concentrations from the inflowing water and outflowing water were collected weekly for both experiments (summer 2019 and spring 2021). For the spring 2021 experiment, N concentrations were also measured within the tank water column and within the media

# Highlight

Floating wetlands are capable of transforming and removing nitrogen from coastal systems.

pores. Water temperature, DO, salinity, specific conductivity, pH, and chlorophyll-a in the tank water column were monitored on a daily basis between 12:00 p.m. and 3:00 p.m. See the supplemental material (Table S1) for average environmental characteristics of the tanks. Hourly precipitation and photosynthetically active radiation (PAR) data were collected from the Chesapeake Biological Laboratory (CBL) monitoring station (https://cblmonitoring.umces.edu/). Plant material accumulation was measured at the beginning and at the end of the experiment, separated into above-ground and below-ground biomass. Denitrification was measured once a month during the course of each experiment.

# 2.2 Experimental Setup

Six mesocosm tanks that were 0.9 m in depth and 1.82 m in diameter were deployed in an outdoor setting at the CBL, Solomons Island, Maryland, United States (38.3194° N, 76.4540° W). The CBL is located in the lower portion of the Patuxent River and is known to be mesohaline (salinity ranges from 5 ppt-18 ppt). Pumped Patuxent River water (222 m from shore) (Figure 1) entered the tanks from the bottom and exited the tanks at the surface through a standpipe (Figure 1). Outflow discharge from each of the tanks went directly back into the Patuxent River. The flow rate was measured and adjusted daily via valves on the inflowing pipes to attain a residence time of 6 h, which is consistent with a semi-diurnal tidal cycle of 6 h. The 6-h residence time was meant to characterize the time it would take a given parcel of water to move through a given location in a tidal estuary with a semi-diurnal tide. In this case the parcel of water is moving through our mesocosm tanks. Three control tanks consisted of unplanted FTW media covering the surface area of the tanks to reduce light availability and associated water column photosynthesis that would lead to mesocosm artifacts. Experimental tanks consisted of the floating media with 19 Spartina patens plants, 88 kg of GardenPro top-soil, and approximately 44 kg of Premier Sphagnum peat moss. As in the control tanks, the FTWs covered the entire surface area of the tanks. During the experiment, the FTWs were designed to be partially submerged to simulate the high-marsh environment common to Spartina patens, commonly known as salt hay. This high-marsh grass was selected in the experiments because it is a common species in the tidal wetlands of the region, has the ability to withstand

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**Fig. 1.** (A) Idealized diagram of the experimental design in triplicate mesocosms tanks with and without floating wetlands. The flow of water enters through the bottom and exits the tanks through a centrally located standpipe. (B) The map shows the research pier at the University of Maryland Chesapeake Biological Laboratory (CBL), Solomons Island, Maryland, United States, where source water for the mesocosm tanks was obtained (Google Maps 2024). (C) The picture on the bottom shows control and experimental tanks on 2021 Jul 2.

partial flooding and a wide range of salinity from 0 ppt -30 ppt (Merino et al. 2010), and is readily available in local nurseries. Recent studies have also shown *S. patens* as a suitable plant for floating wetlands deployed in brackish environments (Landaverde et al. 2024). After the FTWs were installed and planted, they were left for 2 weeks before taking the first measurements, in order for the wetland plants to establish and for conditions within the tanks to stabilize.

# 2.3 Sample Measurements and Analysis

Dissolved ( $NH_4^+$ , DON,  $NO_{2+3}^-$ ) and particulate N (PN) concentrations were measured on a weekly basis from the inflow and outflow for summer 2019. For spring 2021 we included dissolved nutrient measurements from within the floating media (i.e. the porewater within the

media), as well as the water column inside each tank. Porewater was collected using a syringe to extract the water from the media, while inflow and outflow water was collected directly from the pipes just downstream from the valves that controlled water flow in and out of the tanks. Water samples from the different parts of the tanks were collected in 2-L Nalgene bottles that were previously acid washed. Inflowing water was measured at one valve where water entered the plumbing for the entire mesocosm system, and the N concentration at this inflow was assumed to be representative for the water entering at the valves for all tanks. Prior to starting the experiment, dissolved N was measured from 2 different valves to confirm that the inflowing concentration of dissolved N was not substantially different between all tanks (i.e., N was not transformed within the plumbing

system). The mean  $\pm$  standard deviations of measurements for ammonium (NH<sub>4</sub><sup>+</sup>) (0.034  $\pm$  0.0004 mg L<sup>-1</sup>) and nitrate + nitrite  $(NO_{2+3})$  (0.02 ± 0.002 mg L<sup>-1</sup>) of the 2 valves indicate highly comparable concentrations (low standard deviation), indicating no significant difference between the inflow pipes. Samples were sent to the CBL Nutrient Analytical Services for analysis. NH4<sup>+</sup> was analyzed using standard methods (4500-NH3 G-1997, MDL 0.009 mg L<sup>-1</sup>), NO<sub>2+3</sub> was analyzed using the catalyzed enzyme reduction method (ASTM D-7781, MDL 0.0057 mg L<sup>-1</sup>), dissolved organic N (DON) was analyzed following the EPA 365.1 method (MDL 0.05 mg  $L^{-1}$ ), and particulate N (PN) was analyzed following the EPA 440.0 method (MDL 0.0263%). Discrete sampling of water temperature, DO, salinity, specific conductivity, and pH were measured on a daily basis using a YSI Sonde MPS 556 just below the floating matrix. Chlorophyll-a was monitored on a daily basis and analyzed within 28 days of collection using 90% acetone extraction technique and fluorometric analysis (EPA 445.0, MDL 0.68  $\mu$ g L<sup>-1</sup>). Water volumes for chlorophyll-a analysis ranged between 60 mL and 180 mL depending on the location (inflow, outflow, water column and media porewater). YSI Sonde calibration was performed weekly, whereas fluorometric calibration was performed every 60 days. We measured whole-tank above- and belowground biomass at the beginning and end of the experiments. Above-ground and below-ground biomass was collected following Kreeger (2014a; 2014b). Initial above- and below-ground biomass was calculated by randomly selecting 3 plants before planting. Since plants were all the same size, we assumed all 3 tanks started with the same above- and below-ground biomass. Plant material was rinsed with deionized (DI) water and dried at 60 °C to constant weight. Then plant material was ground and analyzed for N content (Zimmermann et al. 1997) (MDL 0.01%) to estimate whole-tank plant N content. To estimate final above-ground biomass, 9 S. patens plants per tank were collected, rinsed with DI, and dried at 60 °C to constant weight and further analyzed for N content. The rest of the above-ground material from each tank was harvested, rinsed with DI water, and dried at 60 °C until the material reached a constant weight. The N content of all sampled plants was averaged and multiplied by the total amount of above-ground biomass in each tank to obtain the total above-ground biomass in mg of N per tank. For final below-ground biomass, 3 randomly selected squares from each floating matrix were cut out and their area was measured (between 40 cm<sup>2</sup> and 40.5 cm<sup>2</sup> per square). All root material was extracted from the matrix in each square, rinsed with DI and dried at 60 °C, and further analyzed for N content. These subsamples are assumed to represent the whole tank, and the subsampled belowground biomass and N content were extrapolated to compute a whole-tank N content.

Denitrification rates (which are actually measured N<sub>2</sub>-N fluxes) were measured in summer 2019 and spring 2021, while nutrient fluxes  $(NH_4^+ \text{ and } NO_{2+3}^-)$  were only measured during spring 2021. These fluxes were measured at 2 randomly selected, pre-defined locations within each FTW media in both the control and experimental mesocosms. Once a month, 2 pre-cut cores of floating wetland matrix from each tank were collected, placed on a 6.5 cm plexiglass core, gently filled with ambient water, and capped with an O-ring-sealed top. One core containing water from the tanks was used as a blank to distinguish between the denitrification happening in the water column and the media. The cores were then placed in the dark in a temperature-controlled water bath to attempt to simulate in-situ conditions. After reaching in-situ conditions, the cores were incubated for 3 hours in the dark. This method is an adaptation of a method used to measure denitrification and nutrient fluxes from bottom sediments (Testa et al. 2022). Every hour, samples were collected in 12-mL exetainers (Labco, UK) from each core and fixed with 60 µL of a saturated mercuric chloride solution (Fulweiler et al. 2007). Samples were analyzed using a membrane inlet mass spectrometer (MIMS, Bay Instruments, Easton, Maryland, United States) and the N<sub>2</sub>/Ar technique (Kana et al. 1994). The N<sub>2</sub>/Ar technique measures the net nitrogen gas (N<sub>2</sub>) production in the sample core, thus denitrification rates and N fixation rates cannot be separated. Thus, the linear increases in N<sub>2</sub> concentration were used to estimate net N<sub>2</sub> production during the experiment as net N<sub>2</sub> flux, or net denitrification (Kana et al. 1994; Cornwell et al. 2016). Detailed calculation on how flux rates were estimated can be found in the supplementary material. Section 1.

### 2.4 Measuring and Removing Periphyton

Mesocosm walls can substantially alter experimental conditions by limiting water exchange, absorbing downwelling light, and providing substrate for a myriad of organisms to grow (Kemp et al. 2009). Because the ratio of wall surface to water column is high in mesocosms, and because periphyton accumulates on tank walls, we sought to directly measure the relative contribution of periphyton to the biomass and N budget of each tank (Chen et al. 1997; Chen et al. 2000). To prevent excess periphyton accumulation, all periphyton in each meso-cosm tank was scrubbed from the walls and collected on a weekly basis. To collect the periphyton, the floating media was lifted using a gantry crane. A 500  $\mu$ m mesh was added to the outflow pipe and then the tank was drained. The wall and tank bottom were scrubbed using a

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clean brush and all material gathered. The collected periphyton was then brought to the laboratory, rinsed with DI water, and dried at 60 °C until a constant weight was reached. All the periphyton collected was analyzed for PC and PN to quantify how much N was being removed by tank artifacts.

# 3. Results

### 3.1 Nutrient Removal

To identify the overall amount of N being removed from the mesocosms, we calculated differences in total N (TN) between the inflow and the outflow rates. TN concentrations were estimated by adding all pools of N together. Overall, experimental mesocosms for summer 2019 and spring 2021 showed the highest removal of TN (i.e., outflow < inflow) when compared to the control mesocosms. Experimental mesocosms removed a total of 23.7 g TN during summer 2019 and 17.3 g TN during spring 2021 experiments. Meanwhile, the control removed a total of 17.1 g TN during summer 2019 experiments and 8.3 g TN during spring 2021 experiments. On 2 occasions during summer 2019 and spring 2021, the control mesocosms became sources of TN, meaning that the concentrations of TN were higher in the outflow than in the inflow (Figure 2).

To further understand N cycling within the tanks, we computed differences between the inflow and the outflow of  $NH_4^+$ ,  $NO_{2+3}^-$ , DON, and PN to determine if there was a net production (outflow>inflow) or net consumption (outflow<inflow) of each analyte. These net production/ consumption calculations quantify the balance of processes that produce and consume each analyte, and thus represent a net transformation, not a permanent removal or production. We hereafter define a net transformation that increases the concentration of an analyte as "production" and a net transformation that decreases the concentration of an analyte as "consumption".

Data suggest a substantial N transformation in both control and experimental mesocosms and years given the observed decrease in  $NH_4^+$  and subsequential increase of  $NO_{2+3}^-$  inside the tanks. During the summer 2019 experiments, the control mesocosms produced 26.4 g  $NO_{2+3}^-$ , while the experimental mesocosms generated 33.0 g  $NO_{2+3}^-$  (Figure 3). In contrast, both treatments showed consumption of DON,  $NH_4^+$ , and PN. Similarly, in the spring 2021 experiments, the control mesocosms produced 16.5 g  $NO_{2+3}^-$  while the experimental mesocosms produced 15.1 g  $NO_{2+3}^-$  (Figure 3). Both control and experimental mesocosms consumed PN



**Fig. 2.** TN weekly removal rates from mesocosm experiments in summer 2019 and spring 2021. Positive values indicate an overall addition and/or production of N in the mesocosm tank, while negative values indicate an overall removal of N. Error bars represent the standard deviation from the mean.

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**Fig. 3.** Weekly removal rates of DON,  $NH_4^+$ ,  $NO_{2+3}^-$  and PN in both control and experimental mesocosms in summer 2019 and spring 2021. Negative values indicate a net removal of PN species. Asterisks indicate where the tanks experienced a production of TN.

and  $NH_4^+$  during the spring. Because there is a substantial amount of  $NO_{2+3}^-$  being produced in the system for both years, we further grouped the dissolved inorganic pools (DIN) (DIN =  $NH_4^+ + NO_2 + NO_3$ ). The results shown in Figure 4 indicate that for both years, the control and experimental mesocosms consumed PN but produced inorganic forms of N. During the spring 2021 experiment (Figure 4), DON was produced in both control and experimental.

During the spring 2021 experiment, measurements of N concentrations from the tank's water column and FTW media porewater suggested temporal variation in N transformations.  $NO_{2+3}$  concentrations from the different parts of the mesocosm for both control and experimental





showed no difference at the beginning of the experiment (initial 20 days), but beginning on Day 20 (mid-May),  $NO_{2+3}$  increased in the porewater (see the supplementary material, Figure S2). The outflow NO2+3 concentration was 0.1 mg L<sup>-1</sup> higher than the inflow during this period (i.e., net production). The opposite pattern was measured for NH4+ in both control and experimental mesocosms, which increased inside the media during the first part of the experiment (and prior to nitrate [NO<sub>3</sub>-N] increase) and then decreased in concentration during the second half of the experiment (~Day 30). The  $NH_4^+$  concentrations at times differed by 0.03 mg L<sup>-1</sup> between the inflow and the media. By the latter half of the experiment,  $NH_4^+$  concentrations were lower in the media than in the water column, while media  $NO_{2+3}$  concentrations were higher than other pools. Lastly, when exploring DON concentrations at different locations in the tanks (see the supplementary material, Figure S2), there was not a substantial difference in concentrations between either tank location or the treatment type.

### 3.2 Nitrogen Transformations and Oxygen Consumption

Denitrification (N<sub>2</sub>-N) rates varied throughout the length

of the summer 2019 and spring 2021 experiments. During the summer 2019 experiments,  $N_2$ -N removal rates increased towards the end of the experiment with the highest flux being 13.4 mg  $N_2$ -N m<sup>-2</sup>h<sup>-1</sup> for the control mesocosm and 10.3 mg  $N_2$ -N m<sup>-2</sup>h<sup>-1</sup> for the experimental mesocosm (Figure 5). The spring 2021 experiments showed a slightly different behavior with the control

mesocosms having the highest N<sub>2</sub>-N removal rate at the end of the experiment (14.71 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup>, this includes an outlier), while the experimental mesocosms had the highest N<sub>2</sub>-N removal rate at the beginning of the experiment (10.9 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup>; Figure 5). Repeated measures ANOVA indicated a significant difference in rates between months (p < 0.05) but no significant

throughout the experiment. Note circles represent outliers.



**Fig. 5.** Denitrification measurements made monthly during the summer 2019 and spring 2021 experiments. Boxplot of denitrification fluxes (thick horizontal bars are median rates) illustrates peak fluxes in September and similarity between control and experimental mesocosms. Spring 2021 denitrification fluxes were similar to the summer rates from 2019, however higher fluxes occurred in April relative to September. Note circles represent outliers.



**Fig. 6.** O<sub>2</sub> consumption fluxes measured for the control and experimental mesocosms

during summer 2019 and spring 2021. Boxplot of the O<sub>2</sub> fluxes (thick horizontal lines

are median rates) measured monthly indicate highest O<sub>2</sub> consumption in the month of September. April fluxes indicate little O<sub>2</sub> consumption however demand increases

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difference between treatments (p > 0.05) for 2019 and 2021.

consumption Oxygen fluxes also varied between the summer 2019 and spring 2021 experiments. During summer 2019 there was an increase in O<sub>2</sub> consumption in both the control and experimental mesocosms over the course of the experiment (Figure 6). However, the variability was lower in the experimental mesocosms. The highest  $O_2$  consumption was reported at the end of the experiment: The control was -391 mg  $O_2$  m-<sup>2</sup>h<sup>-1</sup>, while the experimental mesocosm was -232 mg O<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>. Oxygen consumption fluxes recorded



**Figure 7.**  $NH_4^+$  and  $NO_{2+3}^-$  fluxes measured during 2021 mesocosm experiments. April fluxes indicate an uptake of  $NO_{2+3}^-$ . However, as the experiment continues, fluxes seem to transition from consumption to production and then to almost no flux of nutrients. Error bars represent 1 standard deviation of the mean of 3 tanks and circles are considered outliers.

for spring 2021 (Figure 6) indicated a slightly different behavior than those from 2019. During the month of April, the O<sub>2</sub> fluxes were close to zero, then they increased over May and June. Repeated measures ANOVA indicated a significant difference in O<sub>2</sub> fluxes between months (p < 0.05) but no significant difference between control and experimental mesocosms (p > 0.05) for 2019 and 2021.

In addition to measuring denitrification and O<sub>2</sub> fluxes, we also measured  $NO_{2+3}^{-}$  and  $NH_4^{+}$  fluxes from all the cores in 2021 (Figure 7). During the months of April and June, fluxes of NO<sub>3</sub>-N indicated an overall removal (Figure 7) whereas the month of June indicated an overall production of NO<sub>3</sub>-N. Repeated measures ANOVA indicate a significant difference between months (p = 0.01for  $NO_{2+3}$ ) but no significant differences between treatments. Lastly,  $NH_4^+$  fluxes (Figure 7) also show high variability, but fluxes tended to be negative. Statistical analysis indicated no significant differences in fluxes between months and between treatments (p = 0.55). To quantify metrics of N cycling associated with the media, we derived a series of indices of N transformations (see the supplementary material, Section 1). First, we computed the nitrification needed to support denitrification. This metric assumes that any N that was denitrified in excess of the  $NO_{2+3}$  influx was generated through nitrification. Computed nitrification ranged between 12.5 mg N m<sup>-2</sup>h<sup>-1</sup> and 300 4.2 mg N m<sup>-2</sup>h<sup>-1</sup> for the control and 5.3 mg N m<sup>-2</sup>h<sup>-1</sup> and 10.3 mg N m<sup>-2</sup>h<sup>-1</sup> for the experimental treatment. To further understand these fluxes, we calculated the denitrification efficiency and NH<sub>4</sub><sup>+</sup>

recycling index (see the supplementary material). The computed denitrification efficiency was near 100% for all fluxes, and the  $NH_4^+$  recycling near zero, suggesting a very efficient conversion of available N to  $N_2$ .

### 3.3. Plant Biomass and Periphyton N Assimilation

In terms of N accumulation, above-ground biomass accumulated higher quantities of N when compared to the below-ground (Table 1). This behavior happened during both experiments in 2019 and 2021. Physically, the plants appeared to have developed very tall stems while the root mat development was modest. We removed 5 g – 70 g and <10 g of periphyton in the experimental and control tanks, respectively (see the supplementary material, Figure S3), accounting for less than 0.5 g N/week. A higher mass of periphyton was collected in the experimental tanks, but this material was around 1 % N, compared to 2% - 3% N in the control tanks (see the supplementary material, Figure S3).

#### 4. Discussion

#### 4.1 Nitrogen Removal was N-species Specific

Overall, our results indicate that floating wetlands are capable of removing TN from estuarine-like environments (Figure 2). These results are consistent with and/or similar to previous FTW research that found removal of TN in retention ponds (White and Cousins 2013; Lucke et al. 2019). Previous research has focused on overall TN changes and plant assimilation alone. Our research goes one step further and explores the transformations of different forms of N. Our results indicate that FTW

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Experiment Year	Above/Below	Initial	Final	Accumulation
Summer 2019	Above	0.67 g N	12.6 g N	11.9 g N (±2.6 g N)
Summer 2019	Below	0.98 g N	2.6 g N	1.6 g N (±0.35 g N)
Spring 2021	Above	0.33 g N	1.5 g N	1.2 g N (±0.45 g N)
Spring 2021	Below	0.50 g N	1.3 g N	0.8 g N (±0.17 g N)

**Table 1** Biomass N accumulation for both experiments in summer 2019 and spring 2021. Accumulation includes the standard deviation from the mean. These are overall accumulation values not extrapolated to surface area.

N removal results from N-species specific transformations associated with microbial remineralization, nitrification, and denitrification. Overall, PN, DON, and NH<sub>4</sub><sup>+</sup> were consumed in the tanks, but NO<sub>2+3</sub> was produced. During both summer 2019 and spring 2021, the FTWs seemed to have supported overall nitrification with a decrease in  $NH_4^+$  and a subsequent increase in  $NO_{2+3}^-$  (Figure 3). This apparent production of NO2+3 is not consistent with previous studies, where Messer et al. (2022) reported that FTWs are capable of removing  $NO_{2+3}$  and Chang et al. (2012) showed that under specific retention pond conditions and areal coverage, FTWs can remove 100% NH<sub>4</sub><sup>+</sup>-N and up to 73% NO<sub>3</sub>-N. Our data suggest that transformations of  $NH_4^+$  to  $NO_{2+3}^-$  could be supported by biofilm and rootassociated microbial communities. Root-associated microbial communities have long been an important part of floating wetland technology (Urakawa et al. 2017; Choudhury et al. 2019; Gao et al. 2019), whose activity leads to the cycling and removal of nutrients through assimilation, nitrification and denitrification in concert with microalgae that assimilate inorganic forms of nutrients. Our measured rates of net NH<sub>4</sub><sup>+</sup> production (Figure 7) in the floating media, although variable, remained close to or below zero, which indicates potential nitrification given that we would expect high rates of remineralization in the media (e.g., O<sub>2</sub> consumption was substantial) (Figure 6). Furthermore, the fact that the net NO<sub>2+3</sub> fluxes were positive in May (and somewhat in April) (Figure 7) support the idea of active nitrification, which in turn supports denitrification (Kemp et al. 1990) and nutrient removal.

### 4.2 Denitrification — the Main Nitrogen Removal Pathway

Denitrification in FTWs has previously been assumed to be an important contributor to N removal, but this process has rarely, if ever, been directly measured in FTWs. Researchers have typically used an acetylene inhibition technique to estimate the potential for denitrification in roots. Results from these experiments indicate that root-associated denitrification was the major N removal pathway in floating wetlands in freshwater, sub-arctic regions, removing between 0.4 N<sub>2</sub>O-N m<sup>-2</sup>h<sup>-1</sup> and 30 mg N<sub>2</sub>O-N m<sup>-2</sup>h<sup>-1</sup> (Choudhury et al. 2019). Other studies in freshwater conditions have developed FTWs with carbon amendments to stimulate denitrification (Messer et al. 2022) and thiosulfate additions to stimulate autotrophic and heterotrophic denitrification (Gao et al. 2018). Messer et al. (2022) showed no significant differences in denitrification between the carbon amendment and control. Meanwhile, Gao et al. (2018) indicated that increased denitrification and plant assimilation with a peak TN removal of 636 mg TN m<sup>-2</sup>h<sup>-1</sup> because thiosulfate increases anoxia and allows denitrification. Our results indicate that denitrification played a major role in removing N from our FTWs under mesohaline conditions. The lowest denitrification rate we measured was 4 times higher than the rates reported for tidal and brackish sediment by Cornwell et al. (2016) (0.8 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup>) while our highest denitrification flux was half of those reported for restored oyster reefs by Kellogg et al. (2013) (21.8 mg  $N_2$ -N m<sup>-2</sup>h<sup>-1</sup>). When comparing our results to oligohaline marshes, our rates are also 4 times higher than those reported by Merrill and Cornwell (2000) (0.8 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup>). Regardless of the experimental period (summer 2019 or spring 2021), our denitrification rates suggest that FTWs are efficient at removing N.

Interestingly, our results also indicate that the control (unplanted media) was also able to sustain rates of denitrification that were comparable to wetland-planted mesocosms. Denitrification in the control ranged from 2.5 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup> to 13 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup> in the summer and from 4.1 N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup> to 25.6 mg N<sub>2</sub>-N m<sup>-2</sup>h<sup>-1</sup> in the spring. Past research investigating the contribution of the media to nutrient transformations and/or removal have shown mixed results. Stewart et al. (2008) indicated that Bio-Haven media successfully promoted nitrification and denitrification, removing 10.6 g day<sup>1</sup> of NO<sub>3</sub>-N and 0.2 g d<sup>-1</sup> of NH<sub>4</sub><sup>+</sup>-N. Garcia Chance et al. (2019) reported that their control performed better than the unplanted and planted treatments but acknowledged that periphyton might have played a role in nutrient transformations and assimilation. Wang et al. (2014) reported that their media facilitated the removal of nutrients in a similar fashion to the planted media. Lastly, Hu et al. (2010) found no difference between a media and no media treatment in an ecological sludge floating-bed artificial ecosystem.

Nonetheless, our results indicate that the porous media and associated biofilm in the FTWs we examined has the potential to support an environment ideal for nitrification and denitrification (Garcia et al. 2022).

The high denitrification we measured were also associated with comparably low  $\mathrm{NO}_{2^{+3}}^{-}$  and  $\mathrm{NH_4^{\,+}}$  fluxes, suggesting internal N cycling to support the observed denitrification. For example, the computed denitrification efficiency was near 100%, while the NH<sub>4</sub><sup>+</sup> recycling efficiency was near zero, suggesting very efficient conversion of available N to N<sub>2</sub>. One explanation for this efficiency is the rapid conversion of DON to  $NH_4^+$  and  $NH_4^+$  to oxidized N, and the apparent nitrification flux we derived is of the same order of magnitude as the denitrification rate  $(4,200 \text{ mg m}^{-2}\text{h}^{-1} - 11,200 \text{ mg m}^{-2}\text{h}^{-1})$ . Nitrification measured in estuaries is often higher than in open oceanic waters (Damashek et al. 2016), however it is highly variable within and between estuaries (Damashek et al. 2016). Although nitrification fluxes in wetland sediments have rarely been reported, our apparent fluxes are higher than nitrification measured in estuarine sediments (Kemp et al. 1990) and comparable to water-column nitrification rates measured in the York River and mid-Chesapeake Bay during destratification events (McCarthy et al. 1984), and in nutrient-rich estuaries like San Francisco Bay, California, United States (Damashek et al. 2016).

### 4.3 Variability in Denitrification Fluxes

Although our measured estimates of denitrification are high relative to other estuarine environments, including tidal wetlands, the fluxes were also highly variable. This suggests that there is a range of conditions within the floating wetland media that are more or less supportive of denitrification. Root development (and thus root content) was not evenly distributed throughout the media, and the algal biofilms that developed on the media were patchy. Plant-root presence might have affected the microbial community composition and thus contribute to variability in N cycling. Tanaka et al. (2012) and Urakawa et al. (2017) found the microbial composition of roots to be more diverse than unplanted media, and it was dominated by Alphaproteobacteria, Betaproteobacteria and Cyanobacteria. Thus, plant presence plays a role in selecting and or dictating the microbial composition in the floating media (Urakawa et al. 2017). More research is needed to better understand the differences in microbial-periphyton composition in planted and unplanted media.

# 4.4 Plant N Assimilation

Plant N assimilation is the way stakeholders in the Chesapeake Bay provide credit for the amount of N removed from a retention pond by an FTW (Lane et

al. 2016). Our results show a higher N accumulation in summer 2019 than in the spring 2021 experiment. Spartina patens has been shown to effectively assimilate N from brackish systems when compared to S. alterni*flora* and other wetland plants (Landaverde et al. 2024), making it suitable for use in FTW technology in estuarine-like environments. However, research indicates that although plants assimilate N during the growing season, it will leach back to the system if not harvested entirely (Wang et al. 2014). Even when harvesting twice during the growing season, plant N assimilation is only a small fraction of the N that is transformed and or potentially removed from the retention pond (Wang et al. 2014). As our results indicate, the media is capable of removing higher amounts of N through denitrification for both summer 2019 and spring 2021 (29 g N and 37 g N, respectively), when directly compared to plant N assimilation (15 g N and 1.7 g N, respectively). These results are consistent with previous studies that suggest most of the removal is done by the periphyton and root-associated biofilm (Wang and Sample 2014).

# 4.5 Floating Wetlands: beyond Retention Ponds

FTWs have been implemented as a way to overcome nutrient removal limitations of retention ponds and wastewater treatment plants for decades. Since 2016, FTWs have been certified as a BMP thanks to their removal efficiency and increased contribution to nutrient and sediment removal from multiple sources of runoff and wastewater (Lane et al. 2016). For decades, this technology has been implemented with the idea that plant growth is the main facilitator of nutrient and sediment removal. However, new research has suggested that the bulk of the nutrient removal happens thanks to the associated microbial communities growing in the floating media and dense root system. Our results, combined with previous research, contribute to the idea that bulk N removal happens effectively and permanently through microbial transformations. More importantly, these results are indicative that floating wetlands have capabilities of removal efficiency through plant and microbial communities in tidal systems. These results can help expand the FTW BMP credit and go beyond retention pond installations. Implementation of this technology in tidal systems can help expand and improve nutrient management efforts beyond land nutrient management in coastal areas where shoreline is highly developed and the restoration of wetlands is not an option. More importantly, the installation of these FTWs along developed coastlines can help abate nutrient in an era where population, land development, and temperatures are expected to increase.

# 5. Conclusion and Future Work

Our mesocosm study in estuarine-like environments revealed that FTWs foster an environment suitable for the transformation and removal of N through multiple pathways. The transformation and removal of N was form-specific, where the FTWs removed dissolved organic, particulate, and NH<sub>4</sub><sup>+</sup> but generated high quantities of  $NO_{2+3}$ . The net  $NO_{2+3}$  production appeared to sustain high rates of denitrification in the wetland media in excess of plant tissue uptake. The control treatment (un-planted media) transformed and removed N in ways comparable to the planted media, identifying microbial communities as a major player in the N transformation and removal in the FTW. Thus, this technology seems to foster a suitable environment for N removal beyond plant assimilation and subsequent harvest. These results are encouraging and support the potential for expansion of current BMP crediting and implementation of FTWs in urban tidal systems where restoration of natural wetlands is not an option. Future research could address some of the limitations of this study, including additional studies to understand the performance of floating wetlands in the fall and winter, experimentation with different species, and perhaps longer experiments that include the entire growing season. In situ studies of FTW performance in small estuaries could help develop methods that allow for accurate measurements of N transformations and changes in concentrations in, and around, the FTW. Additionally, future research should also include the evaluation of current implementation methods like anchoring techniques, evaluation of estuarine plant performance, and FTW designs better suited for tidal systems, and thus maximize N transformation and removal potential.

# **Supplementary Material**

The online version of this article contains a link to supplementary material that includes: **Table S1** Environmental characteristics for the mesocosm tanks in summer 2019 and spring 2021; **Figure S1** Active chlorophyll-a, total chlorophyll-a, and phaeophytin collected from the inflow and treatment tanks; **Figure S2** NO<sub>2</sub>+ NO<sub>3</sub>, NH<sub>4+</sub>, DON and PN concentrations within different environments in the mesocosm experiments in 2021; **Figure S3** Periphyton weekly accumulation in the control and experimental tanks.

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# **Author Contributions Statement**

Methodology: ISV, JMT, LAH; data analysis: ISV; writing original draft: ISV; reviewing/editing original draft: JMT, LAH; investigation: ISV; resources: JMT, LAH; supervision: JMT; project administration: LAH; funding acquisition: LAH, JMT. All authors have read and agreed to the published version of the manuscript.

# **Conflict of Interest Statement**

The authors have no conflict of interest to report.

# **Data and Code Availability Statement**

Data will be provided upon reasonable request.

# **Related Publication Statement**

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