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EVALUATION OF FLOATING TREATMENT WETLADS IN STORMWATER RETENTION PONDS ON POULTRY FARMS TO REDUCE NUTRIENT LOADING

by

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THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Joshua K. Lowman, entitled Evaluation of Floating Treatment Wetlands in Stormwater Retention Ponds on Poultry Farms to Reduce Nutrient Loading has been approved by the thesis committee as satisfactorily completing the thesis requirements for the degree Master of Science.

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ABSTRACT

EVALUATION OF FLOATING TREATMENT WETLADS IN STORMWATER RETENTION PONDS ON POULTRY FARMS TO REDUCE NUTRIENT LOADING

Joshua K. Lowman

Best management practices (BMPs) such as stormwater retention ponds are implemented to collect runoff and drainage from poultry farms and provide a possible intervention point for water treatment. Floating treatment wetlands (FTWs) are thought to remove pollutants from surrounding water columns. The majority of research conducted on FTWs focuses on nutrient removal via plant uptake. The results of prior studies conclude that plant uptake of nutrients alone is not a reliable source of nutrient removal because macrophytes and microalgae only temporarily immobilize N; this conclusion suggests that without periodic harvesting of plants, nutrient cycling would occur, while permanent removal of N is dependent upon denitrification. Floating treatment wetlands were evaluated to see if they could be used as a BMP. Denitrification potential was evaluated in the sediment and FTW through a denitrification enzyme assay by measuring the maximum potential of the microbial community at sample collection. Denitrification potentials among the FTWs were 5 to 7 times higher than rates found among pond sediments. Although significant denitrification was present, maximum nutrient removal could only take place if plants were harvested from FTWs in addition to microbial denitrification. The combination of plants and organisms within FTW matrices could potentially reduce nitrogen concentrations within stormwater retention ponds. Floating treatment wetlands provide a new tool that could assist in the uptake of nutrients, especially those associated with eutrophication. Floating treatment wetlands should be further analyzed but could potentially serve as a successful BMP in low flow systems within the Chesapeake Bay Watershed.

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CHAPTER ONE:

Literature Review

Introduction

Nutrient loading to the Chesapeake Bay is a major concern for the health of the Chesapeake. Excess nutrient loads have the potential to cause eutrophication. This process results in an increase in aquatic biomass, low water clarity and low dissolved oxygen levels and has the potential to create dead zones (areas where dissolved oxygen is depleted) and decrease solar penetration throughout the Bay (Pierzynski et al., 2005). Best management practices (BMPs) are implemented within the Chesapeake Bay Watershed to reduce the effects of non-point sources of pollution to surface waters. However, significant nutrient loads are still exhibited even after these practices are implemented (Kleinman et al., 2007); these findings suggest that other sources of nutrient removal must be provided in order to conserve the natural resources that the Bay provides.

In 2008, the USEPA established new regulations designed to reduce nutrient loading from confined animal feeding operations (CAFOs) (USEPA, 2008). These regulations hold CAFOs to a zero discharge standard that does not allow the discharge of excess nutrients (nutrients found in animal manure) into the environment. However, this standard is difficult for CAFOs to obtain.

Stormwater retention ponds are a BMP that collect runoff and drainage (either directly or via drainage ditches) from poultry farms and provide a possible intervention point for the treatment of potentially contaminated water. Floating treatment wetlands (FTWs), also known as floating emergent macrophyte wetlands (Fonder and Headley, 2011), can be used in these stormwater ponds to remove excess nutrients by nutrient uptake as well as microbial transformation. Floating treatment wetlands (FTWs) are man-made recycled plastic (high density polyethylene, HDPE) matrices similar to attached growth media filters (used in aquaculture). Macrophytic plants installed on FTWs allow bacterial and algal colonization (Headley and Tanner, 2006). The combination of organisms removes nutrients from the surrounding water column, effectively reducing their concentrations (Bachard and Home, 2000; Kadlec and Wallace, 2009; Rogers et al., 1991; Smith and Tiedje, 1979).

Anaerobic organisms remove excess soluble nitrogen (N) in the water column through a process called denitrification ($2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO\uparrow \rightarrow N_2O\uparrow \rightarrow N_2\uparrow$) (Brady and Weil, 2008, Groffman et al., 1999). Some of the organisms responsible for denitrification are bacteria such as *Achromobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Thiobacillus denitrificans* (Brady and Weil, 2008).

Drainage ditches typically connected to stormwater management ponds are common on Maryland's Eastern Shore because of the shallow groundwater table and course textured soils (NRCS Soil Survey, 2011). Earlier research has found that subsurface transport is accountable for more than 80% of water flow and phosphorus (P) export into agricultural drainage ditches. Most BMPs designed to reduce P transport focus on overland flow and are therefore of limited usefulness in this landscape (Kleinman et al., 2007).

Phosphorus is the limiting nutrient in freshwater systems and is affected by a number of chemical and mechanical phenomena. For example, mixing, by wind or biota, can place inorganic P back into solution through suspension of soil particulates in the water column. Anaerobic conditions can also effect the availability of P in the presence of iron; as anaerobic conditions increase, iron complexes may become reduced ($Fe^{3+} \rightarrow Fe^{2+}$), making iron-phosphate complexes more soluble, thus releasing P into solution (Brady and Weil, 2008).

Removal of P, unlike removal of N, occurs through adsorption to soil particles, depending on the availability of adsorption sites on soil matrices. This removal process occurs through chelation involving the oxidation of iron followed by the retention of P as ferric phosphate (Pant and Reddy, 2001). Although P removal is possible, this process may not occur if the binding site is already saturated because adsorption is a mechanism that is dependent upon the number of binding sites available for nutrient uptake to occur (Canter et al., 1987). If binding sites are already saturated on a given soil particle adsorption is not likely to take place. This spike in nutrient availability of P then allows for increased algal and plant growth (i.e. eutrophication), which is a common problem within the Chesapeake Bay.

The use of FTWs within stormwater retention ponds attempts to remove nutrients (N and P) through uptake, immobilization and transformation by microbes, algae, and macrophytic plants. Beyond the use of FTWs in CAFO production areas, the information gained through this study provides a basis to reduce surface nutrient pollution for other watershed regions and even for non-agricultural applications. For example, this technology can be adapted to reduce nutrient pollution in farm ponds and intermittent streams outside of the Delmarva Peninsula. In addition, this research can be applied to non-agricultural sects within urban or suburban water retention basins, such as the golf course industry. The knowledge gained from this study can also be utilized in regional BMPs to improve the overall quality of local waterways.

Current Knowledge

Floating treatment wetlands provide a new tool that can be utilized to assist in the uptake of nutrients, especially those that are commonly associated with eutrophication. However, FTWs have only been marginally evaluated as compared to other treatment wetland systems in effectiveness (Headley and Tanner, 2006; Kadlec and Wallace, 2009). The majority of research conducted on FTWs focuses on nutrient removal via plant uptake (Stewart et al., 2008; Tanner and Headley, 2011; Wang et al., 2011). The results of prior studies conclude that plant uptake of nutrients alone is not a reliable source of nutrient removal because macrophytes and microalgae only temporarily immobilize N. Without periodic harvesting of the plants, nutrients would otherwise return to the system through plant decomposition. Therefore, the permanent removal of N is dependent upon denitrification (Brix, 1997; Poe et al., 2003; Rogers et al., 1991). Based on an extensive literature review, current research on FTWs has not attempted to quantitatively analyze denitrification within FTWs. The research reviewed however, suggests losses via denitrification using mass balance equations or mesocosms (Chang et al., 2012; De Stefani et al., 2011; Stewart et al., 2008).

Nutrient Removal Through Plant Uptake

Plant uptake of water soluble nutrients is a viable route of transient nutrient removal, but unfortunately, the process does not ensure permanent nutrient removal (Brix, 1997; Rogers et al., 1991). Brix (1994) and Gumbricht (1993) found the nutrient removal rates measured in typical wetland soil through plant uptake can be in the range of 30 to 150 kg P ha^{-1} $year^{-1}$ and 200 to 2500 kg N ha^{-1} $year^{-1}$ (N: P ratio in the range of

6.7- 16.7); however, the plants in the FTW system have to be harvested in a manner similar to that of agricultural crop removal systems on a yearly to seasonal basis to completely remove the N and P from the system.

Compared to other plants (such as food crops), macrophytes are likely not efficient for successful and complete nutrient removal, since macrophytes are rarely removed in non-agricultural, low maintenance systems. The continual maintenance required by the removal of macrophytes would make this form of N removal difficult to maintain permanently in an agricultural or urban setting and if harvesting was utilized it would require a sufficient amount of additional funding.

Establishing the effectiveness of FTWs by quantifying denitrification that occurs in the system would provide significant evidence for FTWs as a tool to remove excess N in stormwater management ponds. One of the primary objectives of this study was to evaluate the magnitude of N removal via denitrification..

Nutrient Removal Through Denitrification

Although plant uptake by itself is an insufficient method for stable nutrient removal, the system sufficiently supplies denitrifying microbial communities with necessary sources of carbon (C) to carry out denitrification. Tiedje (1988) and Beauchamp et al., (1989) concluded that denitrification is dependent upon various biotic factors such as readily available carbon, pH, N availability, microbial viability, dissolved oxygen concentrations and temperature. Although a difficult process, quantifying the amount of denitrification that occurs in a system through acetylene (C_2H_2) inhibition is an effective and reliable technique. The C_2H_2 inhibition method has been previously criticized in the literature for high temporal and spatial variability, slow diffusion into sediments, decomposition of C_2H_2 by microbes and underestimation of denitrification rates due to inhibition of nitrification which produces nitrate (NO_3^-) during decomposition (Groffman et al., 2006). Despite these criticisms, this method is ideal for the purposes and applications of this particular study because it utilizes available technology to measure the denitrification potential of FTWs and allows for a high volume of samples to be analyzed in a short period of time. Through this quantification method, establishing effective denitrificaiton by the FTWs will suggest the usefulness of this tool as a BMP. As in any study, experimental error of denitrification estimates do occur; however, the ideal methods of measuring denitrification used in this study can be utilized to determine if in fact a significant amount of N removal is occurring within the FTW matrix.

Microbial Nitrogen Removal in Floating Treatment Wetlands

One advantage of FTWs is the large surface area available for microbial communities to thrive. The FTW matrix, along with suspended roots beneath the FTW mat, provides a large amount of surface area for microbial colonization, which forms a biofilm (Brix, 1997). No published studies indicate definite nutrient removal through bio-film uptake; however, numerous studies mention biofilms as a potential removal pathway after nutrient and mass imbalances were uncovered through calculation and analysis (Headley and Tanner, 2006; Stewart et al., 2008; Tanner and Headley, 2011; Wang et al., 2011). These bio-films are likely colonized with bacteria able to perform denitrification.

Tanner and Headley (2011) found that a decrease of copper (Cu) and P as well as a decrease of fine suspended solids occurred in the presence of macrophytic plants within FTWs as compared to FTW matrix material without macrophytic plants. However, their study found only a small amount of Cu and P removal actually occurred through plants; therefore, another phenomenon existed to create the overall decrease in these two nutrients. Biofilms may have decreased the levels of Cu and P in their study, which may indicate another mechanism of nutrient removal in this study involving FTWs.

Objectives

Floating treatment wetlands have the potential to remove nutrients from surface water through plant and microbial uptake and denitrification. However, there is limited quantitative data on the cumulative impact of FTWs on nutrient removal in agricultural storm water ponds. Quantification of N and P removal within a FTW would provide a foundation for implementation of FTWs in the Chesapeake Bay Watershed as a BMP that could be supported by various policy and cost-share mechanisms. This finding would encourage use of FTWs as a tool to improve and maintain water quality in various stormwater management applications. Therefore, our objectives were to: (1) assess total nutrient removal potential of FTWs via plant uptake and attenuation of nutrients on the FTW matrix material; (2) monitor and evaluate denitrification potential within the wetland matrix; and (3) investigate the potential for FTWs to be used as a BMP throughout the Chesapeake Bay Watershed.

CHAPTER TWO:

Monitoring Floating Treatment Wetlands:

Field and Laboratory Techniques

MATERIALS AND METHODS

Site Selection and Design

Site selection for the study was determined in partnership with Perdue Farms Inc. from farms under volunteer grower contracts with Perdue. Four farms were chosen as finalist according to research site needs, farm set-up, and accessibility. The two farms selected for the study had stormwater management ponds to collect stormwater runoff from grassed waterways. These ponds were located parallel to manure storage areas, farm access roads, and chicken house structures. In addition, these sites had ponds that stayed flooded throughout the year and provided ample space to install the FTWs.

The sites were located on broiler farms in Federalsburg, Maryland in Caroline County (38.777347, -75.751474, Site 1) and Church Hill, Maryland in Queen Anne's County (39.103923, -75.89824, Site 2) on Maryland's Eastern Shore. Both chicken farms were relatively new, built within the last three years and were approximately 32 kilometers from one another. Both farms contained predominantly loamy to sandy loam soils and were well to very well drained (Soil Survey Staff, 2011). Both ponds were excavated to provide clay for the floors in the poultry houses on the site and as such had clay bottoms enhancing their ability to retain drainage and runoff water.

The barrier and concrete cinder blocks used in the construction of the base of each chicken house for the foundation sealed the houses from the surrounding environment. This building technique, conducted under Perdue supervision for chicken houses built on the Delmarva Peninsula, protects the surrounding natural environment from nutrient leaching into the soil. This environmental measure potentially prevents contamination of the surface and ground water aquifers.

Each farm pond represented a block; within each block, three floating treatment wetlands (FTWs) represented three separate units of study. Within each unit, three parameters were measured: (1) oxidation reduction potential (ORP, Eh); (2) N and P accumulation among the plants of the FTW matrix; and (3) denitrification potential. Within each pond block, pH and total N and P were measured in water samples as well as in sediment samples collected from each pond during core sampling events. The analysis of these parameters took place both in the field and in the lab according to the methods below.

Floating Treatment Wetland Matrix

The FTWs in this study, also referred to as Biohavens© (Floating Island International™, Montana, USA), were made of post-consumer recycled plastic (high density polyethylene, HDPE) coated in brown latex. The FTWs were intertwined to form a dense mass similar to attached growth media filters used in the aquaculture industry. To give the plastic matrix material added buoyancy and rigidity, various areas throughout the FTW were injected with marine grade polystyrene and PVC framing. The wetland contained four layers approximately 5.08 cm thick bound together for a total thickness of 20.32 cm. The FTWs used in this study were rectangular in shape and measured approximately 2.44 m x 1.52 m x 20.32 cm. Pre-cut 5.08 cm x 10.16 cm diameter holes were provided by the manufacturer to allow for wetland plants to be planted throughout the surface of the FTW (Figure 1).

Figure 1: Photograph representing the floating treatment wetlands used in this study (July 2011).

Floating Treatment Wetland Core Samples

Precut removable cores within the FTW matrix allowed measurement of nutrient accumulation. Prior to launch, each FTW (2.44 m x 1.52 m x 20.32 cm) contained twenty-four 7.62 cm diameter removable cores designated for removal in groups of five to be drawn on Day 1, 30 90, 180, 270 and 460. However, some sampling days had to be split into two days since increased biomass made it difficult to sample the wetland matrix. The project began in April 2011. April 6, 2011 represented Day 1, May 6, 2011 represented Day 30, July 5, 2011 represented Day 90, October 5, 2011 (183 days) and October 7th (185 days) represented Day 180 and January 5, 2012 (275 days) and January 10, 2012 (280 days) represented Day 270. Each core was assigned a number and placed in a sequential manner. Before sampling occurred, each sampling period listed above was assigned five random numbers representing individual core samples. The randomization occurred through Microsoft Excel. The selected samples were collected according to these previously chosen random numbers on the designated dates. The core samples were collected according to the map represented in Figure 2.

Before launch, removable cores were created in the FTWs by drilling 7.62 cm diameter holes with a 7.62 cm hole saw through each of the four FTW layers. Once the cores were removed, the four layers were individually sewn together with yellow 23 kg synthetic fishing line and labeled accordingly. The cores were then replaced back into the FTWs for future sampling. This process was replicated until 144 cores had been created; thus, six FTWs had twenty-four cores each, for a total of 144 cores. On each sampling day, the removal of five cores throughout the four sampling days occurred. Four extra core samples were made as well to account for any unforeseen event or experimental error. An additional 144 blank, non-vegetated cores were then created to replace the cores that were removed on the previous listed sampling days. Three additional FTWs (2.44 m x 1.52 m x 20.32 cm) were required in order to make replacement cores. When marine grade polystyrene or PVC piping was encountered in the extracted cores they were discarded so that the combined core sample only consisted of the FTW matrix material.

After removal of each core sample on each sample date, the cores were replaced by another removable core in the event that core sampling would be continued in the future. This step was also taken in an attempt to return the surrounding FTW matrix to similar conditions before sampling had taken place. Once the cores had been removed from the FTWs, they were placed in plastic bags and stored in a cooler on ice during transportation to the lab. These samples were then cut and separated by individual layer and stored in a freezer until further analysis could take place.

All of the cores above were analyzed in separate DEAs; they were frozen due to equipment restraints at the beginning of this study. However they were not used in this study due to significant loss of microbes due to freezing the samples. Instead, a separate sampling date (Day 460) was created in order to determine the amount of denitrification potential after the FTWs had been in the water for the length of the study. This information was then used to determine a cumulative denitrification potential of FTWs over the course of 460 days.

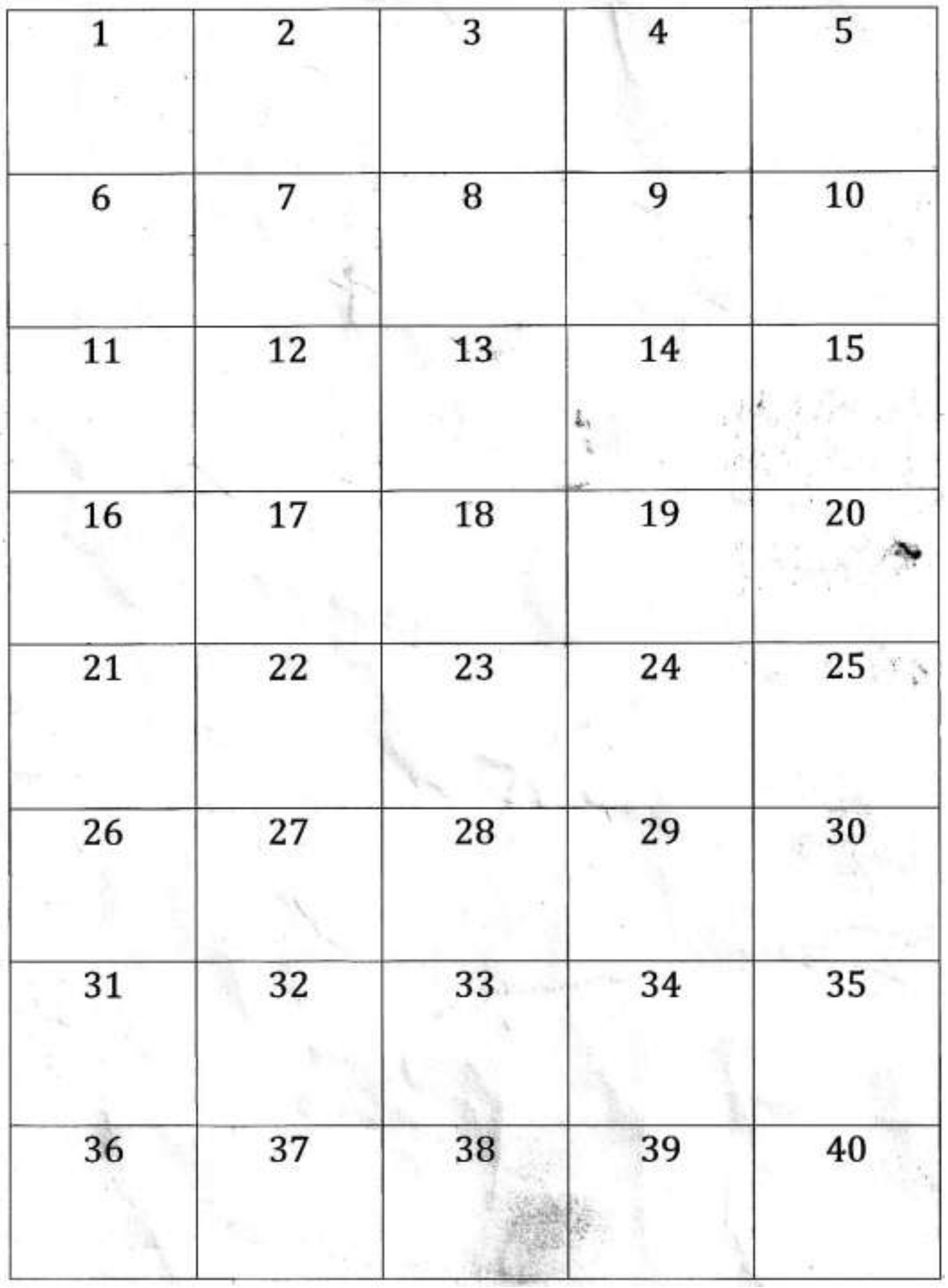


Figure 2: Schematic showing map of plant sampling area.

Wetland Plants and Soil Amendment

Plants native to Maryland's Eastern Shore were planted throughout the FTW to simulate a natural wetland ecosystem common to the U.S. Mid Atlantic region. Prior to FTW launch, one bale of sphagnum peat moss (0.085 cubic meters) was spread throughout the surface of the bare plastic matrix of the FTW. Plant holes and the surface of the FTW were filled and covered with peat moss. The peat moss provided a lightweight starting soil media for the plant material and prevented moisture from escaping from around the plant roots. It also served as protection from UV rays that could cause photo degradation of the FTW recycled plastic matrix.

Prior to planting, wetland plants were started in a greenhouse to ensure successful plant establishment. Baseline samples of plant material were collected prior to the FTW planting. Sixteen of the following native plant species (totaling 80 plants), were planted throughout the surface of each FTW before the project launch: *Carexta lacustris* (Lake Sedge), *Hibiscus moscheutos* (Swamp Mallo), *Asclepias incarnata* (Swamp Milkweed), *Lobelia cardinalis* (Cardinal Flower), and *Scrirpus validus* (Soft Stemmed Bullrush). The FTWs were launched in each pond and watered to soak the FTW matrix and peat moss to prevent peat moss from being blown away. The FTWs were anchored to either side of the pond banks with wooden stakes and rope. This mechanism allowed easy retrieval of the wetlands in shallow water (accessible by chest waders).

Plants were destructively sampled at each sampling period; however, no plants were sampled in the first 30-day sampling period because of concern of minimal plant root establishment. Roots were left after sampling to encourage plant reestablishment for purposes of future evaluation. Plant sampling was conducted according to a 3.72 m^2 (total area of each FTW) block design (Figure 3). Two blocks were selected at random during each sampling event for each of the individual FTWs. To eliminate edge effect, the edge of the FTW was excluded from the plant sampling regime, leaving a total of 1.67 m^2 for plant sampling. In each 30.5 cm x 30.5 cm area that was sampled, every plant was cut level with shears and placed into plastic bags. The plants were weighed, dried in a plant drying oven at 60 ºC, weighed again, ground and run through a two millimeter sieve. The plants were then stored at room temperature in air tight plastic bags for future analysis.

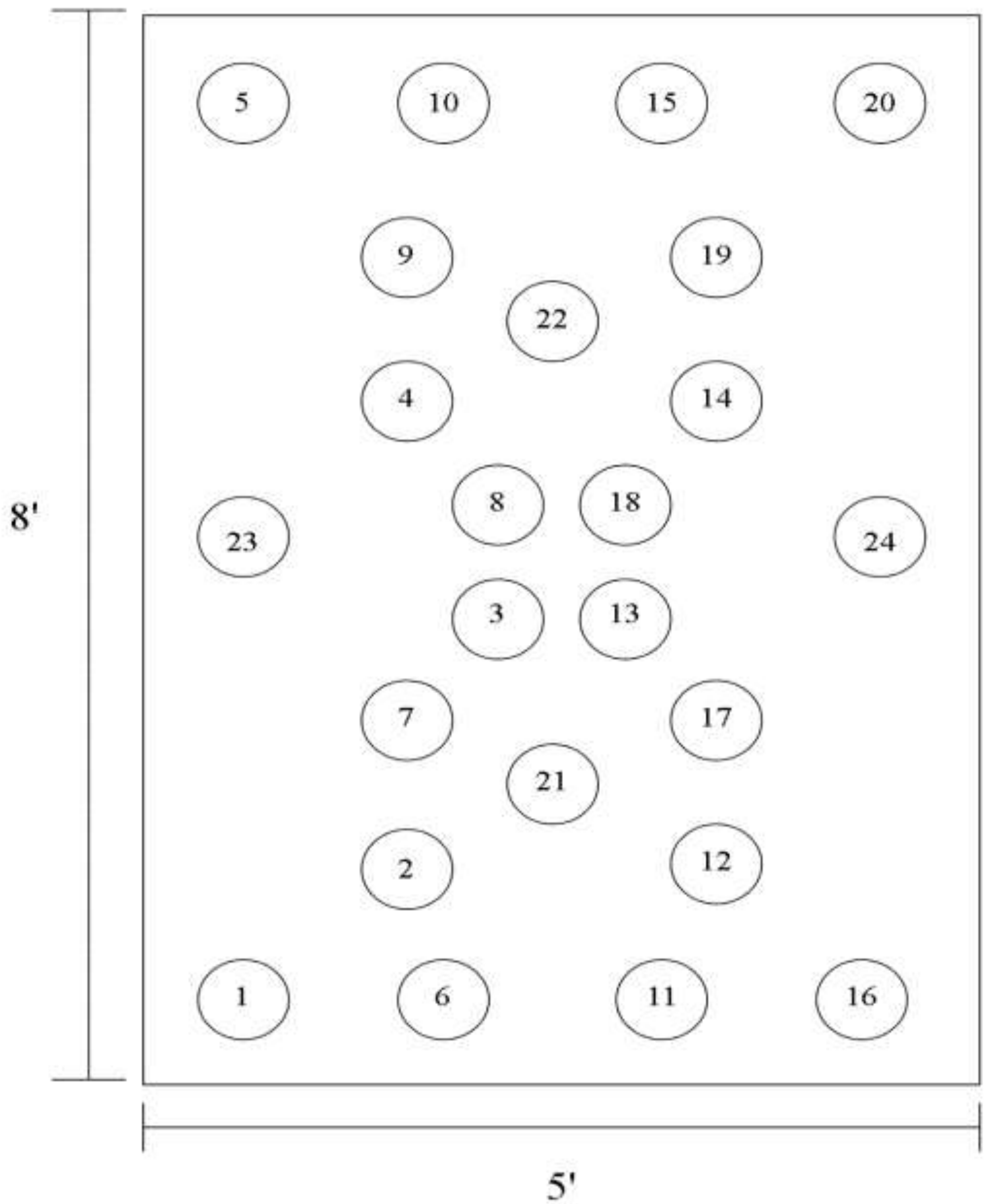


Figure 3: Schematic showing map of size (5 foot by 8 foot) and location of floating treatment wetland core samples on each floating treatment wetland. Three sample areas among floating treatment wetlands: edge (1, 6, 11, 16, 5, 10, 15, and 20), mid (2, 4, 7, 9, 12, 14, 17, 19, 21, and 22), center (3, 8, 12, 18, 23, and 24).

FTW Matrix Denitrification Analysis

Denitrification analysis of the FTW matrix and sediment took place in the lab following collection from the field and a period of incubation. Nitrous oxide (N_2O) was analyzed in the headspace of the incubation container following the addition of acetylene gas (C_2H_2) using a modified Acetylene Inhibition Method and Denitrification Enzyme Assay (DEA) (Casey et al., 2004; Groffman et al., 1999; Smith and Tiedje, 1979). Factors affecting denitrification (ORP and weather) were monitored on site via a Watch Dog Weather Station (Spectrum Technologies, Inc., Illinois, USA) and Global Water Oxidation Reduction Potential (ORP) sensors (Xylem, Inc., Texas, USA).

Floating treatment wetland matrix material and sediment samples were incubated to determine denitrification potential. These samples were taken on Day 461 (Site 2, 7/9/2012) and Day 464 (Site 1, 7/12/2012) after deployment of the FTWs. Five core samples (7.62 cm diameter) from each FTW were collected and divided into five separate bags and each core layer was cut into 1/8ths with shears. The samples were then placed on ice and taken back to the lab were they were left out over night and analyzed the next morning. Three sediment samples were taken from each pond in the same general area of the FTWs.

The day before the denitrification enzyme assay (DEA), the acetylene, gas standards and the DEA solution were prepared. Nitrous oxide (N_2O) standards (0, 10, 50, 100, 250, 500 and 1000 ppm) were prepared using certified gas standards from Air Liquide Specialty Gas (Air Liquide, Inc., Pennsylvania, USA). Standards were created by serial dilutions with ultra high purity nitrogen gas (N_2, Airgas, Inc., Pennsylvania, USA) and a gas tight syringe (Hamilton Company, Nevada, USA); the standards and N_2 gas were combined and stored in air tight one liter gas bags (SKC, Inc., Pennsylvania, USA) that were flushed with N_2 gas and evacuated. Standard concentrations of 10, 100 and 1000 ppm N_2O were directly inserted in the gas bags from the compressed gas bottles (Scotty/Air Liquide, 14 liter bottles). Industrial grade acetylene was purified by passing it through a concentrated sulfuric acid trap and two distilled de-ionized water traps followed by a flask containing desiccant proceeded by a glass wool filter to remove any particulate matter (Groffman et al., 1999; Hyman and Arp, 1987). This purified gas was then directly inserted into a one liter gas bag from the filter apparatus. The DEA solution was created by mixing 0.72 g potassium nitrate, 0.5g glucose and 0.125g chloramphenicol (an antibiotic) to one liter of distilled deionized water (Groffman et al., 1999). Chloramphenicol was used to inhibit the growth of the microbial community so the actual amount of microbes at the time of sample collection could be measured as opposed to measuring the expansion of the microbial community throughout the length of the incubation.

The next morning, approximately 12 hours later, the above standards were injected manually (via gas syringe) in triplicate into a gas chromatograph (Agilent 7890A, Agilent Technologies, California, USA). A detailed list of settings and apparatus used while operating the GC in this experiment can be found in (Table 1). The DEA solution was heated to approximately 100 °C on the morning of the denitrification enzyme assay (DEA) and stirred simultaneously in an attempt to remove all dissolved gases in solution. The solution was then allowed to cool to room temperature (25 °C).

Each of the four layers from the FTW matrix samples were combined so that each core could be evaluated in the assay. The four layers were then weighed and placed in a

125 ml Erlenmeyer flask. The same procedure was performed for the sediment samples with one difference: 10 g of sediment was weighed and placed in to the flasks. Following the above procedure, 75 ml of DEA solution was then added to the flasks with FTW material, and 10 ml of DEA solution was added to the flasks with sediment. Different volumes of solution were used because the sediment and the FTW matrix took up different volumes within the Erlenmeyer flasks. To ensure all the material was submerged, the above volumes of DEA solution were then added. Suba Seal turnover septum stoppers (Sigma Aldrich Part No. Z124664, Missouri, USA) were placed on the Erlenmeyer flasks and the flasks were then evacuated for one minute and flushed with ultra high purity nitrogen for one minute. This particular step was repeated three times.

After all flasks had been flushed and evacuated, 10 ml of C_2H_2 was added individually to each flask. This transitional step marked the start of the assay (T = 0 min.). A 600 µl sample was taken immediately after the injection of C_2H_2 from the Erlenmeyer flask and manually inserted into the gas chromatograph. The flasks were then placed on a wrist shaker and shaken at a moderate speed. Each flask was sampled every 30 minutes for 120 minutes. Once a sample was taken from the flask (starting at 30 minutes), 600 µl of ultra high purity N_2 was placed back into the flasks to maintain a pressurized headspace. Time and temperature were recorded at each sampling point. After the assay was completed, the FTW matrix samples used in the DEA were removed from the flasks, placed in aluminum drying pans and dried in an oven at 50 °C. The same procedure was performed for the sediment samples with one difference: 10 grams of sediment collected from the field was weighed and placed on aluminum drying pans.

Both the FTW samples and the sediment samples were allowed to dry for a week, removed from the oven and weighed. Their dry weights were then recorded.

Gas Chromatograph and Components
Model: Agilent Technologies 7890A
Detector: Electron Capture Detector
Column: GS Carbon Plot J&W 113-3133
30 m X 320 µm X 3 µm
Carrier Gas: Ultra High Purity N_2
Inlet
Heater: 50°C
Pressure: 12.4 psi
Total Flow: 42 ml/min
Septum Purge Flow: 3 ml/min
Mode: Split
Split Ratio: 12:1 @ 36 ml/min
Oven
Temperature: 40°C
Equilibrium Time: 0.25 minutes
Detector
Heater: 250°C
Make-up Flow: N_2 @ 10 ml/min
Signal: 5 Hz/0.04 minutes

Table 1: Gas Chromatograph, Settings and Colum Used.

Monitoring Oxidation Reduction Potential (ORP)

ORP within the FTW matrix was measured via two installed and enclosed glass bulb platinum electrode sensors connected to a central data logger (Global Water, Xylem, Inc., Texas, USA). Measurements were collected every five minutes for 12 months. In each pond, a total of six probes were installed with two probes per FTW. The probes were placed 15.24 cm (three layers in depth atop the forth layer) into the FTW, directly in the center and along the edge of the FTWs. Data from each wetland were downloaded on a bi-weekly basis. A PC computer and compatible software provided by Global Water Instrumentation (Xylem, Inc., Texas, USA) allowed data collection of ORP by taking measurements four times every hour in 5 minute intervals. The probes were calibrated after five months of use using an ORP calibration kit from Sensorex Corporation (California, USA).

Water Samples

Grab water samples were collected at random locations in each pond whenever FTW matrix samples were collected. Water samples were collected in one liter bottles and stored on ice until they were returned to the lab where they were stored in a refrigerator at 2 ºC until further analysis. An automated Isco sampler was also used to take water samples over an eight-week period (Teledyne Isco, Inc.). These samples were collected every 12 hours via the automated sampler and then manually collected from the sampler on a weekly basis. Water samples were then taken back to the lab and an acid persulfate autoclave digestion method was used to digest the water samples. Five milliliters of water was added to 50 mL screw top testubes and 7.5 mL of a premixed solution was also added (this solution contained 13.4 g of reagent grade $K_2S_2O_8$, 3 g of reagent grade NaOH and 1 L of deionized distilled water). The test tubes were then capped and placed in an autoclave set at a temperature of 121 °C and a pressure of 1.20 atm for 30 minutes. The samples were then allowed to cool at room temperature. All water samples were then analyzed (unfiltered and simultaneously) colorimetrically for total N and P (Lachat Instruments, Colorado, USA).

Sediment Samples

Sediment samples were taken at random from each pond during FTW sampling using a bottom dredge sediment sampler attached to a rope. The sampler was repeatedly dropped to the bottom and retrieved until approximately two kilograms of sediment was obtained. The sediment was allowed to settle and excess water was decanted from the sediment sample. The remaining sediment was stored in plastic bags and kept on ice until returned to the lab and refrigerated at 2 ºC. A subsample of the sediment was then weighed and dried in an oven at 60 °C for one week, weighed again, and ground. The remaining sediment that was not dried and ground was frozen until further analysis could take place. The samples were then analyzed colorimetrically for total N and P using an acid persulfate autoclave digestion method similar to the method used above (Lachat Instruments, Colorado, USA). Sample concentrations were then represented in milligrams of N or P per kilogram of sample.

CHAPTER THREE:

Results & Discussion

RESULTS AND DISCUSSION

Denitrification Potential of FTWs and Stormwater Pond Sediment

The purpose of measuring denitrification in this study was to determine if denitrification within FTWs actually occurs. Additionally, this study aimed to compare denitrification rates in FTWs versus denitrification rates in sediments within the same stormwater management ponds. Before data was collected, the hypothesis predicted that nitrate and nitrite would be removed from the system via denitrification; however, the rate of denitrification was predicted to be higher in the sediment samples than the FTWs. This hypothesis was predicted because we assumed that since sediment (i.e. hydric soil) particles contain a greater surface area and more carbon is present, the sediment is more likely to provide more desirable conditions for denitrifying bacteria to thrive as compared to the plastic surface on FTWs.

The FTW matrix exhibited higher denitrification rates than the sediment samples when compared on a dry-weight basis. Quantitatively, within the same stormwater retention ponds, FTW denitrification rates were in the range of 2.10 - 7.60 mg- N_2O-N kg-FTW $^{-1}$ h^{-1}, whereas sediment sample denitrification rates were in the range of 0.30 - 1.40 mg-N_2O-N kg-sediment $^{-1}$ h^{-1} on a dry weight basis (Figure 4).

Differences in denitrification rates between FTWs and sediment may be due to a number of factors. One possible explanation may be the location of these sampling sites within the ponds. Floating treatment wetlands were always in the upper water column of the pond, while the sediment sampled from each stormwater retention pond was continuously submerged throughout the year. As a result, because denitrifying bacteria among the FTWs are exposed to an increased amount of mixing in the upper water column, they are also exposed to more frequent inputs of soluble N. This factor then increases the likelihood for the bacteria to thrive; therefore, FTWs sustain larger denitrifying bacteria communities as compared to the sediment in the pond where less mixing and less continual inputs of N are available.

Initially, our hypothesis predicted that the center of the FTW would encourage more negative oxidation-reduction potential (ORP) readings as compared to the edge of the FTW. This thought was based on the idea that at the center, less mixing would occur and less dissolved oxygen would be present, thereby allowing more reduced conditions to be present and overall increased denitrification. However, this proved incorrect as increased denitrification potentials were more prevalent among the edge and mid sections of the FTW and always less prevalent in the center of the FTW. Therefore, more denitrification may have occurred at the edge of the FTW instead of the center due to mixing and dissolved N availability.

Oxidation Reduction Potential measurements were used to help determine whether or not reduced conditions were present in order for denitrification to occur. Oxidation reduction potential is a qualitative measurement and refers to the potential of an atom to either lose electrons (oxidation) or gain electrons (reduction) and is measured in water as voltage between a platinum wire enclosed inside a glass bulb and reference electrode; as the voltage increases so does the ORP reading (Nordstrom and Wilde, 2005). The sensor sends a signal back to the data logger; the data logger amplifies the voltage created and converts (i.e. corrects) the signal to milliamps (mA) which is then converted to a reading in milivolts (mV). The reading given in millivolts is a standard measurement and converting this voltage is not necessary as the given number is the number used. The more negative an ORP reading, the more reduced the surrounding atmosphere is, while the more positive a reading is the more oxidized the atmosphere is. These differences occur due to the differing electric potentials outside the sensor's glass bulb (Manahan, 2009). The ideal range for denitrification to occur is approximately between -200 and +200 mV (Inniss, 2005).

Throughout the monitoring of the FTWs, we found that ORP measured in the field by the probes confirmed what was being seen in laboratory analysis. Denitrification rates calculated from laboratory analysis support our ORP measurements in the field. The incidence of ORP reading in the desired range (desired range of denitrification, ± 200 mV) per month on a daily basis at both sites occurred 14-27% of the time (Figure 5 a, b and 6 a, b). Within the FTW structure itself, the edge was within the desired range 27% of the time while the center was within the desired range 14% of the time. Therefore, the ORP measurements show that the edge is more likely to provide the conditions ideal for denitrification to occur.

The above reporting of ORP is based upon ORP probes that were only calibrated once throughout their deployment in the field. Calibration of the probes is recommended every six months; however, we only calibrated the sensors once due to funding restraints. When interpreting Figure 12a and 13a, below, it should also be noted that these particular probes have a maximum of +500 mV and a minimum of -500 mV; however, recorded readings were much lower than the -500 mV range. These readings are likely due to the frequency of calibration and thus this factor should be taken into account when interpreting these measurements. When analyzing the data all values that were recorded at an operating voltage below 10 volts were excluded, this precautionary procedure was taken as a quality assurance measure according to the manufacturer's recommendations.

Our results indicate that the FTW edge actually presented higher denitrification potential due to increased aeration from mixing as compared to the center of the FTW. This finding is supported by the ORP reading as stated above. The higher rate of denitrification at the FTW edge could be due to increased microbial colonization and level of function and therefore more desired conditions at the interface of the probe (i.e. where the probe meets the FTW matrix material). The ORP readings found on the edge of the FTWs are an indicator that ideal conditions for denitrification are prevalent and therefore reduction of nitrate and nitrite is occurring. Therefore, FTWs do remove excess N in the stormwater management ponds, primarily through functioning bacteria, by transformation of N from water soluble state to a gaseous state; later, N_2 gas is eventually released into the atmosphere.

The results of this study were compared to those by previous authors in the available literature who used similar procedures, measurements, units of measurement (N_2O-N kg-sediment $^{-1}$ h $^{-1}$) and interpretation of denitrification rates (with the exception of the adaptations used for FTWs in this study). The denitrification rates found in this study were much higher than those in other studies. Casey et al., (2001) found denitrification rates in wetland soils receiving runoff from a golf course to be in the range of 16-32 µg N_2O-N kg-soil $^{-1}$hr $^{-1}$ and Sirivedhin and Gray (2006) found denitrification rates in constructed wetlands to be in the range of 3.5-4 µg N_2O-N kg-soil $^{-1}$day $^{-1}$. However, the systems in those studies did not have the high water concentrations of total N (Figures 9 and 10) as measured on agricultural sites used in this study, so the lower rates in their studies as compared to this study may be relative in terms of lower total N available for denitrification.

It should also be noted that the denitrification enzyme assay method used in this study actually represents the potential for denitrification to occur and not necessarily the actual denitrification occurring in the field. Therefore, the denitrification results should not be interpreted as what would actually occur in a constructed environment or natural setting in the field and may include some error. In the laboratory, microbes are provided with higher concentrations of carbon and N than what would be regularly found in standard environmental conditions. Therefore, these measured and calculated potentials may be providing an over estimation or even underestimation of the amount of denitrification occurring in the field. Other researchers have found that DEAs are likely to underestimate denitrification rates (Groffman et al., 2006; Qin et al., 2012). Qin et al. (2012) found that as much as 11.7% of the N_2O was reduced into N_2 in the presence of acetylene, which accounted for an overall underestimation of the rates that were actually occurring. As a result, the calculated denitrification amounts of the FTWs found through this study may be an over or under estimation of the amount of denitrification occurring in the natural environment based on available methods of measurement.

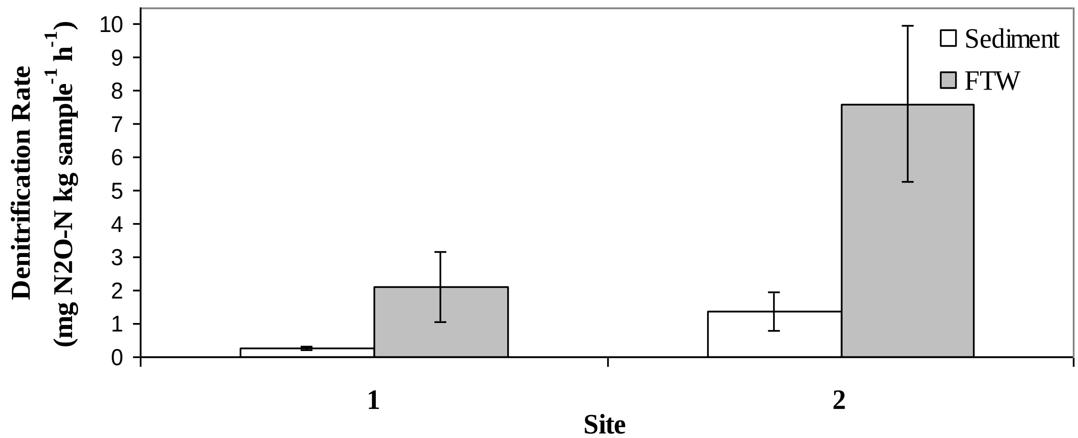
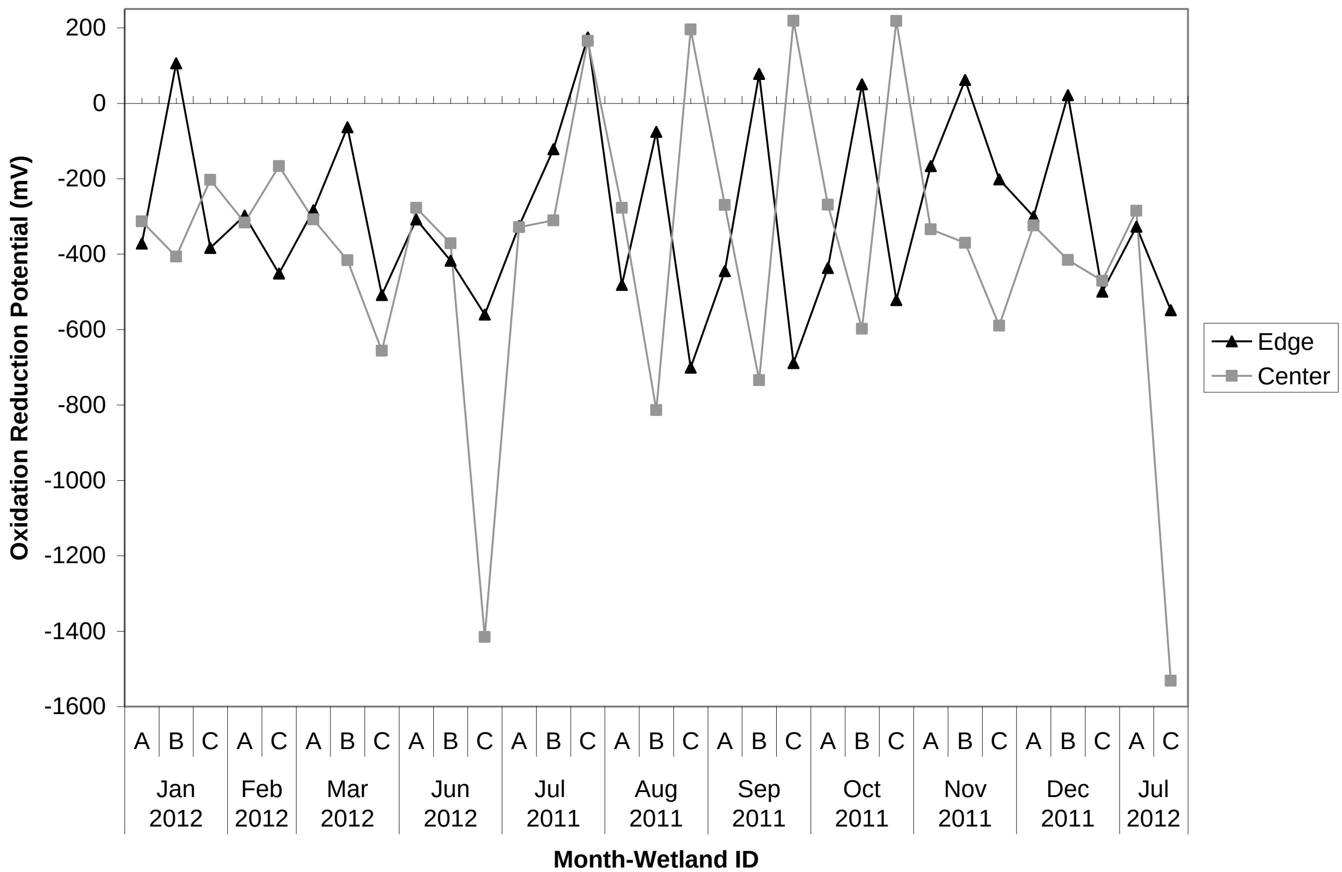


Figure 4: Denitrification enzyme activities of floating treatment wetlands versus pond sediment given the same experimental treatments. The error bars represent one standard deviation around the mean.



Oxidation Reduction Potential (mV)
200
0
-200
-400
-600
-800
-1000
-1200
-1400
-1600
Edge
Center
A B C A C A B C A B C A B C A B C A B C A B C A B C A B C A C
Jan 2012
Feb 2012
Mar 2012
Jun 2012
Jul 2011
Aug 2011
Sep 2011
Oct 2011
Nov 2011
Dec 2011
Jul 2012
Month-Wetland ID

Figure 5a

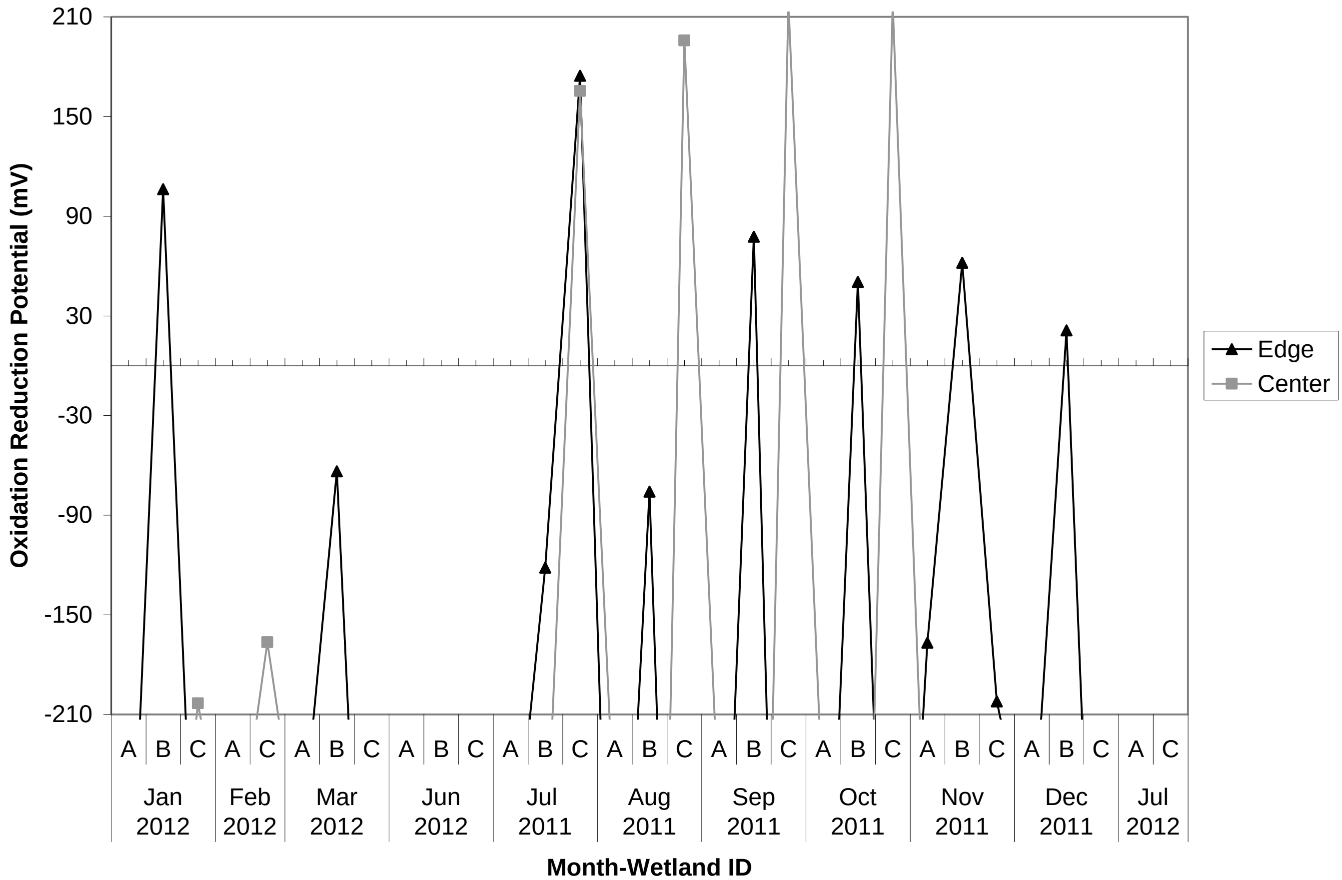
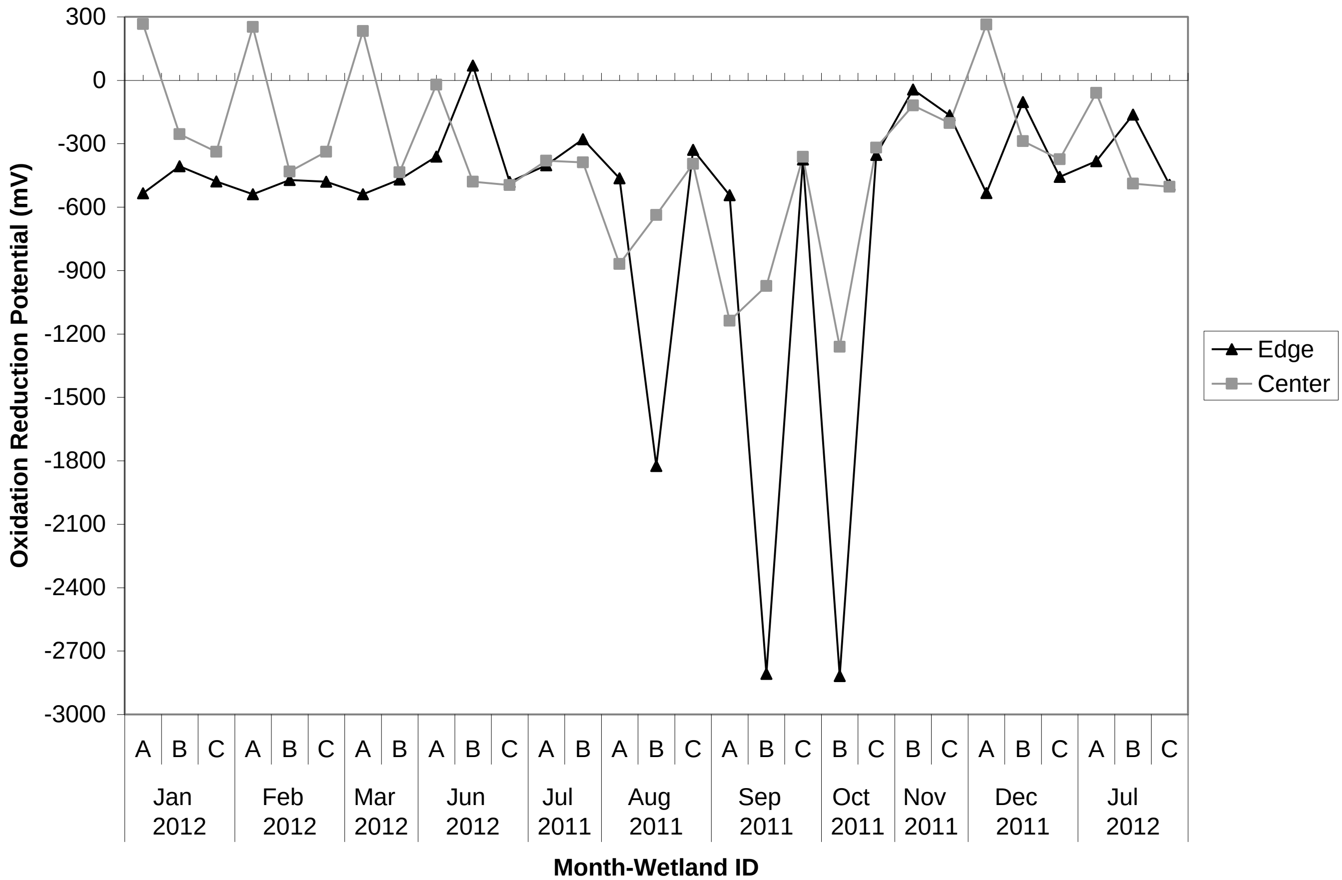


Figure 5b

Figure 5: Oxidation reduction potential monthly mean in millivolts at Site 1 measured from July 2011 to July 2012 with the exception of April 2011-May 2011. A, B and C represent each floating treatment wetland and the numbers indicate the month of each recording.

a) Full range of oxidation reduction potential measurements, b) ideal range of oxidation reduction potential measurements for denitrification to occur ± 10 millivolts.



300
0
-300
-600
-900
-1200
-1500
-1800
-2100
-2400
-2700
-3000
Oxidation Reduction Potential (mV)
A B C A B C A B A B C A B A B C A B C B C B C A B C A B C
Jan 2012
Feb 2012
Mar 2012
Jun 2012
Jul 2011
Aug 2011
Sep 2011
Oct 2011
Nov 2011
Dec 2011
Jul 2012
Month-Wetland ID
Edge
Center

Figure 6a

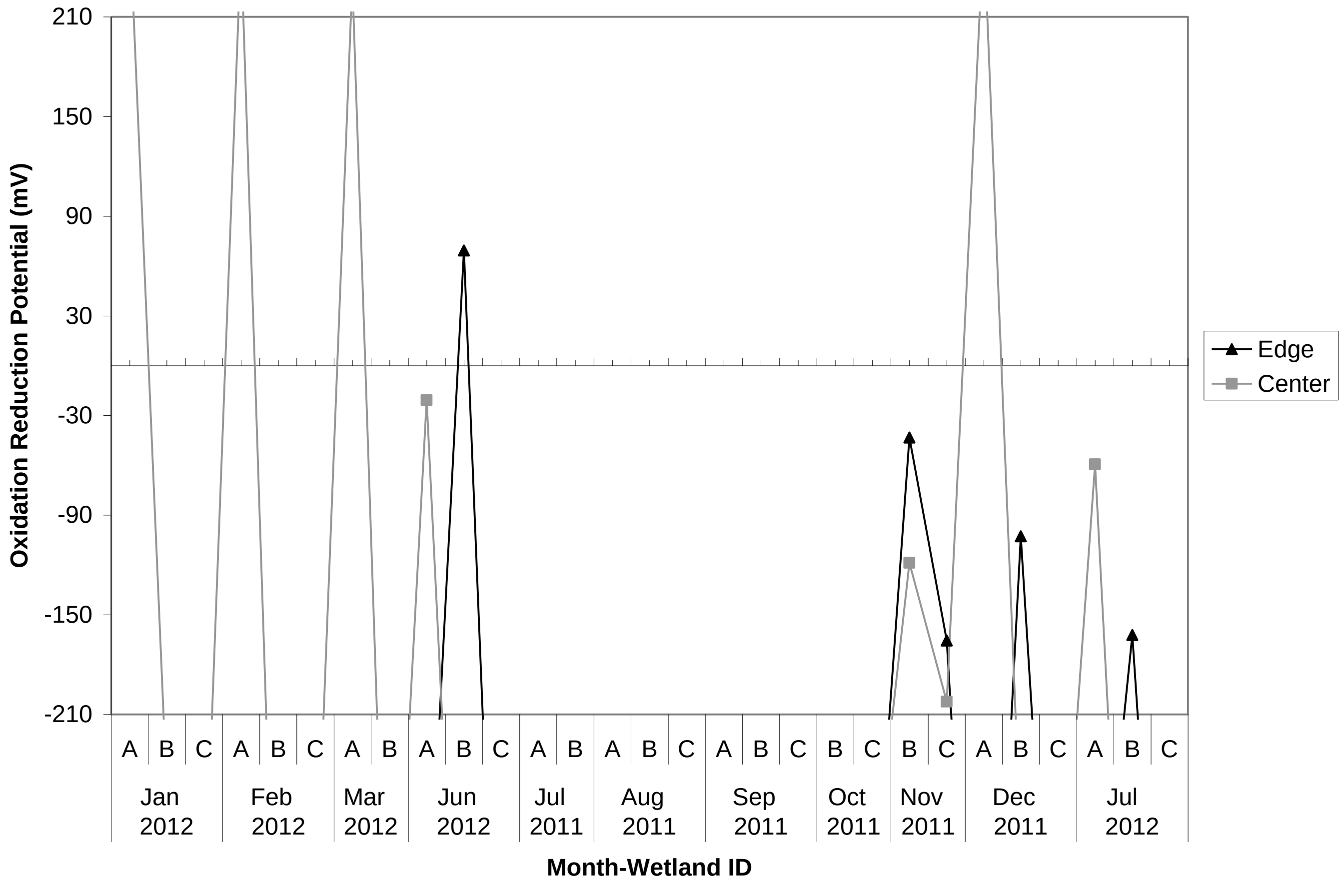


Figure 6b

Figure 6: Oxidation reduction potential monthly mean in millivolts at Site 2 measured from July 2011 to July 2012 with the exception of April 2011-May 2011. A, B and C represent each floating treatment wetland and the numbers indicate the month of each recording. a) Full range of oxidation reduction potential measurements, b) ideal range of oxidation reduction potential measurements for denitrification to occur ± 10 millivolts.

Nutrient Removal via Plant Uptake

Plant samples were collected to evaluate total N and P removal through plant uptake in above ground plant bio-mass on the surface of the FTWs. Before the study began, we predicted that a significant removal of N and P by plants would occur. However, we predicted that the amount of N and P removal by plant uptake would plateau and eventually begin to decrease, thereby releasing these nutrients that were previously consumed back into the surrounding stormwater pond (i.e. nutrient cycling). Later data analysis proved this portion of the hypothesis correct. The process of nutrient cycling is clearly illustrated in Figure 7, both in total N (7b) and P plant uptake (7a). The measurements in Figure 7 illustrate plant cycling; for example, plants matured in the early and late season measurements (between July and October) resulting in increased nutrient and biomass accumulation. Alternatively, the plants began to die off and decompose between October and January, and as a result a decrease in N and P uptake is reported.

Plant uptake values for measured nutrients in this study were in the range of 26-162 kg-N ha $^{-1}$ for total N and 13-69 kg-P ha $^{-1}$ for total P (N: P ratio in the range of 2 - 2.4). Plant biomass totals of each pond also followed the same trend between Site 1 and Site 2.

The trends of total plant biomass can be seen below in Figure 8. In July, the macrophytic plants were still growing and had not met their peak growth state. The largest amounts of total N and P removal and the largest collection of total plant biomass per pond on a wet basis occurred in October at both sites. In January, both sites reported their lowest plant biomass weights, while total N and P at Site 1 had decreased from

October but was not as low as concentrations seen in July. Total N and P concentrations representing the amount by plant removal at Site 2 were at their lowest point in January.

Both Brix (1994) and Gumbricht (1993) reported similar concentrations of nutrient removal in constructed wetland soils to those found in the FTWs in this study. Their reported concentrations of P removal by FTWs were in the range of 30 to 150 kg-P ha $^{-1}$ yr $^{-1}$ and concentrations of N removal were in the range of 200 to 2500 kg-N ha $^{-1}$ yr $^{-1}$ (N:P ratio in the range of 6.7- 16.7) (Brix, 1994; Gumbricht, 1993). Although similar amounts of nutrients were removed in this study, complete nutrient removal is evidently dependent upon harvesting of the plants to prevent cycling back into the system. If the plants were not harvested, the N, P and other nutrients removed by the plants would return to the system via nutrient cycling. Continual build up of nutrients in the system every year in addition to the inputs from runoff collected in the grassed waterways surrounding the poultry farm could lead to increased eutrophication or nutrient releases to local waterways.

In agreement with our findings, Brix (1994) concluded that harvesting was necessary in order for the plant uptake of nutrients to be totally eliminated from the system. However, in their study and the others cited above, FTW maintenance would be ideal if nutrient removal were not dependent upon harvesting of plant biomass. Reducing the maintenance of FTWs would significantly reduce their cost and make this application more practical to be used as a BMP. The estimated cost of a FTW (not including the cost of labor) is \$301.00 m $^{-2}$ (according to verbal communication with Floating Island International, Inc. Licensee); therefore, one 3.72 m $^{-2}$ FTW similar to those used in this study would cost $1120.00. As a result, this method of pollutant removal would be much more feasible and less time-consuming and costly if the plants did not require harvesting.

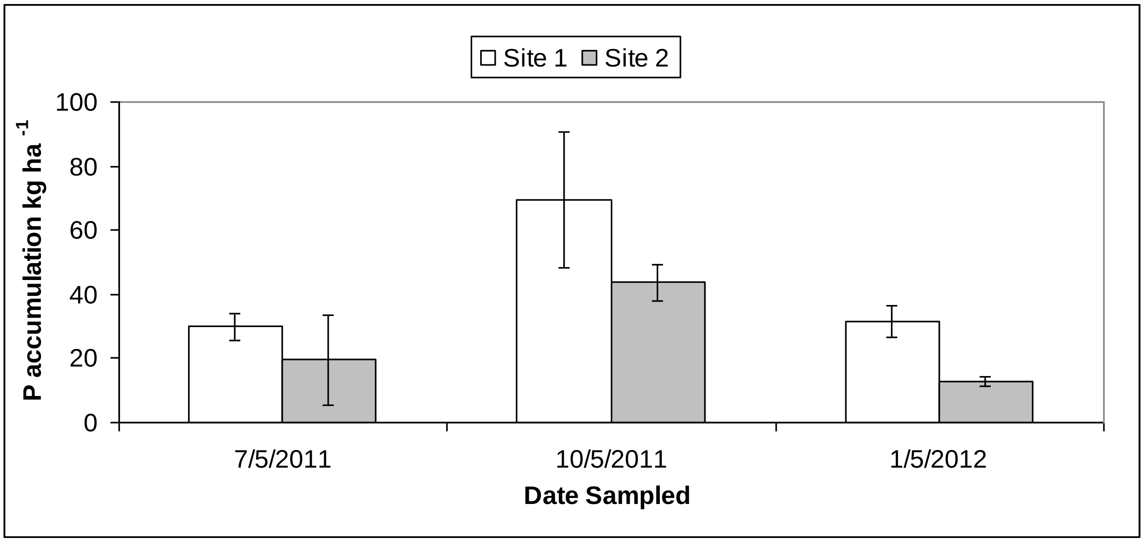


Figure 7a

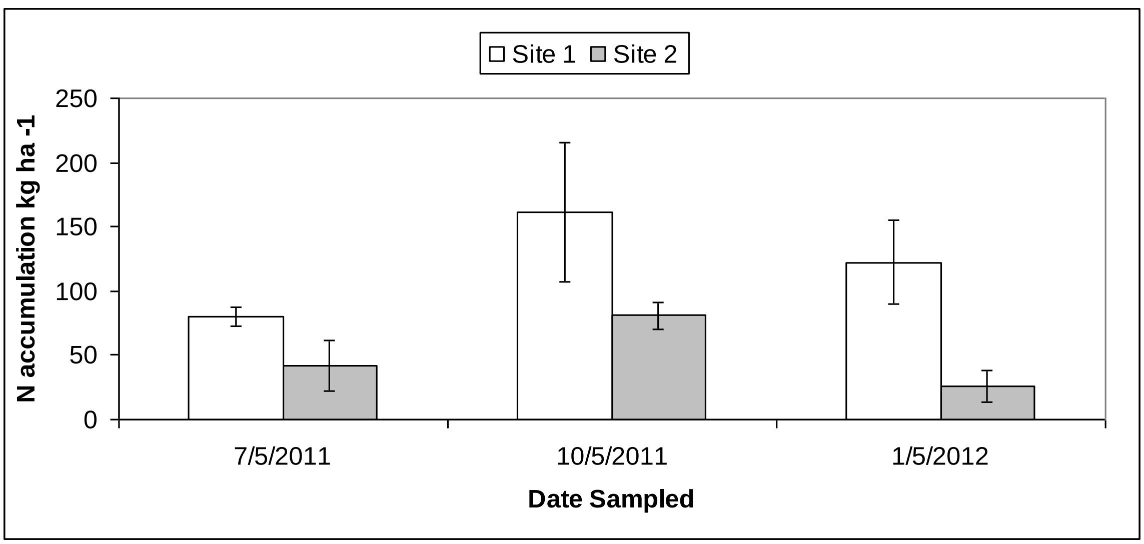


Figure 7b

Figure 7: Nutrient (i.e. nitrogen and phosphorus) accumulation in above ground plant biomass in kg ha^{-1}, (a) Mean total phosphorus values at each site per sample date; plant biomass was sampled from two 30.5 cm ^{2} areas per floating treatment wetland, (b) Mean total phosphorus values at each site per sample date; plant biomass was sampled from two 30.5 cm ^{2} areas per floating treatment wetland. The error bars in the bar graph represent one standard deviation around the mean.

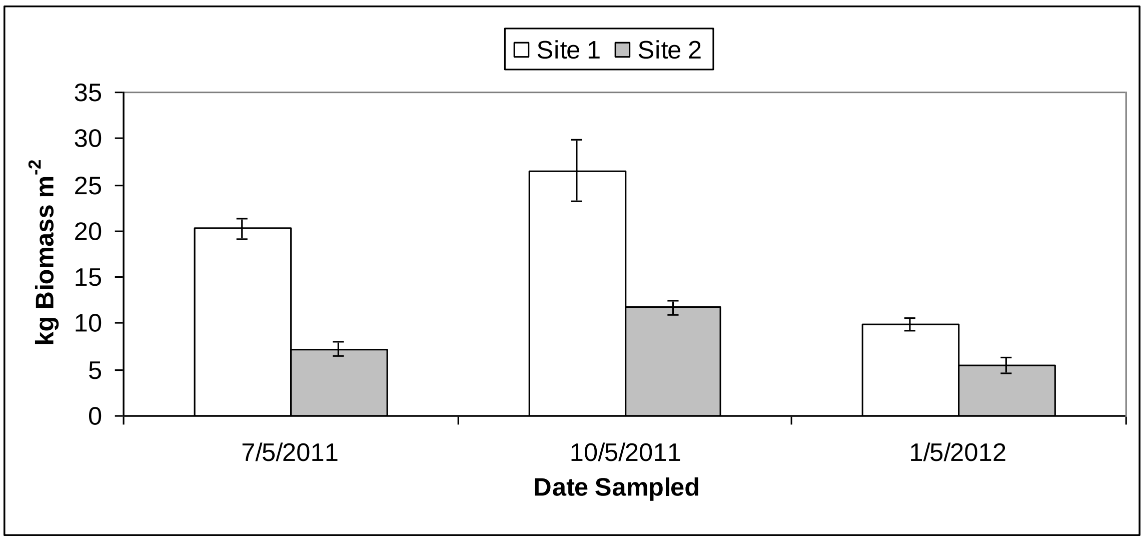


Figure 8: Total above ground plant biomass collected from each pond on each sampling date (on a wet weight basis). The error bars in the bar graph represent one standard deviation around the mean.

Surrounding Environmental Factors

Water and sediment samples were taken to assess the water pH, N and P concentrations within each stormwater management pond. These background concentrations allowed a better understanding of what changes, if any, were occurring in the pond. For example, these measurements allowed us to determine if seasonal fluctuations, nutrient inputs and usual or unusual trends within the stormwater ponds were occurring. Weather monitoring also took place at each site using a portable weather station (Watchdog 2700, Spectrum Technologies, Inc., Illinois, USA).

Two types of water samples were collected and analyzed. Grab samples were collected every time the site was visited for data collection, and automated samples were collected for two months. Both grab and automated water samples were evaluated for pH; however, pH was not consistently monitored throughout the study. Automated water sample pH ranged from 6.52-9.68 with an average pH of 7.30 (among 128 samples where pH was measured). Grab water sample pH ranged from 6.71-9.68 with an average of 8.11 (among 54 samples where pH was measured). After further analysis, we found that higher pH readings were measured among samples that were taken in the middle of the day or afternoon. We believe these higher pH values can be attributed to peak mid-day algae and phytoplankton blooms (eutrophication) and also lower dissolved CO_2 due to increased photosynthesis. Overall, these processes would account for higher pH values by making the pond water more alkaline (Tucker and D'Abramo, 2008).

As seen in Figure 9, a decrease in N and an increase in P levels occurred in the water samples taken from the stormwater ponds between July and August. These fluctuations could be due to a number of factors. One factor was precipitation; rainstorms were prevalent in the region in late July-early August 2011 (Figure 10). An increase in P and a decrease N could also be due to increased nutrient cycling due to microbial growth and other organisms dying off, resulting in decomposition, denitrification and retention of nutrients in organic material or high P inputs from the surrounding area due to stormwater runoff.

Automated water samples (Table 2, Figure 9) and grab water samples (Table 3, Figure 10) were analyzed for total N and P as well. In both types of water samples, we see similar trends as discussed above showing an increase in P in the summer months as well as a decrease in N. The opposite occurred as temperatures dropped as the winter approached; P concentrations decreased and N concentrations increased. This trend could potentially be explained by the suspension of P from sediments in the summer due to microbial action in the sediment as well as increased nutrient cycling and therefore a decrease in N due to uptake from organic material and microbial uptake. As the winter months progressed, a decrease in P occurred due to sorption to sediment; correspondingly a decrease in microbial viability and an increase in N occurred due to nutrient cycling and ground water recharge.

Sediment samples were collected and evaluated for N and P concentrations on four separate sample dates. The sediment was dried and analyzed for total N and P concentrations (Table 4, Figure 11).

Weather data was collected at Site 2 throughout the majority of the study from July 2011 to July 2012. However, the weather station at Site 1 had electronic issues and would not consistently record data; therefore this data was not included. We were comfortable to assume that weather at both sites was very similar due to their close proximity to one another and similarities in topography, which is common on Maryland's Eastern Shore. Temperatures maintained usual seasonal trends while there were some spikes in precipitation; the largest spikes in precipitation were seen during severe weather conditions such as thunderstorms or torrential downpours (Figures 12 and 13).

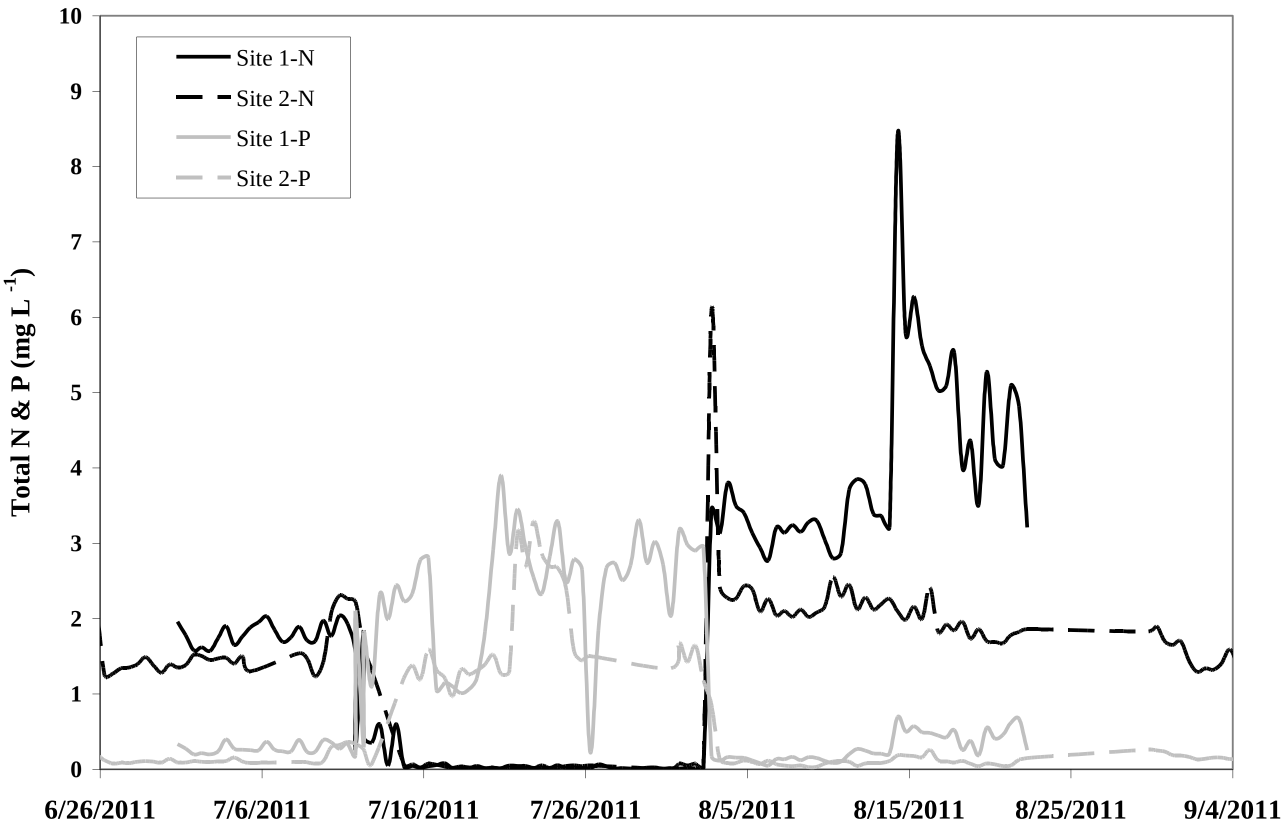


Figure 9: Fluctuation in total nitrogen and phosphorus concentrations within the stormwater management ponds from June-September 2011; water samples were taken via automated ISCO sampling units every 12 hours (7 am and 7 pm).

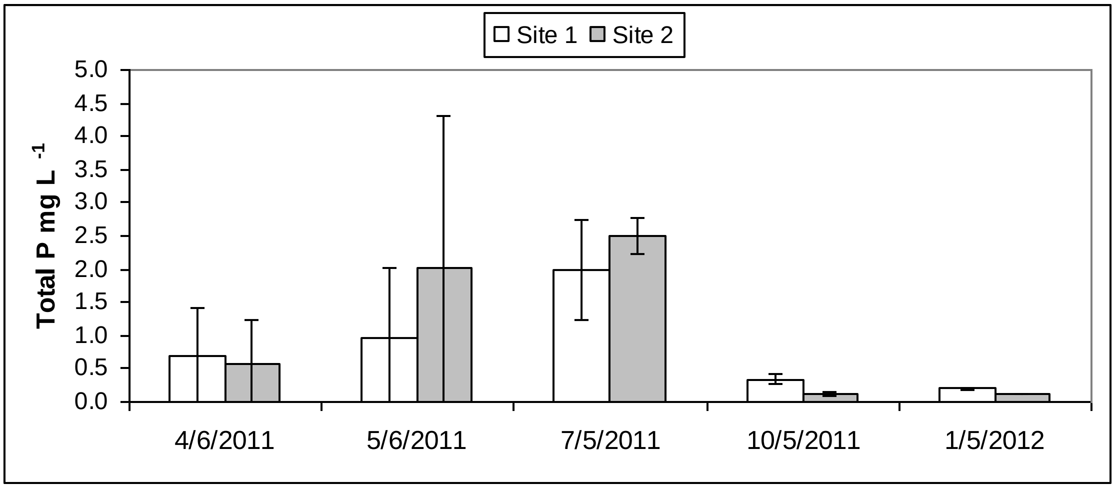


Figure 10a

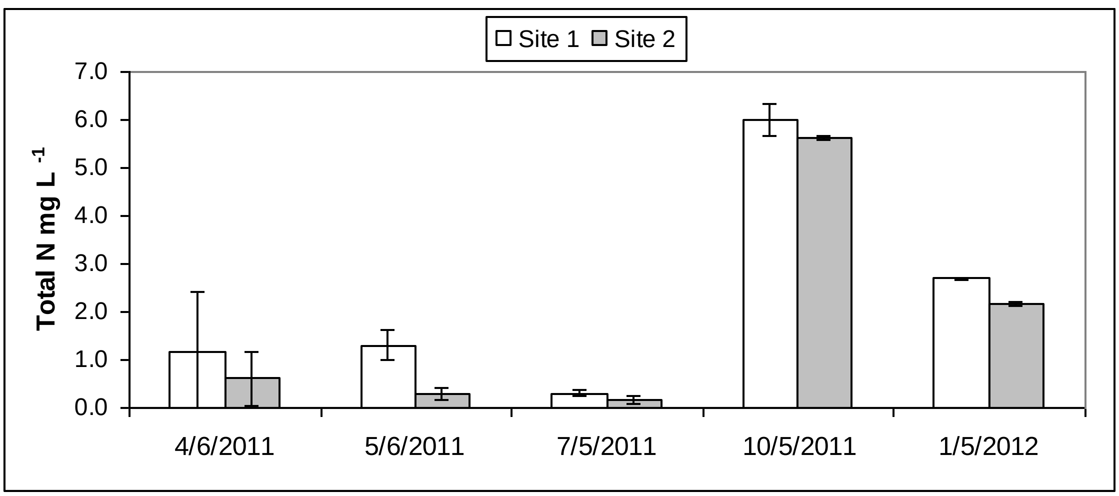


Figure 10b

Figure 10: Mean nutrient concentrations in water samples collected in triplicate from the stormwater management ponds throughout the duration of the study via grab samples: a) total phosphorus in milligrams per liter, b) total nitrogen in milligrams per liter. The error bars in the bar graph represent the standard deviation.

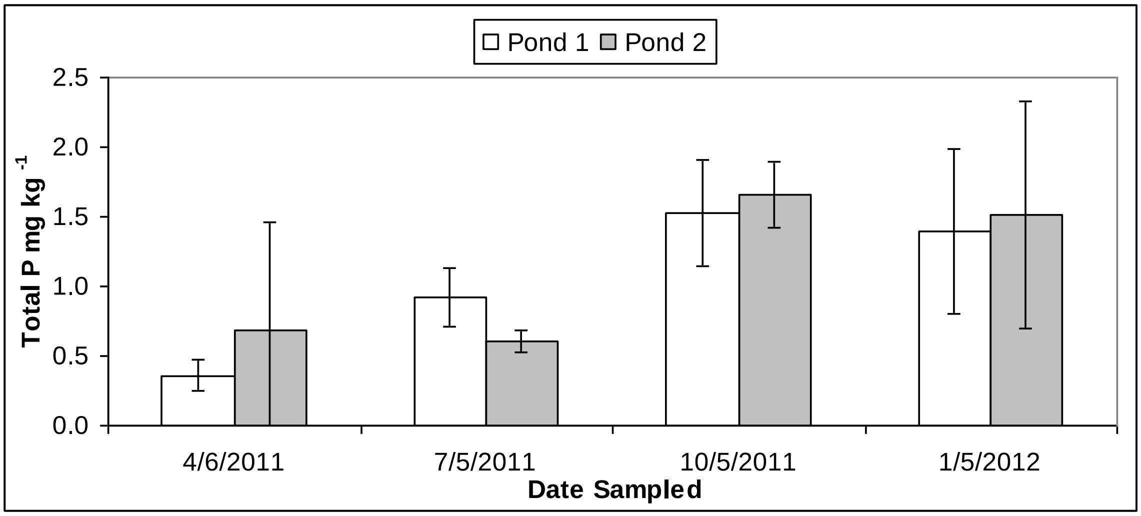


Figure 11a

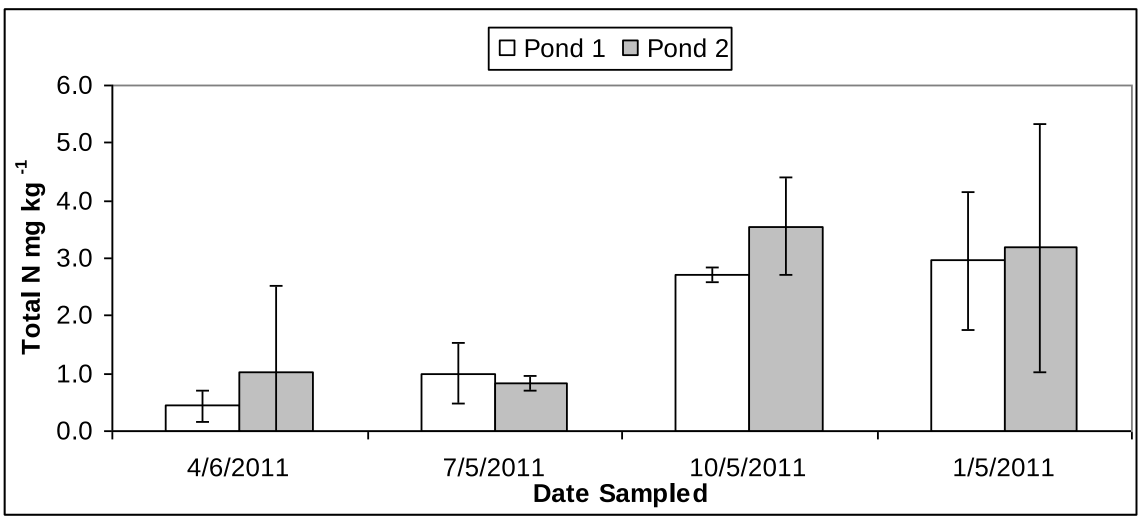


Figure 11b

Figure 11: Total nutrient concentrations in pond sediments sampled from the beginning of the study to Day 270: a) total phosphorus in grams of phosphorus per kilogram of sediment, b) total nitrogen in grams of nitrogen per kilogram of sediment. The error bars in the bar graph represent the standard deviation.

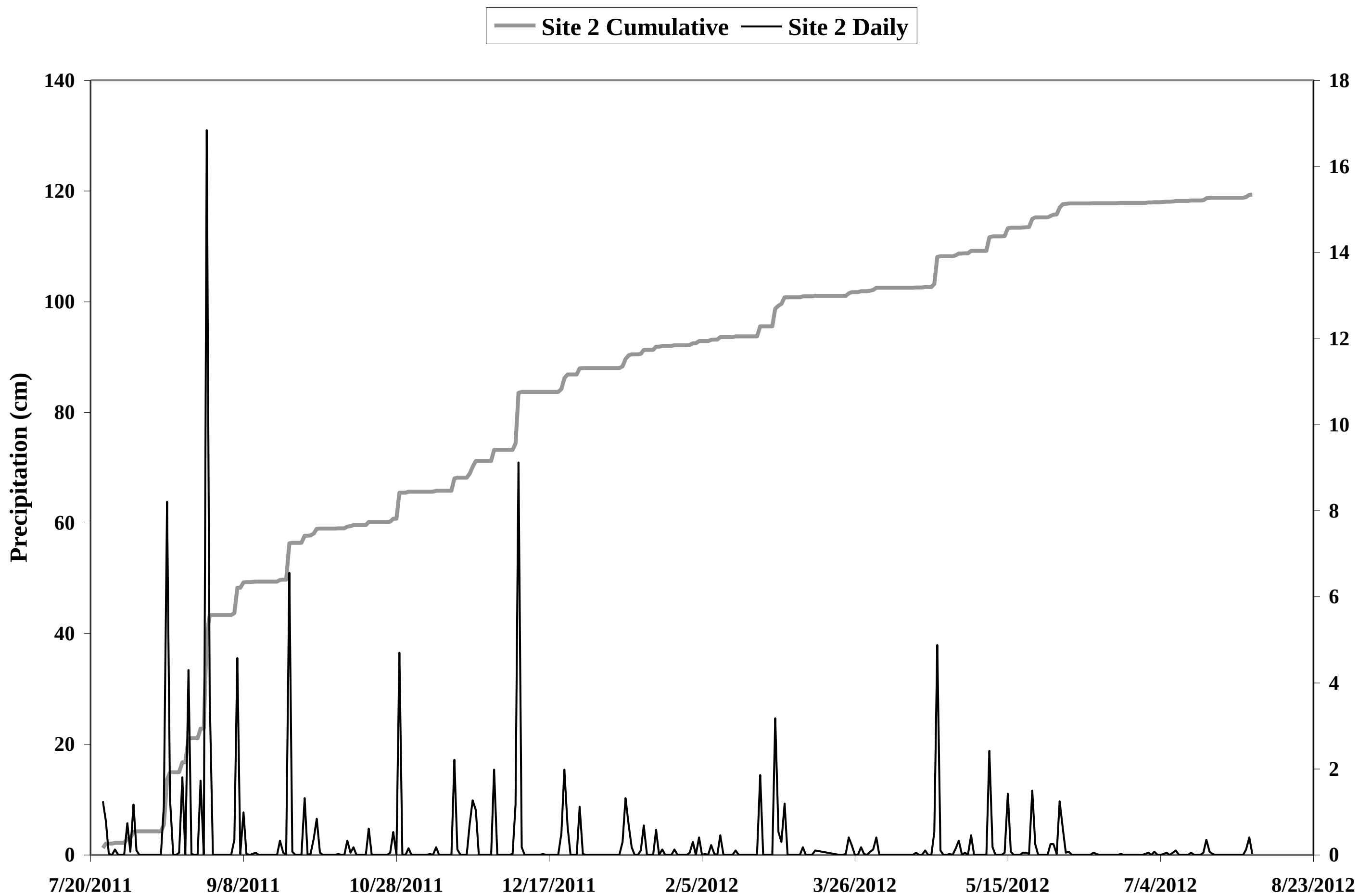


Figure 12: Cumulative and daily measured precipitation at Site 2.

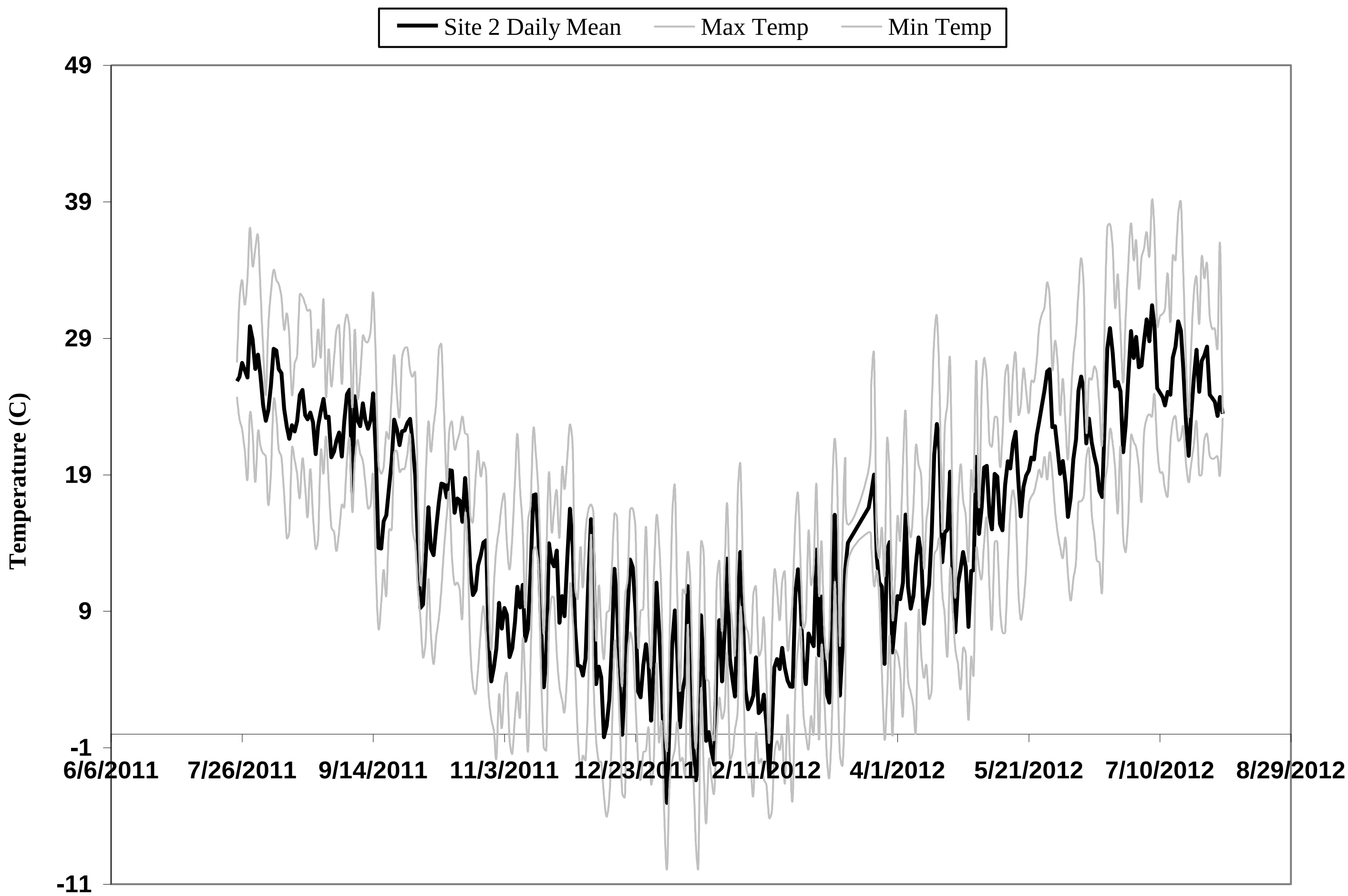


Figure 13: Daily mean, maximum, and minimum temperature at Site 2.

	Site 1-N	*Site 2-N*	*Site 1-P*	*Site 2-P*
	mg L^{-1}			
Mean	2.00	1.38	1.14	1.14
Range	0.003-8.43	0.005-6.11	0.05-3.90	0.028-3.30

Table 2: Automated water sample total nitrogen and phosphorous mean concentrations and concentration ranges taken at Site 1 and Site 2.

	Site 1-N	*Site 2-N*	*Site 1-P*	*Site 2-P*
	$mg\ L^{-1}$			
Mean	2.62	1.52	0.756	0.179
Range	0.002-6.35	0.002-5.70	0.133-2.61	0.072-4.73

Table 3: Grab water samples taken at Site 1 and Site 2 for total nitrogen and phosphorous mean concentrations and concentration ranges.

	Site 1-N	*Site 2-N*	*Site 1-P*	*Site 2-P*
	$\_\_\_\_$ g kg^{-1} $\_\_\_\_$			
Mean	1.78	1.52	1.05	1.12
Range	0.043-2.97	0.83-3.56	0.36-1.53	0.61-1.66

Table 4: Sediment samples taken at Site 1 and Site 2 for total nitrogen and phosphorous mean concentrations and concentration ranges.

FTWs Versus Pond Sediment

When comparing N removal of FTWs and ponds in which the FTWs were launched, only estimates can be given due to the nature of the denitrification measurements used in this study. Estimates were calculated for the FTWs using a density of 0.65 g cm^{-3}. The density of the FTW matrix material was measured on Day 460 to include plant and microorganisms that may have colonized on the FTW matrix material throughout the length of the study. A density of 1.35 g cm^{-3} was used to estimate the sediment in the study. This density was found in northern Chesapeake Bay sediments in a survey study performed by the Maryland Department of Natural Resources (Halka, 2000) and was used since actual sediment densities in this study were not calculated.

The total estimated nitrous oxide N removal via denitrification among the sediment at Site 1 was 841.84 kg N_2O-N yr^{-1} and at Site 2 was 2165.72 kg N_2O-N yr^{-1}. The total estimated nitrous oxide N removal via denitrification among FTWs at Site 1 was 27.24 kg N_2O-N yr^{-1} and was 98.04 kg N_2O-N yr^{-1} at Site 2.

These estimates were determined by first finding the pond area for the sediment including 4 cm in depth (which was the estimated bioactive layer of sediment) which resulted to be an area of 237.16 m^{-3} at Site 1 and 130.80 m^{-3} at Site 2. Then, the total area of the FTWs (which was 2.30 m^{-3} at each site) was converted into liters. Each of the four measurements in liters was individually multiplied by the estimated pond sediment density (1.35 kg L^{-1}) and the measured FTW density (0.65 kg L^{-1}). The amount of pond sediment (320198.73 kg at Site 1 and 176591.31 kg at Site 2) and FTW (1472.64 kg at both Sites) in kilograms was then multiplied by the measured denitrification rate of the pond sediment (0.30 mg-N_2O-N kg-sediment $^{-1}$ h $^{-1}$ at Site 1 and 1.4 mg-N_2O-N kg-sediment $^{-1}$ h $^{-1}$ at Site 2) and the FTWs (2.10 mg-N_2O-N kg-FTW $^{-1}$ h $^{-1}$ at Site 1 and 7.60 mg-N_2O-N kg-FTW $^{-1}$ h $^{-1}$ at Site 2). This number in kg N_2O-N h $^{-1}$ was then multiplied by 24 hours per day multiplied by 365 days per year to get kg N_2O-N yr $^{-1}$ in order to obtain the denitrification rate.

When the FTWs were directly compared to sediments on a yearly basis, the FTWs removed 3% to 5% N_2O-N of the total N_2O-N removed by the pond sediment itself. This study had a total of 11.16 m $^{-2}$ of FTW material within each pond. However, it is recommended that 61.32 m $^{-2}$ FTW per 0.41 hectare or 150 m $^{-2}$ FTW per hectare of a pond or lake be the ratio of FTW per body of water (verbal communication with BioHaven® Licensee). The amount of N removal by the floating treatment wetland could theoretically be increased by doubling the amount of floating treatment wetlands that were used in this study; so, the addition of 22 m $^{-2}$ of FTW could potentially double the amount of N removed. According to these potential estimates, adding at least 11.16 m $^{-2}$ of FTW to a pond will result in an additional 3% to 5% increase in complete soluble N removed from the pond as compared to a pond were no FTWs were used.

From the above estimations, cost analysis results in an estimated cost per kilogram of N removed per year by the FTWs. We know that the FTW material itself (not including labor) cost $301 m $^{-2}$ and, as described above, the estimated removal of nutrients from each pond on a yearly basis according to the measured denitrification Rates. With the area of the FTWs and the density of the FTWs, we can then estimate the cost per kilogram of N removed from each pond per year. The FTWs at Site 1 would cost approximately $123.36 kg N_2O-N year and the FTWs at Site 2 would cost approximately $34.28 kg N_2O-N year.

Fluctuations in Denitrification Rates Due to Seasonal Variations

When considering seasonal fluctuations among the FTWs, differences are more likely to occur in the summer and winter months of the year. If fluctuations do occur, the efficiency of FTWs and areas where denitrification occur also change seasonally (Figure 14). Although our denitrification potential data cannot support this theory because it was only measured once, our ORP readings do give some support for this theory: 83% of the ORP readings during the winter and fall months (6 months) indicate that more negative readings occur at the center of the FTWs compared to the edge of the FTWs, while in the summer (5 months) only 40% of the ORP readings are higher at the center as compared to the edge. This trend indicates variation among the FTWs during the summer and winter seasons, where more desirable denitrification conditions along the edge are more likely in the summer and more desirable denitrification conditions among the center in the winter.

The likelihood for denitrification to occur among FTWs fluctuates throughout the season due to temperature, dissolved oxygen and N concentrations among the ponds in which the FTWs reside (Beauchamp, 1989, Tiedje, 1988). In the summer months the microbial community along the edge of the FTWs takes up dissolved oxygen; once the microbes have removed the dissolved oxygen in the water (acting like an O_2 filter), the N rich water then travels toward the center of the FTW. The further the distance away from the edge of the FTW, the more denitrification is occurring until all dissolved N is depleted as indicated in Figure 14 during the summer. The reducing conditions among the center of the FTW become so low that the microbial community no longer seek out dissolved N (mainly due to its availability) but otherwise use other electron acceptors as sources of respiration such as Mn, Fe, S or C (Vepraskas and Faulkner, 2001)

As the winter approaches, dissolved oxygen concentrations increase and therefore denitrification among the edges of the wetland becomes less likely. The availability of N increases due to ground water inputs and decreased plant and algal uptake. The zone of denitrification within the FTWs becomes localized in the center of the wetland since more dissolved oxygen is available and more of the FTW has to act as an oxygen filter. At this point, denitrification is also temperature dependent and is less likely to occur especially at night when temperatures drop and become much colder.

Understanding the spatial variation within FTWs in denitrification potential and seasonal changes in these spatial patterns is important when designing FTWs for stormwater management ponds. The edge should be maximized but at the same time this variable should be dependent upon the climate in which the FTWs are being implemented. If FTWs are placed in a colder climate, perhaps the design used in this study is appropriate; however if FTWs are being used in a warmer climate, maximizing the edge of the FTW may be more efficient in the removal of dissolved N.

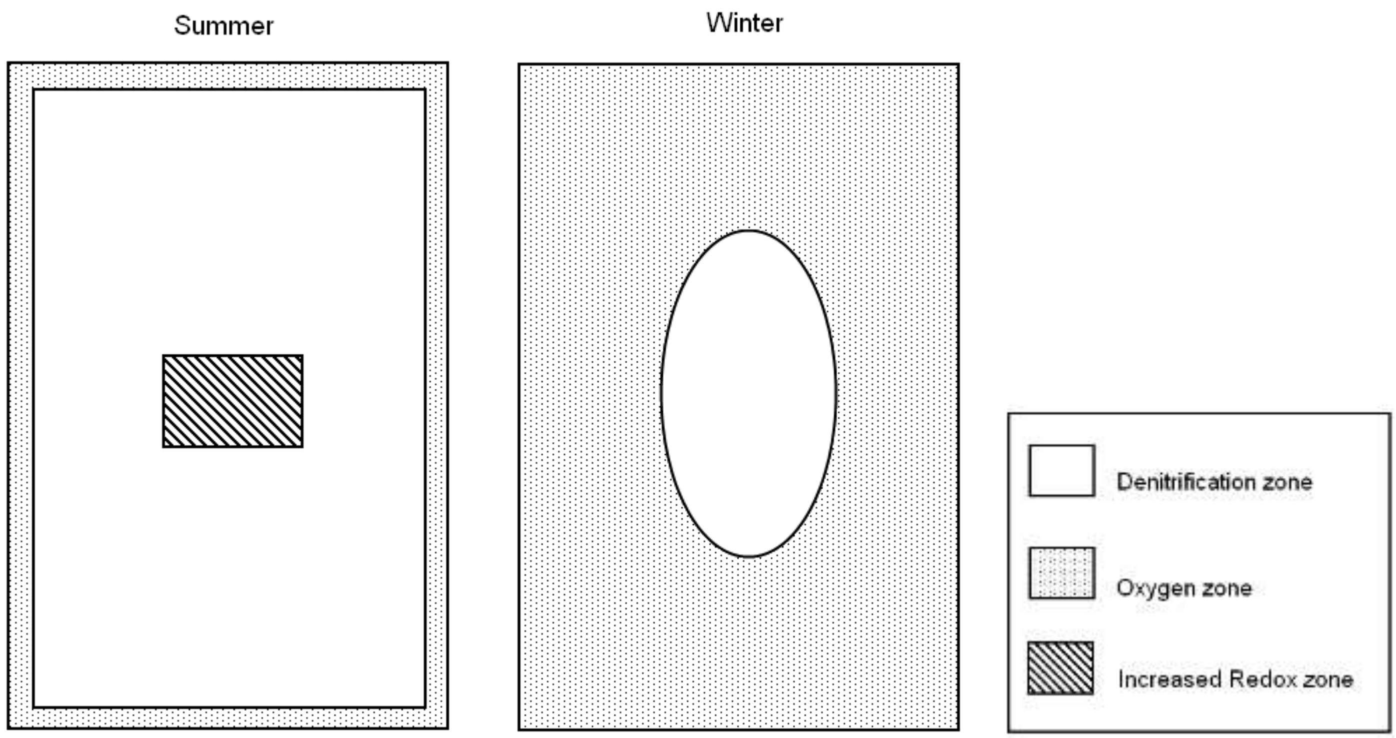


Figure 14: Represents the seasonal variations of denitrification patterns within the floating treatment wetland matrix. The rectangle on the left represents what occurs during the summer months and the diagram on the right represents what occurs during the winter months. As winter approaches more dissolved oxygen is present within the pond water shrinking the zone of denitrification. In the summer a larger denitrification zone is present because less dissolved oxygen is present and the floating treatment wetland perimeter acts like an oxygen filter allowing the more interior sections to perform more denitrification. The closer to the center of the floating treatment wetland, the less soluble nitrogen is available which forces the microbes to rely on other electron acceptors for respiration.

Conclusions

The floating treatment wetlands in stormwater retention ponds evaluated in this study successfully removed considerable amounts of N. Denitrification rates in this study demonstrated N_2O-N removal by FTWs to be 5 to 7 times higher than denitrification rates by sediments of the stormwater management ponds on a dry weight basis. However, when the density of the sediment and area of the pond are taken into account, the pond sediment removes more N_2O-N per year as compared to the FTWs within the ponds. With the addition of more FTWs, this scenario could potentially change. Even though the sediment density was not taken into account, FTWs add an additional environment at the surface of the pond that would otherwise not be present to increase the amount of denitrification that is taking place in ponds.

Through monitoring of ORP to estimate ideal denitrification conditions, we found that reduced conditions within the FTWs remained throughout the study and ideal denitrification (according to the literature and ORP measurements) conditions were more prevalent along the edge of the FTWs. According to the measured denitrification rates, we also found that reduction of soluble N to gaseous N was more likely to occur within areas along the perimeter or closer to the edge of the FTW.

These findings suggest that it may be ideal to engineer FTWs in a way to eliminate the potential for high reduction to occur in the center of the FTW but still allow space for oxygen to be removed (i.e. filtered) along the edge where the wetland is exposed to higher dissolved oxygen concentrations, thereby maximizing the amount of denitrification occurring within the FTW matrix. However, climatic conditions should also be taken into account when determining the shape of the wetlands used in a pond or lake, as the shape of the wetlands used in this study may be beneficial in areas where cold winters persist (like the mid Atlantic). On warm days when denitrification can still occur in the winter, the edges of the FTW become anoxic while the interior remains anaerobic, allowing denitrification to take place during warm periods throughout the winter. When climate parameters are similar to the climate in this study, an increase in the edge of a FTW could potentially decrease the amount of N removal throughout the winter. This observation was not confirmed in this study and should be investigated further in future studies to evaluate FTW geometry and climatic interactions to maximize denitrification.

Plant uptake of N and P was consistent with other FTW and treatment wetland research. However, as concluded by this study as well as other research, complete nutrient removal must take place via harvesting. Otherwise, nutrients will return to the system from which they were removed via nutrient cycling processes. It can also be concluded that the use of FTWs independently as a BMP for removal of P was only feasible through periodic plant harvesting. Overall, FTWs would be a reliable BMP for N removal via denitrification; however, optimal N removal was dependent upon plant harvesting along with naturally occurring denitrification processes.

Floating treatment wetlands that are similar to those used in this study should ideally be used in a closed, low flow system such as lakes, ponds or stormwater management enclosures. Theoretically the potential for pollution from detached plastic material through breakdown of the plastic is more likely to occur in rivers and streams and may potentially contribute to the persistence of plastic particulates in oceans worldwide. Although using manmade materials similar to those used in this study provide an environmental benefit, stringent management practices should be implemented in order to not cause other environmental ramifications that may further impact the environment in the future.

The findings from this study should be further investigated in a more extensive manner to provide additional supportive evidence for the use of FTWs in agricultural settings as well as use in other non-agricultural settings such as waste-water treatment facilities, urban stormwater management ponds or ponds where high nutrient concentrations are present. Floating treatment wetlands should also be further defined and classified by the FTW research community as there are many variations of FTWs (e.g. BioHavens®, Floating Islands International, Inc., vs. Beemats®, Beemats, LLC) that are similar but have different qualities that may potentially create differences among research findings. Once these various FTW systems are classified, specific methodology and use of units used in data presentation should then be set into place to allow direct comparisons between different environmental settings and different FTW types.

In conclusion, FTWs enhanced denitrification in stormwater retention ponds, thereby removing dissolved N. However, FTWs do not provide reliable nutrient removal through plant uptake due to the need for plant harvesting to prevent cycling of N and P back into the system. More research is needed to fully understand the entire potential of this product prior to implementation of the FTWs as a true BMP.

Appendix 1: Denitrification Rate Calculations

Raw Data to % Concentration

$y = mx+b = 59635x+14.129$, $r^2 = 0.9987$

$$\text{Conc.} = \frac{\text{Peak Area} - 14.129}{59635} = \text{\% concentration} = \text{\% concentration}/100$$

Volume of N_2O (L)

- 74 mL = volume of headspace in flask with wetland matrix **plastic**
- 139 mL = volume of headspace in flask with **sediment**

Concentration X mL X 1L / 1000mL = N_2O volume in Liters (L)

Mols of N_2O (in flask headspace)

PV= nRT
P= 1 atm
V= N2O Vol. (L)
n= mols of N2O/unknown
R= 0.08206 L atm/(mol K)
T= Average Temp on day of GC run in Kelvin
1 atm X N2O Vol. (L) = n(0.08206 L atm/(mol K))(297.73 K)

Micrograms of N_2O

- Molecular Weight of N_2O = 44.0128 grams/mol

(mols of N2O) X (44.0128 g/mol) X (10^6 ug / 1 g) = µg of N_2O

Accounting for Dissolved N2O - Bunsen Coefficients

$M = C_g \times (V_g + V_t \times \beta)$
M = Total amount of N_2O in the water plus gas phase
C_g = Concentration of N_2O in the gas phase (mols of N_2O and ug of N_2O)
V_g = Volume of the gas phase = Sediment = 0.139 L = Plastic = 0.074 L
V_l = Volume of liquid phase Sediment = 0.010 L = Plastic = 0.075 L
β = Bunsen Coefficient 0.544 (average room temp of both incubations was 25°C)

Micrograms and mols of N produced

- Using stoichiometry find the amount of N in N2O
  - Molecular weight of N2O is 44.0128 g/mol
  - Molecular weight of N is 14.0067 g/mol

((Total ug of N2O)(28.0134))/(44.0128 g)= ug or mols N produced

Micrograms of N2O-N/kg sample

- The dry weight of each sediment and wetland matrix sample was used
- (ug of N produced/0.00705 kg)
- Use ug N2O-N/kg sample and mols of N2O-N vs. time, to find the rate of N production using the slope of the line

Appendix 2: Pond Denitrification Rates/Year

Pond Sediment (area and density accounted for)

Pond Area ft^2 X 0.125ft (estimated bio-active layer of sediment) = ft^3

ft^3 X 28.316 liters/ ft^3 = L

Liters of Sediment X Density (1.35 kg/l) = kg of sediment

kg of sediment X denitrification rate (N_2O-N/kg/hr) X 10^{-6} mg/1 kg = kg N_2O-N/hr

kg N_2O-N/hr X 24 hours/day X 365 days/year = kg N_2O-N/yr

FTWs (area and density accounted for)

5 ft X 8 ft X 8 in (0.666ft) = 26.6 ft^3

26.6 ft^3 X 3 FTWs/pond = 80 ft^3

80 ft^3 X 28.316 Liters/1 ft^3 = L

Liters of FTW X Density of FTW (0.65 kg/l) = kg

kg of FTW X denitrification rate (N_2O-N/kg/hr) X 10^{-6} mg/1 kg = kg N_2O-N/hr

kg N_2O-N/hr X 24 hours/day X 365 days/year = kg N_2O-N/yr

References

Bachard P.A.M., Home A.J. (2000) Denitrification in constructed free-water surface wetlands: . Effects of vegetation and temperature. Ecological Engineering 14:17-32.

Beauchamp E.G., Trevors J.T., Paul J.W. (1989) Carbon sources for bacterial denitrification. Advances in soil sciences 10.

Bowden, W.B. 1987. The biogeochemistry of nitrogen in freshwater wetlands. Biogeochemistry 4:313-348.

Brady N.C., Weil R.R. (2008) The nature and properties of soils. 14 ed. Pearson Prentice Hall.

Brix H. (1994) Functions of macrophytes in constructed wetlands. Water Science and Technology 29:71-78.

Brix H. (1997) Do macrophytes play a role in constructed treatment wetlands? Water Science and Technology 35:11-18.

Campbell, C.R. 1992. Determination of total nitrogen in plant tissue by combustion. Plant analysis reference procedures for the southern US Southern Coopertive Series Bulletin 368:20-22.

Canter L.W., Knox R.C., Fairchild D.M. (1987) Ground water quality protection CRC.

Casey R.E., Taylor M.D., Klaine S.J. (2004) Localization of denitrification activity in macropores of a riparian wetland. Soil Biology and Biochemistry 36:563-569.

Casey R.E.T., Klaine M.D., Stephen J. (2001) Mechanisms of nutrient attenuation in a subsurface flow riparian wetland. Journal of Environmental Quality 30:1732.

Chang N.B., Islam K., Marimon Z., Wanielista M.P. (2012) Assessing biological and chemical signatures related to nutrient removal by floating islands in stormwater mesocosms. Chemosphere 88:736-743.

De Stefani G., Tocchetto D., Salvato M., Borin M. (2011) Performance of a floating treatment wetland for in-stream water amelioration in NE Italy. Hydrobiologia 674:157-167.

Faulwetter, J.L., M.D. Burr, et al. 2011. Floating treatment wetlands for domestic wastewater treatment. Water science and technology 64:2089-2095.

Fonder N., Headley T. (2011) Systematic Classification, Nomenclature and Reporting for Constructed Treatment Wetlands. Water and Nutrient Management in Natural and Constructed Wetlands:191-219.

Forshay, K.J., and E.H. Stanley. 2005. Rapid nitrate loss and denitrification in a temperate river floodplain. Biogeochemistry 75:43-64.

Grob, R.L., and E.F. Barry. 2004. Modern practice of gas chromatography. Wiley-Interscience.

Groffman P.M., Altabet M.A., Böhlke J.K., Butterbach-Bahl K., David M.B., Firestone M.K., Giblin A.E., Kana T.M., Nielsen L.P., Voytek M.A. (2006) Methods for measuring denitrification: diverse approaches to a difficult problem. Ecological Applications 16:2091-2122.

Groffman P.M., Holland E.A., Myrold D.D., Robertson G.P., XiaoMing Z., Coleman D.C., Bledsoe C.S., Sollins P. (1999) Denitrification. Standard soil methods for long-term ecological research.:272-288.

Gumbricht T. (1993) Nutrient removal processes in freshwater submersed macrophyte systems. Ecological Engineering 2:1-30.

Halka J. (2000) Deposition and Distribution of Bottom Sediment in the Chesapeake Bay, The Impact of Susquehanna Sediments on the Chesapeak Bay, Scientific and Technical Advisory Committee Workshop Report, Chesapeake Bay Program. pp. 1-29.

Headley T.R., Tanner C.C. (2006) Application of floating wetlands for enhanced stormwater treatment: a review, Aukland Regional Council Technical Publication, National Institute of Water & Atmospheric Research Ltd., Hamilton, New Zealand. pp. 1-94.

Hyman M.R., Arp D.J. (1987) Quantification and removal of some contaminating gases from acetylene used to study gas-utilizing enzymes and microorganisms. Applied and environmental microbiology 53:298-303.

Hopfensperger, K.N., S.S. Kaushal, et al. 2009. Influence of plant communities on denitrification in a tidal freshwater marsh of the Potomac River, United States. Journal of environmental quality 38:618-626.

Hwang, L., and B.A. LePage. 2011. Floating islands an alternative to urban wetlands. Wetlands:237-250.

Inniss, E.C. 2005. Use of redox potentials in wastewater treatment. p. 399. Water encyclopedia.

Kadlec R.H., Wallace S. (2009) Treatment wetlands. CRC Press, Boca Raton.

Kleinman P.J.A., Allen A.L., Needelman B.A., Sharpley A.N., Vadas P.A., Saporito L.S., Folmar G.J., Bryant R.B. (2007) Dynamics of phosphorus transfers from heavily manured Coastal Plain soils to drainage ditches. Journal of Soil and Water Conservation 62:225.

Manahan S.E. (2009) Fundamentals of Environmental chemistry CRC.

Moret-Ferguson, S., K.L. Law, et al. 2010. The size, mass, and composition of plastic debris in the western North Atlantic Ocean. Marine Pollution Bulletin 60:1873-1878.

Mosier, A.R., and L. Mack. 1980. Gas chromatographic system for precise, rapid analysis of nitrous oxide. Soil Science Society of America Journal 44:1121-1123.

Nordstrom D.K., Wilde F.D. (2005) REDUCTION-6.5 OXIDATION POTENTIAL (ELECTRODE METHOD), in: U. S. G. Survey (Ed.), Field Measurements-Reduction-Oxidation Potential. pp. 1-22.

NRCS Soil Survey N.R.C.S., United States Department of Agriculture. (2011) Official Soil Series Descriptions.

Pant H.K., Reddy K.R. (2001) Phosphorus Sorption Characteristics of Estuarine Sediments under Different Redox Conditions. Journal of Environmental Quality 30:1474-1480.

Pierzynski G.M., Sims J.T., Vance G.F. (2005) Soils and environmental quality CRC press.

Poe A.C., Piehler M.F., Thompson S.P., Paerl H.W. (2003) Denitrification in a constructed wetland receiving agricultural runoff. Wetlands 23:817-826.

Qin S., Hu C., Oenema O. (2012) Quantifying the underestimation of soil denitrification potential as determined by the acetylene inhibition method. Soil Biology and Biochemistry 47:14-17.

Richardson, J.L., and M.J. Vepraskas. 2000. Wetland soils: Genesis, hydrology, landscapes, and classification. CRC.

Rogers K.H., Breen P.F., Chick A.J. (1991) Nitrogen removal in experimental wetland treatment systems: evidence for the role of aquatic plants. Research Journal of the Water Pollution Control Federation 63:934-941.

Sirivedhin T., Gray K.A. (2006) Factors affecting denitrification rates in experimental wetlands: field and laboratory studies. Ecological Engineering 26:167-181.

Smith M.S., Tiedje J.M. (1979) Phases of denitrification following oxygen depletion in soil. Soil Biology and Biochemistry 11:261-267.

Soil Survey Staff N.R.C.S., United States Department of Agriculture. (2011) Web Soil Survey.

Stewart F.M., Mulholland T., Cunningham A.B., Kania B.G., Osterlund M.T. (2008) Floating islands as an alternative to constructed wetlands for treatment of excess nutrients from agricultural and municipal wastes-results of laboratory-scale tests. Land Contamination & Relclamation 16:25-33.

Tanner C.C., Headley T.R. (2011) Components of floating emergent macrophyte treatment wetlands influencing removal of stormwater pollutants. Ecological Engineering 37:474-486.

Tiedje J.M. (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. Biology of anaerobic microorganisms:179-244.

Tucker C.S., D'Abramo L.R. (2008) Managing high pH in freshwater ponds, Southern Regional Aquaculture Center. pp. 1-5.

USEPA. (2008) Revised National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitations Guidelines for Concentrated Animal Feeding Operations in Response to the Waterkeeper Decision, in: U. S. E. P. Agency (Ed.), Federal Register. pp. 70418-70486.

Vepraskas M.J., Faulkner S.P. (2001) Wetland soils: genesis, hydrology, landscapes, and classification; Redox Chemistry of Hydric Soils CRC Press.

Wang C.Y., Sample D.J., House G. (2011) Application of Floating Treatment Wetlands to Stormwater Management–A Pilot Mesocosm Study, ASABE Meeting Presentation, Louisville, KY.

Weragoda, S.K., K.B.S.N. Jinadasa, et al. 2012. Tropical application of floating treatment wetlands. Wetlands 32:1-7.

Xin, Z., X. Li, et al. 2012. Effect of stubble heights and treatment duration time on the performance of water dropwort floating treatment wetlands (ftws). Ecological Chemistry and Engineering S 19:315-330.

Zhao, F., S. Xi, et al. 2012. Purifying eutrophic river waters with integrated floating island systems. Ecological Engineering 40:53-60.

Zhu, L., Z. Li, et al. 2011. Biomass accumulations and nutrient uptake of plants cultivated on artificial floating beds in china's rural area. Ecological Engineering 37:1460-1466.

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Education:

- B.S. Natural Resource Sciences, University of Maryland, College Park, MD, 2009
- M.S., Environmental Science, Towson University, Towson, MD, expected January 2013

Employment History:

- Graduate Research Assistant, University of MD, Jan. 2010 to Aug. 2012. Tasks included: Evaluation on FTWs to assist in the Maryland Department of Natural Resources (MD-DNR) best management practice (BMP) implementation process. Prepared gas phase samples and operated a gas chromatograph to measure denitrification in plastic wetland matrix and sediment samples. Collected water, plant and sediment samples.
- Biological Science Technician, National Park Service, Washington, DC May 2010 to September 2010. Tasks included: Controlled and eradicated invasive exotic plants within parks of the National Capital Region of the NPS. Inventoried and mapped exotic vegetation to help develop and maintain various ecological strategies. Managed and applied various chemical herbicides as a tool for exotic plant management; responsible for disposal and storage of unused herbicides.
- Research Assistant, UMD College Park, MD, July 2007 to December 2009. Tasks included: Installing phosphorus recovery filters in ag drainage ditches to improve water quality. Collected water samples using automated water sampling equipment and computer software. Performed an incubation study to investigate the efficacy of nitrogen stabilizers. Acquired skills in soil and environmental laboratory methods and procedures.

Presentations:

- Presented Research Presentation Titled: "Evaluation of Floating Treatment Wetlands in Stormwater Retention Ponds on Poultry Farms to Reduce Nutrient Loading" in Cincinnati, OH at the American Society of Agronomy 2012 International Meeting, October 2012.