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Assessment of Non-Anthropogenic Addition of Uric Acid to a Water Treatment Wetlands

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Abstract: Artificial water-treatment wetlands can reduce nitrogen and phosphorous nutrient concentrations in wastewater effluent to improve water quality and decrease eutrophication in natural waters. The Orlando Easterly Wetlands (OEW) is an engineered wetland that polishes 57 million liters of wastewater per day, lowering the total nitrogen and phosphorous concentrations through biological, physical, and chemical processes. In addition to purifying the water, the wetlands provide habitat for avian, mammalian, reptilian and macroinvertebrate species. Previous research has shown that avian species affect the eutrophication of agricultural reservoirs near their roost. The research herein quantifies uric acid in avian and reptilian excretory product and tracks its concentration profile throughout the OEW over a seven-month period. This measure of the non-anthropogenic contribution to nitrogen within the park includes winter months when large numbers of migratory birds occupy the wetland. The enzymatic decomposition of uric acid and the subsequent fluorimetric analysis were used to quantify uric acid throughout the flow train of the OEW. High concentrations of 2–4 mg/L uric acid were found in the influent, but drastically declined to concentrations below 0.2 mg/L in the effluent.

Keywords: treatment wetland; uric acid; fluorimetric analysis; waterfowl

1. Introduction

Effluent from wastewater treatment facilities contains high concentrations of nitrogen (N) and phosphorus (P). When water from these facilities flows directly into rivers and streams, the presence of N and P can result in the rapid growth of algae, eutrophication, and the death of animal life due to depleted oxygen in the water. The treatment of wastewater with biotic and abiotic processes in dedicated wetlands is a frequently employed water polishing method [1]. Heterotrophic microbes [2], emergent and submerged aquatic vegetation (SAV) [3], the accretion of organic matter [4] redox [5], precipitation, and sorption [6], each help to remove nutrients, including nitrogen and phosphorus. The Orlando Easterly Wetlands (OEW) is a treatment wetland located in east Orange County, Florida, in the United States. Prior to wetland construction, the area was a cattle pasture. It began receiving the effluent from a wastewater treatment plant (WWTP) in 1987 [7]. The OEW was created for the secondary water treatment of effluent from a nearby municipal WWTP, which serves approximately 400,000 residents of central Florida. The site has been successful in its intended purpose of diminishing nutrients from the water; the total nitrogen concentration decreases from 2.31 mg/L (influent) to 1.0 mg/L (effluent). The phosphorous concentration decreases from 0.13 mg/L (influent) to 0.03 mg/L

(effluent). The OEW has provided a platform for several water treatment studies including hydraulic analysis [8], prescribed burns [9] and analytical chemistry educational research [10].

Prior to the construction of the wetland, surveys from the mid 1800s indicate that the site was a wet prairie. It was subsequently drained for agricultural use in the mid-20th century. Since the construction of the wetland in the 1980s, an unintended additional benefit of the OEW is the restoration of animal habitat to the area that served as a cattle pasture for decades. Over 200 species of birds (some threatened or endangered) have found a home at the OEW, as well as mammals, reptiles, fish, macroinvertebrates and insects. The presence of these species makes the water-polishing capabilities of the wetlands even more remarkable than it at first appears, since the guano of birds and reptiles is an abundant source of nitrogen and phosphorus. Quantifying the total N and total P from the inflow to the outflow provides an oversimplified view of the park's efficacy, and the amount of nutrients added by animals and then remediated by the park have not been previously quantified.

This research reports the analysis of the concentration of uric acid (hereafter abbreviated UA, chemical structures shown in Figure 1) present in surface waters throughout the flow path of an engineered wetland, the OEW. In the metabolic processing of foodstuffs, nitrogen-containing protein and nucleic acids are broken down to form ammonia, a toxic substance. Human metabolic processes subsequently convert ammonia primarily to urea, and in smaller quantities, to uric acid which is excreted in urine [11]. Birds and reptiles convert ammonia almost exclusively into urate salts [12] and uric acid (two species in pH-dependent equilibrium, see Figure 1), which is excreted in guano. Uric acid has in the past been suggested as a possible indicator of pollution within a body of water. O'Shea and Bunch [13] examined uric acid from human waste as a pollution indicator and suggested that avian uric acid could be used for a similar purpose. It is noteworthy that some measures of uric acid specify only the acidic form, while others use the term "uric acid" to refer to the formal concentration of all ionized and uncharged species of the form. Herein, we used the term uric acid to refer to the formal concentration.

The U.S. state of Florida, where the OEW is located, has a large transient migratory bird population with many species roosting during the winter. Roosting species have been shown to contribute to eutrophication of waters below their nest. One study within the closed ecosystem of the Maji Agricultural Reservoir in Wonju, Gangwond-do, South Korea found that cormorants can contribute heavily to the eutrophication of the waters below their roost [14]. In more open waters, such as Lake Balaton in Hungary, bird droppings were still considered to be a potential source of eutrophication, even from only a few hundred nests [15]. For the research reported herein, the quantification of ammonia and uric acid at the inflow was used to determine the anthropogenic contribution of uric acid concentration in the wastewater that had persisted through the WWTP. The stratified heterogeneous sampling of the water throughout the flow train of the park allowed the quantification of ammonia and uric acid added due to animal (non-anthropogenic) sources.
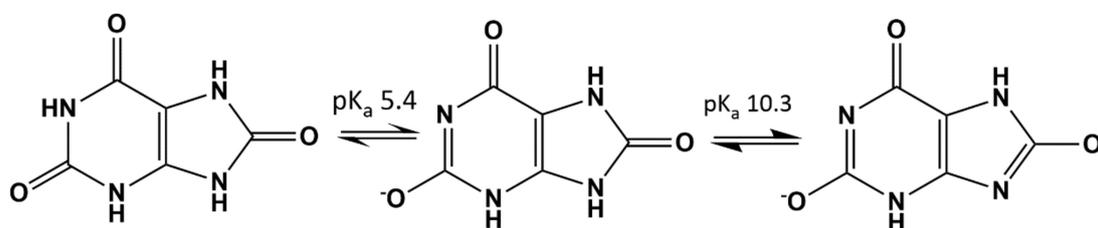
**Figure 1.** Chemical structure of uric acid and urate conjugate bases.

A variety of uric acid quantification methods are presently published. Early in the 20th century, colorimetric methods were published to quantify uric acid in the blood of human medical patients in an effort to diagnose gout [16]. Spectrophotometric methods were subsequently refined [17] and more recently, sensitive enzyme-based fluorescence methods have been published [18]. Fluorescence methods are more sensitive than spectrophotometric methods and were therefore used in this study. Enzymatic

fluorimetric analysis was utilized with two enzymatic reactions: the uricase catalyzed reaction of uric acid to form allantoin with the production of hydrogen peroxide, followed by the horseradish peroxidase-catalyzed reaction of hydrogen peroxide with Amplex Red, to form the fluorescent product, resorufin. In addition to uric acid concentration data, the total nitrogen and total phosphorous levels were obtained by the Iron Bridge WWTP in-house laboratory, along with the bird counts obtained within a week of the water sampling dates to determine if any correlation existed between uric acid and the amount of wildlife inhabiting the park at the time. An analysis of avian, fish, and alligator excreta was also conducted to determine the uric acid additions to the wetlands. The time frame reported herein extends from September 2017 to March 2018, and includes a sampling immediately following an extreme weather event, Hurricane Irma, in September 2017.

2. Materials and Methods

2.1. Structure of the Park and Sampling

OEW receives the WWTP effluent and distributes it throughout a 4.9 km^2 area partitioned into seventeen cells. Before flowing into the St. John's River, the water passes through three main vegetative regions: a 1.6 km^2 deep marsh populated with cattail reedy marsh plants and bulrush sedge (where most nutrient removal takes place), a 1.5 km^2 mixed marsh, and a 1.6 km^2 hardwood swamp with a lake. The influent water divides and follows three main paths (referred to as North, Central, and South, see Figure 2 at the influent control structure. The water flows under gravity with its flow rate restricted by water control structures and berms to maintain an approximately 30 day residence in the wetland.
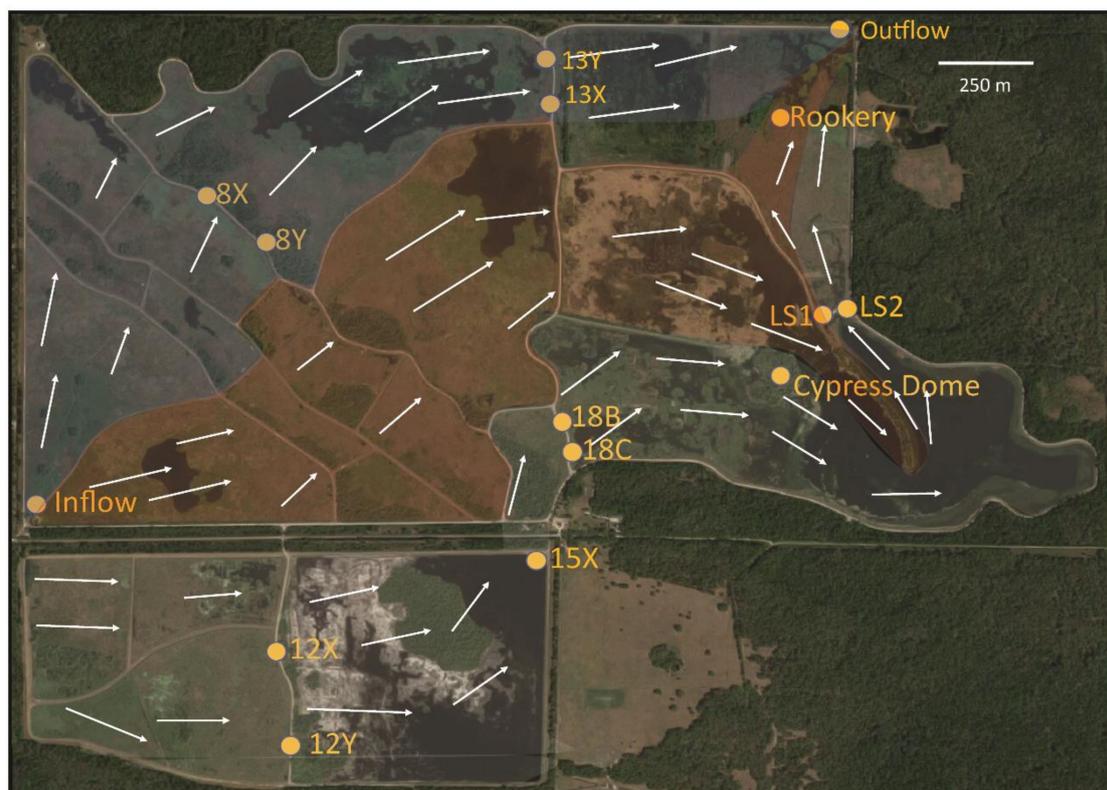
Figure 2. Orlando Easterly Wetlands (OEW) water flows through three paths: the north flow path (shaded light blue), central flow path (shaded red), and the south flow path (shaded yellow).

The concentration profile was expected to vary as a function of the sampling location, just as nitrogen and phosphorus decrease from the influent to the effluent canal. Therefore, a stratified

heterogeneous sampling procedure was employed to characterize the variation in uric acid. For example, along the north flow train, 500 mL samples were collected at the inflow control structures, at a water control structure past the deep marsh and 1.2 km from the influent (site 8x in Figure 2), in the mixed marsh (site 13X in Figure 2, 2.5 km from the influent), and in the outfall canal (3.4 km from the influent). Similar approaches were used along the central and south flow paths. Water near a roosting site (labeled Rookery in Figure 2, pictured in Figure 3) near the outflow canal, and a cypress dome (pictured in Figure 3) which serves as a habitat for birds, were sampled from a boat. At the time of the sample collection, the pH, conductivity, dissolved oxygen, and temperature of the water were measured. The dates of collection were, 17 September 2017, 1 December 2017, and 1 March 2018. The September 2017 sampling date occurred 7 days following Hurricane Irma, which passed over central Florida as a category 1 hurricane on 11 September 2017.

Figure 3. (**A**) Photograph of a rookery OEW site with the outflow canal control structure in the back. (**B**) Birds exit cypress dome at sunrise during bird count in December 2017.

2.2. Uric Acid Chemical Analysis

An Amplex™ Red Uric Acid/Uricase Assay Kit from Molecular Probes was used to prepare the solutions for the fluorescence measurements. Solutions to conduct the enzymatic assay were frozen at −20 °C when not in use. A 10 mM solution of Amplex Red was created by dissolving 0.26 mg Amplex Red in 100 μL DMSO. A 100 U/mL of solution of horseradish peroxidase (HRP) was prepared by dissolving 20 U of HRP in 200 μL of a reaction buffer at pH 7.2. The buffer was composed of 0.5 M Tris-HCl and 5 mM $CaCl_2$. The uricase enzyme (10 U) was dissolved in 100 μL of deionized H_2O to create a 100 U/mL solution. An enzymatic reaction mixture was prepared using 50 μL of the Amplex Red solution, 20 μL of the HRP solution, 20 μL of the uricase solution, and 4.91 mL of the reaction buffer.

The avian, fish, and alligator excreta samples were analyzed using the same enzymatic assay but required prior preparation. For these samples, a small quantity of dry, frozen excreta was ground with a pestle and mortar, and added to 100 mL of a 0.1 M arginine buffer (pH = 9.3). The solutions were mixed for 1 h and then the particulates settled under gravity. Water samples and extracted excreta samples were filtered using Whatman #43 filter paper, and 50 μL of each sample was placed in a cell on the microplate. A deionized water blank, and a 20 mM H_2O_2 solution (positive control) were dispensed in separate microplate cells, and the uric acid standard solutions (1–10 μM) were also placed in microplate cells. The enzyme reaction mixture (50 μL) was added to all the standard solutions and samples, and the data recordings were taken after the solutions were incubated at 37 °C for 30–40 min with the enzyme mixture before analysis.

The total nitrogen and ammonia concentrations in wetland surface water were measured using the total Kjeldahl nitrogen method and colorimetric analysis, respectively. Quantification was performed by a local government laboratory in the city of Orlando, using Environmental Protection Agency (EPA) methods.

2.3. Instrumentation

An Infinite 200 PRO Tecan microplate reader was used to quantify the uric acid, using Tecan i-control software. The instrument was operated in fluorescence mode, with an excitation wavelength of 510 nm and an emission wavelength 590 nm. The excitation bandwidth was 9 nm and the emission bandwidth was 20 nm, with an integration time of 5 ms. The measurements made by the Iron Bridge in-house laboratory included the total Kjeldahl nitrogen (TKN), and the colorimetric nitrate–nitrite analysis by approved Environmental Protection Agency (EPA) methods. EPA 351.2 was used for the TKN, and EPA 353.2 was used for the NO_x N.

3. Results

3.1. Uric Acid Concentrations

Uric acid in OEW surface waters was measured using the Amplex Red fluorimetric assay; the limit of detection for the assay was measured to be 0.2 ppm uric acid. The concentration profiles for the park are shown in Figure 4. The average influent concentration for the three dates is 2.2 ± 1.2 ppm. The effluent concentration for each sampling date was below the limit of detection (i.e., below 0.2 ppm). The decrease in anthropogenic uric acid was noteworthy and indicated that the treatment in the wetlands was functioning as intended. In the case of the September 2017 measurements, for which sampling occurred within a week of a category 1 hurricane, the concentrations of uric acid at most sites following the inflow were below the limit of detection, with few exceptions. This is likely due to the large volume of rain that was flushed through the park during the storm event, simultaneously diluting the uric acid present in the cells and increasing the rate of effluent discharge. Typically, 57 million liters per day are discharged, however, the average for September 2017 was 157 million liters per day. The rainfall resulting from that storm event can be compared to the average rainfall data for the preceding two years and for the following year, shown in Figure 5A. Conversely, the rainfall during the months of the December 2017 and March 2018 were much lower, near 5 cm.
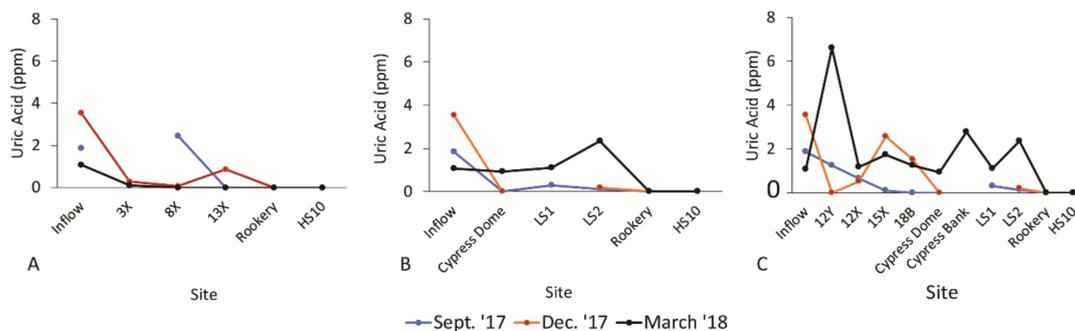
Figure 4. Concentration profiles for uric acid from influent to effluent (designated HS10) along the north flow path (**A**), central flow path (**B**) and the south flow path (**C**). Sites referred to as x axis labels can be found in the map in Figure 2 and are listed on the axis in the order through which the water passes. HS10 refers to the effluent canal.

Given the presence of wildlife in the park, one of the aims of this project was to determine if uric acid contributed by animal excreta could be quantified in the water. Evidence of animal uric acid can be seen at several sites in the plots in Figure 4. For both the September 2017 and December 2017 data, the majority of the sites do not contain quantities of uric acid measurable by the methods used. Notable exceptions include discrete regions such as 8× (deep marsh water control structure, north flow path) in September, and 13× (mixed marsh water control structure, north flow path) in December, where the concentrations of uric acid exceeded those of the preceding sites. The March 2018 data show considerably higher concentrations of uric acid due to non-anthropogenic sources, especially along

the central and south flow path. Importantly, by the time the water reaches the outfall canal (HS10), the concentrations are once again below the limit of detection.
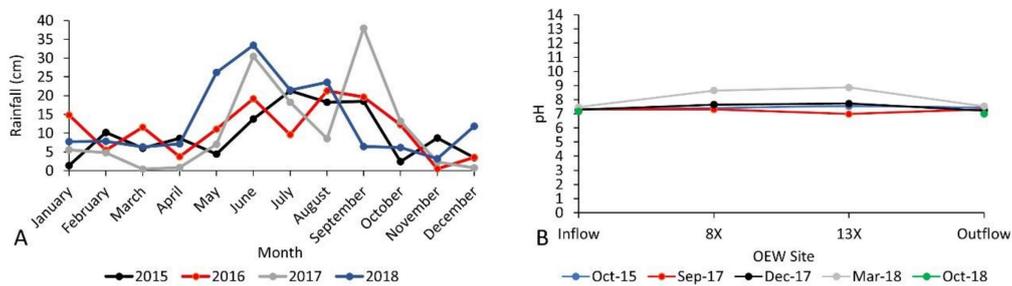
Figure 5. Contextual data from the OEW. (**A**) Monthly rainfall for the OEW during the years preceding and the year following the sampling dates. (**B**) Measured pH at the inflow and outfall canal from the sampling dates reported herein, as well as a date two years preceding and a date following, showing a largely invariant pH. The site locations refer to those labeled on the map in Figure 2.

3.2. Animal UA Analysis and Bird Population

Uric acid was quantified in the excreta from reptiles (*Alligator mississippiensis*, collected from site 8×), largemouth bass (*Micropterus salmoides*, collected from site 18C), and avian guano (unknown species, scraped from leaves in the cypress dome). The uric acid composition of each was found to be 0.439%, 0.972%, and 5.5% by dry mass, respectively. Given these percentages, the avian excreta in particular seem likely contributors to the uric acid added to the wetland. To assess the likelihood waterfowl increasing the uric acid, two avian specialists volunteered to conduct a ground survey of the birds present in the wetland. Each survey occurred at sunrise when roosting birds leave the cypress dome. The bird counts and identities were estimated by one bird specialist and independently confirmed by a second. The species of birds present and their estimated numbers are shown in Figure 6.
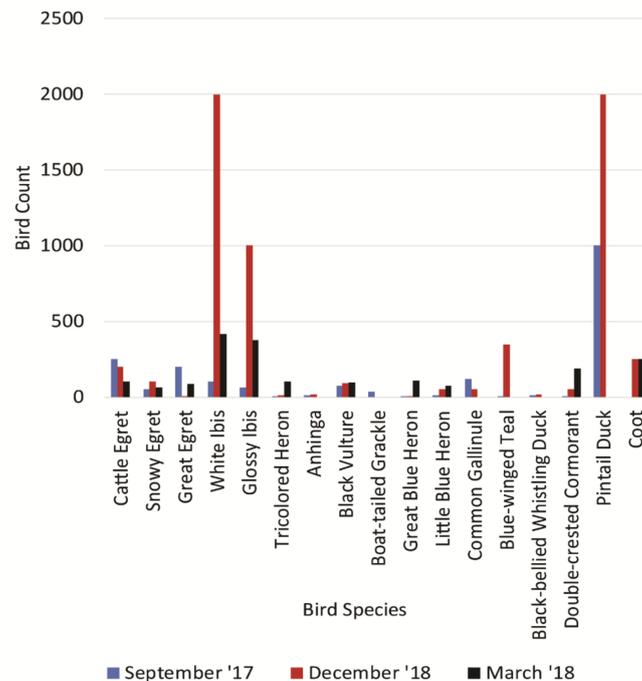
Figure 6. Water-fowl count data occurring within a week of the water sampling dates.

In 1993, the Environmental Protection Agency published bird count data [19] for wading birds that were collected from low altitude aerial surveys. The totals reported then included great egret, snowy egret, white ibis, great blue heron, glossy ibis, little blue heron, tri-colored heron, black-crowned night heron, wood stork and cattle egret. The data from that study, along with the counts reported here, are shown in Table 1. It is apparent from the data from 34 years preceding the data in Figure 6, that the average number of wading birds per hectare has risen dramatically. Given that the previous study was conducted within 6 years of the OEW conversion from cattle pasture to wetland, it is perhaps not surprising that the wildlife numbers immediately following the construction would be low initially but rise over the intervening years. In the 1993 report, there was seasonal variation in the bird count that varied by more than a factor of 4 (55 wading birds counted in May 1992 and 267 in Oct 1991), while a similar seasonal variation was also observed for the more recent data reported herein.

Table 1. Combined bird count data from the present study and the 1993 Environmental Protection Agency (EPA) report.

Date	GE	SE	WI	GB	GI	LB	TH	BH	WS	CE	Total	Density Birds/ha
Oct 1991 *	107	88	6	1	0	30	17	0	0	13	267	0.54
Dec 1991 *	10	33	0	10	15	8	1	0	0	0	77	0.04
Feb 1992 *	42	11	59	11	0	1	0	1	0	0	129	0.26
Mar 1992 *	16	3	106	8	0	2	2	0	1	0	138	0.28
Apr 1992 *	38	26	35	5	1	1	4	0	19	0	130	0.26
May 1992 *	18	4	9	8	0	4	0	0	0	4	55	0.11
Sept 2017	200	50	100	4	65	10	7	0	0	250	686	1.37
Dec 2017	8	100	2000	8	1000	50	10	0	0	200	3376	6.77
Mar 2018	83	62	415	109	374	72	103	0	0	103	1321	2.65

GE = great egret, SE = snowy egret, WI = white ibis, GB = great blue heron, GI = glossy ibis, LB = little blue heron, TH = tricolored heron, BH = black-crowned night heron, WS = Wood stork, CE = cattle egret, ha = hectare. * These data are reproduced from the 1993 EPA aerial bird count.

It is noteworthy from Figure 6 that the largest number of birds present was counted at the time of the December sampling, when transient migratory birds were more likely to be present in the subtropical climate. However, surprisingly, December uric acid samples did not show the highest UA concentrations. The March bird count data show the fewest numbers of birds, and yet also present the highest consistent concentrations of uric acid. There may be several contributing variables to this disparity. One possibility may be that the March sampling data included the highest number of cormorants, piscivorous water birds which have been reported to excrete high concentrations of uric acid below their roost [14]. A contributing scenario is likely the presence of reptilian and fish species, with excrement-containing uric acid. Other confounding factors include the solubility of uric acid as a function of temperature and pH, discussed in the following paragraphs.

4. Discussion

4.1. Uric Acid Solubility

The effect of solubility on concentrations of UA, particularly as a rationale for the low concentration of uric acid in the surface waters when the transient bird populations are higher and water temperatures are colder (December data, Figure 4), was investigated. The precipitation and sedimentation of UA may be a rational for its low aqueous concentrations. The solubility of uric acid depends on its ionization state and the temperature of the solutions. The ionization is pH-dependent, with the un-ionized form exhibiting pK_a 5.5, above which, the monobasic urate predominates. The monobasic form can be further ionized at higher pH, with pK_a 10.3. The fraction of the various forms of uric acid are shown in Figure 7. The pH measured at the time of the water sampling varied throughout the wetland

between 6.69 and 8.98, with an average pH of 7.4 ± 0.6 (see Figure 5B). The pH of the water entering and exiting the wetland shows largely invariant values over time, with some small increase near the mixed marsh during the spring 2018 sampling, when aquatic vegetation (SAV) would be rapidly growing. Photosynthesis removes carbon dioxide from water, which is in equilibrium with carbonic acid. The removal of CO_2 tends to have the effect of slightly increasing the pH of the water. Within the measured pH range, the monobasic urate form represents at least 97% of the formal concentration of uric acid. It is, therefore, the solubility of the basic form that is the most relevant to determining if precipitation is a major mechanism of the depletion of uric acid in solution.
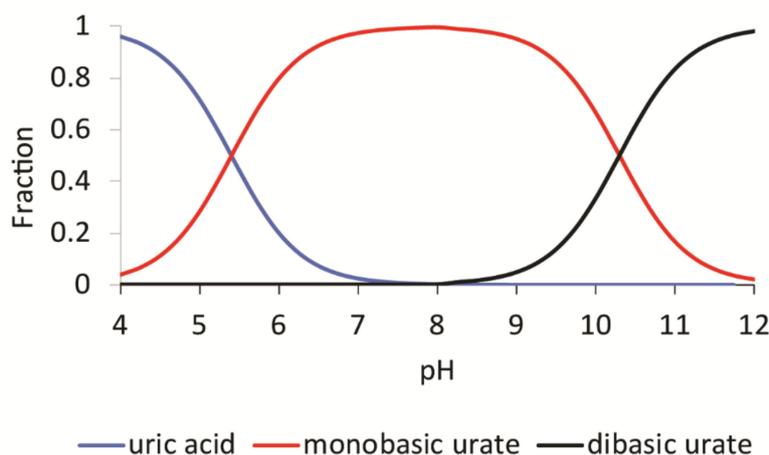

Figure 7. Fraction of uric acid and urate salts as a function of pH.

Wilcox and Khalaf [20] conducted a thorough analysis of the solubility of uric acid and urate salt as a function of pH, temperature and ionic strength, with all the relevant equilibria expressions. They acknowledge the potential confusion caused by the use of the term, uric acid, to mean all the protonated and deprotonated forms of the species. In this work, we similarly use the term uric acid to specify the formal concentration of all the protonated states. The solubility of uric acid increases as a function of temperature and ionic strength and decreases with increasing pH. Wilcox et al. [20] report the solubility of monosodium urate at 37 °C, pH 7.4 and ionic strength 0.16 (conditions relevant to human physiology) to be 5.4×10^{-4} M, but the temperatures of the wetland water were significantly colder and the ionic strength lower. Wilcox provides a theoretical equation, derived from Arrhenius plots, for which the solubility of monobasic urate can be calculated as a function of temperature (T, Kelvin) and ionic strength (I):

$$K_{sp} = 5120 exp^{(1.09 \sqrt{I} - \frac{5700}{T})} \tag{1}$$

To obtain a theoretical value of monobasic urate solubility using equation (1), the ionic strength of the wetland water was first estimated from the electrical conductivity of the water that was measured at the time of sampling, which ranged from 414–536 µS/cm. The linear relationship between conductivity and ionic strength, reported by Griffin and Jurinak [21], resulted in ionic strength conditions of 0.06–0.08 M. Therefore, under the temperature and ionic strength conditions measured at the wetland, the solubility of monobasic urate is calculated to range from 0.003 M–0.007 M (or 0.6–1.3 g/L, for monosodium urate). These solubilities are well above all of the concentrations of UA measured at the wetland, even for those colder temperatures for December 2017 sampling dates, so the loss of UA from the water is not likely to be due to precipitation and sedimentation.

4.2. Uric Acid Degradation

One possible explanation for the removal of UA from wetland water is the presence of microorganisms [22] with the capability to degrade uric acid with the subsequent formation of

ammonia. Both aerobic [23] and anerobic [24] species of bacteria have been shown to decompose uric acid. Anaerobic bacteria have been shown to convert UA to xanthine, with the subsequent decomposition forming ammonia, formic acid, carbon dioxide and glycine. Aerobic bacteria mineralize UA to allantoin, allantoic acid, urea and eventually, ammonia. The ammonia produced by either process may be dissolved in water, volatilized, or further oxidized to nitrate and nitrite. Evidence for microbial degradation in the wetland could then be sought in the total nitrogen and ammonia concentrations. Figure 8 shows the concentrations of total nitrogen and ammonia concentrations for the water sampled at the same sites, dates, and times where the water was collected for UA analysis.
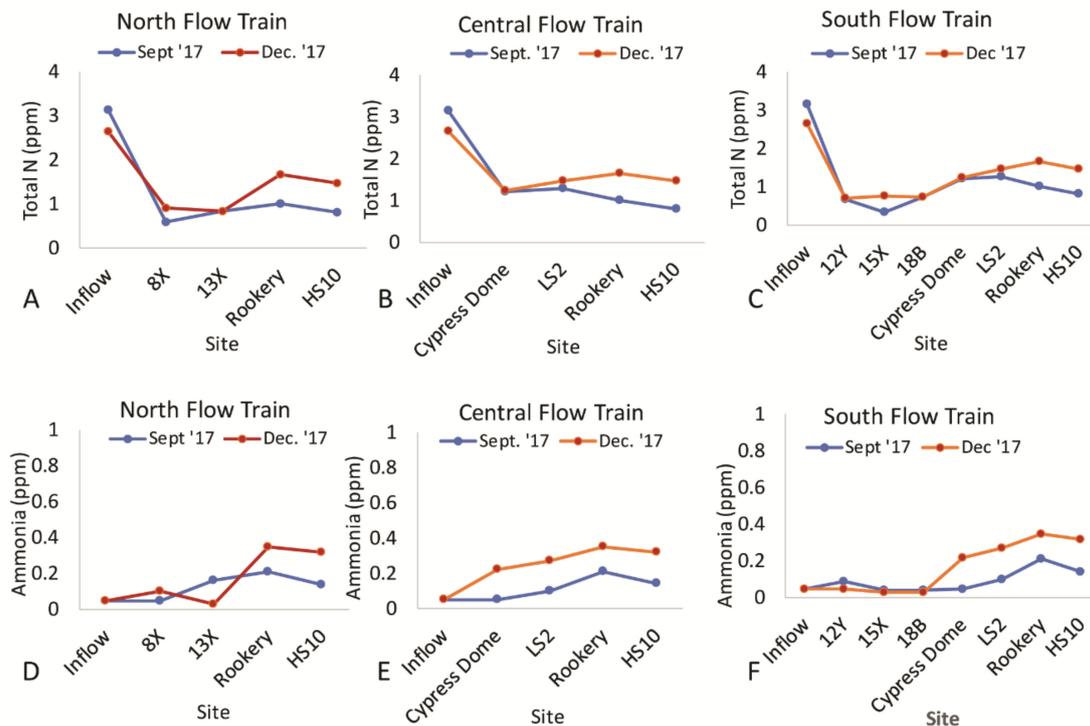
Figure 8. Concentrations of total nitrogen (**A–C**) and ammonia (**D–F**) along the three flow trains of the wetland. The site designations occur in order from inflow to outfall (HS10) and other labels can be correlated with sites in the map in Figure 2.

The north and south flow train (Figure 8, graphs A and C) show an initial decline in the total N through the first cells of the deep marsh, populated with bulrush sedge and cattail plants. This initial decline is not observed in the central flow path (Figure 8, graph B) because the samples were not collected near the site of the deep marsh but sampled the inflow and cypress dome and later sites. As the water traverses the cypress dome and rookery, where more birds are expected to be present, the total nitrogen concentrations are higher than at the deep marsh sampling sites (8X, 12Y, 15X, 18B, Figure 2). The ammonia concentrations at those sites are higher than the inflow concentrations for all flow paths. This increase in ammonia and total nitrogen indicates the contribution of additional nutrients to the water from the wildlife, and the increasing ammonia in particular, is consistent with the microbial degradation of uric acid that is added to the water beyond the deep marsh. Although the nitrogen was only measured for two of the sample collection days, over thirty years of data concerning the total nitrogen entering and exiting the OEW are publicly available and are consistent with the sampling data in Figure 8. As an example, the total nitrogen average for the year 2018 is plotted in Figure 9 [25]. These data show a trend of first dramatically decreasing, then slightly increasing total nitrogen content, consistent with those observed during the specific sampling points of this study.
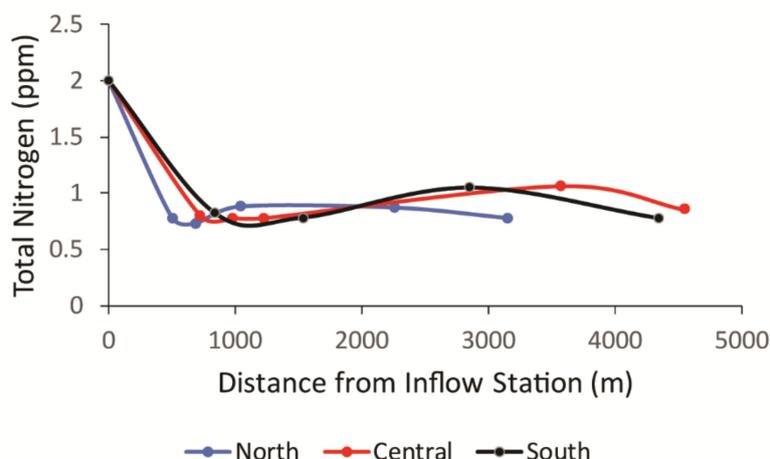
Figure 9. Average total Kjeldahl nitrogen concentration as a function of the distance traveled from inflow to outfall for 2018 along each of the three flow paths.

4.3. Nutrient Balance

The strikingly low burden of avian uric acid that was quantified in this study merits consideration relative to the uric acid burden inherent to the wastewater treatment conditions. Considering the averages of the UA influent concentration (Figure 4), the OEW receives an average of 2.2 UA mg·L^{-1}. With an average influent volume of 57 million liters per day, on average the park receives 120 kg UA per day. To estimate if the added UA from avian species would significantly compare to this, the average mass of excretions from the birds was approximated from literature reports of cormorant, egret, coots and heron. Mukherjee and Borhad [26] measured the dry masses and contents of excreta for those species; with the assumption that they represent all of those counted at the OEW (Figure 6), an input of 140 kg·day^{-1} UA (September 2017), 450 kg·day^{-1} UA (December 2017) and 130 kg·day^{-1} UA (March 2018) would be added to the park. These quantities would be expected to be measurable since they are similar to or larger than that of the influent, but that approximation assumes that birds exclusively excreted in the water near their roost. Those conditions might explain the higher UA concentrations observed on the South flow train during the March sampling. However, Mukherjee [26] points out that there are conditions for which waterfowl may improve the quality of freshwater by providing a mechanism for the removal of autochthonous nutrients. For example, a bird species can consume nutrients at one freshwater site and fly elsewhere when the product is excreted. Hahn et al. [27] explored this notion further and developed a mathematical model to predict the allochthonous nutrient input of freshwater birds. Their estimates considered that the herbivorous species of birds can consume 38.2 mg N·g^{-1}, from roots, seeds and foliage, and Mukjherjee reports that the egret and heron consumption of insects and cormorant consumption of fish can also provide a mechanism for the movement of nutrients. Hahn et al. estimated that only 12–26% of feces would be excreted near the roosts, and these estimates provide a possible rationale for the remarkably low concentrations of both uric acid and total nitrogen profiles at the wetland.

5. Conclusions

The uric acid concentrations at the wetland show a generally decreasing trend, and in all cases the effluent UA concentrations are below the limit of detection for the enzymatic fluorimetric assay. Isolated spikes in uric acid concentrations occur at various sites throughout the wetland, likely resulting from the excreta of alligator, fish, or avian species; these isolated high UA concentrations are uncorrelated with bird count data. The rapid decline in concentrations geographically following most elevated measurements indicate that the UA is mitigated by variables within the park. Precipitation and sedimentation are excluded as factors that could deplete the concentration. Evidence of the

mineralization of uric acid, possibly by bacterial species, is seen in the increased concentrations of total nitrogen and ammonia at sites where the UA is diminished. The lowest UA concentrations were observed within 1 week of a category 1 hurricane, when substantial rainfall increased the volume and flow rate of the effluent.

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