

**Vegetation and Metal Impacts on Soil Phosphorus Forms in Stormwater
Treatment Area 2, Florida**

**By
Taylor Smith
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1. Abstract

The Stormwater Treatment Areas (STAs) of the Everglades were designed and are operated to retain phosphorus (P) and reduce P concentrations in surface waters in accordance with water quality standards. One way to reduce P in surface water is assimilation through vegetation uptake and burial through soil accretion. Characterizing the relative lability or recalcitrance of assimilated P can be important in determining the potential for soils to release P back into the water column. Also, understanding metal (Ca, Mg, Fe, and Al) interactions with P can be useful in meeting the P removal guidelines. The submerged aquatic vegetation (SAV) cell within STA 2 exhibits more inorganic P enrichment, while the emergent aquatic vegetation (EAV) cell exhibits greater organic P enrichment. The SAV cell shows influence from abiotic and biotic factors, which could account for relatively more inorganic P enrichment compared to the EAV cell which is more influenced by biotic factors, such as litter production and accretion of organic matter. As P moves through the SAV cell and becomes transformed, a strong positive correlation with Fe:Al occurs due to more inorganic P enrichment, while the EAV cell shows no correlation with metals by the time outflow is reached. Thus, vegetation and metals do impact P cycling within the STA cells.

KEYWORDS: Everglades restoration, metal interactions with phosphorus, nutrient removal, phosphorus retention, wetland, wetland vegetation

2. Introduction

2.1 History of the Everglades and the stormwater treatment areas (STAs)

Before the draining occurred in the late 1800's, the Everglades extended from Lake Okeechobee to Florida Bay and was 160 km long, 65 – 80 km wide, and consisted of approximately 10,000 km² (Light and Dineen, 1994). Agricultural and urban development, changes to hydrology, and eutrophication from stormwater over the past 100 years have threatened the integrity of this historic ecosystem (Chimney and Goforth, 2001). A decline in water quality as well as other environmental disturbances were noted as early as 1938 (Beard, 1938). In 1947, Everglades National Park was established to conserve what remained of the natural landscape and prevent further degradation. In 1948, the Central and South Florida Project was initiated to protect urban and agricultural lands from flooding and to provide a source of freshwater for the booming urban population. This project included the construction of 3 Water Conservation Areas (WCAs). It also created what is called the Everglades Agricultural Area (EAA) after improving drainage to the south of Lake Okeechobee. The EAA's farm runoff generally flowed directly into the WCAs, carrying high levels of phosphorus (P) with it (Hand et al., 1986). This runoff caused some pockets of eutrophication within the WCAs, especially along peripheral areas of these wetlands closest to water-inflow structures.

The Central and South Florida Project was established in good faith, but ultimately damaged the Everglades in the name of flood protection and water supply. With the hyper-management that was occurring, some areas became excessively flooded while other areas became over-drained (Kushlan, 1989), which caused significant disturbances to the Everglades' natural hydropatterns and hydroperiods. These disturbances had further reaching consequences

as wading bird populations declined and sawgrass/slough habitats became replaced with cattail (Rutchev and Vilchek, 1994; Wu et al., 1997).

In 1972, the Florida Flood Control District was renamed the South Florida Water Management District, and given a different, more encompassing mission: in addition to flood control and providing freshwater, they were tasked with ensuring water quality, water supply, and environmental protection. This marked the first time of managing not for human use, but for the environment. In 1988, a 1515 ha tract of land was proposed to be used for constructed wetlands to allow water to be treated before discharge in the Everglades. This was the beginning of what would be called the Everglades Nutrient Removal Project (ENRP). The ENRP had three main objectives: (1) remove nutrients from EAA inflow before the water was discharged to the Arthur R. Marshall Loxahatchee National Wildlife Refuge, (2) provide the District with operational experience and design data needed to maximize long-term nutrient removal, and (3) develop and implement optimal nutrient removal technologies. In 1991, the Everglades Protection Act was passed, which began implementation of stormwater treatment areas (STAs) to restore and protect Everglades water quality (Chimney and Goforth, 2001).

Constructed wetlands are used to treat influent water before discharging cleaner water into another downgradient water body (Kadlec and Wallace, 2009). Wetlands can foster biogeochemical transformations of pollutants to inert forms in a relatively inexpensive and effective manner. These newly formed chemical compounds can exit the system via volatilization (nitrogen) or can remain stored in the soil in their inert forms (phosphorus). When P enters a wetland with inflowing water, biotic and abiotic processes are utilized to remove that P from the flowing water. This can be achieved by uptake from vegetation, precipitation reactions in the water column, and sequestration or burial into the soil (Reddy, et al., 1999,

Vymazal, 2007, Kadlec, 2009). With all these retention mechanisms at work, constructed wetlands may have a finite capacity to store P, which is controlled by the continual formation of non-reactive P (NRP) forms. The proportion of reactive P (RP) to NRP in the soil can be useful to assess performance as well as predict operational time remaining.

Originally, there were 6 constructed wetlands in south Florida built for the purpose of P removal from drainage waters destined for the Everglades Protection Area. Phase I of this project was to achieve an outflow total P (TP) of 50 µg/L, with a long-term goal of achieving 10 µg/L. Since the STAs were sized to achieve the 50 µg/L objective (Walker, 1995), STA research has now focused on technological advances to meet the 10 µg/L long-term goal (Goforth, 2001). This research has produced three ways in which we may improve STA performance: (1) the size/area of the STAs, (2) internal hydraulic efficiencies, and (3) dominant vegetation communities within the STAs (Newman and Lynch, 2001; Dierberg et al., 2002). For the purposes of this report, we will focus on the differing vegetation communities within the STAs and how they influence P cycling.

2.2 STA Vegetation and Associated P Dynamics

The STAs of the Everglades have been managed to facilitate two dominant vegetative communities: emergent aquatic vegetation (EAV) and submerged aquatic vegetation (SAV) (Goforth, 2005). The EAV includes species such as *Typha latifolia* and *T. domingensis* (cattail), *Schoenoplectus californicus* (giant bulrush) and *Nymphaea odorata* (water lily) (Figure 1). The SAV includes species such as *Najas quadalupensis* (southern naiad), *Potamogeton illinoensis* (pondweed) and *Hydrilla verticillata* (hydrilla) (Figure 2).

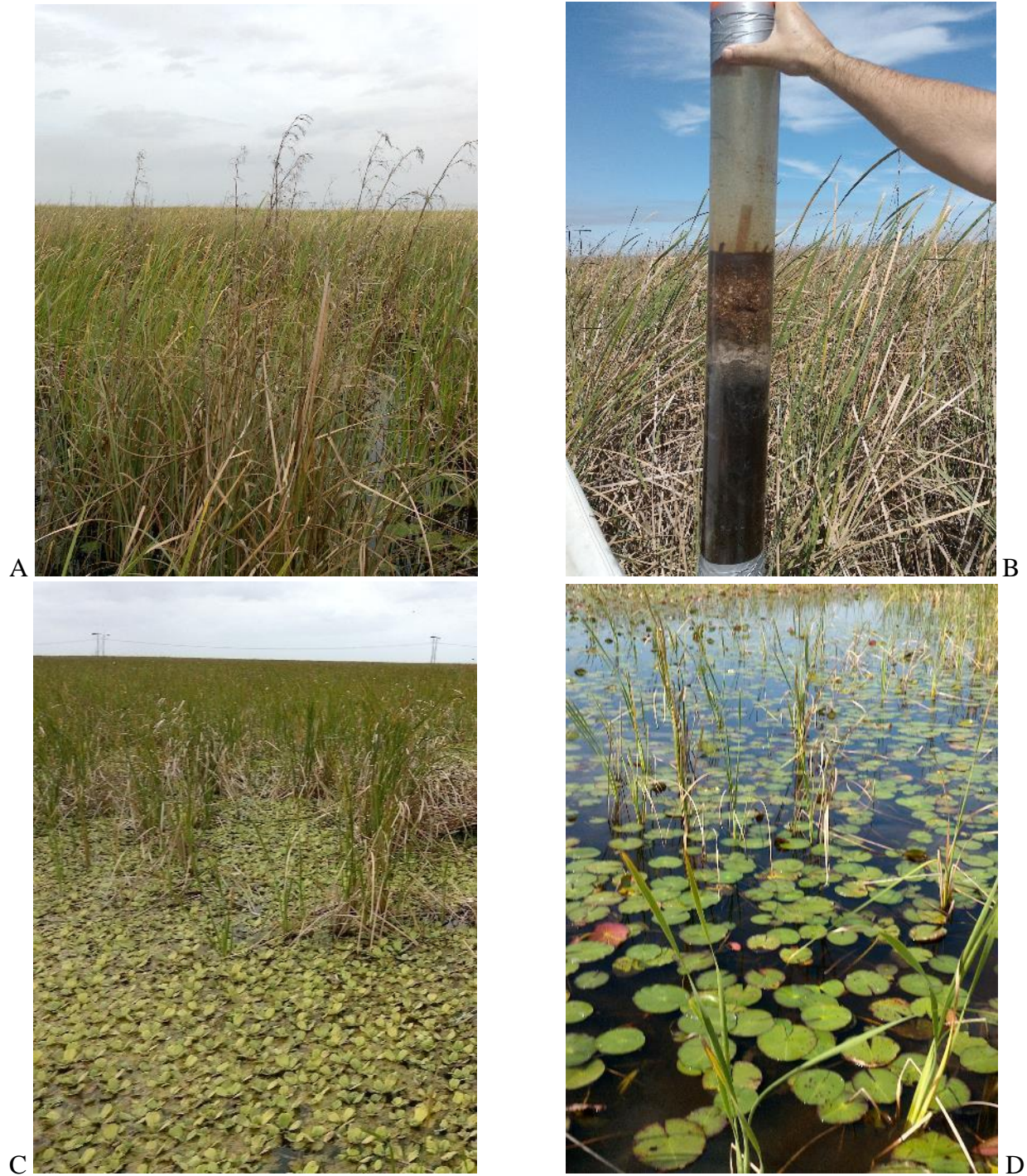


Figure 1. STA 2 Cell 1 – Representative soil core and commonly observed vegetation at sampling stations (February 9-11, 2016). A) Cattail (*Typha domingensis*) and sawgrass (*Cladium jamaicense*), B) Intact soil core, cattail, C) Cattail and water lettuce (*Pistia stratiotes*), and D) Water lily (*Nymphaea odorata*) and cattail.



Figure 2. STA 2 Cell 3 – Representative soil core and commonly observed vegetation at sampling stations (October 13-15, 2015). A) Illinois pondweed (*Potamogeton illinoensis*) and Southern naiad (*Najas guadalupensis*), B) Intact soil core, water lilies (*Nymphaea spp.*) and southern naiad, C) Southern naiad and Periphyton, and D) Intact soil core and southern naiad.

In south Florida, high P inputs into lakes, rivers, and streams are linked to shifting vegetation communities in the Everglades, causing historical ecosystem attributes to dwindle (Noe, et al., 2001). The Everglades Stormwater Treatment Areas were constructed in part to solve this problem by removing P from influent waters before being discharged into the WCAs (Chimney, et al., 2006). The reactivity/stability of this P is pertinent to the ongoing effectiveness of the STAs and to the satisfaction of state-mandated P levels entering the Everglades. Many environmental factors influence the reactivity of P forms in treatment wetlands such as: hydrologic conditions, redox and pH fluctuations, temperature, microbial communities present, ions present, available electron acceptors, and anthropogenic effects (Qualls and Richardson, 1995, Fisher and Reddy, 2001, Pant and Reddy, 2001, Vymazal, 2007). Also, the chemical form of P and the amount of these forms relative to other P forms can influence P reactivity via equilibria and mass action principles.

Wetland vegetation plays a role in both storage and transformation of P. Depending on the dominant vegetation community, pathways for P storage and transformation can vary. Both EAV and SAV offer different P storage mechanisms and subsequently sequester different P forms. Though EAV has great potential to store P due to extensive root networks/rhizomes and high ratio of below- to above-ground biomass, little water column P is directly taken up by these plants (Richardson and Marshall, 1986). The EAV can increase hydraulic resistance, thereby promoting increased settling of P from the water column. This also creates an opportunity for periphyton communities to uptake P if these communities are available. This settled, particulate P can be incorporated into the organic matter and become accreted as organic P (Po), which shows the biotic processes at work to sequester P in these environments. The EAV is useful for reducing relatively high water column P levels compared to SAV cells. In the SAV community,

P is generally taken up directly from the water column by vegetation, which allows for removal of P at lower P concentrations (14 – 100 µg/L) (Dierberg et al., 2002). These plants eventually undergo senescence, depositing the held P in an organic form. The SAV can also alter the physicochemical environment of surrounding water to cause P precipitation (Reddy et al., 1987). This inorganic P (Pi) can precipitate with iron (Fe), aluminum (Al), calcium (Ca), and magnesium (Mg), which demonstrates the SAV's ability to use biotic and abiotic processes to retain P. The makeup of vegetation within constructed wetlands can thus be important for meeting floodwater P removal goals.

Phosphorus dynamics in the STAs are no different than those found in natural wetlands, though understanding these dynamics can help to maximize P retention and minimize P release. The reactivity of sequestered P forms is operationally defined and is determined by assessing the solubility of P in acid and alkali solutions (Richardson and Reddy, 2013). These sequestered P forms are generally split into four groups: 1. Dissolved inorganic phosphorus (DIP) or soluble reactive phosphorus (SRP), 2. Dissolved organic phosphorus (DOP), 3. Particulate inorganic phosphorus (PIP), and 4. Particulate organic phosphorus (POP). These groups are used to understand the specific retention mechanisms. Reactive P is generally considered labile and will exit the system relatively sooner compared to non-reactive P, which tends to remain in the system from decades to centuries (Reddy, et al., 2002). The SRP is considered bioavailable while particulate and organic P typically must first be transformed into dissolved inorganic P before being reactive and bioavailable. Particulate P can be critical when considering physical retention mechanisms (sedimentation/resuspension) and SRP can be important when studying biotic retention processes. With this in mind, EAV vegetation is likely to retain relatively more particulate forms of P, while SAV is more likely to retain relatively more dissolved or soluble

forms of P. With the goal of treating influent water, NRP forms are ideal to keep downstream waterbodies more pristine.

Once the P is retained in the wetland, either through biotic or abiotic processes, other mechanisms can (and often do) re-release this P back into the water column, causing internal loading. This internal loading of P can cause major problems even if external loading is reduced significantly, as the internal load can negate the reduction and potentially prevent satisfying water quality criteria (Søndergaard et al., 2001). Processes that release particulate P include resuspension via wind and current, biological resuspension, and sloughing of periphyton into the water column (Reddy et al., 1999; Søndergaard et al., 2001). The SRP can be released by biotic and abiotic mechanisms including: decomposition of vegetation, desorption/dissolution of precipitates/complexes, and presence of a sharp diffusion gradient. Change in physicochemical factors can also affect the release rate of SRP (Gächter and Meyer, 1993). High pH, high temperature, and/or reduced redox often accelerates SRP release into the water column of wetlands (Reddy et al., 1999).

This study focuses on the quantification of various P fractions within the soil of an Everglades STA and the influence of vegetation and metals on said P fractions. Relative size and stability of these P fractions was determined in different soil layers by depth to assess the effects of vegetation. The hypothesis is that SAV will exhibit more inorganic P enrichment in soils than organic P, while EAV will exhibit relatively more P_o enrichment than P_i . This result is attributed to the influence of both biotic and abiotic factors in the SAV-dominated ecosystem, while the EAV-dominated ecosystem is mainly influenced by biotic factors. This knowledge is important for understanding P dynamics in wetlands and manipulating the environment to favor P sequestration.

3. Materials and Methods

3.1 Site Description

STA 2 Cell 1 and Cell 3 were chosen for study, and source water properties for both cells are the same. STA 2 is part of a 6-piece network of treatment wetlands below the Everglades Agricultural Area and has been operating for 17 years and is considered to be one of the better performing treatment wetlands.

The STA 2 is subdivided into treatment cells, which have differing vegetation. STA 2 Cell 1 is predominately populated with EAV while STA 2 Cell 3 primarily is populated with SAV. In both STA 2 Cell 1 and Cell 3, a transect was established corresponding to the hydrologic flow path, from which 11 stations per cell were identified and sampled. In Cell 1, the stations are 34, 51, 69, 86, 104, 121, 138, 156, 173, 191, and 208, with benchmark sites at 34, 121, and 208 (Figure 3). In Cell 3, the stations are 20, 38, 56, 74, 92, 110, 128, 146, 164, 182, and 200, with benchmark sites at 20, 128, and 200 (Figure 4).

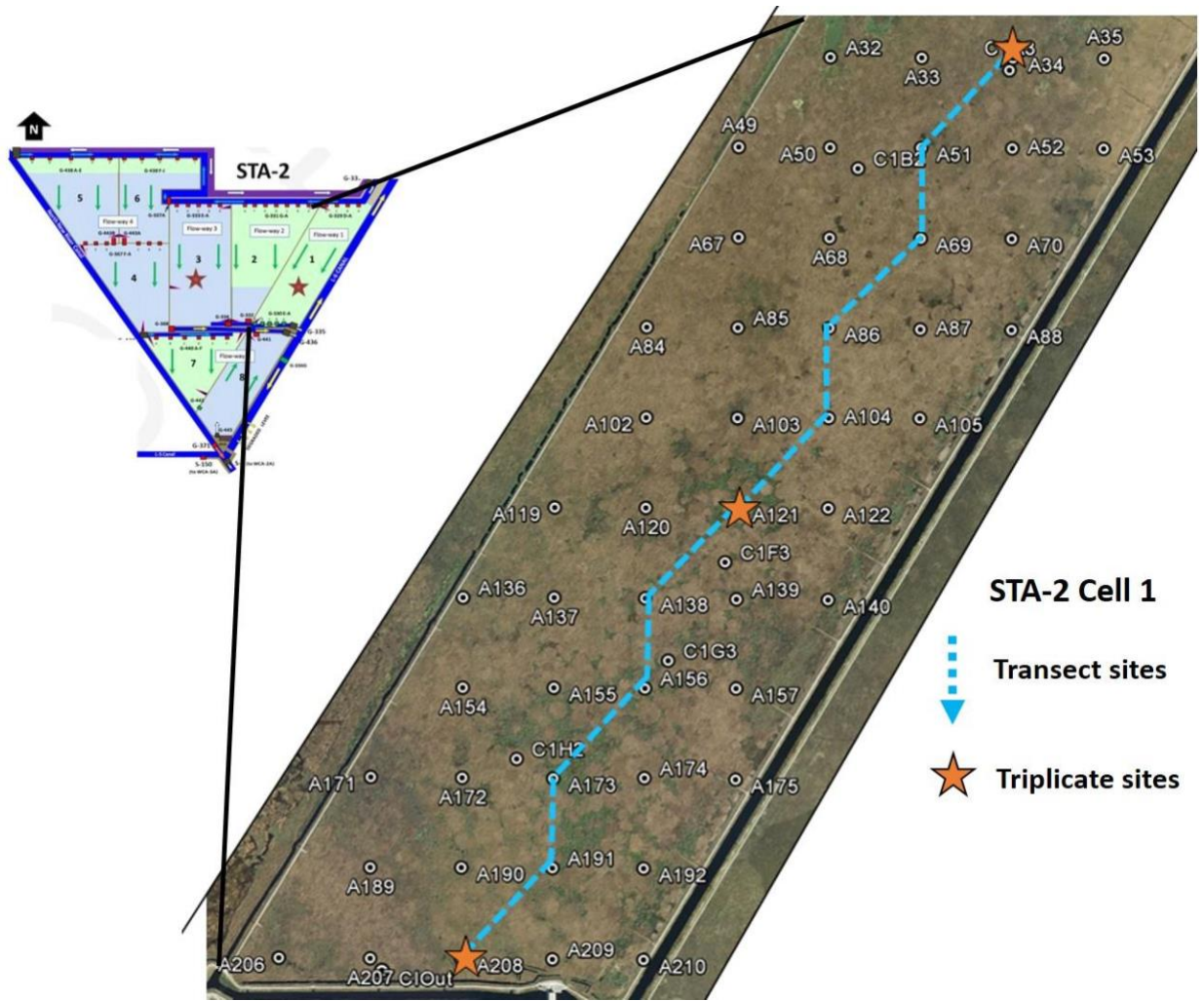


Figure 3. STA 2 Cell 1 – Soil and surface water sampling locations.

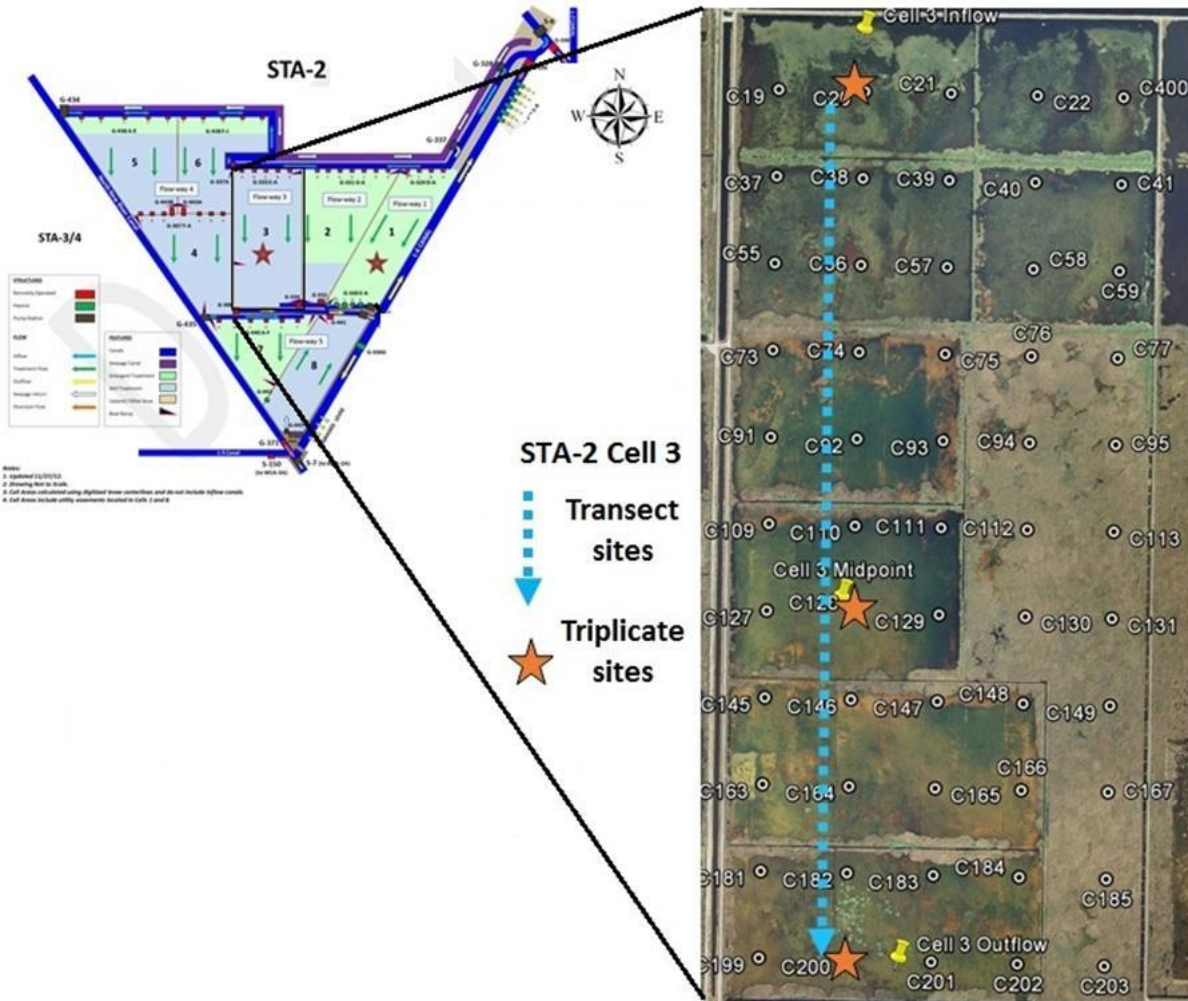


Figure 4. STA 2 Cell 3 – Soil and surface water sampling locations.

Both STA 2 Cell 1 and Cell 3 are operated in a parallel configuration, so both cells receive similar inflow water, so this configuration is conducive to studying the effects of differing vegetation communities.

3.2 Soil Sampling

Intact soil cores (n=18) were taken from STA 2 Cell 1 and Cell 3 in September of 2016. Triplicate samples were taken from three benchmark sites along a transect at set distances from the surface water inflow (inflow, midflow, and outflow) of each cell. Soil samples were collected

using a tube (10.2 cm internal diameter; and 0.2 cm thickness). While in the field, soil cores were transferred into clear cellulose acetyl butyrate tubes for containment and transported to the laboratory on ice for storage at 4°C. Soil core processing required the sectioning of soil cores into four distinct layers – floc, recently accreted soil (RAS), pre-STA 1 soil, and pre-STA 2 soil. Floc depth was measured by allowing flocculent material to settle in a plastic core tube for 3-4 hours. Floc was obtained by pouring off into a bag after recording its depth. The remainder of each soil core were divided up into RAS, pre-STA 1 soil and pre-STA 2 soil. The RAS (consolidated accrued layer) was sectioned off from the pre-STA soil (antecedent). Pre-STA soil was then divided into pre-STA 1 and pre-STA 2, with pre-STA 1 always being the top 5 cm of antecedent soil, and pre-STA 2 being everything below the initial 5 cm. Depth of soil cores ranged from 20.9 – 32.4 cm in EAV and 17.7 – 34.3 cm in SAV, determined by the depth to underlying bedrock.

3.3 Soil and Chemical Analysis

Soil pH was determined using a soil:water ratio of 1:1 and a glass electrode. A subsample of wet soil was dried at 70°C to determine dry weight and moisture content. The bulk density was determined by calculating the dry weight of the sample and dividing it by the volume of the corer. Loss on ignition was determined by ignition of a known amount of oven-dried soil at 550°C, with results expressed on a percentage of oven-dried soil basis. Total carbon (TC) and total nitrogen (TN) were determined on dried, ground samples using a Costech Elemental Analyzer (Model 4010, Costech Analytical Industries, Inc., Valencia, CA), with results expressed on an oven-dried basis. Total P (TP) represents the amount of organic P and inorganic P in soil samples and was determined by a combination of ignition at 550°C and acid digestion to convert P_o into P_i , followed by analysis for P_i by ascorbic acid techniques using an autoanalyzer

(USEPA, 1993; Method 365.1). Total inorganic P in soil was extracted with 1.0 M HCl (soil to solution ratio = 1:50; 3-hour extraction time), and filtered solutions (0.45 μm filter) were analyzed for P using an autoanalyzer (USEPA, 1993; Method 365.1). Extractable metals (Ca, Mg, Fe, and Al) in soils were extracted with 1.0 M HCl (soil to solution ratio = 1:50; 3-hour extraction time) and filtered solutions were analyzed for metals using inductively coupled argon plasma spectrometry (ICAP) (USEPA, 1993; Method 200.7). The mass of total phosphorus in each soil profile (Floc, RAS, pre-STA 1, and pre-STA 2) and as a whole soil was calculated using TP concentrations, bulk density, and soil depth. The amount of phosphorus stored was expressed on a mass per unit area basis. At the 11 transect sites in each cell, water column attributes such as temperature, pH, dissolved oxygen, and specific conductivity were measured using handheld YSI. Water depth was determined using a graduated pole. Water samples were submitted to the District lab for chemical analysis, where the surface water samples were analyzed for TP, total dissolved P, SRP, ammonium, nitrate, dissolved organic nitrogen, TN, dissolved organic carbon, total organic carbon, Ca, Mg, and sulfate.

3.4 Soil Phosphorus Fractionation

The distribution of P in soil was determined using a modified version of a sequential chemical fractionation procedure (Hedley et al., 1982; Ivanoff et al., 1998; Reddy et al., 1998) (Figure 5). This procedure produces P fractions that are operationally defined by the reagent in which the P is released into solution. Total inorganic P was determined by sequential extraction with 0.5 M NaHCO_3 followed by 1.0 M HCl, and summing the two parts. Inorganic P was separated into loosely bound (labile) P_i , Fe/Al bound P, and Ca/Mg bound P. Total organic P was determined by sequential extraction with 0.5 M NaHCO_3 , 1.0 M HCl, and 0.5 M NaOH, as well as a final digest for TP (U.S. EPA, 1993, Method 365.1), and summing the four parts.

Organic P was separated into loosely bound (labile) Po, microbial biomass P (MBP), fulvic acid P, humic acid P, residual P. All fractionations were performed on 136 samples at the selected sampling locations.

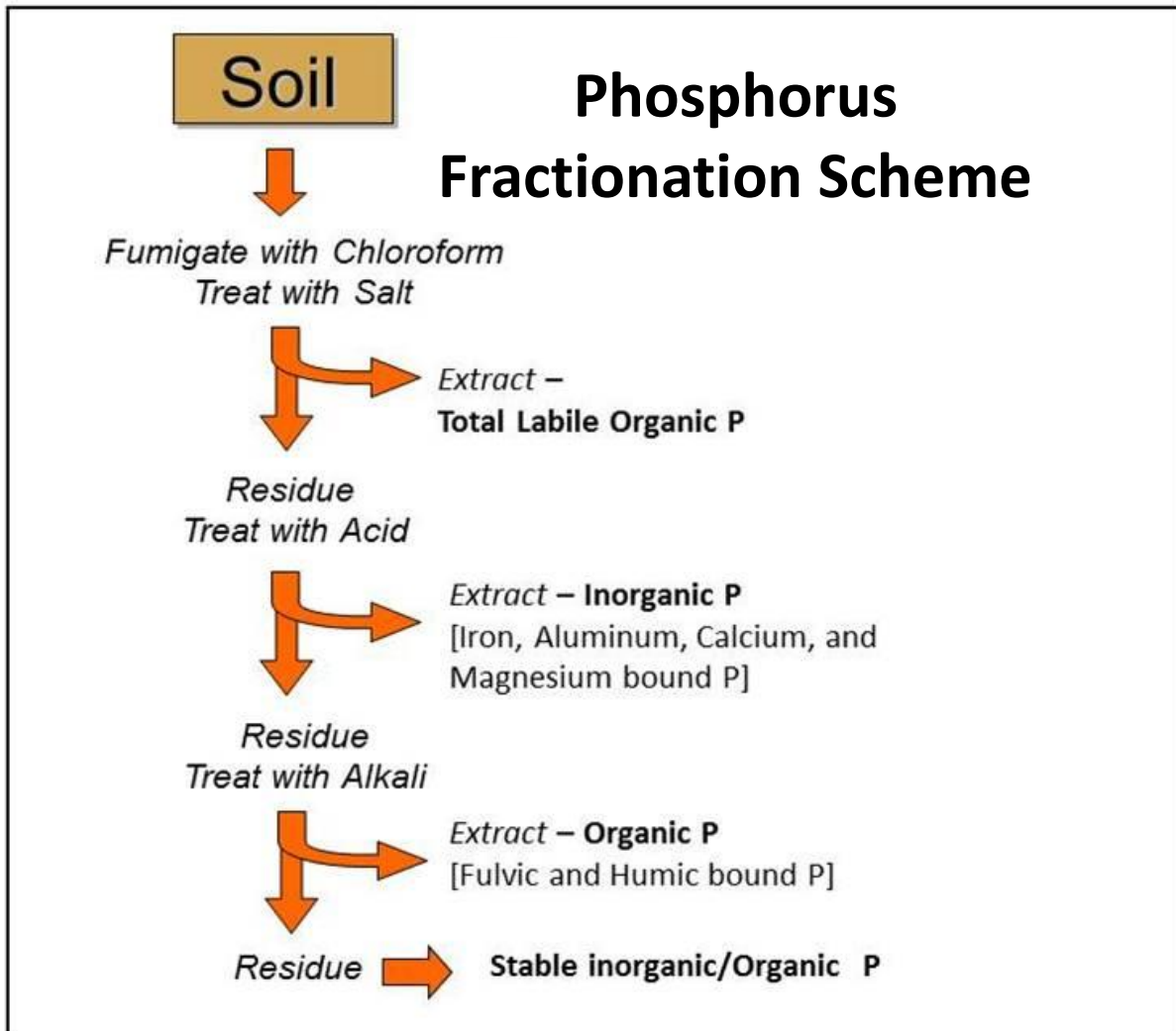


Figure 5. Reddy, K.R., Chua, T., and Richardson, C.J. (2013). Organic Phosphorus Mineralization in Wetland Soils. Chapter 35, page 683-700. In: R.D. DeLaune, K.R. Reddy, C.J. Richardson, and P.J. Megonigal, eds. Methods in Biogeochemistry of Wetlands.

Sodium bicarbonate (0.5 M NaHCO₃) extractable P: Two identical wet samples were weighed out (0.5 g dry-weight equivalent) into 40 mL polycarbonate tubes. One set was subjected to chloroform fumigation for 18 – 24 h to determine MBP. The MBP is assumed to be

the difference between fumigated $\text{NaHCO}_3\text{-TP}$ and nonfumigated $\text{NaHCO}_3\text{-TP}$. After chloroform fumigation, both fumigated and nonfumigated samples were treated with 25 mL of 0.5 M NaHCO_3 (1:50 dry soil-to-solution ratio), buffered to pH 8.5 with NaOH, and shaken for 14 – 18 h on a reciprocating mechanical shaker. After shaking, samples were centrifuged at 6000 rpm for 10 minutes and filtered using a 0.45 μm membrane filter. Filtered solutions were analyzed for both SRP (U.S. EPA, 1993, Method 365.2) and a separate portion extract digested for TP (U.S. EPA, 1993, Method 365.1). These fractions are referred to as $\text{NaHCO}_3\text{-Pi}$ and $\text{NaHCO}_3\text{-TP}$, respectively, while $\text{NaHCO}_3\text{-Po}$ is assumed to be the difference between $\text{NaHCO}_3\text{-TP}$ and $\text{NaHCO}_3\text{-Pi}$. Total labile organic P (TLOP) is defined as the sum of MBP and $\text{NaHCO}_3\text{-Po}$. The fumigated residual soil was used for the following sequential extraction.

Hydrochloric acid (1.0 M HCl) extractable P: The residual soil was then treated with 25 mL of 1.0 M HCl (1:50 dry soil-to-solution ratio) and allowed to equilibrate for a period of 3 h on a mechanical shaker, followed by centrifugation and filtration as described above. The filtered solutions were analyzed for both SRP using a Shimadzu Spectrophotometer UV-1800 (USEPA, 1993, Method 365.1) and TP using inductively coupled plasma mass spectrometry (ICP-MS) (USEPA, 1993; Method 200.7). These fractions are referred to as HCl-Pi and HCl-TP. HCl-Po is assumed to be the difference between HCl-TP and HCl-Pi.

Sodium hydroxide (0.5 M NaOH) extractable P: Residual soil for the above extraction was treated with 25 mL of 0.5 M NaOH (1:50 dry soil-to-solution ratio) and allowed to equilibrate for a period of 14 – 18 h on a mechanical shaker, followed by centrifugation and filtration as described above. Filtered solutions were analyzed for both SRP and a separate aliquot extract is digested for TP (USEPA, 1993, Method 365.1). These fractions are referred to as NaOH-Pi and NaOH-TP, respectively, with NaOH-Pi considered to represent fulvic acid P.

The difference between NaOH-TP and NaOH-Pi was assumed to be organic P (NaOH-Po) associated with humic acids.

Residual P: The residual soil from the above extraction was combusted at 550°C for 4 h. The ash was dissolved in 6.0 M HCl, followed by analysis using a Shimadzu Spectrophotometer (Anderson, 1976; USEPA, 1993, Method 365.1). A similar method was used to analyze total P of the original soil.

3.5 Statistical Analysis

The P fractions from different distances away from inflow were compared within each respective cell and between each vegetation community. A one-way ANOVA test and LSD values at $\alpha=0.05$ were determined using R in RStudio, and used for comparison between distance from inflow, P fractions, vegetation, and metals.

The P fractions within different soil sections were compared between an EAV (Cell 1) and a SAV (Cell 3) cell of STA 2. A one-way ANOVA test and R^2 values at $\alpha=0.05$ were determined using RStudio, and used for comparison between vegetation, metals (Ca, Mg, Fe and Al) and P fractions.

4. Results and Discussion

4.1 Soils

Key soils data is shown in concentrations (Table 1) and storages (Table 2). Total C was significantly affected by vegetation type and distance from inflow, specifically for floc (Table 3). For floc, EAV had 53% higher TC than SAV, but the TC difference between EAV and SAV diminishes further down in the profile. The TC generally increased with depth in the profile, with an average increase of 13% and 59% from floc to pre-STA 2 in EAV and SAV, respectively.

Both floc and RAS showed a significant increase in TC with distance from inflow, with an average of 7% and 10% increase in floc, and 8% and 21% increase in RAS, from inflow to outflow in EAV and SAV, respectively.

STA 2	Sample type	Depth	pH	Bulk Density	P	N	C	S	Ca
Cell 1		(cm)		(g/cm³)	(mg/kg)	(g/kg)	(g/kg)	(g/kg)	(g/kg)
EAV	Floc	4.7 ± 2.4	7.5 ± 0.2	0.04 ± 0.02	1740 ± 499	33.3 ± 3.9	414.8 ± 55.8	11.9 ± 1.9	91.8 ± 52.6
EAV	RAS	3.1 ± 1.4	7.7 ± 0.07	0.08 ± 0.02	1100 ± 193	29.6 ± 3.7	436.2 ± 48.9	13.1 ± 1.1	73.2 ± 35.3
EAV	Pre-STA 1	5 ± 0	7.4 ± 0.2	0.2 ± 0.03	411 ± 72	30.4 ± 1.9	478.6 ± 11.5	13.5 ± 1.1	47 ± 6.9
EAV	Pre-STA 2	13.9 ± 1.2	7.2 ± 0.09	0.2 ± 0.02	234 ± 39	31.7 ± 1.7	500.6 ± 6.5	10.0 ± 1	44.8 ± 3
Cell 3									
SAV	Floc	7.3 ± 1.8	7.6 ± 0.2	0.2 ± 0.04	697 ± 280	11.5 ± 2.7	193.5 ± 31.3	3.8 ± 1.1	288.5 ± 29.3
SAV	RAS	3.4 ± 1.3	7.7 ± 0.06	0.3 ± 0.08	597 ± 204	12.2 ± 3.8	232.2 ± 56.0	4.8 ± 1.3	258.4 ± 39.4
SAV	Pre-STA 1	5 ± 0	7.5 ± 0.07	0.3 ± 0.04	412 ± 69	28.3 ± 1.9	473.5 ± 15.2	9.4 ± 0.6	65.5 ± 12.1
SAV	Pre-STA 2	11.6 ± 3.8	7.2 ± 0.1	0.3 ± 0.05	387 ± 63	29.8 ± 2.5	488 ± 32.9	7.2 ± 1	44.5 ± 6

Table 1. Soil properties from different vegetation communities in STA 2 Cell 1 and Cell 3 (mean ± Std. Error). There are 17 samples from each depth and each vegetation community. Total concentrations of various nutrients are shown. STA = Stormwater treatment area, EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, P = Phosphorus, N = Nitrogen, C = Carbon, S = Sulfur, and Ca = Calcium.

STA 2	Sample type	Depth	P	N	C	S	Ca
Cell 1		(cm)	(g/m²)	(g/m²)	(g/m²)	(g/m²)	(g/m²)
EAV	Floc	4.7 ± 2.4	2.9 ± 1.4	53.1 ± 19.4	654.7 ± 236.4	19.1 ± 7.5	163 ± 118
EAV	RAS	3.1 ± 1.4	2.6 ± 1.8	66.7 ± 34.1	980 ± 516.8	29.5 ± 16.2	208.7 ± 231.2
EAV	Pre-STA 1	5 ± 0	3.2 ± 0.6	242.2 ± 47	3815.2 ± 682.6	106.7 ± 14.7	370.5 ± 62
EAV	Pre-STA 2	13.9 ± 1.2	5.5 ± 1.2	745.7 ± 69.5	11797.5 ± 1119.1	236.4 ± 32.5	1058.8 ± 150.6
Total storage			14.2	1107.7	17247.4	391.7	1801
Cell 3							
SAV	Floc	7.3 ± 1.8	8.2 ± 5.7	124.9 ± 58.2	2144.7 ± 979.5	42.5 ± 22	3198.3 ± 1301.5
SAV	RAS	3.4 ± 1.3	6.7 ± 6.1	102.2 ± 47.9	2022.1 ± 1049.4	41.6 ± 22.8	2593.9 ± 1828.6
SAV	Pre-STA 1	5 ± 0	6.4 ± 1.2	437.9 ± 64.7	7344 ± 1129	144.8 ± 20	1008.5 ± 200.1
SAV	Pre-STA 2	11.6 ± 3.8	14.2 ± 6.7	1048.4 ± 367	17243.7 ± 6226.5	252.7 ± 95.8	1608.9 ± 691.9
Total storage			35.5	1713.4	28754.5	451.6	8409.6

Table 2. Mass storages of key elements in different layers of soil profile within STA 2 Cell 1 and Cell 3 (mean ± Std. Error). EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area, P = Phosphorus, N = Nitrogen, C = Carbon, S = Sulfur, and Ca = Calcium.

Location	Floc TC (mg/kg)			RAS TC (mg/kg)			Pre-STA 1 TC (mg/kg)			Pre-STA 2 TC (mg/kg)		
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05
Inflow	403 a	187 a	*	376 a	196 a	*	485 a	475 a	*	500 NS	507 a	NS
Midflow	421 a	170 b	*	450 b	218 a	*	460 b	484 a	*	497 NS	499 a	NS
Outflow	475 b	251 c	*	434 b	328 b	NS	481 a	455 b	*	496 NS	488 b	NS
Mean	433	203	*	420	247	*	475	472	*	498	498	NS

Table 3. Total carbon (TC) concentrations for various profile depths, vegetation types, and distance from the water inflow point. EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area. Letters in columns indicate a significant difference between transect locations, and asterisks (*) indicate a significant difference between EAV and SAV, for each profile depth interval at $\alpha=0.05$.

Total N was significantly affected by vegetation type and depth in the profile, with distance from inflow playing a minor role (Table 4). For floc, EAV has 65% higher TN than SAV, whereas RAS EAV has 54% higher TN than SAV. SAV TN showed an increase of 60% from floc to pre-STA 2, with EAV TN remaining relatively constant. Both floc and RAS displayed significant increases in TN from inflow to outflow, with a 14% and 31% increase in floc, and 11% and 44% increase in RAS, in EAV and SAV, respectively.

Location	Floc TN (mg/kg)					RAS TN (mg/kg)					Pre-STA 1 TN (mg/kg)					Pre-STA 2 TN (mg/kg)				
	EAV		SAV		P<0.05	EAV		SAV		P<0.05	EAV		SAV		P<0.05	EAV		SAV		P<0.05
Inflow	31	a	11	a	*	25	a	10	a	*	28	a	27	a	NS	30	a	30	ab	NS
Midflow	36	b	10	a	*	29	b	12	a	*	29	ab	30	b	NS	33	b	32	a	NS
Outflow	36	b	16	b	*	28	b	18	b	NS	31	b	26	c	*	31	ab	29	b	*
Mean	34		12		*	28		13		*	29		28		NS	31		30		NS

Table 4. Total nitrogen (TN) concentrations for various profile depths, vegetation types, and distance from the water inflow point. EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area. Letters in columns indicate a significant difference between transect locations, and asterisks (*) indicate a significant difference between EAV and SAV, for each profile depth interval at $\alpha=0.05$.

For a majority of samples, TP had no significant correlation to C:N ratios by soil layer, with only SAV RAS showing a significant positive correlation (Table 5). A negative correlation between TP and C:N ratio by distance from inflow was observed primarily in the EAV cell at all distances, but was only present in outflow samples of the SAV cell (Table 6).

STA 2	C:N
EAV	
Floc TP	ns
RAS TP	ns
Pre-STA 1 TP	ns
Pre-STA 2 TP	ns
SAV	
Floc TP	ns
RAS TP	0.49
Pre-STA 1 TP	ns
Pre-STA 2 TP	ns

Table 5. Pearson correlation coefficients for C:N ratios in STA 2 Cell 1 and Cell 3. All soil layers were divided (floc, RAS, pre-STA 1, and pre-STA 2) and used to generate values ($\alpha=0.05$; ns= not significant). STA = Stormwater treatment area, EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, C = Carbon, N = Nitrogen, and TP = Total Phosphorus (mg/kg).

STA 2	C:N
EAV	
Inflow	-0.93
Midflow	-0.8
Outflow	-0.84
SAV	
Inflow	ns
Midflow	ns
Outflow	-0.76

Table 6. Pearson correlation coefficients for total phosphorus (TP) correlated to C:N ratios in STA 2 Cell 1 and Cell 3. All Distances from inflow (inflow, midflow, and outflow) were divided and used to generate values ($\alpha=0.05$; ns= not significant). STA = Stormwater treatment area, EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, C = Carbon, and N = Nitrogen.

4.2 Total Phosphorus

The TP was significantly affected by vegetation type, depth in the profile, and distance from inflow (Table 7). The EAV- dominated cell had significantly higher TP than the SAV- dominated cell for floc and RAS, but not for pre-STA 1 or pre-STA 2. On average, the EAV had 159% and 94% higher TP than the SAV for floc and RAS, respectively. For pre-STA 2 samples, SAV had 59% significantly higher TP than EAV. The TP concentrations for SAV for all depths varied significantly with distance from the inflow, but only for floc and pre-STA 2 samples in the EAV- dominated cell. For SAV, TP decreased by 52% and 55% from inflow to outflow for floc and RAS, respectively. For pre-STA 1 and pre-STA 2 of SAV, TP increased by 45% and 51% from inflow to outflow, respectively. For EAV, floc TP decreased by 50% from inflow to outflow, while pre-STA 2 TP increased by 45%. The TP concentrations generally decreased with depth in the profile in both dominate vegetation types as well, with an average of 88% and 49% decrease from floc to pre-STA 2 in EAV and SAV, respectively.

Location	Floc TP (mg/kg)					RAS TP (mg/kg)					Pre-STA 1 TP (mg/kg)					Pre-STA 2 TP (mg/kg)				
	EAV		SAV		P<0.05	EAV		SAV		P<0.05	EAV		SAV		P<0.05	EAV		SAV		P<0.05
Inflow	2137	a	1117	a	*	1113	NS	825	a	NS	315	NS	284	a	NS	190	a	274	a	*
Midflow	1861	b	332	b	*	1114	NS	368	b	*	312	NS	381	b	*	167	a	317	ab	*
Outflow	1133	c	535	c	*	814	NS	375	b	*	452	NS	412	b	NS	275	b	415	b	NS
Mean	1710		661		*	1014		523		*	360		359		NS	211		335		*

Table 7. Total phosphorus (TP) concentrations for various profile depths, vegetation types, and distance from the water inflow point. EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area. Letters in columns indicate a significant difference between transect locations, and asterisks (*) indicate a significant difference between EAV and SAV, for each profile depth interval at $\alpha=0.05$.

4.3 Metals

Positive correlations were observed between Ca and Mg and all fractions (Pi, Po, NRP, and TP) in both EAV and SAV cells (Table 8). A negative correlation was shown between N and all fractions in both cells as well. Aluminum had negative correlations with all fractions in EAV, but no significance in SAV. Carbon had negative correlations with all fractions in SAV, but also showed a positive correlation with EAV Pi (Table 8). Fe was not significant for all fractions except SAV NRP, where a negative correlation was observed. Metal concentrations for all depths individually and all distances from inflow are reported below (Table 9). All metals from all depths change significantly from inflow to outflow except Mg in EAV pre-STA 2. Mean Fe concentrations are significantly different between EAV and SAV at all depths. Mean Al, Ca, and Mg concentrations are significantly different between EAV and SAV at all depths except pre-STA 2.

STA 2	Ca	Mg	Fe	Al	N	C
EAV (count = 68)						
Inorganic P (Pi)	0.45	0.35	ns	-0.42	-0.54	0.24
Organic P (Po)	0.44	0.36	ns	-0.41	-0.59	ns
Non-reactive P (NRP)	0.54	0.53	ns	-0.26	-0.63	ns
Total P (TP)	0.46	0.37	ns	-0.42	-0.58	ns
SAV (count = 68)						
Inorganic P (Pi)	0.55	0.6	ns	ns	-0.59	-0.6
Organic P (Po)	0.4	0.45	ns	ns	-0.41	-0.37
Non-reactive P (NRP)	0.73	0.79	-0.31	ns	-0.77	-0.77
Total P (TP)	0.55	0.61	ns	ns	-0.58	-0.58

Table 8. Pearson correlation coefficients for key parameters in STA 2 Cell 1 and Cell 3. Data from all soil layers (floc, RAS, pre-STA 1, and pre-STA 2) was used to generate values ($\alpha=0.05$; ns= not significant). STA = Stormwater treatment area, EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, P = Phosphorus, Ca = Calcium (g/kg), Mg = Magnesium (mg/kg), Fe = Iron (mg/kg), Al = Aluminum (mg/kg), N = Nitrogen (g/kg), and C = Carbon (g/kg).

A.

Location	Floc Fe (mg/kg)			RAS Fe (mg/kg)			Pre-STA 1 Fe (mg/kg)			Pre-STA 2 Fe (mg/kg)										
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05								
Inflow	3039	a	2229	a	*	2957	a	1888	a	*	1786	a	2110	a	*	1309	a	1992	a	*
Midflow	744	b	268	b	*	1058	b	856	b	*	2182	b	2717	b	*	1642	b	2647	b	*
Outflow	623	c	908	c	*	1005	b	1785	c	*	2491	c	3040	c	*	2747	c	3702	c	*
Mean	1469		1135		*	1673		1510		*	2153		2622		*	1899		2780		*

B.

Location	Floc Al (mg/kg)			RAS Al (mg/kg)			Pre-STA 1 Al (mg/kg)			Pre-STA 2 Al (mg/kg)										
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05								
Inflow	2524	a	1929	a	*	2232	a	1675	a	*	1552	a	1822	a	*	1759	a	1760	a	NS
Midflow	352	b	201	b	*	380	b	565	b	*	1503	a	1966	b	*	2036	b	2028	b	NS
Outflow	499	c	606	c	*	722	c	1316	c	*	2638	b	2211	c	*	2575	c	2503	c	NS
Mean	1125		912		*	1111		1185		NS	1898		2000		*	2123		2097		NS

C.

Location	Floc Ca (mg/kg)			RAS Ca (mg/kg)			Pre-STA 1 Ca (mg/kg)			Pre-STA 2 Ca (mg/kg)										
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05								
Inflow	87	a	280	a	*	127	a	278	a	*	52	a	65	a	*	47	NS	40	a	NS
Midflow	89	a	314	b	*	75	b	281	a	*	52	a	56	b	NS	42	NS	49	b	NS
Outflow	36	b	240	c	*	31	c	193	b	*	42	b	77	c	*	47	NS	52	b	NS
Mean	71		278		*	78		250		*	49		66		*	45		47		NS

D.

Location	Floc Mg (mg/kg)			RAS Mg (mg/kg)			Pre-STA 1 Mg (mg/kg)			Pre-STA 2 Mg (mg/kg)										
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05								
Inflow	7920	a	10065	a	*	6745	a	9145	a	*	5460	a	5103	a	*	5158	NS	4717	a	*
Midflow	4841	b	9161	b	*	3826	b	7936	b	*	4377	b	4392	b	NS	4998	NS	4333	b	*
Outflow	3870	c	8008	c	*	3709	b	7464	b	*	4689	c	6114	c	*	5075	NS	5960	c	*
Mean	5544		9078		*	4760		8182		*	4842		5203		*	5077		5003		NS

Table 9. Metal concentrations for various profile depths, vegetation types, and distance from the water inflow point. A, B, C, and D show iron (Fe), aluminum (Al), calcium (Ca), and magnesium (Mg) concentrations, respectively. EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area. Letters in columns indicate a significant difference between transect locations, and asterisks (*) indicate a significant difference between EAV and SAV, for each profile depth interval at $\alpha=0.05$.

Positive correlations were also found between EAV pre-STA 2 and both Ca:Mg and Fe:Al ratios (Table 10). This could represent a relationship that was important before STA 2 was built, as only the deepest soil layer shows significance. Negative correlations were observed between SAV floc and Fe:Al ratios, as well as SAV RAS and Fe:Al ratios. Due to the relationship being present only in the top two soil layers, it could be an indication that this correlation started occurring after the establishment of STA 2. In the EAV cell, both inflow and midflow samples displayed a positive correlation to Fe:Al ratios, while only midflow samples correlated to Ca:Mg ratios (Table 11). In the SAV cell, inflow samples showed a positive correlation to Ca:Mg ratios, while outflow samples were positively correlated to Fe:Al ratios. This could potentially be a result of the SAV cell biogeochemically transforming P forms and consequently changing the related metal ratio from Ca:Mg to Fe:Al.

STA 2	Ca:Mg				Fe:Al			
	Floc	RAS	Pre-STA 1	Pre-STA 2	Floc	RAS	Pre-STA 1	Pre-STA 2
EAV (count = 17)								
Total P (TP)	ns	ns	ns	0.57	ns	ns	ns	0.78
SAV (count = 17)								
Total P (TP)	ns	ns	ns	ns	-0.53	-0.72	ns	ns

Table 10. Pearson correlation coefficients for key metal ratios in STA 2 Cell 1 and Cell 3. Data was divided by soil layer (Floc, RAS, pre-STA 1, and pre-STA 2) and used to generate values ($\alpha=0.05$; ns= not significant). STA = Stormwater treatment area, EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, and P = Phosphorus. Ca:Mg = Calcium : Magnesium and Fe:Al = Iron : Aluminum, unit less ratios.

STA 2	Ca:Mg	Fe:Al
EAV		
Inflow	ns	0.58
Midflow	0.78	0.6
Outflow	ns	ns
SAV		
Inflow	0.9	ns
Midflow	ns	ns
Outflow	ns	0.72

Table 11. Pearson correlation coefficients for key metal ratios in STA 2 Cell 1 and Cell 3. Data was divided by distance from inflow (inflow, midflow, and outflow) and used to generate values ($\alpha=0.05$; ns= not significant). STA = Stormwater treatment area, EAV = Emergent aquatic vegetation, and SAV = Submerged aquatic vegetation. Ca:Mg = Calcium : Magnesium and Fe:Al = Iron : Aluminum, unit less ratios.

4.4 Phosphorus Fractions

Total inorganic P was significantly affected by vegetation type and distance from inflow (Table 12). All soil layers except for RAS showed a significant difference between EAV and SAV, with EAV having 22% more Pi than SAV in floc, but SAV having 47% and 75% more Pi than EAV in pre-STA 1 and pre-STA 2, respectively. Both floc and RAS displayed significantly

decreases in Pi with distance from inflow, with a 34% and 65% loss in floc, and 50% and 65% loss in RAS, in EAV and SAV, respectively. It is important to note that floc and RAS are decreasing with distance from inflow, but pre-STA 1 and pre-STA 2 seem to be increasing. Total Pi generally decreased with depth in the profile, with an average decrease of 93% and 66% from floc to pre-STA 2 in EAV and SAV, respectively. Total Pi typically represents about 24% of TP in the EAV- dominated cell but represented around 47% of TP in SAV-dominated cell averaged across all depths and distances from inflow. Averaged across all sites the Pi generally represented 32% of TP.

Location	Floc Pi (mg/kg)			RAS Pi (mg/kg)			Pre-STA 1 Pi (mg/kg)			Pre-STA 2 Pi (mg/kg)										
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05								
Inflow	514	a	685	a	*	313	a	544	a	*	55	NS	119	a	*	24	a	122	a	*
Midflow	517	a	140	b	*	275	a	152	b	*	57	NS	62	b	NS	35	b	81	b	*
Outflow	339	b	239	c	NS	157	b	188	b	NS	65	NS	153	a	*	32	b	164	c	*
Mean	456		355		*	249		295		NS	59		111		*	31		122		*

Table 12. Total inorganic phosphorus (Pi) concentrations for various profile depths, vegetation types, and distance from the water inflow point. EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area. Letters in columns indicate a significant difference between transect locations, and asterisks (*) indicate a significant difference between EAV and SAV, for each profile depth interval at $\alpha=0.05$.

Total organic P was significantly affected by vegetation type and distance from inflow, with drastic differences displayed in floc and RAS (Table 13). In floc, RAS, and pre-STA 1, EAV had 76%, 70%, and 18% higher Po than SAV, respectively. All depths displayed a significant difference with distance from inflow, with an average decrease of 51% and 31% for floc, and 18% and 33% in RAS, but with an average increase of 48% and 57% in pre-STA 1, and 46% and 65% in pre-STA 2, in EAV and SAV, respectively. Total Po generally decreased with depth in the profile, with an average decrease of 86% and 31% from floc to pre-STA 2 in EAV

and SAV, respectively. Total Po typically represented less than 76% of TP in the EAV-dominated cell but represented about 53% of TP in the SAV-dominated cell averaged across all depths and distances from inflow. Averaged across all sites, Po represented less than 68% of TP.

Location	Floc Po (mg/kg)			RAS Po (mg/kg)			Pre-STA 1 Po (mg/kg)			Pre-STA 2 Po (mg/kg)		
	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05	EAV	SAV	P<0.05
Inflow	1623 a	432 a	*	800 a	280 a	*	261 a	165 a	*	166 a	152 a	NS
Midflow	1344 b	192 b	*	838 a	216 b	*	255 a	319 b	*	132 a	236 b	*
Outflow	794 c	296 c	*	657 b	187 b	*	386 b	259 c	*	243 b	251 b	NS
Mean	1254	307	*	765	228	*	301	247	*	180	213	NS

Table 13. Total organic phosphorus (Po) concentrations for various profile depths, vegetation types, and distance from the water inflow point. EAV = Emergent aquatic vegetation, SAV = Submerged aquatic vegetation, RAS = Recently accreted soil, STA = Stormwater treatment area. Letters in columns indicate a significant difference between transect locations, and asterisks (*) indicate a significant difference between EAV and SAV, for each profile depth interval at $\alpha=0.05$.

5. Conclusions

The SAV cell has exhibited more inorganic P enrichment in floc and soils than organic P, while the EAV cell has exhibited relatively more organic P enrichment than inorganic P in the same soil layers. Phosphorus enrichment in SAV is primarily driven by a combination of biotic and abiotic factors. High calcium and magnesium activity in this system could account for the increased inorganic P enrichment. Phosphorus enrichment in EAV is primarily driven by litter production and accretion of organic matter, facilitating inflow particulate P to be incorporated into the soil.

As P moves through the SAV cell and becomes transformed, a strong positive correlation with Fe:Al is evident due to more inorganic P enrichment, while the EAV cell shows little correlation with metals. This could indicate that in the SAV cell, phosphorus has been

biogeochemically transformed and consequently changed the related metal ratio from Ca:Mg to Fe:Al, as the P moved through the cell. This could also be evidence of SAV altering the physicochemical environment of the water column, either by reducing the pH or increasing the redox potential, thereby changing the precipitation agent from calcium to iron. Further investigation into surface water quality, and its ties to soil chemistry in this system, would be a logical next step. This study could help illustrate the complex interactions between water column P and soil P transformations.

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