University of Florida College of Agriculture and Life Sciences Honors Thesis

Using Properties of Dissolved Organic Matter to Trace Phosphorus Sources, Flows and Transformations in the Everglades Stormwater Treatment Areas

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Introduction:

The Florida Everglades is an interconnected system of wetlands whose nutrient cycles support a highly diverse community including many endangered and threatened species (Mitsch et al., 2014; Guardo et al., 1995). The Everglades wetland system also aids in the provision of clean water, recreation, enhances the commercial fishing industry and has benefits such as carbon sequestration (Richardson et al., 2014). Stormwater runoff rich in nutrients from the Everglades Agricultural Area (EAA) and Lake Okeechobee watershed poses a threat to the unique habitat through alterations of the natural nutrient profiles (Mitsch et al., 2014). This flow of stormwater is mainly controlled by a system of canal implemented by the South Florida Water Management District (Guardo et al., 1995). Prior to the intensive agricultural development in the EAA north of the Everglades, the system traditionally had oligotrophic conditions, partial to a community with dense sawgrass (Mitsch et al., 2014; Guardo et al., 1995).

The ability of natural wetlands to reduce nutrient loads without costly treatment facilities led to the construction of manmade wetland systems for the inflow of nutrients to the Everglades system from the EAA and Okeechobee watershed (Pietro and Ivanoff 2015; Kadlec, 2016). These constructed wetlands are referred to as Stormwater Treatment Areas or STAs (Pietro and Ivanoff, 2015), and are used primarily to reduce phosphorus (P) loads, through storage, transformation, sorption and precipitation with calcium carbonate (Pietro and Ivanoff 2015; Guardo et al., 1995). As of 2014, there were six STAs in The Everglades used for this purpose, with government regulations limiting the total P (TP) contained in outflow to approximately 10 parts per billion (ppb) (Mitsch et al., 2014).

While there are numerous instances of constructed wetlands and STAs reducing the nutrient loads associated with inflows, the goal of 10 ppb reliably over long periods of time is not easily achieved (Mitsch et al., 2014). There are various means by which P is removed, including accumulation in floc and litter layers, biological utilization, and bio-accretion (Kadlec, 2016). However, based on the biota involved in some P cycling mechanisms, namely the vegetation which takes up P and incorporates it into biomass, some of the P in the outflow may have originated within the system itself through plant decomposition (Chan et al., 2015). In order for a system to remain effective it must be maintained in such a way that peat accumulation, and the build-up of decaying biota present in the system do not contribute to higher TP levels in the water, as this results in lower removal efficiency (Kadlec, 2016). The sediments which accumulate in the system increase the P found in floc and soil, which, in turn, can be released to the water when the concentration of P in the water is reduced so that it is below that of the soil sediments present (Diaz et al., 2006).

Outflow P is composed of more organic than inorganic P, which may be the result of transformations to soluble reactive phosphorus (SRP) through biomass uptake or simply because it has not yet been removed by the associated processes (Dierberg & Dusk, 2008). SRP is generally the first form of P to be assimilated into by biomass, while dissolved organic P (DOP) is generally a long-term source of P in the system (Dierberg & Dusk, 2008). Similarly, variations in vegetation types, such as submerged aquatic vegetation (SAV) or emergent aquatic vegetation (EAV) may also result in differences in DOP due to light availability and differences in plant type and composition (Dierberg & Dusk, 2008). These two factors combined may be able to determine the potential sources of P in outflow water which may be tracked through the system (Larsen et al., 2009).

Along with P removal, other functions and parameters such as dissolved organic matter (DOM), and dissolved organic carbon (DOC) or total dissolved nitrogen (TDN) can be indicators of transformations of P and organic matter (OM). DOM is produced through the decomposition of organic material, including roots, leaf litter, microbes and other living sources in the system, and includes DOC, dissolved organic nitrogen (DON), as well as DOP and other components (ElBislawi and Jaffe 2015). DOC specifically, is frequently produced during the treatment process of constructed wetlands as nutrients fuel plant growth and development which ultimately leads to decay, again reintroducing C and nutrients back into the system (Villa et al., 2014). Starting in 1934 with Redfield, it was determined that species have a given C:N:P ratio which can be used to determine the levels of decomposition of DOM (Sardans, Rivas-Ubach & Penuelas 2012; Redfield, 1934). DOM can be tracked through the system because there are structural differences in various organic molecules, which absorb and reflect light in different, measurable ways (Weishaar et al., 2003).

Additionally, DOM can be used to determine flow patterns and concentrations at locations further along the system flow path (Noe et al., 2007). Determining the composition of DOM can reveal potential sources of the material, and how it is able to interact in the environment based on the concentration of bioavailable forms and decomposition stages (Hansen et al., 2016). Using known optical properties of DOM and thus through determining the levels of specific ultraviolet absorbance and various wavelengths DOM can be traced through systems (Hansen et al., 2016). The optical properties determined from spectral analysis can be used to establish relationships between DOM and nutrient content as well as bioavailability of specific compounds (Stedmon et al. 2003).

Relating DOM to the P concentrations in oligotrophic environments such as the natural Everglades, is key to understanding vital biogeochemcial transformations which occur. Utilizing the information from UV absorbance and optical properties, and C:N:P ratios, this study will explore the differences between EAV and SAV constructed wetlands through the treatment process to determine the sources and transformations of P in the Everglades STAs. Light availability between EAV and SAV systems vary, which influences the processes which occur, the microbes present and the hydrolysis or photolysis of P compounds (Moore et al., 2010). EAV dominated wetlands, have less light availability than SAV systems, which may impact the DOM and nutrient ratios present in each system (Moore et al., 2010). Analyses of the DOM components in the STA systems will potentially lead to a better understanding of how to reliably reduce outflow P concentrations to ensure they are below the current guidelines. It is expected that the optical properties of DOM and the DOM nutrient ratio stoichiometry will demonstrate a decrease in bioavailable P from inflow to outflow and an increase in organic P which is less bioavailable.

Methods:

Water Sampling and Processing

Surface water samples were obtained from STA Cells 1 and 3 on October 11, 2017. STA Cell 1 contains predominantly EAV, while Cell 3 is comprised predominantly of SAV, the locations of these cells can be seen in Images 1 and 2 below which describes where the samples were collected from. The samples obtained from both cells were collected at three different points, the inflow, mid-flow and outflow sections of the associated cells, this is outlined below in Table 1. Inflow points, represent areas where stormwater is flowing into the cells, the mid-flow points are located at approximately the midpoint of the flowpath, and the outflow reflects the location where water exits the cell. The pH of the water samples was recorded, and the samples were stored in refrigerated conditions of approximately 2-5^{o} C until being processed for nutrient analysis which occurred in stages over a 6 week period.

Table 1 Descriptions of the sample site identification including STA cell, which is described in the images 1 and 2. The site ID which includes the position of the sample site along the flowpath, as well as the sample name as referenced for the completion of this research are also determined.

Sampling Date	STA Cell	Site ID	Sample Name
10/10/2017	1	Inflow-C1	17-1347
10/10/2017	1	Mid-flow-C1	17-1348
10/10/2017	1	Outflow-C1	17-1349
10/10/2017	3	Inflow-C3	17-1350
10/10/2017	3	Mid-Flow-C3	17-1351
10/10/2017	3	Outflow-C3	17-1352

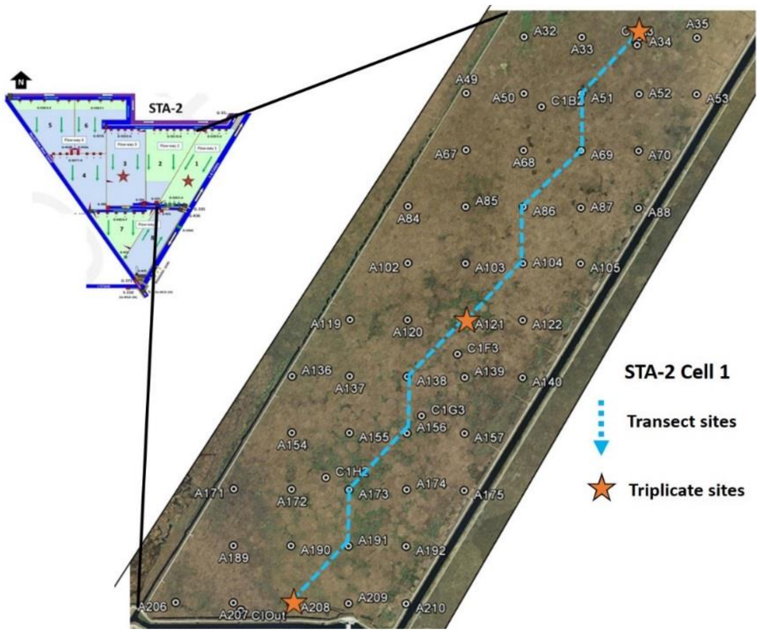


Image 1 This image reflects the location and orientation of STA 2-Cell 1 from which three water samples were obtained. The stars indicate the sites from which water samples were taken and the blue indicates the location of the transect within the STA cell.

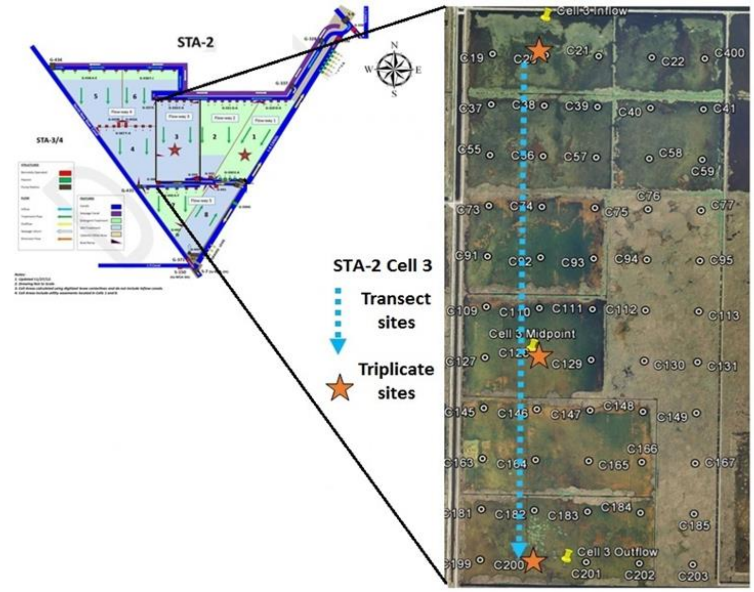


Image 2 This image reflects the location and orientation of STA 2-Cell 3 from which three water samples were obtained. The stars indicate the sites from which water samples were taken and the blue indicates the location of the transect within the STA cell.

Nutrient Analysis

The samples were analyzed for their concentrations of TP, total organic carbon (TOC), total nitrogen (TN) as well as SRP. Samples were filtered through a 0.2 µm membrane filter to ensure the nutrients present were within the dissolved size fraction (Larsen et al., 2010; Twardoswski et al., 2004).

TP was measured using a persulfate block digestion, while SRP was measured using the ascorbic acid method and color reagents (Pant et al, 2002; USEPA 1993). TOC and TN were determined using color reagents and absorbance measurements as were the SRP analyses (USEPA 1993). Dissolved organic phosphorus, (DOP), was then calculated by subtracting SRP from TP. Due to the filter used, the TOC, and TN represent the dissolved portions of C and N, respectively, and are shown as dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). These values were then used to determine the C:N:P ratios of each of the samples being analyzed.

DOM Spectral Analysis

The DOM spectral analysis was conducted using a Shimadzu UV-1800 Spectropotometer using standard preparations (Hansen et al., 2016; Helms et al., 2008; Yamin et al., 2017 & Li & Hur, 2017). Hansen (2016) outlines specific wavelengths which should be analyzed. The absorption coefficient (a) was calculated as: a=2.303A/l, where A = absorbance, and l = path length in meters (Helms et al., 2008). The path length in this case is 2 cm, or 0.02 m. The specific absorbance, or SUVA was then calculated for each wavelength using the following calculation; L*mg C^{-1} * m^{-1} = (a)/(TOC concentration in mg/L) (Helms et al., 2008). Specifically, $SUVA_{254}$ is frequently associated with aromaticity of carbon compounds (Weishaar 2003) and values for the samples are provided in Table 2.

Table 2 Basic absorbance data which was calculated using the formulas described in the text above in the process of initial data processes for the DOM sample analysis

Sample	Absorption Coefficient (m^{-1})	$SUVA_{254}$ (L*mg-C^{-1}*m^{-1})
EAV cell 1 inflow	399.34	10.75
EAV cell 1 mid-flow	342.57	9.33
EAV cell 1 outflow	374.81	12.5
SAV cell 1 inflow	354.20	9.36
SAV cell 1 mid-flow	359.95	9.87
SAV cell 1 outflow	375.38	9.95

Spectral slopes ($S_{275-295}$, $S_{350-400}$) were also calculated for wavelengths between 275-295 nm, and 350-400nm (Helms et al., 2008). Before the spectral slope can be determined, the log-transformed absorption coefficients must be calculated, then the slope of the linearly fitting formula may be determined. These values were then used to create spectral slope ratios as $S_{275-295}$ to $S_{350-400}$ (S_R) (Helms et al., 2008; Sihi et al., 2016) as shown in Table 3.

Table 3 The values of spectral slope and slope ratios calculated using the formulas provided by (Helms et al., 2008).

Sample	$S_{275\text{-}295}$	$S_{350\text{-}400}$	S_R
EAV cell 1 inflow	0.02423	0.01971	1.22923
EAV cell 1 midflow	0.02163	0.01893	1.142599
EAV cell 1 outflow	0.0227	0.02011	1.128903
SAV cell 3 inflow	0.01789	0.0195	0.917391
SAV cell 3 midflow	0.02484	0.0195	1.273599
SAV cell 3 outflow	0.02021	0.01894	1.067159

Results and Discussion:

DOP is the portion of dissolved P exclusive of SRP, which is readily assimilated by various species of plants and algae. Understanding how DOP is transformed and how it moves through STA systems is key to understanding how to better reduce the P present in outflow waters. One way to do so, is to track DOM and compare these values to the nutrient ratios at different points along the flow paths of the STA.

$SUVA_{254}$

$SUVA_{254}$ is used to determine the DOM aromatic content as well as the estimated molecular weight of the DOM and is therefore commonly used to determine the properties of DOM (Hansen et al., 2016; Helms et al., 2008; Weishaar et al., 2003). As $SUVA_{254}$ increases, the aromatic content of the carbon molecules increases and therefore the reactivity of the molecule decreases (Helms et al., 2008; Larson et al., 2009). Microorganisms utilize non-aromatic carbon first, and often transform non-aromatic forms of carbon into aromatic forms as part of their metabolic processes (Hur et al., 2011).

The $SUVA_{254}$ values for cell 1, which is dominated by EAV initially decreased between inflow and midflow, then increased between midflow to outflow regions. However, for cell 3 which contains mostly SAV, the $SUVA_{254}$ values changed minimally along the flowpath as described in Images 1 and 2. As organic carbon compounds are decomposed and broken down the recalcitrance increases, which then increases the stability of these residual molecules (Hur et al., 2011). Therefore, since the $SUVA_{254}$ values are increasing across the flowpath, meaning outflow values were higher than inflow, it likely indicates that decomposition is occurring along the flowpath, as well. Since SAV cells showed a smaller increase in SUVA values, there is potentially more OM being put into the system which reduces the overall concentration of recalcitrant or aromatic C. This could be further supported with information from the nutrient concentrations present in each of the two cells. Specifically, the ratios of C:N:P, as well as the concentrations of each of these nutrients which can be used to further evaluate the differences between the cells based on the concentration of recalcitrant materials present in EAV and SAV.

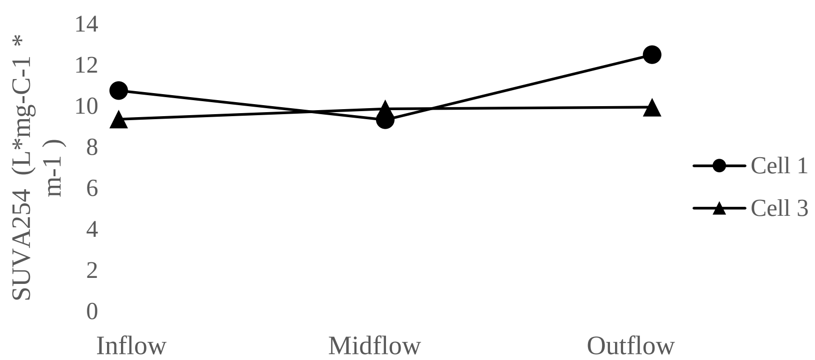


Figure 1 Differences in SUVA values between EAV in Cell 1 and SAV in Cell2, across the transect at three points; inflow, midflow and outflow.

Spectral Slope

Similar to the $SUVA_{254}$ values, spectral slope is commonly used to determine properties of DOM as it changes depending on the molecular weight characteristics of the organic matter (Roccaro et al., 2015; Helms et al., 2008). Specifically, spectral slope examines the effects of level of humification or potential ages of the DOM (Hansen et al., 2016). Spectral slope values are different across various sample types, since the starkest differences occur between 275-295 nm wavelengths and 350-400 nm wavelengths it is common to determine slope, and slope ratios between these wavelengths (Helms et al., 2008). Slopes in these ranges generally have a more negative relationship or slope with increasing molecular weight of the DOM in the sample

(Roccaro et al., 2015). According to Hur (2011), this indicates that as slopes decrease, the DOM contains a higher concentration of higher molecular weight or more humified, and older molecules.

The log-transformed absorbance coefficients have variations present in the slope values between wavelengths of 275-295nm, and between 350-400nm as seen in Figure 2. The spectral slope values at those wavelengths are generally used to determine differences in the molecular weights of the DOM which can be indicators of photobleaching and the breakdown of OM (Helms et al. 2008).

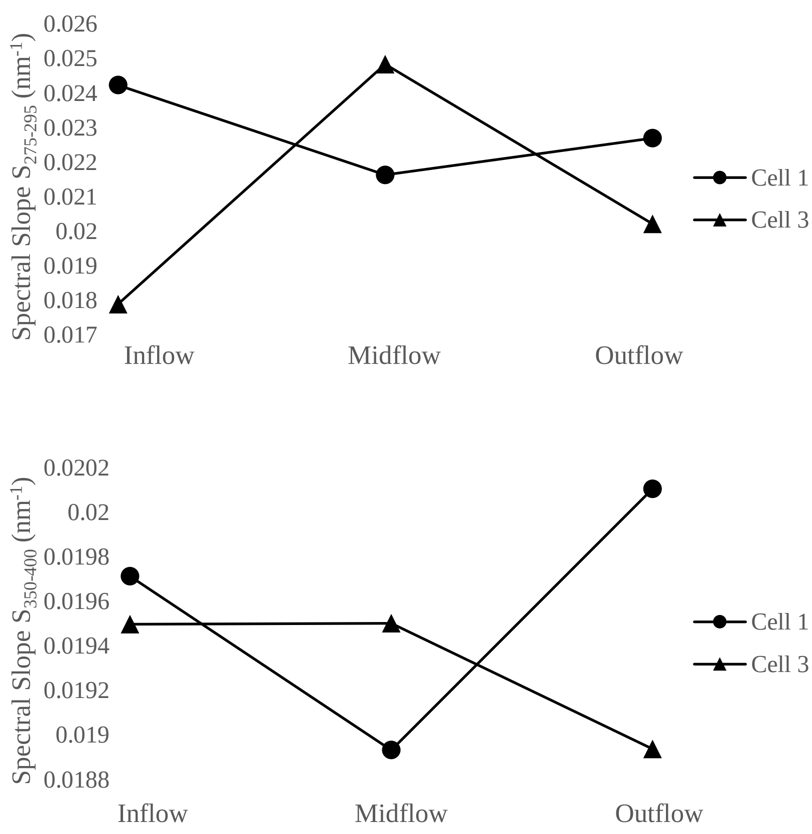


Figure 2 The spectral slope values found at each site, across the flow path for 275-295 nm wavelengths (top) as well as 350-400 nm wavelengths (bottom).

Higher spectral slopes between 275 and 295 nm signal the effects of photobleaching, which results in low molecular weight molecules (Helms et al., 2008). In cell 1, with EAV species, there is a decrease in slope between inflow and midflow as well as a slight increase between midflow and outflow. However, there is relatively little change here. On the contrary, higher spectral slopes between 350 and 400 nm indicate humification (Helms et al., 2008). Cell 1 shows a steep decline in slope between inflow and midflow and steep increase from midflow to outflow. These points suggest that there is more humification in cell 1 than photobleaching and light degradation. Since the cell has emergent vegetation with little light penetration, this determination is representative.

However, the opposite is shown in cell 3 with SAV species. In regard to wavelengths between 275 and 295 nm, there is a steep increase in slope indicating high photobleaching between inflow and midflow and a smaller decrease between midflow and outflow. Additionally, within the wavelengths between 350-400 nm, there is a relatively little change between inflow and midflow and a small decrease from midflow to outflow. This indicates that there is more photobleached material in cell 3 which considering the high light conditions is representative as cell 1.

Spectral Slope Ratios

In addition to the calculation of the spectral slopes, the ratio between $S_{275\text{-}295}$ and $S_{350\text{-}400}$ was calculated as it is relevant in determining the level of humification in the samples, based on molecular weight (Helms et al., 2008; Chow et al., 2013; Twardowski et al., 2004). In cell 1, the spectral slope ratio of DOM decreased between all transect points, however, the decline was minimal between midflow and outflow as shown in figure 3. In contrast, for cell 3, there was an increase between inflow and midflow followed by a decrease between midflow and outflow. This was similar to the previous DOM measures regarding the molecular weight of the carbon molecules present.

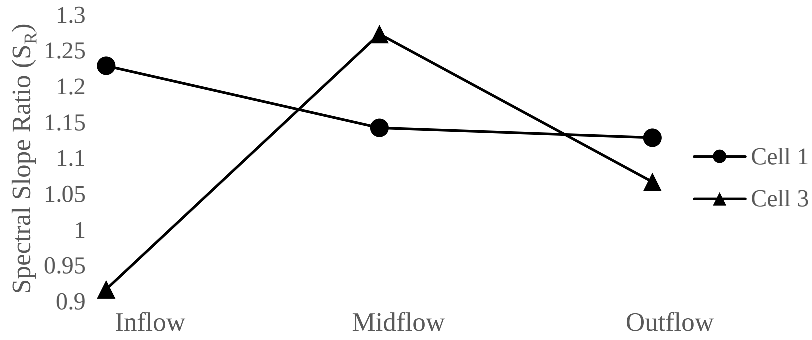


Figure 3 Variations in spectral slope ratio between the two cells over the three sites, inflow, midflow and outflow

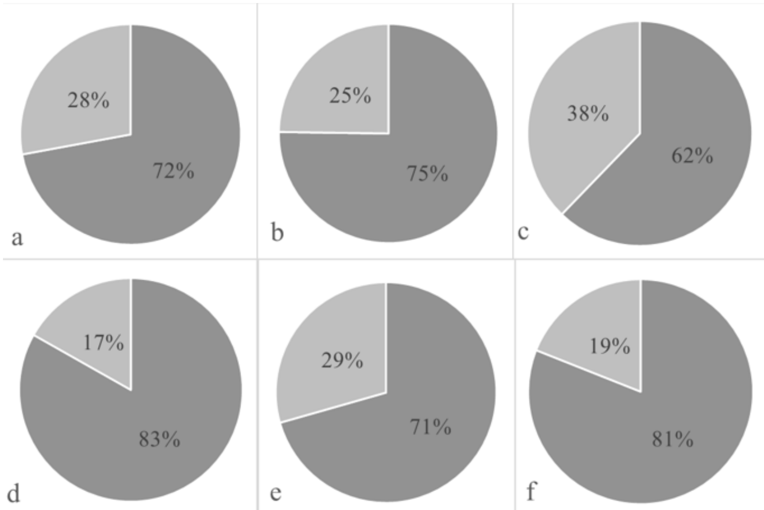
Stoichiometry

Nutrient ratios can also be used to determine the source of DOM (Chow et al., 2013). When comparing the concentration of DOC, TDN and DOP over the inflow, midflow and outflow regions, the SAV cell remained relatively constant or even increased in nutrient concentrations, while the EAV cell showed a more typical constructed wetland pattern of decrease in concentration of nutrients as the water progressed along the cell flowpath seen in Table 4.

Table 4 The nutrient concentrations present in the DOM as measured in order to determine the nutrient ratios present in the DOM. DOC, TDN, TP and SRP were measured and DOP was calculated by subtracting SRP from TP. This was calculated for each of the sample sites.

Nutrient	Concentration (mg/L)					
	EAV			SAV		
	inflow	midflow	outflow	inflow	midflow	outflow
Dissolved Organic Carbon DOC	37.14	36.7	29.92	37.83	36.45	37.69
Total Dissolved Nitrogen TDN	2.408	1.974	1.803	3.318	2.79	2.812
Total Dissolved Phosphorus TDP	0.129	0.117	0.045	0.119	0.136	0.137
Soluble Reactive Phosphorus SRP	0.093	0.088	0.028	0.099	0.096	0.111
Dissolved Organic Phosphorus DOP	0.036	0.029	0.017	0.02	0.04	0.026

While these values indicate changes in nutrient concentrations along the flowpaths of both cells, the changes in proportions are easier to understand using figure 4.



a) EAV cell inflow d) SAV cell inflow
b) EAV cell midflow e) SAV cell midflow
c) EAV cell outflow f) SAV cell outflow

Figure 4 The transformations of the proportions of SRP and DOP across the sample sites for the two cells. The dark grey indicates the percentage or SRP and the light grey indicates DOP.

Figure 4 shows that in the EAV cell, the first two sites, inflow and midflow experienced relatively little change in the proportion of SRP and DOP (figure 4). Within the third site however, the outflow point showed an increase in proportion of DOP and showed less SRP. Therefore, as P is transformed across the system, it increases in recalcitrant or nonbioavailable forms. This could be do to further decomposition and a lack of inputs of new material. However, the SAV cell showed an increase in the proportion of DOP from inflow to midflow, then a decrease from midflow to outflow sites. This demonstrates an increase in nonbioavailable P from inflow to midflow followed by an increase in bioavailable P. Therefore, this shows there is an increase in fresh OM between the midflow and outflow of cell 3 generating more SRP. Dierberg and Debusk (2008) provide results similar to that of cell 1 as the proportion of SRP decreases from inflow to outflow.

The C:N:P ratios present in OM vary based on species, whether they are living or non-living specimens (Scharler et al., 2015; Di Palo). Ratios between SAV and EAV species have been shown to vary, where the C:N and C:P ratios are higher for the EAV and the N:P ratio is relatively similar (Xia et al., 2014). The changes in concentration of nutrients which occurred indicate that there are changes to the composition of OM. This is supported by the DOM calculations as well. Increases in ratios of dissolved nutrients in the middle of the flowpath indicates that the nutrients are being transformed or added from the biota within the system itself. When there are small changes in ratios, it may indicate that decomposition is not occurring as quickly, or that inputs from the system match what is being decomposed.

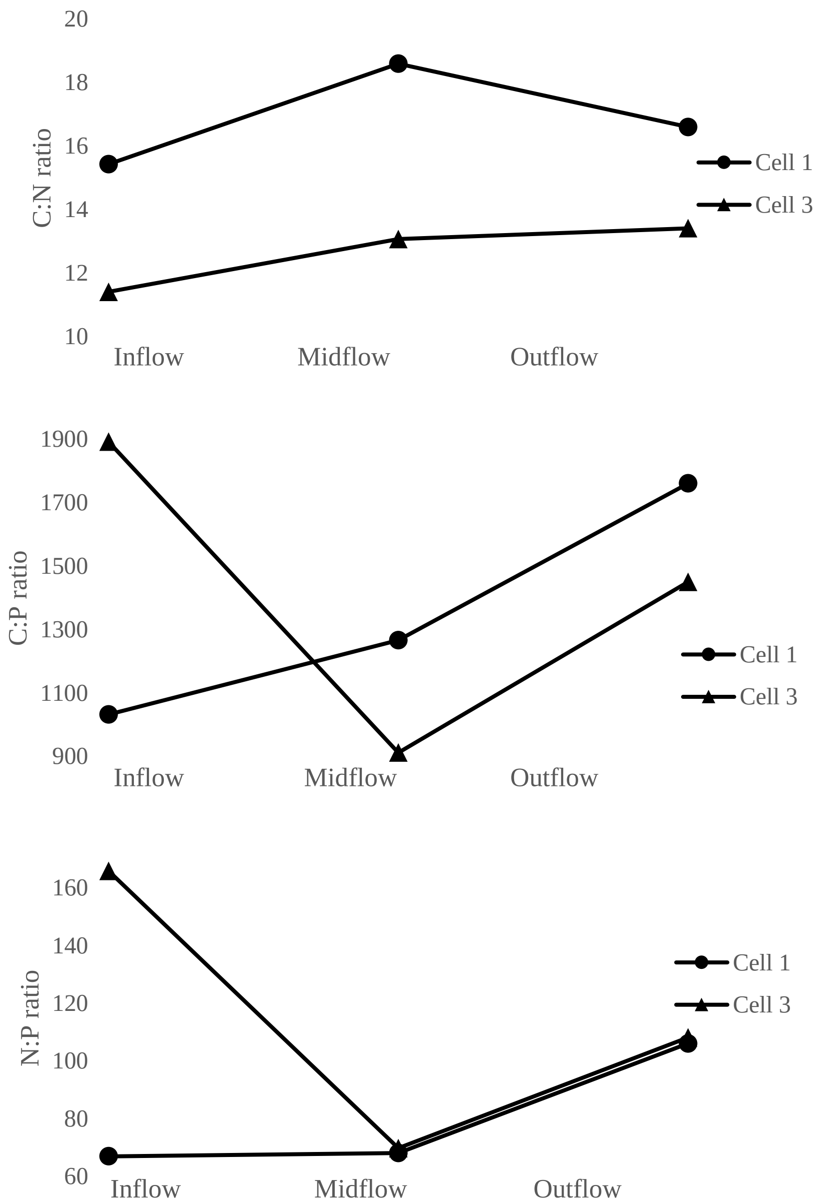


Figure 5 These graphs indicate the ratios of nutrients present in DOM, over the inflow, midflow and outflow sites of the two cells being studied.

The EAV cell, cell 1, showed an increase from 15 to 19 in C:N ratio from inflow to midflow, but then showed a decrease from 19 to 17 from midflow to outflow. However, for the C:P and N:P ratios, this cell showed an increase along all points, however a more drastic increase is seen between midflow and outflow in both. In cell 3, there was less consistency. The C:N ratio increased from 11 to 13 between inflow and midflow then remained constant between midflow and outflow. However, the C:P ratio decreased by more than half, from 1892 to 911, between inflow and midflow, then increased to 1450 between midflow and outflow. The N:P ratio acted similarly to the C:P, in both cases although it increased from midflow to outflow, the final ratio was still lower than that of the inflow site.

Conclusions:

Constructed wetlands are often used to reduce P and other nutrients in runoff and wastewater directed into the Florida Everglades. However they are often unable to consistently reach the goal of 10 ppb of TP in outflow waters (Mitsch et al., 2014). Understanding the processes which transform and remove P from the system is vital to understanding how P can better be removed from the system. By investigating the optical properties of DOM in water samples collected along the flowpath of two cells, as well as the nutrient ratios of C, N and P, it was possible to determine likely sources of the nutrients in outflow waters.

The two cells examined were an EAV cell and a SAV cell. There were clearly differences in the effectiveness and traits of these two cells. EAV cells generally, have an increase in heavy weight molecules between inflow and midflow and an increase in low molecular weights from midflow to outflow (Hansen et al., 2016). In contrast, the SAV cell, showed an increase in low molecular weight from inflow to midflow and an increase in weight from midflow to outflow (Hansen et al., 2016). Therefore, in the EAV cell, the material showed an increase in humification initially, followed by a decrease after midflow. While, in the SAV cell humified material was either lost or maintained, then increased after midflow. Similarly, the photobleaching and photolysis increased in the SAV cells than in the EAV cells due to the increase in light availability.

The $SUVA_{254}$ values calculated also showed differences between the cells and along the flow path. In the EAV cell, $SUVA_{254}$ values decreased between inflow and midflow and increased between midflow and outflow. In contrast, the $SUVA_{254}$ values for the SAV cell remained relatively stable. This indicates that the EAV cell experienced a decrease in aromatic carbon initially, followed by an increase in aromatic compounds (Weishaar et al., 2003). More aromaticity means less biologically active carbon, showing that as treatment progressed carbon became less biologically active (Helms et al., 2009 & Weishaar et al., 2003). The SAV cell however, showed increases and then decreases in $SUVA_{254}$ values from inflow to midflow and from midflow to outflow, respectively. This suggests that there is an increase in biologically active carbon, followed by a decrease in these molecules (Weishaar et al., 2003).

The nutrient values and ratios indicate similar findings. For all sites along the EAV cell flow path, DOC, TDN, and DOP decreased in concentration, though to varying amounts. However, the SAV cell showed a decrease initially, followed by an increase in nutrients for all except DOP. DOP in the SAV cell increased from inflow to midflow and decreased from midflow to outflow. This increase suggests that in the SAV cells, there is an input of nutrients from the system itself. This was not expected. Due to the increased light availability it was expected that the SAV cells would more efficiently remove nutrients. However, due to the increased microbial and plant life in this cell, it is possible that the concentration of nutrients in the cell was impacted by this life.

In conclusion, the optical properties and nutrient ratios indicate that there is an addition of fresh organic matter along the flow path, especially notable in the SAV cells, which decreases the aromaticity of the carbon present and increases the biologically active portions of carbon. Similarly, through the tracking of DOM, the data indicates that the P present in the outflow likely comes from, in part, the system itself. Based on the data collected, it appears as though there is an input of P from within the system itself. There are differences in the nutrient transformations between each of the cells, due to the differences in vegetation types between SAV and EAV properties. Additionally, the presence of microbial decomposition compared to light decomposition through photolysis is also different between the cells. More research is necessary into how the P can be better removed from the system, however, changing the EAV and SAV properties of the cells with different areas along the flow path may be part of a solution.

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