Title: Evaluation of nutrient stoichiometric relationships amongst ecosystem compartments of a subtropical treatment wetland. Fine-scale analysis of wetland nutrient stoichiometry.

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Abstract

Background: Evaluation of carbon (C), nitrogen (N) and phosphorus (P) ratios in aquatic and terrestrial ecosystems can advance our understanding of biological processes, nutrient cycling and the fate of organic matter (OM) in aquatic ecosystems. Eutrophication of aquatic ecosystems can disrupt the accumulation and decomposition of OM which serves as the base of the aquatic food web, and is central to the effectiveness of a treatment wetland. This study investigated nutrient stoichiometry within and between wetland ecosystem compartments (i.e. water column, vegetation, flocculent and soil) of two treatment flow-ways (FWs) in the Everglades Stormwater Treatment Areas located in south Florida (USA). These FWs include an emergent aquatic vegetation cell dominated by *Typha spp.*(cattail) and a submerged aquatic vegetation cell composed of species such as *Chara* spp. (muskgrass) and *Potamogeton* spp. (pondweed). The primary objective of this evaluation was to determine if nutrient stoichiometry is consistent within and between ecosystems and compartments to understand biogeochemical cycling and controls of nutrient removal within a treatment wetland ecosystem.

Results: This study demonstrates that C, N, and P stoichiometry can be highly variable among ecosystem compartments and between differing wetland ecosystems. Generally, total P declined along the length of each treatment FW in all ecosystem compartments, whereas trends in total N and C trends were more variable. These changes in C and nutrient concentrations result in variable nutrient stoichiometry along treatment FWs signaling potential changes in absolute and relative nutrient availability and biogeochemical processes.

Conclusions: Assessment of wetland nutrient stoichiometry between and within ecosystem compartments suggest decoupling of C:N:P relationships likely as a consequence of differential external nutrient supply, differences in primary producer communities and differential decomposition of organic matter. However, stoichiometry varies often monotonous along the flow paths, likely exhibiting a response to nutrient loading. Differences in C:N:P ratios in primary producers, light availability, microbial immobilization in the early stage of decomposition as well as nutrient mining during decomposition of OM are likely feedback mechanisms that lead to deviations from fixed stoichiometry, which in turn may have considerable influence on nutrient removal rates. This information could be used to further understand water treatment performance with respect to stoichiometric processes and OM decomposition.

Keywords: decomposition, mineralization, Everglades, treatment wetlands

Introduction

The study of nutrient stoichiometry, pioneered by Redfield (1934, 1958), laid the foundation of two important biogeochemical principles that later became basic tenets of ecological stoichiometry: (1) organisms have consistent carbon (C), nitrogen (N) and phosphorus (P) molar ratios and (2) the abundance of C, N and P in organisms is regulated by interactions between them and their environment. The basic premise of the Redfield ratio is that ratios of C:N:P are well constrained based on the similarity of measured N and P concentrations in marine plankton relative to the nitrate (NO_3) to phosphate (PO_4) ratio in deep ocean water. The Redfield ratio describes the ratio of NO_3, PO_4 and non-calcite inorganic C (i.e. inorganic C, N and P) in deep seawater (Redfield 1934, 1958; Lenton and Watson 2000; Geider and La Roche 2002). The stoichiometric values of the Redfield ratio thus describe the average composition of marine OM and the necessary oxygen required to decompose the organic matter (OM) via respiration. Since its acceptance, the Redfield ratio has been debated and revisited frequently in light of new analytical methods, more data, and clarification of the frequent misrepresentations of the notable Redfield ratio (Lenton and Watson 2000; Geider and La Roche 2002). Furthermore the Redfield ratio concept has been extended beyond marine ecosystems into freshwater and terrestrial systems where C and nutrient concentrations are not part of a homogenous reservoir, are more variable between ecosystems and their dynamics significantly (Dodds et al. 2002; Dodds 2003; Cleveland and Liptzin 2007; Xu et al. 2013).

The fate of C in ecosystems is regulated by mass-balance principles and the availability of other key nutrients such as N and P (Hessen et al. 2004). In general, wetlands are net C sinks that store a large amount of the global C driven by accumulation of C via plant productivity that exceeds export from the decomposition of organic matter (Billett and Moore 2008; Kayranli et al. 2010). Decomposition of OM involves a stepwise conversion of complex organic molecules into simple constituents through physical leaching and fragmentation, extracellular hydrolysis, and catabolic activities by microbes (Reddy and DeLaune 2008). Anthropogenically mediated nutrient loading to otherwise pristine aquatic ecosystems has a potential to disrupt the ecological balance and substantially affect nutrient (i.e. N and P) cycling through the disruption of both plant OM production and OM decomposition. Long-term nutrient enrichment in aquatic ecosystems can affect plant composition and OM decomposition, which in turn alters supply and demand for nutrients in different ecosystem compartments (plants, soil OM, microbial biomass) (Davis 1991; Reddy et al. 1999; Wright and Reddy 2001). Therefore, excessive external inputs of nutrients to an ecosystem can lead to disruption in the stoichiometric balance of ecosystem compartments by preferential assimilation, turnover and mineralization.

In the Everglades ecosystem, nutrient enrichment of natural systems has significantly influenced OM accumulation via larger ecosystem shifts in plant and algal communities (Davis and Ogden 1994; Pan et al. 2000). Eutrophication of aquatic ecosystems allows for nutrient tolerant species to establish and thrive while reducing the overall coverage of nutrient sensitive species leading to a cascading effect on the entire ecosystem structure and function (Davis and Ogden 1994; Newman et al. 1998). Stormwater run-off enriched with nutrients has accelerated soil accretion dynamics (Craft and Richardson 1993; Reddy et al. 1993). Overall, Everglades flora and fauna have been significantly impacted through widescale encroachment of cattails, loss of calcareous periphyton and other ecological changes within the Everglades marsh (Davis and Ogden 1994). In an effort to restore the biological integrity of the system, the State of Florida and the US Federal government initiated restoration and control efforts. One such effort is the construction of treatment wetlands to improve the quality of agricultural runoff water originating in the Everglades Agricultural Area (EAA) prior to entering the downstream Everglades ecosystem (Chen et al. 2015). These treatment wetlands, referred to as the Everglades stormwater treatment areas (STAs) were constructed with the primary objective of removing P from surface water prior to discharge to the Everglades Protection Area. The STAs are composed of several treatment cells which use natural wetland communities to facilitate the removal of P from the water column by leveraging natural wetland processes including nutrient storage in vegetative tissues and soils (Kadlec and Wallace 2009).

Prior studies have suggested that C:N:P ratios in soil and soil microbial biomass are tightly constrained providing a Redfield-like stoichiometric ratio across forested, grassland and natural wetland ecosystems (Cleveland and Liptzin 2007; Xu et al. 2013). Redfield (1958) observed both the concentrations and ratio of elements in ocean water were constrained, indicating that, despite large variability in nutrient concentration and amount nutrient ratios remain unchanged in a specific ecosystem compartment (e.g. vegetation groups, algae, soil, microbial biomass). Constrained stoichiometry thus suggests close interactions and feedbacks between organisms and their environment. In contrast, decoupling of stoichiometry can occur when e.g. imbalanced nutrient loads induce changes in element stoichiometry in specific compartments. Given the primary purpose of the STAs and the role they play as biogeochemical hotspots (McClain et al. 2003), this study will evaluate stoichiometry relationships within treatment wetlands that receive relative high nutrient loading, and that show, through in-wetland nutrient uptake, differences in nutrient concentration and loading along their flow path. The objectives of this study were to investigate nutrient stoichiometry in two treatment wetland ecosystems characterized by different dominant vegetation, to understand changes in C, N and P pools within ecosystem compartments. The first objective of this study was to evaluate nutrient relationships (i.e. C x N, C x P and N x P) within surface water, soil flocculent material (floc), recently accreted soil (soil) and vegetation live aboveground biomass (AGB) between two flow ways (FWs), one dominated by emergent aquatic vegetation (EAV) and the other by submerged aquatic vegetation (SAV). The first hypothesis is within a given FW cell, overall nutrient stoichiometry in these ecosystem compartments will be tightly constrained despite differences in dominant vegetative communities as suggested by previous studies (Cleveland and Liptzin 2007; Xu et al. 2013). The second objective of this study was to assess changes in nutrient stoichiometry in surface water, floc and soil ecosystem compartments along each FW transect in the direction of flow, essentially evaluating if nutrient stoichiometry within ecosystem compartments significantly change with distance downstream (and thus with nutrient loading). The second hypothesis is that due to external loading, shifts in nutrient stoichiometry are likely to occur along a given flow path, causing a decoupling of stoichiometric relationships, In essence, utilization and storage of excess nutrients supersede internal nutrient regulation and feedbacks resulting in strong gradients of C:N:P stoichiometry from inflow-to-outflow.

Methods

Study Area

Everglades STAs reduce surface water P loads in an effort to preserve and protect the remaining Everglades ecosystem (Chimney 2017). A total of six STAs with an approximate area of 231 km^2 are located south of Lake Okeechobee in the southern portion of the EAA (Fig 1). Prior land uses within the current STA boundaries include natural wetlands and agricultural land use dominated by sugarcane. The primary source of inflow water to the STAs is agricultural runoff originating from approximately 284 km^2 of farmland upstream. Everglades STA treatment cells are comprised of a mixture of EAV and SAV communities in several configurations including EAV and SAV treatment cells arranged in parallel or in series (Chen et al. 2015).

Stormwater Treatment Area-2 has been in operation since June 1999 with an effective treatment area of approximately 63 km^2 divided into eight treatment cells. This study was conducted in two cells, which are conventionally called FWs 1 and 3, respectively. The vegetative community of FW 1 is comprised predominately of EAV vegetation including *Typha domingensis* Pers. (cattail) and *Cladium jamaicense* Crantz (sawgrass) while FW 3 is dominantly SAV including *Chara* spp. (muskgrass), *Potamogeton* spp. (pondweed) and *Najas guadalupensis* Spreng (southern naiad) with approximately a third of the FW occupied by EAV species. Furthermore, prior to STA-2 construction FW 1 was a historic natural wetland while approximate two-thirds of FW 3 was previously farmed and is now managed as a SAV system (Juston and DeBusk 2006).

Data Source

Data used in this study were collected by South Florida Water Management District and University of Florida staff and was a part of a larger project within the overall South Florida Water Management District's Restoration Strategies Science Plan to evaluate performance and optimization of the Everglades STAs (South Florida Water Management District 2013). Data from one of the main studies of the Science Plan to evaluate P-sources, form, fluxes and transformation process in the STAs was used for this study and can be found in University of Florida Wetland Biogeochemistry Laboratory (2017). Water quality monitoring locations were established along two flow paths within STA-2 along a transect running from inflow to outflow of the FW. Weekly surface water grab samples were collected at monitoring locations within FWs 1 and 3 to characterize changes in nutrient concentrations and availability during prescribed/semi-managed flow events (Fig 1). When adequate water was available within the water management systems, prescribed flow events were scheduled and cycled through various flow/no-flow sequences for FWs 1 and 3. Surface water grab samples were collected just under the water surface at a depth of 10-15 centimeters. Water column parameters such as total P (TP), total N (TN), and dissolved organic C (DOC) were analyzed on these water samples. In the Everglades system, the organic C pool is predominately composed of the DOC fraction with very low particulate OC concentrations (*Julian, unpublished data*, Julian et al. 2017), therefore it is assumed that DOC is approximately equivalent to total OC concentrations to reflect other total nutrient stoichiometry. Soil samples were also collected along the flow transects twice during the dry and wet seasons between 2015 and 2016. Soils were sampled using the push-core method consistent with methods used in prior wetland soil studies (Bruland et al. 2007; Osborne et al. 2011; Newman et al. 2017). Samples were extruded from the soil core tube and partitioned into floc and soil. Floc was characterized as the suspended unconsolidated material on top of the consolidated soil. It was poured into a secondary sampling container, allowed to settle for 4 hours, supernatant water was removed via syringe and remaining floc material was analyzed accordingly. The remaining consolidated soil was segmented with the 0 - 5 cm segment retained for analyses. Floc and soil samples were analyzed for percent ash, TP, TN, and TC. Loss-on-ignition (LOI) was calculated using percent ash values subtracted by 100 percent. Live and senescent AGB were sampled from dominant vegetation in FW 1 while only live-AGB was sampled from FW 3 at the end of the 2015 (November 2015) and 2016 (September 2016) wet seasons. Additional details regarding floc and soil sampling is discussed by University of Florida Wetland Biogeochemistry Laboratory (2017). Vegetation samples were collected from four to eight randomly placed 0.25 m^2 quadrats adjacent to the identified sampling locations. Vegetation sampling locations were located at inflow, mid and outflow regions of the FWs within close proximity to the surface water and soil monitoring locations (Fig 1). Dry homogenized vegetation samples were analyzed for TP, TN and TC content consistent with U.S. Environmental Protection Agency approved methods (Table 1). Surface water inflow volume and TP concentrations were retrieved from the South Florida Water Management District (SFWMD) online database (DBHYDRO; www.sfwmd.gov/dbhydro) for each FW. For purposes of this data analysis and summary statistics, data reported as less than method detection limit (MDL; Table 1) were assigned a value of one-half the MDL, unless otherwise noted.

Data Analysis

Hydraulic and P loading rates (HLR and PLR, respectively) were calculated based on methods by Kadlec and Wallace (2009). Weekly surface water grab total phosphorus samples were collected at inflow and outflow structures and used to estimate inflow and outflow P-load amounts. Phosphorus loading rates were estimated using daily TP load divided by FW area. Hydraulic loading rates were estimated by dividing flow volume and FW area. Surface water nutrient concentrations were converted from mass of nutrient per volume concentration (i.e. mg L^{-1}) to a molecular concentration (i.e. mM). Soil, floc and vegetation nutrient concentrations were converted from a mass of nutrient per mass of soil (i.e. mg kg^{-1}) to moles per mass of soil or vegetation biomass (i.e. mmol kg^{-1}).

Nutrient stoichiometric relationships within each ecosystem compartment (i.e. surface water, soil, floc and vegetation) were examined by evaluating power law slopes using standardized major axis (SMA) estimation (’smatr’ package; Warton et al. 2006) consistent with Cleveland and Liptzin (2007). Unlike standard regression techniques which are used to predict one variable from another, SMA regression assesses the best fit line between two variables. Molar nutrient concentrations were log-transformed and slope of the SMA regression was evaluated against the null hypothesis that the slope was not different from one ($\beta \neq 1$). Power law $\left(y = kx^{\beta}\right)$ and its linearized form $(\log(y) = \beta\log(x) + \log(k))$ are used to evaluate the degree of proportional scaling between two variables. Scaling relationships and power-law distributions are key to understanding fundamental ecological relationships and processes in the natural system such as energy acquisition and transformation, biomass-growth relationships and evaluation of watershed chemostasis (Brown et al. 2002; Marquet et al. 2005; Wymore et al. 2017). In this analysis if the SMA regression results indicate a statistically significant relationship (i.e. $\rho<0.05$), indicating that the slope is significantly different from one, then the variables are independent and do not strictly proportionally scale (i.e. allometric growth). If the slope is not statistically different from one (i.e. $\rho>0.05$), then the variables exhibit proportional changes (i.e. isometric growth). Analytically, the concept of a constrained stoichiometric relationship can be assessed by the R^2 of the SMA regression. Low R^2 values reflect high stoichiometric variability resulting in a loosely constrained relationship while high R^2 values reflect low stoichiometric variability resulting in a tightly constrained relationship. This same approach was used in this study to test if the proportional relationships (i.e. C x P) are preserved as concentrations change in the various nutrient pools within each compartment similar to Cleveland and Liptzin (2007). To evaluate the degree of correlation between nutrients in a given compartment and to provide another metric to determine a relationships degree of constraint, Spearman's rank sum correlation was used to evaluate molar nutrient concentrations (no-transformation).

Surface water (DOC:TP, DOC:TN and TN:TP), soil and floc molar ratios (TC:TP, TC:TN and TN:TP) were compared between FWs by Kruskal-Wallis rank sum test. To characterize the relationship between floc and soil along the two-flow path transects, fractional distance downstream was broken into two categories, inflow to mid-point region (<0.5) and mid-point to outflow region (>0.5). These distance categories were identified as these regions of the FW are very different with respect to nutrient loading and in-situ nutrient concentrations (Juston and DeBusk 2011). Soil and floc TN:TP were compared between FWs and distance downstream categories by Kruskal-Wallis rank sum test, separately. Floc and soil TN:TP were also compared by Spearman's rank sum correlation by flow path separately.

Longitudinal change point analysis was performed on mean surface water, floc and soil nutrient stoichiometric ratios for data collected in FWs 1 and 3. Surface water nutrient ratios considered were TN:TP, DOC:TP, and DOC:TN, similarly floc and soil nutrient ratios include N:P, C:P and C:N. Mean nutrient ratios were compared to fractional distance downstream by Spearman's rank sum correlation and rate of change was evaluated using Theil-Sen slope estimator ('zyp' package; Bronaugh and Werner 2013). Nutrient stoichiometry change-point detection along the flow way transects was evaluated using Davies' difference-in-slope test comparing values to fractional distance downstream ('segmented' package; Vito and Muggeo 2003). Detection of a changepoint is a simple way of assessing gradients downstream and would potentially indicate regime shifts under monotonous decreasing nutrient loads. All statistical operations were performed with R© (Ver 3.1.2, R Foundation for Statistical Computing, Vienna Austria), unless otherwise stated all statistical operations were performed using the base R library. The critical level of significance was set at $\alpha = 0.05$.

Results

A total of five prescribed/managed flow events occurred between August 10th, 2015 and November 22nd, 2016 with events ranging from 35 to 63 days in FWs 1 and 3 within STA-2.

During the flow events, daily HLR ranged between 0 (no inflow) to 11.5 cm d^{-1} with FW 3 receiving a relatively higher mean HLR of 3.0 ± 0.3 cm d^{-1} (compared to 0.9 ± 0.1 cm d^{-1}) and higher maximum daily HLR of 11.5 cm d^{-1} (3.9 cm d^{-1} in FW 1). Observed daily PLR values ranged from 0 (no loading) to 9.5 mg m^{-2} d^{-1} with FW 3 receiving a higher relative load with a mean PLR of 1.9 ± 0.2 mg m^{-2} d^{-1} and experiencing the highest daily maximum PLR rate. Flow-way 1 achieved a mean PLR of 0.4 ± 0.06 mg m^{-2} d^{-1} with a daily maximum PLR of 1.6 mg m^{-2} d^{-1} (complete summary of flow event characteristics can be found in the Supplemental Material; Table S1 and Fig S1). The daily HLR and PLR observed during this study was consistent with historic operational loading rates experienced for these FWs to date (Chen et al. 2015).

Water Column C:N:P relationships

Overall, mean DOC, TN and TP concentrations were relatively comparable (Table 2) between the FWs as expected since they received the same source water but experienced different loading (HLR and PLR; Table S1) regimes and dominant vegetative communities. All surface water stoichiometric relationships resulted in statistically significant relationships with slopes significantly different from one (Table 3) indicating that nutrient pools scaled independently. The models between the two FWs diverge drastically in most cases except for the DOC-TN relationship for FW 1 for which the high correlation suggests some degree of a constraint (i.e. moderate R^2 Fig 2 and Table 3). Stoichiometric relationships associated with TP (i.e. DOC:TP and TN:TP) were not as tightly constrained as indicated by SMA $R^2 < 0.60$ and Spearman r-values <0.80 (Table 3) driven largely by the extreme variability in weekly measurements (Table 2). Stoichiometric ratios of DOC:TP, DOC:TN and TN:TP (Table 4) were significantly different between the two FWs with FW 3 experiencing greater DOC:TP and TN:TP values and lower DOC:TN values (Fig 3 and Table 2).

Total P, TN and DOC concentrations gradually decline in concentrations along the STA-2 FW 1 flow way transect (Fig 4). Along with declines in concentrations along the flow path, DOC:TN and TN:TP significantly increased (Table 5) with no significant change points detected (ρ=0.59 and ρ=0.22, respectively) along the flow way transect. However, DOC:TP did not significantly change along the FW1 (Table 5) and no change point was detected (ρ=0.19) despite having the largest rate of increase (based on Theil-Sen slope) between stoichiometric ratios. Similar to FW 1, FW 3 TP and TN were decreased with distance downstream with TN with largest decline along the transects (Fig 4). Meanwhile, in FW 3 DOC remained relatively constant with distance downstream (Fig 4). All surface water stoichiometric ratios were positively correlated with distance downstream along FW 3 at a similar rate as FW 1 (Table 5) and no change points detected for any of the stoichiometric ratios (DOC:TP ρ=0.08; DOC:TN ρ=0.21; TN:TP ρ=0.12).

Floc C:N:P relationships

Percent organic matter (OM), as indicated by LOI, ranged from 12.1 to 91.4 % across the FWs with FW 1 having higher OM in the floc material (Table 2). In addition to having lower OM, FW 3 floc material was more mineral as indicated by lower LOI values than FW 1 (Table 2). Among TC, TP and TN in floc, TP concentration was generally the most variable parameter between and within sites with coefficients of variance as high as 56.2 % at any given site but overall FW 3 exhibited the highest overall coefficient of variance with 40.3% spatial and temporal variability (Table 2).

All floc stoichiometric relationships resulted in statistically significant relationships with log-transformed SMA slopes significantly different from one, except for TC-TN in FW 1 (Table 3). Relationships where slopes were significantly different from one indicate that these nutrient pools independently scale. The TC-TN relationship of FW 1 resulted in a slope not significantly different from one suggesting that TC and TN in the floc compartment of FW 1 proportionally scale with some degree of constraint (Table 3). In addition to not scaling proportionally, floc TC-TP and TN-TP for both FWs were not significantly correlated thereby suggesting these nutrients pools are not constrained and vary widely (Table 3). Despite scaling independently, floc TC-TN for FW 3 was highly correlated (Fig 2 and Table 3). Floc TC-TN relationships are very similar between FWs and appear to fall along a continuum (Fig 2) with FW 1 generally having greater TN concentrations in the floc compartment (Table 2). Floc TC-TP and TN-TP relationships in FW 3 appear to have broken out into two distinct populations with a discernible break-point between populations occurring at approximately 800 mg P kg^{-1} (Fig 2), above which TN and TC concentrations remain relatively low. These points mostly correspond to sampling locations in the inflow region of the FW. Floc TC:TP and TC:TN stoichiometry significantly differ between FWs (Table 4) with FW 3 having higher TC:TN and TC:TP ratio (Fig 3), suggesting TP and TN are less constrained relative to TC when compared across different systems. Meanwhile, floc TN:TP was not significantly different between FWs (Table 4) suggesting that even though TP concentrations are highly variable between and within FWs TP and TN are tightly constrained across systems.

Similar to the surface water compartment, floc TP concentration declined with distance downstream along the FW 1 transect (Fig 4). Meanwhile, floc TC and TN did not indicate an appreciable change with distance downstream (Fig 4). Floc TC:TP and TN:TP molar ratios were positively correlated with distance downstream while TC:TN molar ratio did not exhibit a significant correlation with distance downstream (Table 5). Furthermore, no significant change points were detected for any stoichiometric ratios along the FW 1 flow way transect (TC:TP ρ=0.13; TC:TN ρ=0.78; TN:TP ρ=0.07). Along the FW 3 transect TC:TN was not significantly correlated with distance downstream (Table 5). While, floc TC:TP and TN:TP were positively correlated with distance downstream at rates of change comparable to that of FW 1 (Fig 4 and Table 5). Moreover, no change points were detected in along the FW 3 transect for TC:TP, TC:TN or TN:TP (TC:TP ρ=0.12; TC:TN ρ=0.16; TN:TP ρ=0.22).

Soil C:N:P relationships

Much like the floc compartment, LOI values in the soil compartment range from 16.5 to 91.0 percent across the study FWs with FW 1 having a higher observed mean LOI value for soil (81.2 ± 1.4 %; Table 2). Additionally, FW 3 soils were more mineral in nature with less apparent OM to FW 1 soils (Table 2). Generally, soils in FW 1 are more enriched with nutrients as indicated by qualitatively greater average concentrations (Table 2). Soil nutrient ratios were generally greater than those observed in the floc compartment with TC:TP (Table 2). Much like the floc compartment, TP was the most variable parameter as indicated by intra- and inter-site coefficients of variance reaching as high as 47.8 % at any given site. Overall, FW 1 exhibited the highest overall TP coefficient of variance with 42.1 % spatial and temporal variability (Table 2).

Soil TC-TP for FW 3 and TC-TN for FW 1 resulted in slopes not significantly different from one suggesting that these nutrient pools in the soil compartment scale proportionally (Table 3). All other relationships in the soil compartment resulting in slopes significantly different from one, indicating independent scaling between nutrients regardless of FW (Fig 2 and Table 3) with varying levels of constraint between pools. Much like in the floc compartment, TC-TN in FW 3 was highly correlated and had relatively high R^2 values suggesting low stoichiometric variability and a relatively tightly constrained relationship, albeit different than a 1:1 relationship. The soil TN-TP relationship of FW 1 was not significantly correlated with a high degree of variability between nutrient pools (Table 3). Much like in the floc compartment, two distinct populations in the soil TC-TP and TN-TP relationships for FW 3 were apparent (Fig 2) with a discernible break-point occurring at approximately 600 mg P kg^{-1} (19.4 mmol P kg^{-1}), above which both TC and TN concentration remained relatively low. These points correspond to samples taken from inflow regions of FW 3.

Similar to the floc compartment, soil TP concentration declined with distance downstream while TC and TN did not exhibit an appreciable decline with distance downstream (Fig 4). Both TC:TP and TN:TP stoichiometry were not significantly correlated with distance downstream along the FW 1 flow way transect with no significant change point detected (TC:TP ρ=0.59; TN:TP ρ=0.46). Soil TC:TN was negatively correlated with distance downstream along the FW 1 flow way transect (Table 5) with no change point detected (ρ=0.30). Along the FW 3 flow transect, TN and TC seem to increase with with distance downstream while TP was declined with distance downstream at a comparable rate of change downstream relative to FW 1 suggesting similar P-removal rates between FWs (Fig 4). Soil TC:TP was positively correlated with distance downstream (Table 5) and a significant change point was detected along the FW 3 flow way transect (Estimate=0.78, $\rho<0.05$). Soil TC:TN was negatively correlated with distance downstream (Table 5) and a significant change point was detected along the FW 3 flow way transect (Estimate=0.71, $\rho<0.05$). Soil TN:TP was not significantly correlated with distance downstream (Table 5), however a significant change point was detected along the FW 3 flow way transect (Estimate=0.71, $\rho<0.05$).

Floc and soil N:P molar ratios were significantly correlated for sites within FW 3 (r=0.85, $\rho<0.01$). In FW 3, the distribution of the data formed what appears to be an S-shaped curve with sharp transition in floc N:P ratio when soil N:P is approximately 100 (Fig 5), but not in FW 1. Furthermore, both floc (χ^2 =20.41, $\rho<0.01$) and soil (χ^2 =4.67, $\rho<0.05$) N:P molar ratios were significantly different along FW 3 between the two distance categories. Within FW 1, floc and soil N:P was not significantly correlated (r=0.28, $\rho=0.21$) and qualitatively the floc-soil relationship within FW 1 appears to be different than that of FW 3 where the upper arm of the S-curve is missing for FW 1 (Fig 4). The lack of an “upper arm” in the FW 1 floc-soil relationship could indicate the floc compartment has not reached saturation or microbial decomposition dynamics differ. Despite the difference in the floc-soil N:P relationships between the two FWs, N:P molar ratios were significantly different between the two distance classes in FW 1 for floc (χ^2 =19.44, $\rho<0.01$) and soil (χ^2 =4.35, $\rho<0.05$).

Vegetation C:N:P relationships

During the vegetation sampling within FW 1 three EAV species were sampled including cattail, sawgrass and *Nymphaea odorata* Aiton (water lily) with cattails accounting for most of the samples collected. Within FW 3, a mix of SAV species were sampled including muskgrass, pondweed and southern naiad with muskgrass being the most common. Plant tissue TP concentrations from both FWs 1 and 3 ranged approximately five-fold with vegetation within FW 3 having higher absolute tissue TP concentrations (Table 2). Plant tissue TN concentrations variability was larger than TP (more than ten-fold range) while the variability in vegetation TC concentrations was smaller (approximately three-fold) (Table 2). Live-AGB in FW 1 shows higher TC:TP and TC:TN ratios but FWs 3 has higher TN:TP ratios (Table 2). Much like the other compartments, variability in TP was greatest amongst the other nutrient parameters with an overall coefficient of variance of 82.0 % while between FWs, FW 1 had a higher coefficient of variance with 74.0 % and FW 3 having a coefficient of variance of 57.4 %.

Much like the other ecosystem compartments live-AGB TC-TP, TC-TN and TN-TP models for FWs 1 and 3 resulted in slopes significantly different than one suggesting that nutrient concentrations within vegetation scale independently with vary levels of constraint (Table 3). TC-TP and TC-TN differ between flow ways at low nutrient concentration but appear to converge as concentration increase. On the other hand, the relationship between TN-TP models between FWs seem to gradually diverge from one another (Fig 2). Live-AGB TP and TN concentration were negatively correlated while TC concentration was positively correlated with distance downstream along the FW 1 flow way transect (Table 5). Both TC-TP and TC-TN relationships were negatively correlated in the vegetation compartment in FW 1 with a higher degree of correlation between TC and TP. Additionally, TC-TP and TC-TN were positively correlated in FW 3 with a higher degree of correlation between TC-TN. Despite these relative differences of TC-TN and TC-TP correlations between FWs, positive correlations were apparent between TN-TP for both FWs.

Discussion

Surface Water Stoichiometric Relationships

Redfield (1958) focused on the composition of inorganic fractions of C and nutrients of deep ocean waters, a relative homogenous reservoir of inorganic nutrients in the deep ocean (Guildford and Hecky 2000). However, in freshwater ecosystems nutrient availability can be highly variable, analytical methods can be tenuous and interpreting inorganic nutrient data can be problematic (Guildford and Hecky 2000; Dodds 2003). Furthermore, biogeochemical cycling of nutrients in freshwater ecosystems is influenced by the ability of some biota to utilize dissolved and particulate inorganic and organic fractions with the organic fractions being utilized via enzymatic hydrolysis (Bergström 2010). However, some studies have successfully used total nutrient fractions to indicate nutrient limiting status and trophic dynamics across the freshwater-to-marine aquatic continuum (Downing 1997; Guildford and Hecky 2000).

The Everglades STAs are optimized to effectively remove P from the water column (Chen et al. 2015). This process is completed through a combination of physical removal and biological uptake. Physical removal of P is done through settling and entrainment of particulate P while biological uptake removes P from the water column through metabolic uptake along a given flow path (Kadlec and Wallace 2009). Therefore, changes in nutrient stoichiometry within and between FWs (Fig 2 and 3) can be the result of biological or physical configurations of the treatment system. Flow-way 3 of STA-2 is a SAV dominated system where the sequestration of P is facilitated partly by the co-precipitation of calcite (Dierberg et al. 2002). Meanwhile, FW 1 is an EAV dominated system where particle entrainment and subsequent P uptake by plant and microbes are dominant nutrient removal and mineralization mechanisms (Reddy and DeLaune 2008). Interestingly, in FW3 the P decrease (or net removal) is more pronounced relative to C and N along the flow path.

In the surface waters of the Everglades STAs, all stoichiometric relationships in both FWs scaled independently but were significantly correlated at varying levels of constraint (Table 3). Most relationships were positively correlated with the exception of DOC-TP relationship in FW 3, which exhibited a negative slope (Fig 2 and Table 3). The observed slope for the DOC-TP relationship for both FWs shows a relative (slope < 1 FW 1) or absolute (slope < 0) depletion of DOC with increasing TP concentrations. Several mechanisms could potentially explain this general trend, the first and most probable is that samples with high P concentrations are run-off water near the inflow, most of which is composed of inorganic-P and low DOC. Perhaps, high C relative to P is maintained along the flow-path, the inorganic forms of P are taken up by phytoplankton while organic forms are preferentially deposited, leading to overall reduction in P, but not in C (which is photosynthetically fixed). Given that FW 3 is an SAV dominant system, a feedback via light availability is possible, preliminary data suggests that light attenuation is lower (i.e. higher light penetration) in FW 3 than FW 1 (University of Florida Wetland Biogeochemistry Laboratory 2017) (Julian and Bhomia 2017). Spears et al. (2008) documented such a potential feedback mechanism in shallow lakes where benthic microalgae under high light disproportionally remove limiting nutrients, and subsequently affect phytoplankton stoichiometry.

Soil Stoichiometric Relationship

Soils are long term integrators of environmental conditions where nutrient concentrations and availabilities are driven by external inputs (i.e. loading) and the interaction of biota and microbial communities dictate biogeochemical cycling (DeBusk and Reddy 2003). Moreover, nutrient concentrations and availability can significantly vary along environmental gradients (i.e. flow, vegetations, soil type) and spatial scales. Variability of nutrient stoichiometric estimates are apparent between local (i.e. STA; this study), ecosystem (i.e. Natural Wetland; Xu et al. 2013) and global scales (Cleveland and Liptzin 2007; Xu et al. 2013).

The stoichiometric relationships apparent in the Everglades STAs soils lack the degree of proportional scaling observed in other studies and diverges somewhat from the “Redfield-like” relationships demonstrated in previous studies at an ecosystem and global scale (Cleveland and Liptzin 2007; Xu et al. 2013). However, this study provides a unique case for applying stoichiometric relationships to understand nutrient cycling and transformations between soil and water column along an enriched nutrient gradient. Moreover, this study allows the comparison of soil stoichiometry within a narrow geographic and geomorphic range (Everglades wetland systems) with large and systematic changes in nutrient loads (distance downstream), and vegetation (EAV vs. SAV). Unlike relationships presented by prior studies C and P concentrations were not well-constrained (Fig 2). Further, stoichiometric relationship in this study exhibits a strong non-linear relationship along the FW despite sharing the same source water. The response of C and P in the Everglades ecosystem is driven largely by the P limiting nature of the natural system combined with high loading of P from upstream sources (Chen et al. 2015). Perhaps, the negative relationship particularly in the SAV system may indicate P mining via benthic algae. Higher P demand per unit increase in C or N in the vegetation in FW 3 can create higher P demand and therefore higher plant and microbial activity to extract P from soil organic matter. In that case we should expect depletion of P relative to C and N along the flow-way. Indeed, P depletion relative to C and N downstream is more pronounced in FW 3 than FW 1, although the significance threshold for the downstream gradient is not always achieved. Meanwhile, soil C and N relationships are somewhat constrained across the combined SAV and

EAV systems and follow a near linear relationship (Fig 2). Overall the non-linear scaling (and often negative) relationships between C, N and P suggests the relative decoupling of P during OM accumulation driven by the rapid consumption of inorganic P (Corstanje et al. 2016), and the utilization of organic P as further indicated by changes in enzyme activities along the treatment FW (University of Florida Wetland Biogeochemistry Laboratory 2017). This decoupling could potentially indicate a more efficient use of P release from the organic pools along the flow path gradients and/or the interaction of mineral and organic pools as in the case of STA-2 FW 3 (Fig S2, S3 and S4). More specifically, the slope between total organic C:total inorganic C (i.e. TOC:TIC) and TN:TP in the soil compartment varies dramatically (Fig S4). This decoupling of P cycling from OM accumulation is also hypothesized to occur in forested ecosystems where P is often limiting (Johnson et al. 2003; Cleveland and Liptzin 2007).

The stoichiometric relationships observed in this study are consistent with relationships observed within the Everglades marsh (Julian et al. 2016a). In addition to direct loading, internal nutrient cycling via the plant-litter-mineralization pathway can play a significant role in soil biogeochemistry, especially toward the back-end of the treatment FW where external inputs are reduced. The role of litterfall and its decomposition plays a significant role in internal nutrient cycling by enriching the soil OM through initially increasing both C and N concentration. Litter N is known to be conserved during decomposition processes ultimately enriching the soil below while C is lost due to microbial respiration. As litter decomposes soil TN concentrations increase, thereby soil TC:TN ratios decrease (Melillo et al. 1989; Julian et al. 2016a). During this study soil and floc TC:TN varied along flow path (Table 4 and Fig 4) where soil TC:TN values begin to increase toward the back third of the FW 3 suggesting a change in biogeochemical drivers such as enzyme activity, microbial composition (University of Florida

Wetland Biogeochemistry Laboratory 2017), substrate composition and deposition environment (i.e. redox, electron acceptor availability, etc.).

Unlike the conservative nature of N, P is rapidly cycled with internal nutrient regeneration from OM decomposition being more important than external inputs (Verhoeven et al. 2006). Phosphorus enrichment reduces net nutrient regeneration from senescent litter but increases nutrient regeneration in soil (Newman et al. 2001) suggesting that P-enrichment accelerates decomposition by increasing microbial activity. Newman et al. (2001) also observed soil TC:TN values indicating the potential for N mineralization which with the addition of P would alleviate P-limited microbial activity thereby releasing inorganic N via mineralization of organic matter. As demonstrated by changes along the flow paths (Table 5) and the variability of nutrient availability (Table 2 and Fig 4), biogeochemical cycling and contributions from different ecosystem compartments can be variable along the flow-paths as P concentrations are reduced lowering the enrichment potential. Furthermore, soil C:N values significantly decline (Table 5) along the flow paths suggesting the potential for N-mineralization which could imply differential nutrient limitations and mineralization processes along the flow path. This is especially apparent in FW 3, an SAV dominated community suggesting vegetation dynamics drive can influence biogeochemical cycling of nutrients when comparing the two flow paths.

Soil – Floc Interaction and Stoichiometry

Floc is a complex matrix of microbes, organic particles (i.e. cellular detritus) and inorganic particles (clays and silts) with substantial inter-floc spaces analogous to pore space in soils are formed from a variety of physical, chemical and biological processes (Droppo 2001). In the

Everglades system, floc material is largely derived from senescing periphyton and macrophytes with very little to no terragenic sediments (Noe et al. 2001, 2003). This matrix of biologic, chemical and geologic material is ecologically important in aquatic systems due to its ability to act as a source or sink of nutrients to the overlying water column. Furthermore, floc represents the beginning of soil OM diagenesis in natural wetland ecosystems (Noe et al. 2003; Neto et al. 2006). As floc is in this sandwich position, we expect stoichiometry to reflect a mixture between the end-points of soil and water.

Much like surface waters, most stoichiometric relationships in the floc layer were independent (i.e. do not proportionally scale, Table 3). Furthermore, the relationship of TP with TN and TC was not tightly constrained as indicated by rank correlation and SMA R^2 suggesting high stoichiometric variability. Floc is likely subjected to the direct result of P-enrichment altering the depositional environment. Alternatively, P-enrichment can also accelerate primary productivity (GPP) and ecosystem respiration (ER) rates (Hanson et al. 2003; Hagerthey et al. 2010) influencing C metabolism in the water column. It is worth noting that concurrent with a strongP gradient in ecosystem compartment (Fig 4) a productivity gradient (i.e. GPP and ER) is also apparent with higher productivity at the inflow regions (Julian and Bhomia 2017). The soil TC-TP relationship could also be the result of variable C and P burial and cycling due to the decay of plant material facilitated by high biomass turnover (i.e. litterfall) and litter decay rates (Chimney and Pietro 2006; Julian et al. 2016a). The strong positive correlation between TC-TN in both floc and soil (Table 3), the relatively high N concentration of live-AGB in FW 3 and the soil and floc TC:TN values observed in this study indicate that vegetation is a strong contributor to the soil composition potentially influencing the relationship of TC to TP through variability in cycling of nutrients in the soil compartment (Table 2).

As previously observed, floc represents a stoichiometric intermediary between primary producers and the microbially reworked compounds in soils organic matter. Neto et al. (2006) evaluated floc and soil C:N ratios along a freshwater-to-marine transect suggesting a mixed source of OM to these compartments along with a decoupling of C to N suggesting variable remineralization rates in soil relative to floc. Conceptually, primary producers, in particular higher plants, exhibit relative high C to nutrient ratio, which may even widen in fresh litter because of redistribution of nutrients. Relatively active microbial activity act to immobilize (or retain) nutrient during early decomposition stages, while C is respired, resulting in nutrient enrichment. Secondary microbial residues that are relatively recalcitrant may still be stripped essential nutrients, in particular P. therefore it is expected that N relative to C to increase during the transition from vegetation to floc to soil, C:P ratios are expected to decline from vegetation to the microbially active floc layer, but then increase again as P mining becomes the dominant P cycling mechanism in the soil. Nitrogen to P ratios may be expected to be similar between vegetation and floc as ratios in vegetation and microbes are in the same range, but in soils N is again expected to be enrich with respect to P due to plant mining in these P limited systems. The ratios in FW 1 and FW 3 roughly follow this pattern but there are some nuances: nutrients are fairly enriched SAV systems, therefore we don’t find further narrowing in the floc layer. Furthermore, C:N ratios in floc and soil show little difference, suggesting that immobilization is occurring early and vigorously in the decomposition continuum. Moreover, the narrow C to nutrient ratio in the floc layer is likely further be fostered by sedimentation of DOM.

Additionally, this transition is accompanied by a co-variate of distance downstream (Fig 4). While N:P ratios are always more narrow in floc than in soil, floc TN:TP values in the inflow-to-mid region of the treatment FW (fractional distance <0.5) are generally lower than TN:TP values in the mid-to-outflow region (fractional distance >0.5). Interestingly, P becomes more enriched relative to N in FW3, indicating additional P accretion as floc transitions into the soil, a pattern not seen in FW 1, potentially indicating shifts in OM deposition and/or decomposition (Fig 5). In FW 1, floc and soil TN:TP lacked any correlation potentially indicating selective removal or variable nutrient remineralization rates. In FW 3 floc and soil TN:TP were significantly correlated again suggesting variability in nutrient removal processes but also potential variability in N or P remineralization rates driven by different microbial communities and enzyme activities (University of Florida Wetland Biogeochemistry Laboratory 2017).

Conclusion and Further Research

Prior studies of stoichiometry suggest that the relationship between C and nutrients is tightly constrained and C:N:P stoichiometric relationships are relatively constrained and consistent with elemental composition of dominate phototrophs (i.e. algae and phytoplankton) in the water column and microbial biomass in soil. However, at a finer scale exemplified in this study nutrient stoichiometric relationship within treatment wetlands are potentially decoupling through processes such as enrichment; disruption of biotic-feedback loops; variable mineralization/immobilization rates; selective removal of water column constituents via biotic uptake or; physical settling, or active mining for limiting nutrients in soils. As a consequence, stoichiometric relationships vary between ecosystems, their compartment, and along gradients of nutrient loading. At the onset of this study three hypotheses were suggested, the first hypothesis that nutrient stoichiometry was tightly constrained across ecosystem compartments was rejected as nutrient concentrations did not scale proportionally in each ecosystem compartment or study FW with a few exceptions (FW 3 Floc TC-TP and FW 1 Floc and Soil TC-TN). The second hypothesis related to observed shifts in nutrient stoichiometry due to ecosystem level biogeochemical processes was accepted due to the significant changes of nutrient stoichiometry relative to distance downstream and between flow paths were apparent. The final hypothesis that stoichiometric relationships between ecosystem compartments differed significantly between flow paths was accepted as each flow path driven by its biota and retained nutrients differently, especially relative to C and N dynamics.

Evaluation of stoichiometric relationships within ecosystem compartments provides a greater understanding of how nutrients and OM cycle through a system relative to one another (i.e. relative enrichment or depletion) such as the diametrically opposed relationships between DOC and TP in the water column along each flow-way. Moreover, scaling properties of nutrients in a stoichiometric framework provide an understanding of fundamental relationships and processes in natural systems and variability in these relationships can offer insight into underlying biogeochemical mechanisms (Brown et al. 2002; Marquet et al. 2005; Wymore *Unpublished Data*). The divergent relationship suggesting process related differences in how nutrients are retained within the ecosystem and as such could he used to understand treatment wetland performance or expectations given the stoichiometric relationships identified in this study. In the context of the Everglades STAs, optimization for P-retention must go beyond a focus on P and P-forms (i.e. organic versus inorganic) but also focus on other continents including C and N. Building from this work, future studies should address the potential for preferential removal and utilization of nutrients from different substrates and organism (uptake and mining in macrophytes, immobilization of nutrients) to further understand ecosystem nutrient uptake and retention. Moreover, perhaps this work coupled with a modeling study that addresses the resulting accumulation in vegetation, floc and soil following the nutrient driven changes in biogeochemical cycles.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Authors' Contributions

PJ performed data analyses including necessary calculations and statistical analyses, and wrote the manuscript. SG and ALW contributed to data interpretation and writing. RB and TZO were involved with data collection and writing. JK, MP and JD were involved in data collection. All authors read and approved the final manuscript.

References

Bergström A-K (2010) The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. Aquat Sci 72:277–281. doi: 10.1007/s00027-010-0132-0

Billett MF, Moore TR (2008) Supersaturation and evasion of CO2 and CH4 in surface waters at Mer Bleue peatland, Canada. Hydrol Process 22:2044–2054. doi: 10.1002/hyp.6805

Bronaugh DB, Werner A (2013) Zhang + Yue-Pilon trends package. CRAN R-Project

Brown JH, Gupta VK, Li B-L, et al (2002) The fractal nature of nature: power laws, ecological complexity and biodiversity. Philos Trans R Soc B Biol Sci 357:619–626. doi: 10.1098/rstb.2001.0993

Bruland GL, Osborne TZ, Reddy KR, et al (2007) Recent Changes in Soil Total Phosphorus in the Everglades: Water Conservation Area 3. Environ Monit Assess 129:379–395. doi: 10.1007/s10661-006-9371-x

Chen H, Ivanoff D, Pietro K (2015) Long-term phosphorus removal in the Everglades stormwater treatment areas of South Florida in the United States. Ecol Eng 79:158–168. doi: 10.1016/j.ecoleng.2014.12.012

Chimney M (2017) Performance of the Everglades Stormwater Treatment Areas. In: 2017 South Florida Environmental Report. South Florida Water Management District, West Palm Beach, FL

Chimney MJ, Pietro KC (2006) Decomposition of macrophyte litter in a subtropical constructed wetland in south Florida (USA). Ecol Eng 27:301–321. doi: 10.1016/j.ecoleng.2006.05.016

Clesceri LS, Greenberg AE, Eaton AD (eds) (1998) Standard Methods for the Examination of Water and Wastewater. American Public Health Association

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85:235–252. doi: 10.1007/s10533-007-9132-0

Corstanje R, Grafius DR, Zawadzka J, et al (2016) A datamining approach to identifying spatial patterns of phosphorus forms in the Stormwater Treatment Areas in the Everglades, US. Ecol Eng 97:567–576. doi: 10.1016/j.ecoleng.2016.10.003

Craft CB, Richardson CJ (1993) Peat Accretion and Phosphorus Accumulation along a Eutrophication Gradient in the Northern Everglade. Biogeochemistry 22:133–156

Davis S, Ogden JC (1994) Everglades: The Ecosystem and Its Restoration. CRC Press, Boca Raton, FL

Davis SM (1991) Growth, decomposition, and nutrient retention of Cladium jamaicense Crantz and Typha domingensis Pers. in the Florida Everglades. Aquat Bot 40:203–224. doi: 10.1016/0304-3770(91)90059-E

DeBusk WF, Reddy KR (2003) Nutrient and hydrology effects on soil respiration in a northern Everglades marsh. J Environ Qual 32:702–710

Dierberg FE, DeBusk TA, Jackson SD, et al (2002) Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: response to hydraulic and nutrient loading. Water Res 36:1409–1422

Dodds WK (2003) Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. J North Am Benthol Soc 22:171–181

Dodds WK, Smith VH, Lohman K (2002) Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Can J Fish Aquat Sci 59:865–874. doi: 10.1139/f02-063

Downing JA (1997) Marine nitrogen: phosphorus stoichiometry and the global N: P cycle. Biogeochemistry 37:237–252

Droppo IG (2001) Rethinking what constitutes suspended sediment. Hydrol Process 15:1551–1564. doi: 10.1002/hyp.228

Geider R, La Roche J (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 37:1–17. doi: 10.1017/S0967026201003456

Guildford SJ, Hecky RE (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnol Oceanogr 45:1213–1223. doi: 10.4319/lo.2000.45.6.1213

Hagerthey SE, Cole JJ, Kilbane D (2010) Aquatic metabolism in the Everglades: Dominance of water column heterotrophy. Limnol Oceanogr 55:653–666

Hanson PC, Bade DL, Carpenter SR, Kratz TK (2003) Lake metabolism: Relationships with dissolved organic carbon and phosphorus. Limnol Oceanogr 48:1112–1119. doi: 10.4319/lo.2003.48.3.1112

Hessen DO, Ågren GI, Anderson TR, et al (2004) Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85:1179–1192. doi: 10.1890/02-0251

Johnson AH, Frizano J, Vann DR (2003) Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. Oecologia 135:487–499. doi: 10.1007/s00442-002-1164-5

Julian P, Bhomia R (2017) Transect Study: Surface Water Quality Monitoring and Analysis. In: Reddy KR (ed) Evaluation of Soil Biogeochemical Properties Influcing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs): 2016-17 Annual Report. University of Florida, Gainesville, FL, pp 317–444

Julian P, Gerber S, Wright AL, et al (2017) Carbon pool trends and dynamics within a subtropical peatland during long-term restoration. Ecol Process 6:43–57. doi: 10.1186/s13717-017-0110-8

Julian P, Gu B, Wright AL (2016) Mercury Stoichiometric Relationships in a Subtropical Peatland. Water Air Soil Pollut 227:472. doi: 10.1007/s11270-016-3180-9

Juston J, DeBusk TA (2006) Phosphorus mass load and outflow concentration relationships in stormwater treatment areas for Everglades restoration. Ecol Eng 26:206–223. doi: 10.1016/j.ecoleng.2005.09.011

Juston JM, DeBusk TA (2011) Evidence and implications of the background phosphorus concentration of submerged aquatic vegetation wetlands in Stormwater Treatment Areas for Everglades restoration. Water Resour Res 47:. doi: 10.1029/2010WR009294

Kadlec RH, Wallace SD (2009) Treatment wetlands. CRC Press, Boca Raton, FL

Kayranli B, Scholz M, Mustafa A, Hedmark Å (2010) Carbon Storage and Fluxes within Freshwater Wetlands: a Critical Review. Wetlands 30:111–124. doi: 10.1007/s13157-009-0003-4

Lenton TM, Watson AJ (2000) Redfield revisited: 1. Regulation of nitrate, phosphate, and oxygen in the ocean. Glob Biogeochem Cycles 14:225–248

Marquet PA, Quinones RA, Abadas S, et al (2005) Scaling and power-laws in ecological systems. J Exp Biol 208:1749–1769. doi: 10.1242/jeb.01588

McClain M, Boyer E, Lisa Dent C, et al (2003) Biogeochemical Hot Spots and Hot Moments At the Interface of Terrestrial and Aquatic Ecosystems. Ecosystems 6:301–3012. doi: 10.1007/s10021-003-0161-9

Melillo JM, Aber JD, Linkins AE, et al (1989) Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Ecol Arable Land — Perspect Chall 53–62

Neto RR, Mead RN, Louda JW, Jaffé R (2006) Organic Biogeochemistry of Detrital Flocculent Material (Floc) in a Subtropical, Coastal Wetland. Biogeochemistry 77:283–304. doi: 10.1007/s10533-005-5042-1

Newman S, Kumpf H, Laing JA, Kennedy WC (2001) Decomposition responses to phosphorus enrichment in an Everglades (USA) slough. Biogeochemistry 54:229–250. doi: 10.1023/A:1010659016876

Newman S, Osborne TZ, Hagerthey SE, et al (2017) Drivers of landscape evolution: multiple regimes and their influence on carbon sequestration in a sub-tropical peatland. Ecol Monogr 87:578–599. doi: 10.1002/ecm.1269

Newman S, Schuette J, Grace JB, et al (1998) Factors influencing cattail abundance in the northern Everglades. Aquat Bot 60:265–280. doi: 10.1016/S0304-3770(97)00089-2

Noe GB, Childers DL, Jones RD (2001) Phosphorus Biogeochemistry and the Impact of Phosphorus Enrichment: Why Is the Everglades so Unique? Ecosystems 4:603–624. doi: 10.1007/s10021-001-0032-1

Noe GB, Scinto LJ, Taylor J, et al (2003) Phosphorus cycling and partitioning in an oligotrophic Everglades wetland ecosystem: a radioisotope tracing study. Freshw Biol 48:1993–2008. doi: 10.1046/j.1365-2427.2003.01143.x

Osborne TZ, Bruland GL, Newman S, et al (2011) Spatial distributions and eco-partitioning of soil biogeochemical properties in the Everglades National Park. Environ Monit Assess 183:395–408. doi: 10.1007/s10661-011-1928-7

Pan Y, Stevenson RJ, Vaithiyanathan P, et al (2000) Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, U.S.A. Freshw Biol 44:339–353. doi: 10.1046/j.1365-2427.2000.00556.x

Reddy KR, DeLaune RD (2008) Biogeochemistry of wetlands: science and applications. CRC Press, Boca Raton, FL

Reddy KR, DeLaune RD, DeBusk WF, Koch MS (1993) Long-term nutrient accumulation rates in the Everglades. Soil Sci Soc Am J 57:1147–1155

Reddy KR, Kadlec RH, Flaig E, Gale PM (1999) Phosphorus Retention in Streams and Wetlands: A Review. Crit Rev Environ Sci Technol 29:83–146. doi: 10.1080/10643389991259182

Redfield AC (1934) On the proportions of organic derivations in seawater and their relation to the composition of plankton (reprint). Benchmark Pap Ecol 1:

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:230A–221

SFWMD (2015) Chemistry Laboratory Quality Manual. South Florida Water Management District, West Palm Beach, FL

South Florida Water Management District (2013) Restoration Strategies Regional Water Quality Plan: Science Plan for the Everglades Stormwater Treatment Areas. South Florida Water Management District, West Palm Beach, FL

Spears BM, Carvalho L, Perkins R, Paterson DM (2008) Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. Water Res 42:977–986. doi: 10.1016/j.watres.2007.09.012

University of Florida Wetland Biogeochemistry Laboratory (2017) Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment areas: 2016-2017 Annual Report. University of Florida, Gainesville, FL

Verhoeven JTA, Arheimer B, Yin C, Hefting MM (2006) Regional and global concerns over wetlands and water quality. Trends Ecol Evol 21:96–103. doi: 10.1016/j.tree.2005.11.015

Vito M, Muggeo R (2003) Estimating regression models with unknown break-points. Stat Med 22:3055–3071. doi: 10.1002/sim.1545

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biol Rev 81:259–291. doi: 10.1017/S1464793106007007

Wright AL, Reddy KR (2001) Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. Soil Sci Soc Am J 65:588–595

Wymore AS, Brereton RL, Ibarra DE, et al (2017) Critical zone structure controls concentration-discharge relationships and solute generation in forested tropical montane watersheds. Water Resour Res 53:6279–6295. doi: 10.1002/2016WR020016

Xu X, Thornton PE, Post WM (2013) A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Glob Ecol Biogeogr 22:737–749. doi: 10.1111/geb.12029

Figures and Tables

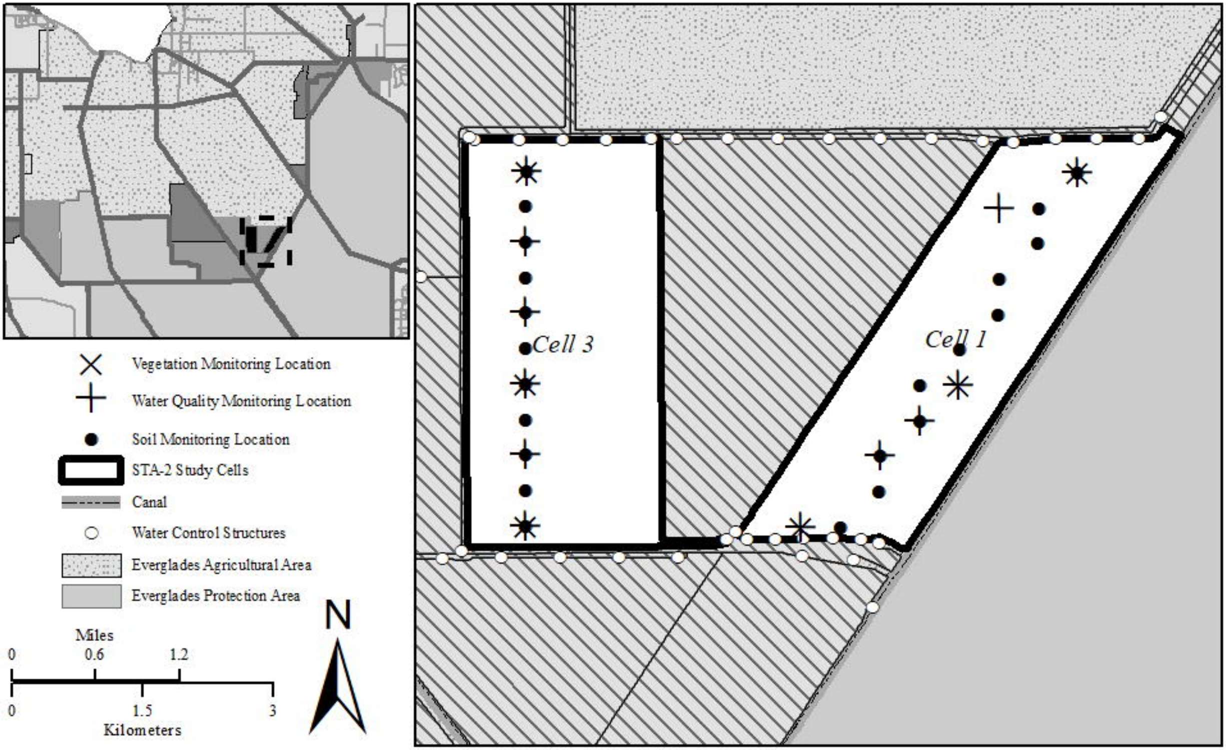
Figure 1. Surface water, soil and vegetation monitoring locations within Everglades Stormwater Treatment Area-2 cells 1 (right) and 3 (left). Cell 1 is predominately emergent vegetation and Cell 3 is predominately submerged aquatic vegetation. Operationally these cells are identified as flow-way 1 and 3, respectively.

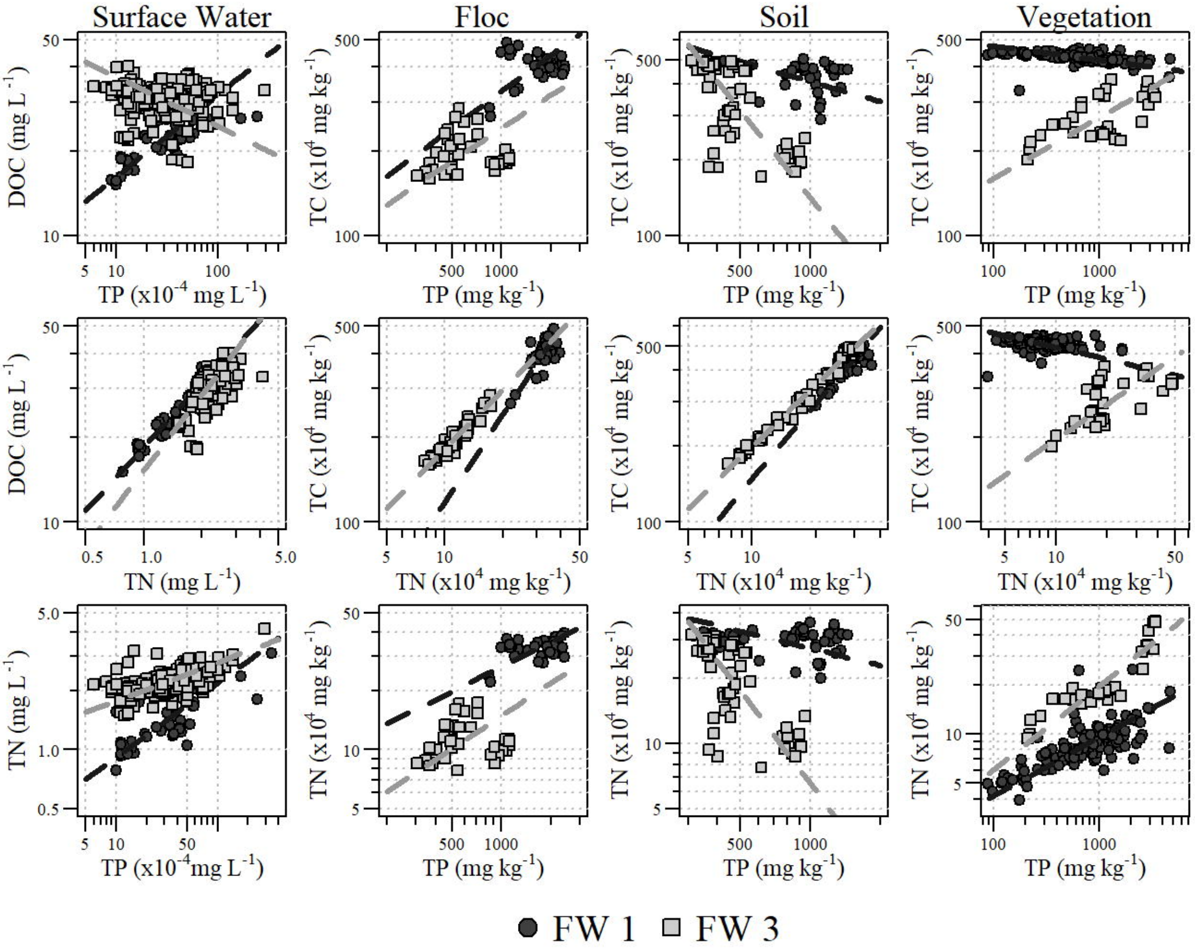
Figure 2. Relationships between carbon (organic carbon in surface water), nitrogen and phosphorus in surface water, soil flocculent material (floc), recently accreted soil, and dominate vegetation live aboveground biomass for STA-2 flow-ways (FWs) 1 and 3. Standardized major axis (SMA) regressions indicated by dashed-lines through the data. Data are represented as mass per volume (surface water only) and mass per mass (floc, soil and vegetation) for ease of viewing, all analyses were performed on molecular concentrations (i.e. mM or mmol kg^{-1}. Values can be converted to molecular concentration by dividing each value by its respective conversion factor (C = 12.01; N = 14.00; P = 30.97).

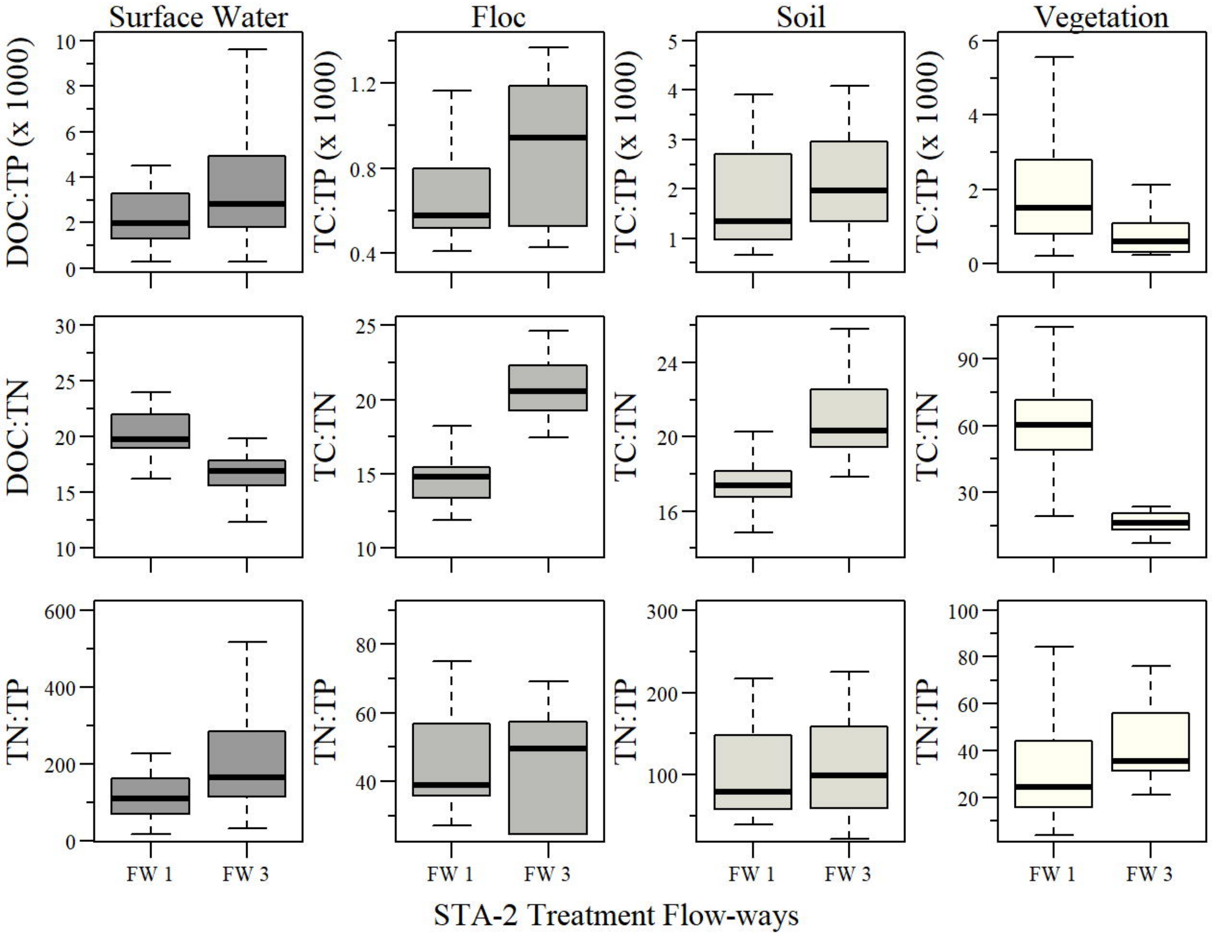
Figure 3. Boxplot comparing stoichiometric relationships between study flow-ways (FWs) for surface water, soil flocculent material (floc), recently accreted soil, and dominate vegetation live aboveground biomass.

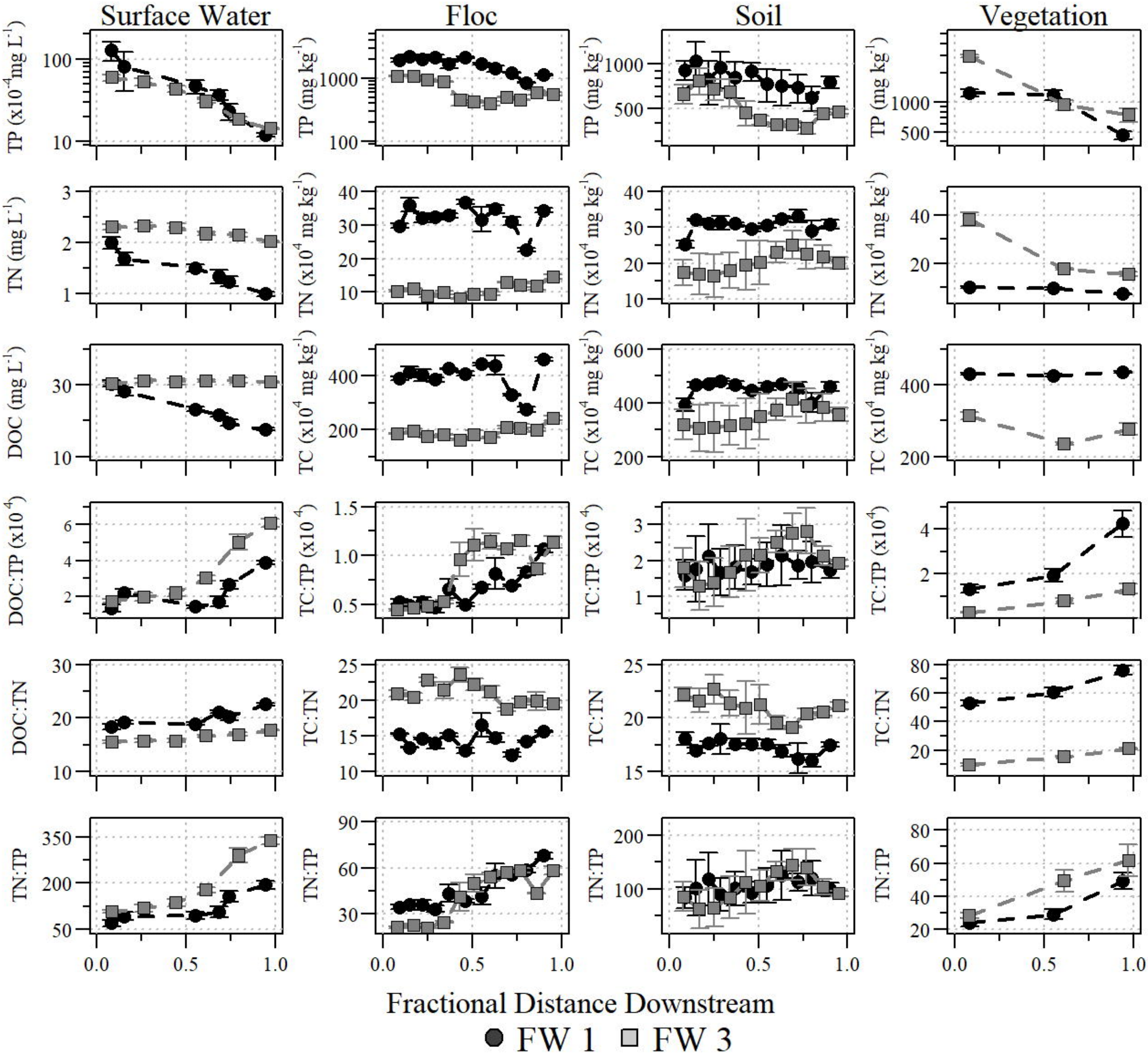
Figure 4. Mean ± standard error phosphorus (TP), nitrogen (TN) and associated molar ratios by fractional distance downstream for STA-2 flow-ways (FWs) 1 and 3. Carbon is expressed as dissolved organic carbon (DOC) for surface water and total carbon (TC) for soil flocculent material (floc), recently accreted soil and vegetation live aboveground biomass. Note scales differ across each ecosystem compartment and parameter.

Figure 5. Comparison of floc and soil TN:TP molar ratio with location along the flow-way identified by size of point (i.e. larger point further down flow path) and distance categories.


Cell 3
Cell 1
Vegetation Monitoring Location
Water Quality Monitoring Location
Soil Monitoring Location
STA-2 Study Cells
Canal
Water Control Structures
Everglades Agricultural Area
Everglades Protection Area
Miles
0
0.6
1.2
0
1.5
3
Kilometers
N


Surface Water
Floc
Soil
Vegetation
DOC (mg L^{-1})
TC (x10^4 mg kg^{-1})
TN (mg L^{-1})
TN (x10^4 mg kg^{-1})
TP (x10^{-4} mg L^{-1})
TP (mg kg^{-1})
TN (mg L^{-1})
TN (x10^4 mg kg^{-1})
50
10
500
100
5
10
100
500
1000
0.5
1.0
5.0
50
FW 1
FW 3


Surface Water
Floc
Soil
Vegetation
DOC:TP (x 1000)
TC:TP (x 1000)
TC:TP (x 1000)
TC:TP (x 1000)
DOC:TN
TC:TN
TC:TN
TC:TN
TN:TP
TN:TP
TN:TP
TN:TP
0
2
4
6
8
10
0.4
0.8
1.2
1
2
3
4
5
0
2
4
6
10
15
20
25
30
10
15
20
25
16
20
24
30
60
90
0
200
400
600
40
60
80
100
200
300
20
40
60
80
100
FW 1
FW 3
STA-2 Treatment Flow-ways


Surface Water
Floc
Soil
Vegetation
TP (x10^{-4}mg L^{-1})
TP (mg kg^{-1})
TN (mg L^{-1})
TN (x10^{4} mg kg^{-1})
DOC (mg L^{-1})
TC (x10^{4} mg kg^{-1})
DOC:TP (x10^{4})
TC:TP (x10^{4})
DOC:TN
TC:TN
TN:TP
0.0
0.5
1.0
Fractional Distance Downstream
● FW 1
□ FW 3

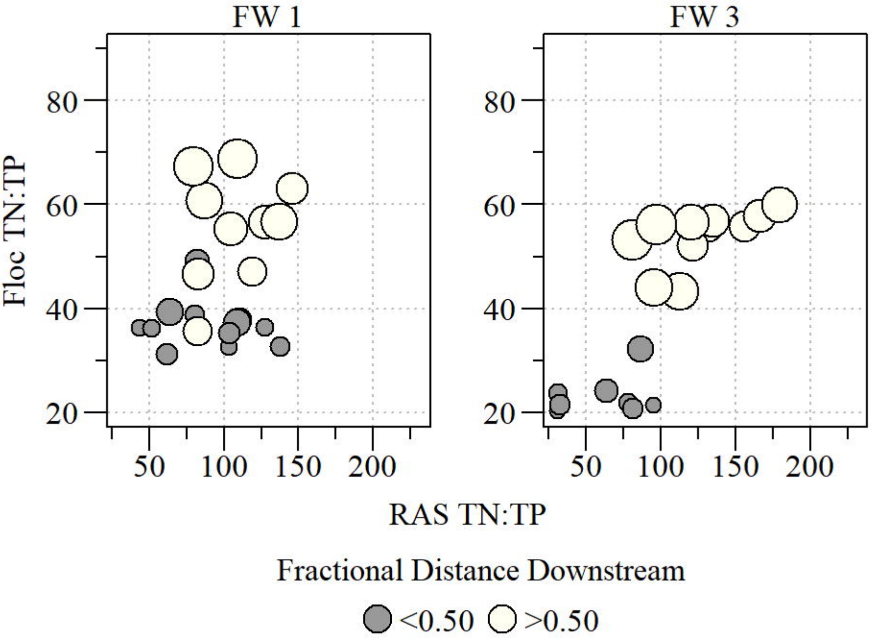

FW 1
FW 3
Floc TN:TP
80
60
40
20
50
100
150
200
RAS TN:TP
Fractional Distance Downstream
<0.50
>0.50

Table 1. Summary of parameters, matrices, analytical methods and minimum detection limit (MDL) used for this study. Additional parameters were collected as part of the larger study University of Florida Wetland Biogeochemistry Laboratory (2017) but not used in this study. All analytical methods are consistent with Florida Department of Environmental Protection or U.S. Environmental Protection Agency Standard Operating Procedures and methods.

Matrix	**Parameter**	**Abbreviation**	**Analytical Method**	**Minimum Detection Limit**	**Method**
Surface Water	Total Phosphorus	TP	SM4500PF	2 µg P L^{-1}	Clesceri et al. (1998)
	Total Nitrogen	TN	SM4500NC	0.02 mg N L^{-1}	Clesceri et al. (1998)
	Dissolved Organic Carbon	DOC	SM5310B	0.8 mg C L^{-1}	Clesceri et al. (1998)
Soil and Vegetation	Loss-on-ignition[1,2]	LOI	Calculation[2]	1.0 %	---
	Total Phosphorus	TP	SM4500PF	16 mg P kg^{-1}	Clesceri et al. (1998)
	Total Nitrogen	TN	SFWMD 3200	2 g N kg^{-1}	SFWMD (2015)
	Total Carbon	TC	SFWMD 3200	2 g C kg^{-1}	SFWMD (2015)

[1] Loss-on-ignition was assessed for soil components only.

[2] Loss-on-ignition was calculated from the difference between 100% and percent ash determined by the analytical method identified as SFWMD 1610 (SFWMD 2015).

Table 2. Summary statistics for parameters and matrices used in this study of samples collected along the Flow ways 1 and 3 flow-path transect within Stormwater Treatment Area-2. Summary statistics include mean, standard error, range, and coefficient of variance. Matrices include surface water, soil flocculent material, recently accreted soil and living aboveground biomass of sampled vegetation. Stoichiometric ratios are expressed as molar ratios and are unitless. (DOC = Dissolved Organic Carbon; TP = Total Phosphorus; TN = Total Nitrogen; TC = Total Carbon; LOI = Loss-On-Ignition).

		Flow-way 1			**Flow-way 3**		
Matrix	**Parameter (Units)**	**Mean ± SE**	**Range**	**CV (%)**	**Mean ± SE**	**Range**	**CV (%)**
Surface Water	DOC (mg L^{-1})	23.2 ± 0.7	15.1 - 35.6	23.6	30.9 ± 0.2	18.2 - 40.2	10.7
	TP (µg L^{-1})	57.4 ± 9.7	9.0 – 378	142.1	35.0 ± 1.8	6.0 – 293	82.3
	TN (mg L^{-1})	1.5 ± 0.06	0.8 - 3.1	32.4	2.2 ± 0.02	1.5 - 4.1	13.7
	DOC:TP	2271± 138	280 - 4527	51.1	3545 ± 141	290 - 14613	66.3
	DOC:TN	20.1 ± 0.3	12.8 – 24.0	10.8	16.6 ± 0.1	9.3 – 19.8	10.4
	TN:TP	118 ± 7.7	16.3 - 342	54.4	206 ± 7.7	31.2 - 788	60.4
Floc	LOI (%)	76.4 ± 2.1	38.8 – 91.4	15.8	22.7 ± 1.2	12.1 - 40.5	32.1
	TC (g kg^{-1})	406 ± 8.6	265 – 488	12.3	199 ± 5.4	160 – 283	15.9
	TP (mg kg^{-1})	1698 ± 81.8	844 – 2436	28.1	642.1 ± 43.8	307 - 1118	40.3
	TN (g kg^{-1})	32.8 ± 0.7	22.0 - 39.4	11.9	11.4 ± 0.4	7.8 - 17.4	22.5
	TC:TP	671 ± 38.6	410 - 1165	33.5	919 ± 55.0	426 - 1369	35.4
	TC:TN	14.5 ± 0.2	11.9 – 18.2	9.3	20.7 ± 0.3	17.4 – 24.6	8.6
	TN:TP	46.0 ± 2.3	27.1 -74.9	29.5	44.9 ± 2.8	20.4 -69.2	36.3
Soil	LOI (%)	81.2 ± 1.4	48.6 – 91.0	12.0	54.2 ± 3.4	16.5 - 85.3	44.4
	TC (g kg^{-1})	445 ± 7.1	290 – 504	11.4	350 ± 16.4	171 – 495	33.4
	TP (mg kg^{-1})	826 ± 48.7	318 – 1449	42.1	509 ± 25.6	312 – 947	35.9
	TN (g kg^{-1})	29.9 ± 0.5	19.9 - 36.2	11.7	20.2 ± 1.1	7.7 - 31.8	39.4
	TC:TP	1777 ± 141	671 - 3917	56.8	2069 ± 145	534 – 4091	50.0
	TC:TN	17.4 ± 0.2	13.6 - 21.1	7.8	20.9 ± 0.3	17.9 – 25.8	9.5
	TN:TP	100 ± 7.5	39.5 – 217	53.2	103 ± 8.0	22.3 – 225	55.1
Vegetation	TC (g kg^{-1})	431 ± 1.7	329 - 464	4.3	272 ± 8.5	186 – 360	18.1
	TP (mg kg^{-1})	989 ± 76	87.2 - 4694	83.1	1435 ± 182	210 – 3378	72.7
	TN (g kg^{-1})	8.9 ± 0.3	4.0 - 24.6	36.7	22.5 ± 1.9	9.4 - 48.7	49.2
	TC:TP	2457 ± 243	205 – 13013	107	845 ± 116	237 - 2487	79.1
	TC:TN	63.0 ± 1.9	19.3 – 117	31.9	16.1 ± 0.8	7.5 – 23.7	30.1
	TN:TP	33.7 ± 2.3	3.9 – 126	74.0	48.1 ± 4.8	12.3 – 120	57.4

Table 3. Standardized major axis (SMA) results of log transformed molar nutrient concentrations and Spearman's rank sum correlation results of molar nutrient concentrations within each ecosystem compartment along flow-ways (FWs) 1 and 3 within Stormwater Treatment Area-2. Bold ρ-values indicate SMA models where slope was not significantly different than one and Spearman's rank sum correlation results that are not statistically significant.

					SMA			**Spearman's**	
Y	**X**	**Compartment**	**Flow Way**	**R^2**	**Slope**	**Intercept**	**ρ-value**	**r**	**ρ-value**
DOC	TP	Surface Water	FW 1	0.50	0.29	2.65	<0.01	0.74	<0.01
			FW 3	0.02	-0.18	-0.30	<0.01	-0.14	<0.05
TC	TP	Floc	FW 1	0.04	0.43	8.71	<0.01	-0.20	**0.27**
			FW 3	0.001	0.39	8.55	<0.01	0.19	**0.27**
		Soil	FW 1	0.16	-0.26	11.35	<0.01	-0.52	<0.01
			FW 3	0.44	-1.17	13.43	**0.15**	-0.60	<0.01
		Vegetation	FW 1	0.23	-0.05	10.64	<0.01	-0.69	<0.01
			FW 3	0.41	0.22	9.24	<0.01	0.55	<0.01
DOC	TN	Surface Water	FW 1	0.89	0.75	2.42	<0.01	0.94	<0.01
			FW 3	0.34	0.89	2.61	<0.01	0.58	<0.01
TC	TN	Floc	FW 1	0.55	1.04	2.35	**0.73**	0.55	<0.01
			FW 3	0.90	0.70	5.01	<0.01	0.93	<0.01
		Soil	FW 1	0.65	1.00	2.82	**0.96**	0.40	<0.01
			FW 3	0.99	0.82	4.36	<0.01	0.98	<0.01
		Vegetation	FW 1	0.03	-0.14	11.39	<0.01	-0.40	<0.01
			FW 3	0.42	0.42	6.93	<0.01	0.62	<0.01
TN	TP	Surface Water	FW 1	0.57	0.37	0.31	<0.01	0.69	<0.01
			FW 3	0.28	0.20	-0.49	<0.01	0.50	<0.01
		Floc	FW 1	0.18	0.42	6.10	<0.01	0.31	**0.07**
			FW 3	0.0004	0.56	5.03	<0.01	0.18	**0.30**
		Soil	FW 1	0.01	-0.26	8.49	<0.01	-0.008	**0.96**
			FW 3	0.45	-1.43	11.11	<0.01	-0.58	<0.01
		Vegetation	FW 1	0.54	0.36	5.28	<0.01	0.74	<0.01
			FW 3	0.81	0.51	5.47	<0.01	0.88	<0.01

Table 4. Comparison by Kruskal-Wallis rank sum test of stoichiometric ratios between flow-ways 1 and 3 for surface water, soil flocculent material (floc) and recently accreted soil ecosystem compartments. Statistically significant comparison identified by italicized ρ-values.

Ecosystem Compartment	Ratio	χ^2	ρ-value
Surface Water	DOC:TP	10.9	*<0.01*
	DOC:TN	74.3	*<0.01*
	TN:TP	22.1	*<0.01*
Floc	TC:TP	7.89	*<0.01*
	TC:TN	50.66	*<0.01*
	TN:TP	0.0005	0.98
Soil	TC:TP	1.76	0.18
	TC:TN	0.13	0.72
	TN:TP	60.12	*<0.01*

Table 5. Flow way assessment of carbon (dissolved organic carbon [DOC] for surface water and total carbon [TC] for floc and soil), total nitrogen (TN) and total phosphorous (TP) stoichiometric ratios along flow-ways 1 and 3 of STA-2. Spearman's rank sum correlation and Thiel-Sen Slope estimate results summarized by flow way and ecosystem compartment.

		Flow-way 1			Flow-way 3		
Compartment	**Parameter**	**Spearman's ρ**	**ρ-value**	**Theil-Sen Slope**	**Spearman's ρ**	**ρ-value**	**Theil-Sen Slope**
Surface Water	DOC:TP	0.8	0.06	198	1.0	<0.01	326
	DOC:TN	0.9	<0.01	0.3	0.9	<0.05	0.2
	TN:TP	1.0	<0.05	133	1.0	<0.01	260
Floc	TC:TP	0.8	<0.01	536	0.8	<0.01	799
	TC:TN	0.1	0.86	0.2	-0.6	0.7	-2.6
	TN:TP	0.9	<0.01	41.7	0.9	<0.01	54.9
Soil	TC:TP	0.4	0.25	225	0.6	<0.05	1389
	TC:TN	-0.7	<0.05	-1.2	-0.7	<0.05	-1.8
	TN:TP	0.4	0.18	32.9	0.6	0.06	90.9