Title: Stoichiometric relationships amongst ecosystem compartments of a subtropical treatment wetland.

Running Header: Wetland Stoichiometry

Paul Julian II[1], Stefan Gerber[2], Rupesh Bhomia[2], Jill King[3], Todd Z. Osborne[4,5], Alan L. Wright[1]

[1] University of Florida, Soil and Water Sciences Department, Ft. Pierce, FL 34945
*Corresponding Author: pjulian@ufl.edu; ORCID: 0000-0002-7617-1354
[2] University of Florida, Soil and Water Sciences Department, Gainesville, FL, 32611

[3] South Florida Water Management District, Water Quality Treatment Technologies, West Palm Beach, FL, 33406

[4]University of Florida, Whitney Laboratory for Marine Bioscience, St Augustine, FL 32080

Abstract

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 decomposition of organic matter. This study investigated wetland nutrient stoichiometry within water column, soil flocculent material, recently accreted soil, and dominate vegetation within two treatment cells of the Everglades Stormwater Treatment Area, south Florida (USA). These cells 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 *Potamgeton* spp. (pondweed). Unlike results of prior studies, this study demonstrated that C, N, and P stoichiometry can be highly variable within and between wetland ecosystem compartments in a P-limited ecosystem. Generally, total P declined along the flow path in all ecosystem compartments, whereas trends in total N and C were not consistent. Meanwhile, TN:TP relationships increased as C:N and C:P varied in various compartments along the treatment flow paths. Assessment of wetland nutrient stoichiometry between and within ecosystem compartments suggest decoupling of organic matter decomposition from nutrient mineralization which may have significant influences on nutrient removal rates and contrasting dominate vegetation communities. This information could be used to further understand water treatment performance of these constructed wetlands.

Keywords: decomposition, mineralization, Everglades, treatment wetlands

Introduction

The study of nutrient stoichiometry, pioneered by Redfield (1934) and Redfield (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 C:N:P is 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).

In general, wetlands are net C sinks that store a large amount of the global C driven by a disproportionate accumulation of C via plant productivity and export from decomposition of organic matter (Billett and Moore 2008). 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 wetlands has a potential to disrupt the ecological balance and substantially affect nutrient cycling. Long-term

nutrient enrichment in wetlands can affect OM decomposition rates by increasing microbial productivity leading to accelerated rates of C mineralization and nutrient cycling (Qualls and Richardson 2000). Microbial growth on low-nutrient substrates (i.e. high C:N or C:P) generally results in the net immobilization of growth-limiting nutrients through scavenging nutrients from the ambient environment (Reddy and DeLaune 2008). External inputs of nutrients such as P can cause alteration in stoichiometric balance of an ecosystem and could decrease N to P ratio in ecosystem compartments (Zhan et al. 2017) and subsequently altering microbial decomposition of OM.

Cultural enrichment of natural systems significantly influences OM accumulation and overall systems ecology through shifts in plant and algal communities which in turn alters the OM feedback loop through changes the dynamics of the litterfall-decomposition continuum (Pan et al. 2000). Enrichment allows for nutrient tolerant species to establish and thrive while reducing the overall coverage of nutrient sensitive species leading to cascading effects on entire ecosystem structure and functions. In the Everglades ecosystem, stormwater run-off has accelerated soil accretion dynamics, and the spread of *Typha* spp. (cattail) have been well documented. Because of nutrient inputs into these wetlands, Everglades flora and fauna have been significantly impacted through widescale encroachment of cattails, loss of calcareous periphyton which were indicative of oligotrophic conditions, 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 in an effort to improve water quality conditions for drainage waters entering the remnant Everglades (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 water entering the Everglades ecosystem from the Everglades Agricultural Area (EAA). The STAs are configured as treatment cells using 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). Previous 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). Given the role of OM accumulation and decomposition in wetland ecosystems nutrient stoichiometry is an important to understand the cycling and controls of nutrient removal. Given the primary objective of the STAs and the role they play as biogeochemical hotspots, this study will evaluate stoichiometry relationships within treatment wetlands. The studies objectives are:

(1) Evaluate surface water, soil flocculent material (floc), recently accreted soil (RAS) and vegetation living aboveground biomass (AGB) stoichiometric relationship within two treatment wetland cells, one dominated by emergent aquatic vegetation (EAV) and the other dominated by submerged aquatic vegetation (SAV).
(2) Evaluate changes in nutrient stoichiometry in surface water, floc and RAS along each flow way transect.

During the evaluation of the studies objectives several hypotheses were developed to further understand the stoichiometric processes within and between treatment wetlands. The studies hypotheses are:

(1) Along a treatment cell, ecosystem compartment stoichiometry will be tightly constrained due to the accumulation and decomposition of organic matter.

(2) Shifts in stoichiometry is likely to occur along the flow path due to differential removal processes and storage processes.

(3) Due to differences in vegetation and associated aquatic biogeochemistry, nutrient stoichiometry in surface water, floc, RAS and living-AGB will differ between flow paths.

Methods

Study Area

Large freshwater treatment wetlands, known as the Everglades STAs, utilized to reduce P loads entering the Everglades ecosystem to preserve and protect the remaining Everglades ecosystem (Chimney 2017). A total of six STAs with an approximate area of 18,000 ha are located south of Lake Okeechobee in the southern portion of the EAA (Fig 1). Prior land uses within the current STA boundary 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 284,000 ha of farmland. Everglades STA treatment cells are comprised of mixtures of EAV and SAV communities in several configurations including EAV and SAV treatment cells arranged in parallel or serial (Chen et al. 2015).

Stormwater Treatment Area-2 has been in operation since June 1999 and is designed to provide a total effective treatment area of approximately 6,270 ha, comprised of a total of eight cells. This study was conducted in two cell, cells 1 and 3. The vegetative community of cell 1 is comprised predominately of EAV vegetation including *Typha domingensis* Pers. (cattail) and *Cladium jamaicense* Crantz (sawgrass) while cell 3 is dominantly SAV vegetation including *Chara* spp. (muskgrass), *Potamgeton* spp. (pondweed) and *Najas guadalupensis* Spreng (southern naiad) with approximately a third of the cell occupied by EAV species Furthermore, prior to being a

part of STA-2, cell 1 was an historic natural wetland while approximate two-thirds of cell 3 was previously farmed and is now managed as a SAV system (Juston and DeBusk 2006).

Data Source

Monitoring locations were established along two flow paths within STA-2 along a transect running from inflow to the outflow of the cell. Weekly surface water grab samples were collected at monitoring locations within cell 1 and 3 to characterize changes in nutrient concentrations and availability during prescribe/semi-managed flow events (Fig 1). When adequate water was available within the water management systems, flow events were scheduled and cycled through various flow/no-flow sequences for cells 1 and 3. Water column parameters assessed for this study include total P (TP), total N (TN), and dissolved organic carbon (DOC). Soil samples were also collected along the flow transects twice during the dry and wet seasons throughout the course of this study. Soils were sampled using the push-core method by a 50-cm long polycarbonate coring tube (10-cm internal diameter) consistent with methods used in prior wetland soil studies (Newman et al. In Press; Bruland et al. 2007; Osborne et al. 2011). Samples were extruded from the soil core tube and partitioned into soil flocculent material (floc) and recently accreted soil (RAS). Soil samples were analyzed for loss-on-ignition, TP, TN, TC and total calcium (TCa). Living and senescent aboveground biomass (AGB) were sampled from dominant vegetation in cell 1 while only living aboveground biomass was sampled from cell 3 at the end of the 2015 (November 2015) and 2016 (September 2016) wet seasons. Vegetations samples were collected from four to eight randomly placed 0.25 m^2 quadrats adjacent to the identified sampling location. Vegetation sampling locations were located in inflow, mid and outflow regions of the cells within close proximity to the surface water and soil monitoring

locations (Fig 1). Dry homogenized vegetation was analyzed for TP, TN and TC 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 cell. For purposes of this data analysis and summary statistics, data reported as less than method detection limit (MDL) were set to the MDL.

Data Analysis

Hydrologic and P loading were calculated consistent with Kadlec and Wallace (2009). Inflow and outflow structure daily discharge rate was determined using the difference between head-water (upstream) and tail-water (downstream) stage elevations in combination with structure geometry. Weekly surface water grab total phosphorus samples were collected at inflow and outflow structures and used to estimate inflow and outflow load amounts. Hydraulic loading rate (HLR) was estimated by dividing flow volume and cell area for each cell. Phosphorus loading rate (PLR) was estimated using daily TP load divided by cell area.

C:N:P Stoichiometric Relationships

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 a molecular concentration per mass of soil (i.e. mmol kg^{-1}). Relationships among surface water, soil, floc and vegetation nutrients were examined by log-log regression using Siegel repeated median's linear model (Siegel 1982) ('mblm' package). All parameters were log transformed

prior to analysis. Log-Log regression is commonly used in allometric analyses of growth and biomass where a slope equal to one indicates isometric or linear scaling. 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).

Surface water (DOC:TP, DOC:TN and TN:TP), soil and floc molar ratios (TC:TP, TC:TN and TN:TP) were compared between cells by Kruskal-Wallis rank sum test. To characterize the relationship between floc and soil along the treatment cells, fraction distance downstream was broken into two categories, inflow to mid-point regions (<0.5) and mid-point to outflow regions (>0.5). Soil and floc TN:TP were compared between cells and distance downstream by Kruskal-Wallis rank sum test, separately. Floc and RAS TN:TP were also compared by spearman's rank sum test by region separately.

Flow path Gradient Analysis

Longitudinal change point analysis was performed on the mean surface water, floc and soil nutrient stoichiometric ratios for data collected in cells 1 and 3. Surface water nutrient ratios considered were TN:TP, OC:TP, and OC:TN, similarly floc and RAS 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). Stoichiometry change-point along the flow way transect was evaluated using Davies' difference-in-slope test ('segmented' package)(Vito and Muggeo 2003; Muggeo 2008). Despite only three sampling locations along the flow path transect (Fig 1), spearman's rank sum correlation test was used to assess changes in absolute nutrient concentrations (i.e. TP, TN and TC) and molar ratio (TC:TP, TC:TN and

TN:TP) along each respective flow path in the vegetation living-AGB of vegetation sampled along the transect.

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 α=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 between cells 1 and 3 within STA-2. During the flow events, daily HLR ranged between 0 (no inflow) to 33.7 cm d^{-1} with cell 3 receiving relatively higher mean HLR of 3.2 ± 0.1 cm d^{-1} and higher maximum HLR of 33.7 cm d^{-1}. Cell 1 achieved a mean HLR of 2.7 ± 0.07 cm d^{-1} with a maximum HLR of 23.7 cm d^{-1}. Observed daily PLR values ranged from 0 (no loading) to 92.9 mg m^{-2} d^{-1} with Cell 3 received a higher relative load with a mean PLR of 3.2 ± 0.1 mg m^{-2} d^{-1} and experiencing the highest maximum PLR rate. Cell 1 achieved a mean PLR of 2.6 ± 0.01 mg m^{-2} d^{-1} with a maximum PLR of 66.1 mg m^{-2} d^{-1}. A summary of flow event characteristic can be found in Supplemental Table 1. The daily HLR and PLR observed during this study was consistent with historic operational loading rates experienced for these cells to date (Chen et al. 2015).

Water column C:N:P dynamics

During this study DOC ranged from 15.1 to 40.2 mg C L^{-1}, TP ranged from 6 to 378 µg P L^{-1} and TN ranged from 0.78 to 4.14 mg N L^{-1} between the two study cells. Molar ratios of DOC to TP ranged from 18.7 to 974.3, DOC to TN ranged from 0.6 to 1.6 and TN to TP ranged from 16.3 to

788.7 (Table 2). Qualitatively, mean DOC, TN and TP concentrations were relatively comparable (Table 2), as expected since these cells receive the same source water but experience slightly different loading (HLR and PLR) 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 none of the nutrient pools proportionally scale. The models between the two cells diverge drastically in most cases except for the DOC-TN relationship which is relatively constrained (Fig 2) as indicated by the similar slopes and intercepts between the two cells (Table 3). Stoichiometric relationships associated with TP (i.e. DOC:TP and TN:TP) were not tightly constrained driven largely by the extreme variability in weekly measurement (Table 2). Stoichiometric ratios of DOC:TP, DOC:TN and TN:TP (Table 4) were significantly different between the two cells with Cell 3 experiencing greater DOC:TP and TN:TP values and lower DOC:TN values (Table 2 and Fig 3).

Total P, TN and DOC concentrations were negatively correlated with distance downstream indicating a gradual decline in concentrations along the STA-2 Cell 1 flow way transect (Table 5 and Fig 4). Dissolved OC decline the most along the flow way transect followed by TN and TP (Table 5). Along with significant declines in concentrations along the flow path DOC:TN and TN:TP significantly increased with no significant change points detected (ρ=0.59 and ρ=0.22, respectively) along the flow way transect. (Table 5). However, DOC:TP did not significantly change along the flow path and no change point was detected (ρ=0.19) despite having the largest rate of increase between stoichiometric ratios (Table 5). Similar to cell 1, cell 3 TP and TN were negatively correlated with distance downstream with TN having the largest decline along the transects (Table 5). Meanwhile, in cell 3 DOC was not significantly correlated with distance downstream (Table 5). All surface water stoichiometric ratios were positively correlated with

distance downstream at a similar rate as cell 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 dynamics

Percent organic matter (OM), as indicated by loss-on-ignition (LOI) ranged from 8.6 to 87.9% across the cells with cell 3 having higher observed OM in the floc material (Table 2). Despite the higher OM, cell 3 floc material was more mineral as indicated by TCa concentrations several orders of magnitude greater the than concentrations observed in cell 1 (Table 2). Meanwhile, floc TC concentrations ranged from 160 to 448 g kg^{-1} (13,321 to 40,630 mmol kg^{-1}), TP concentrations ranged from 307 to 2,436 mg kg^{-1} (9.9 to 78.6 mmol kg^{-1}) and TN concentration ranged from 7.8 to 39.4 g kg (651 to 3,280 mmol kg^{-1}) across cells 1 and 3. Floc molar ratios of TC to TP ranged from 410.9 to 1,369.2, TC to TN ranged from 10.2 to 21.1 and TN to TP ranged from 23.8 to 87.4 (Table 2). Generally, TP concentration was the most variable parameter between and within sites with coefficients of variance reaching as high as 78% at any given site but overall cell 3 exhibited the highest overall coefficient of variance with 27% spatial and temporal variability (Table 2).

Floc TC-TN and TN-TP models for both cells and the TC-TP model for cell 3 resulted in slopes significantly different from one while the TC-TP slope for cell 1 was not significantly different than one, indicating TC and TP scale proportionally (Table 3). Similar to surface water stoichiometry models, floc stoichiometry models deviate drastically except for the TC-TN relationships which are nearly identical between the two cells and fall along a continuum (Fig 2) with cell 1 generally having greater TN concentrations in the floc compartment (Table 2). Floc TC:TP and TC:TN stoichiometry significantly differ between cells (Table 4) with cell 3 having higher TC:TN and TC:TN ratio (Fig 3), suggesting TP and TN are less constrained relative to

TC. Meanwhile, floc TN:TP stoichiometry was not significantly different between cells (Table 4) suggesting that even though TP concentrations are highly variable between and within cells TP and TN are tightly constrained.

Floc TP concentration was negatively correlated with distance downstream along the cell 1 transect at a rate of -1531.0 mg kg^{-1} $Distance^{-1}$ (Table 5) indicating a significant decline in TP concentration along the flow path. Meanwhile, floc TC and TN were not significantly correlated with distance downstream (Table 5). 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 cell 1 flow way transect (TC:TP ρ=0.13; TC:TN ρ=0.78; TN:TP ρ=0.07). Along the cell 3 flow way transect, TP, TN, TC, TC:TN were not significantly correlated with distance downstream (Table 5). While, floc TC:TP and TN:TP was positively correlated with distance downstream at rates of change comparable to that of cell 1 (Table 5 and Fig 4). Moreover, no change points were detected in along the cell 3 flow 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 dynamics

Much like the floc compartment, LOI values in the RAS compartment range from 9.0 to 83.5% across the study cells with cell 3 having a higher observed mean LOI value for RAS (45.8 ± 3.4 %; Table 2). Additionally, cell 3 soils were more mineral in nature with greater TCa concentrations relative to cell 1 soils (Table 2). Soil TC concentration tanged from 171 to 504 g kg^{-1}, TN ranged from 7.7 to 38.2 g kg^{-1} and TP ranged from 312 to 1449 mg kg^{-1} across cells 1 and 3 with cell 1 being generally more enriched with nutrients as indicated by qualitatively greater average concentrations (Table 2). Soil nutrient molar ratios are generally greater than

those observed in the floc compartment with TC:TP values ranging from 534.2 to 4,061.4, TC:TN ranged from 11.6 to 22.2 and TN:TP ranged from 26.0 to 262.8 (Table 2). Much like the floc compartment, TP was the most variable parameter with between and within site coefficients of variance reaching as high as 58% at any given site but overall cell 1 exhibited the highest overall coefficient of variance with 42% spatial and temporal variability (Table 2).

Soil TC-TP and TC-TN models for both cells and the TN-TP model for cell 3 resulted in slopes significantly different from one while the TN-TP model for cell 1 was not significantly different than one, indicating TN and TP for cell 1 scale proportionally (Table 3). Soil TC:TN stoichiometry significantly differed between cells (Table 4) with cell 3 having a greater TC:TN ratio (Fig 3). Meanwhile, TC:TP and TN:TP were not significantly different between cells (Table 4) indicating that despite the lack of proportional scaling for TC-TP and TN-TP, P is somewhat constrained along the flow path between the two cells.

Soil TP concentration was negatively correlated with distance downstream with no change point was detected within cell 1 (Table 5). Within cell 1, soil TC and TN were not significantly correlated with distance downstream (Table 5). Both TC:TP and TN:TP stoichiometry was not significantly correlated with distance downstream along the cell 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 cell 1 flow way transect (Table 5) with no change point detected (ρ=0.30). Along the cell 3 flow transect, TN and TC were positively correlated with distance downstream with a greater rate of change than cell 1 while TP was negatively correlated with distance downstream at a comparable rate of change downstream relative to cell 1 (Table 5). Soil TC:TP was positively correlated with distance downstream (Table 5) and a significant change point was detected along the cell 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 cell 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 cell 3 flow way transect (Estimate=0.71, $\rho<0.05$)

Floc and soil N:P molar ratios were significantly correlated for sites within cell 3 (r=0.85, $\rho<0.01$) with the data forming what appears to be an S-shaped potentially suggesting an pseudo equilibrium dynamics between floc and soil nutrient stoichiometry (Fig 5). 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 cell 3 between the two distance categories. Within cell 1, floc and soil N:P was not significantly correlated (r=0.28, ρ=0.21) and qualitatively the floc-soil relationship within cell 1 appears to be different than that of cell 3 where the upper arm of the S-curve is missing for cell 1 (Fig 5). The lack of an "upper arm" in the cell 1 floc-soil relationship could indicate the floc compartment has not reached saturation. Despite the difference in the floc-soil N:P relationships between the two cells, N:P molar ratios were significantly different between the two distance classes in cell 1 for floc (χ^2=19.44, $\rho<0.01$) and soil (χ^2=4.35, $\rho<0.05$).

Vegetation C:N:P dynamics

During the vegetation sampling within cell 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 cell 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 cells 1 and 3 ranged from 87.2 to 4,693.5 mg kg^{-1} with vegetation within cell 3 having higher absolute tissue TP concentrations (Table 2). Plant tissue TN

concentrations ranged from 4.0 to 48.7 g kg^{-1} and TC concentrations ranged from 186.0 to 464.0 g kg^{-1} with cell 3 having higher tissue TN and cell 1 having higher tissue TC concentrations (Table 2). Living AGB molar ratios of TC to TP ranged from 205.4 to 13,012.0, TC to TN ranged from 6.4 to 100.0 and TN to TP ranged from 4.6 to 146.4 with cell 1 having higher TC:TP and TC:TN ratios and cells 3 having 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 cells, cell 1 had a higher coefficient of variance with 83.1% and cell 3 having a coefficient of variance of 72.7%.

Much like the other ecosystem compartments living-AGB TC-TP, TC-TN and TN-TP models for cells 1 and 3 resulted in slopes significantly different than one (Table 3) suggesting that nutrient concentrations within vegetation do not proportionally scale. Between cells, TC-TP and TC-TN models appear to converge at the higher TP and TN concentrations, respectively (Fig 2). While TN-TP models between cells seem to gradually diverge from one another (Fig 2).

Living-AGB TP and TN concentration was negatively correlated while TC concentration was positively correlated with distance downstream along the cell 1 flow way transect (Table 5). Meanwhile, living-AGB TC:TP, TC:TN and TN:TP along the cell 1 flow wat transect was positively correlated with distance downstream (Table 5 and Fig 4). Similarly, living-AGB TP and TN concentration were negatively correlated with distance downstream along the cell 3 flow way transect (Table 5). Living-AGB TC concentration along the cell 3 flow way transect was not significantly correlated with distance downstream (Table 5). As observed along the cell 1 flow way transect TC:TP, TC:TN and TN:TP were positively correlated with distance downstream (Table 5 and Fig 4).

Discussion

Surface Water Stoichiometric Relationships

Redfield (1958) focused on the composition of inorganic fractions of C and nutrients of deep ocean waters. This emphasis on inorganic nutrients have carried forward because of the 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 of inorganic nutrient data can be problematic (Guildford and Hecky 2000; Dodds 2003). Furthermore, biogeochemical cycling of nutrients in freshwater ecosystems is influence by the ability of some biota to utilize both dissolved inorganic portion and the remaining fraction via enzymatic hydrolysis (Bergström 2010). Therefore 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)

Surface water stoichiometric relationships can be highly variable due to transport of nutrient into and through aquatic ecosystems. Aquatic stoichiometry is subject to landscape scale processes such as run-off where water column nutrients stocks can vary (i.e. increase-P), atmospheric depositional inputs and dilution from hydrologic inputs such as rainfall and hyporheic recharge. Furthermore, internal process such as DOC leaching, soil to water column nutrient fluxes, groundwater upwelling, and selective uptake also has the potential to influence aquatic stoichiometry. The total load of nutrients is considered to limit algal production and biomass while nutrient form (as discussed above) and the proportion (i.e. stoichiometry) can affect the species composition. Biotic feedbacks and regulatory mechanisms manifested as individual organisms' ability to maintain their stoichiometry under mass balance constraints while at the ecosystem level, growth of different species also modifies the physical and chemical

environment through alterations in physical habitat (e.g. turbidity, flow), light, oxygen, pH, as well as substrate availability (Glibert 2012). Therefore, it is expected that to some degree aquatic stoichiometry should be relatively constrained and consistent. However, biogeochemical feedbacks and regulatory processes interacting with the physical system alter stoichiometric relationships over very short spatial and temporal scales as see in this study (Fig 2).

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 flow ways (Fig 2 and 4) can be the result of biological or physical configurations of the treatment system. The extreme variability in aquatic stoichiometric relationships, especially DOC:TP could be attributed to vegetation mediated dynamics, nutrient uptake and OM decomposition (Fig 2). Cell 3 of STA-2 is a SAV dominated system where the sequestration of P is facilitated partly by the co-precipitation of calcite is a strong removal mechanism (Dierberg et al. 2002). Meanwhile, cell 1 is an EAV dominated system where particle entrainment and P mining from soils by vegetation and microbial decomposition are dominate removal mechanisms (Reddy and DeLaune 2008). This difference in P removal mechanisms could explain the variability in the water column DOC:TP relationship and to some extent TN:TP where cell 3 is less reliant on microbial decomposition but rather SAV-geochemical mediated P removal (Fig 2). Another line of evidence suggesting differential removal resulting in variable stoichiometric relationships is the distance downstream trend in DOC concentrations where DOC declines along the flow path in cell 1 while cell 3 remains relatively constant (Fig 4) indicating microbial

consumption of DOC trough decomposition of dissolved OM (DOM) within cell 1 (Qualls and Haines 1992; Cleveland et al. 2004).

Soil Stoichiometric Relationships

Redfield (1958) concluded that the elemental composition of plankton was *"uniform in a statistical sense..."*. Several studies have taken this approach to explain the relatively elemental composition in other ecosystems and ecosystem compartments (Cleveland and Liptzin 2007; Xu et al. 2013). Cleveland and Liptzin (2007) determined that much like marine plankton and oceanic water, soil and soil microbial biomass are well-constrained at the global scale across forest and grassland ecosystems. Despite significant differences in nutrient stoichiometry between forest and grassland ecosystems, Cleveland and Liptzin (2007) concluded that similarities in soil and microbial element ratios among sites and across large scales were more apparent than different. Xu et al. (2013) presented comparable results with a larger dataset spanning several other ecosystems including natural wetlands and concluded that soil and microbial element stoichiometry vary widely among ecosystems. Cleveland and Liptzin (2007) estimated the "Redfield-like" stoichiometric ratios of C, N and P in soil as 186:13:1, while Xu et al. (2013) estimated a global average soil C:N:P of 287:17:1. Additionally, Xu et al. (2013) demonstrated the soil stoichiometric ratios of natural wetlands to be 1347:72:1. It is abundantly clear that stoichiometric relationships in soil is variable between ecosystems as presented by Cleveland and Liptzin (2007) and Xu et al. (2013) with C being the most variable constituent in the stoichiometric realm.

The stoichiometric relationships apparent in the Everglades STAs lacks the degree of proportional (allometric) scaling (this study) and diverges from the "Redfield-like" relationships demonstrations 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. Unlike relationships presented by prior studies C and P were not well-constrained (Fig 2) between EAV and SAV communities and exhibit a strong non-linear relationship 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 system combined with high loading of P from upstream sources (Chen et al. 2015). Meanwhile, C and N dynamics are somewhat constrained (Table 4) and following a near linear relationship (Fig 2) in the soil compartment. Within the Everglades the majority of TN is comprised of organic nitrogen as indicated by the strong near-linear relationship with C and N for most compartments (Fig 2) (Julian et al. 2016b). Variability in N concentrations is driven largely by biological N_2 fixation, N mineralization and burial (Inglett et al. 2011). Overall the relationships between C, N and P suggested the relative decoupling of P as OM accumulation driven by the rapid consumption of inorganic P (Corstanje et al. 2016), the decomposition of OM and the utilization of labile organic P as indicated by changes in enzyme activities along the treatment cell (*Inglett, et al., unpublished data*). This decoupling could potentially indicated 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 Cell 3 (Supplemental Fig 2). 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 apparent in the STAs are consistent with relationships observed within the Everglades marsh (Julian et al. 2016a). In addition to direct loading, nutrient inputs in the form of senescent plant material can play a significant role in soil biogeochemistry,

especially toward the back-end of the treatment cell. 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, as biomass decomposes soil OM characteristics change. Litter N is known to be conserved during decomposition processes ultimately enriching the soil below while C is lost due to microbial degradation, assimilation and respiration in terrestrial ecosystems. 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 cell 3 suggesting a change in biogeochemical drivers and deposition environment.

Unlike the conservative nature of N, P is rapidly cycled with internal nutrient regeneration from OM decomposition has been hypothesized to be more important than external inputs (Verhoeven et al. 1988). 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 and altering P recycling dynamics. 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 towards N-mineralization thereby potentially altering nutrient limitations and mineralization processes

especially in cell 3 an SAV dominated community suggesting vegetation dynamics pointing to differences in biogeochemical cycling of nutrients between these two flow paths.

Soils are long term integrators of environmental conditions (DeBusk and Reddy 2003). However nutrient concentrations and availability as indicated by stoichiometric ratios can significantly vary along environmental gradient (i.e. flow, vegetations, soil type) at the local (i.e. STA) and ecosystem (i.e. Natural Wetland; Xu et al. 2013) level to the global level (Cleveland and Liptzin 2007; Xu et al. 2013) as referenced by the variability of nutrient stoichiometric estimates.

Soil – Floc Stoichiometric Relationship

Floc is a complex matrix of microbes, organic particles (i.e. cellular detritus), inorganic particles (clays and silts) and substantial inter-floc spaces analogous to pore space in soils and is 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 overlaying water column ultimately influencing a variety of biogeochemical processes including nutrient cycling primarily through microbial activity. Furthermore floc represents the beginning of soil OM diagenesis (Noe et al. 2003; Neto et al. 2006).

As observed elsewhere, floc and soil represent stoichiometric intermediaries between microbial and higher plant end-members. Neto et al. (2006) evaluated floc and soil C:N ratios along a freshwater-to-marine transect indicating a mixed source of OM to these compartments with a decoupling of C to N at one site suggesting variable remineralization rates in soil relative to floc.

In our study, floc and soil follow the same pattern as Neto et al. (2006) in that N:P ratios of floc and soil area mixture of microbial biomass (TN:TP < 14; Xu et al. 2013) and EAV (TN:TP > 45; Table 2) or SAV (TN:TP > 60; Table 2). Additionally, this mixing is accompanied by a co-variate of distance downstream (Fig 5) where floc TN:TP values in the front of the treatment cell (fractional distance <0.5) are generally lower than TN:TP values towards the back-end (fractional distance >0.5) potentially indicating shifts in OM decomposition or selective nutrient removal processes (Fig 5). Furthermore, in cell 1 floc and soil TN:TP lack any correlation potentially indicating selective removal or remineralization of P and N quicker than that of N while in cell 3 floc and soil TN:TP.

Conclusion and Further Research.

Prior studies of stoichiometry suggest that the relationship between C and nutrients is tightly constrained and C:nutrient 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, based on this study nutrient stoichiometric relationship within treatment wetlands are potentially decouple through enrichment processes, disruption of biotic-feedback loops or selective removal of water column constituents. These processes translate across floc, soil and vegetation where stoichiometric relationship vary between compartments driven by biotic and physical processes. Additional study is needed to understand and evaluate biotic-feedback loops and their role in mediating nutrient stoichiometry, the role that biogeochemical and physical processes that regulate stoichiometric relationships and how does the Everglades ecosystem and STAs related to local and global stoichiometric relationships.

Acknowledgements

We would like to SFWMD and UF Wetland Biogeochemistry Laboratory staff members for field and analytical support. We would also like to thank Dr. Mark Brenner, Dr K. Ramesh Reddy, the anonymous peer reviewer(s) and editor(s) for their efforts and constructive review of this manuscript. Financial support for this project was provided by the South Florida Water Management District (Contract #4600003031).

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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

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

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

Cleveland CC, Neff JC, Townsend AR, Hood E (2004) Composition, Dynamics, and Fate of Leached Dissolved Organic Matter in Terrestrial Ecosystems: Results from a Decomposition Experiment. Ecosystems 7: . doi: 10.1007/s10021-003-0236-7

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

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

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

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

Glibert PM (2012) Ecological stoichiometry and its implications for aquatic ecosystem sustainability. Curr Opin Environ Sustain 4:272–277 . doi: 10.1016/j.cosust.2012.05.009

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

Inglett PW, Rivera-Monroy VH, Wozniak JR (2011) Biogeochemistry of Nitrogen Across the Everglades Landscape. Crit Rev Environ Sci Technol 41:187–216 . doi: 10.1080/10643389.2010.530933

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

Julian P, Payne GG, Xue SK (2016b) Chapter 3A: Water Quality in the Everglades Protection Areas. In: 2016 South Florida Environmental Report. South Florida Water Management District, West Palm Beach, FL

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

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

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

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

Muggeo VM (2008) Segmented: an R package to fit regression models with broken-line relationships. R News 8:20–25

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 (In Press) Drivers of landscape evolution: multiple regimes and their influence on carbon sequestration in a sub-tropical peatland. Ecol Monogr. doi: 10.1002/ecm.1269

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

Qualls RG, Haines BL (1992) Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Sci Soc Am J 56:578–586

Qualls RG, Richardson CJ (2000) Phosphorus enrichment affects litter decomposition, immobilization, and soil microbial phosphorus in wetland mesocosms. Soil Sci Soc Am J 64:799–808

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

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

Siegel AF (1982) Robust regression using repeated medians. Biometrika 69:242–244

US EPA (2007) Method 6010C: Inductively coupled plasma-atmoic emission spectrometry. US Environmental Protection Agency, Washington DC

Verhoeven JTA, Kooijman MM, Wirdum GV (1988) Mineralization of N and P along a trophic gradient in a freshwater mire. Biogeochemistry 6:31–43 . doi: 10.1007/BF00002931

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

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

Zhan S, Wang Y, Zhu Z, et al (2017) Nitrogen enrichment alters plant N: P stoichiometry and intensifies phosphorus limitation in a steppe ecosystem. Environ Exp Bot 134:21–32 . doi: 10.1016/j.envexpbot.2016.10.014

Figures and Tables

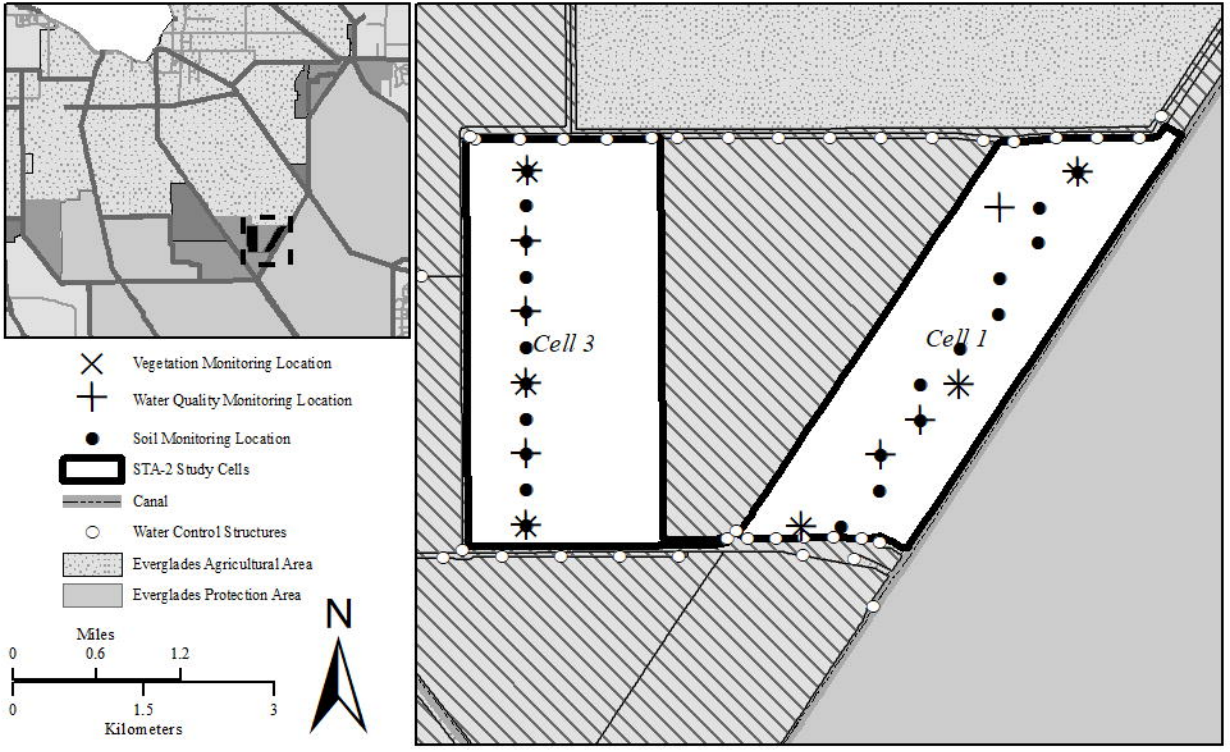
Figure 1. Surface water, soil and vegetation monitoring locations within Everglades Stormwater Treatment Area-2 Cells 1 (right) and 3 (left). Cell 1 is predominately emergent vegetation and Cell 3 is predominately submerged aquatic vegetation.

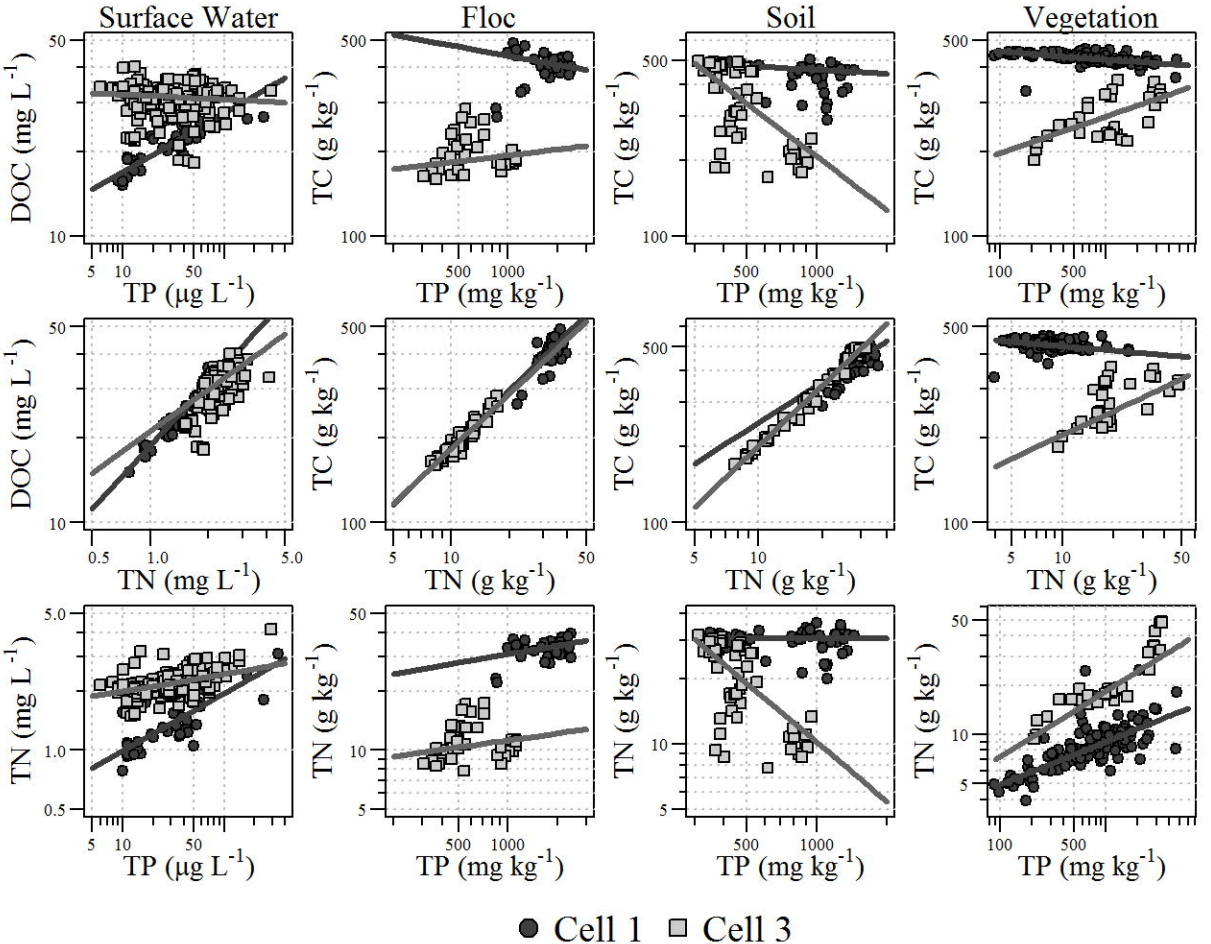
Figure 2. Relationships between carbon (organic carbon in surface water), nitrogen and phosphorus in surface water, soil flocculent material (floc), recently accreted soil, and dominate vegetation aboveground living biomass for STA-2 Cells1 and 3. Relative correlation from Spearman's rank sum correlation indicated by lines through the data by Thiel-Sen linear model estimate.

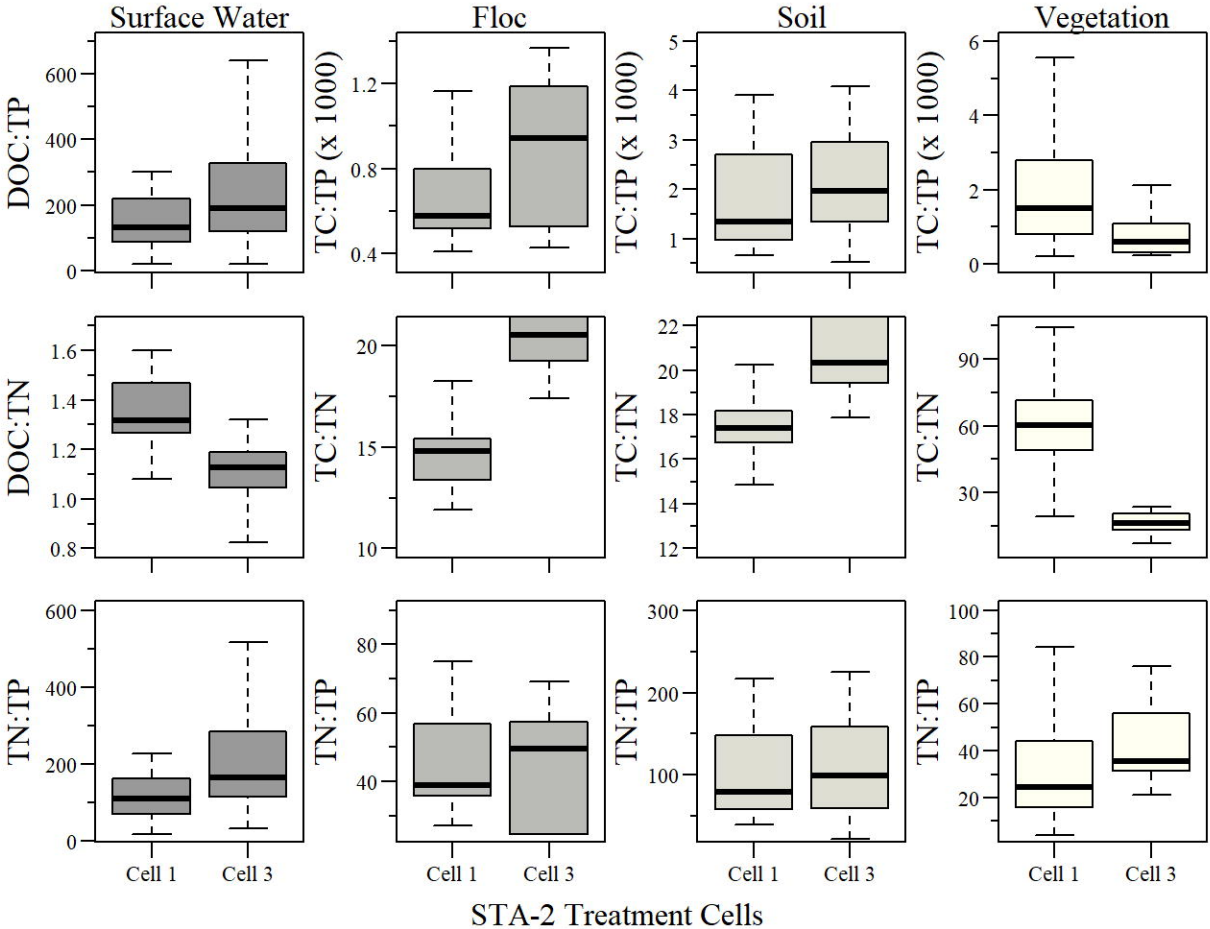
Figure 3. Boxplot comparing stoichiometric relationships between study cell for surface water, soil flocculent material (floc), recently accreted soil, and dominate vegetation aboveground living biomass.

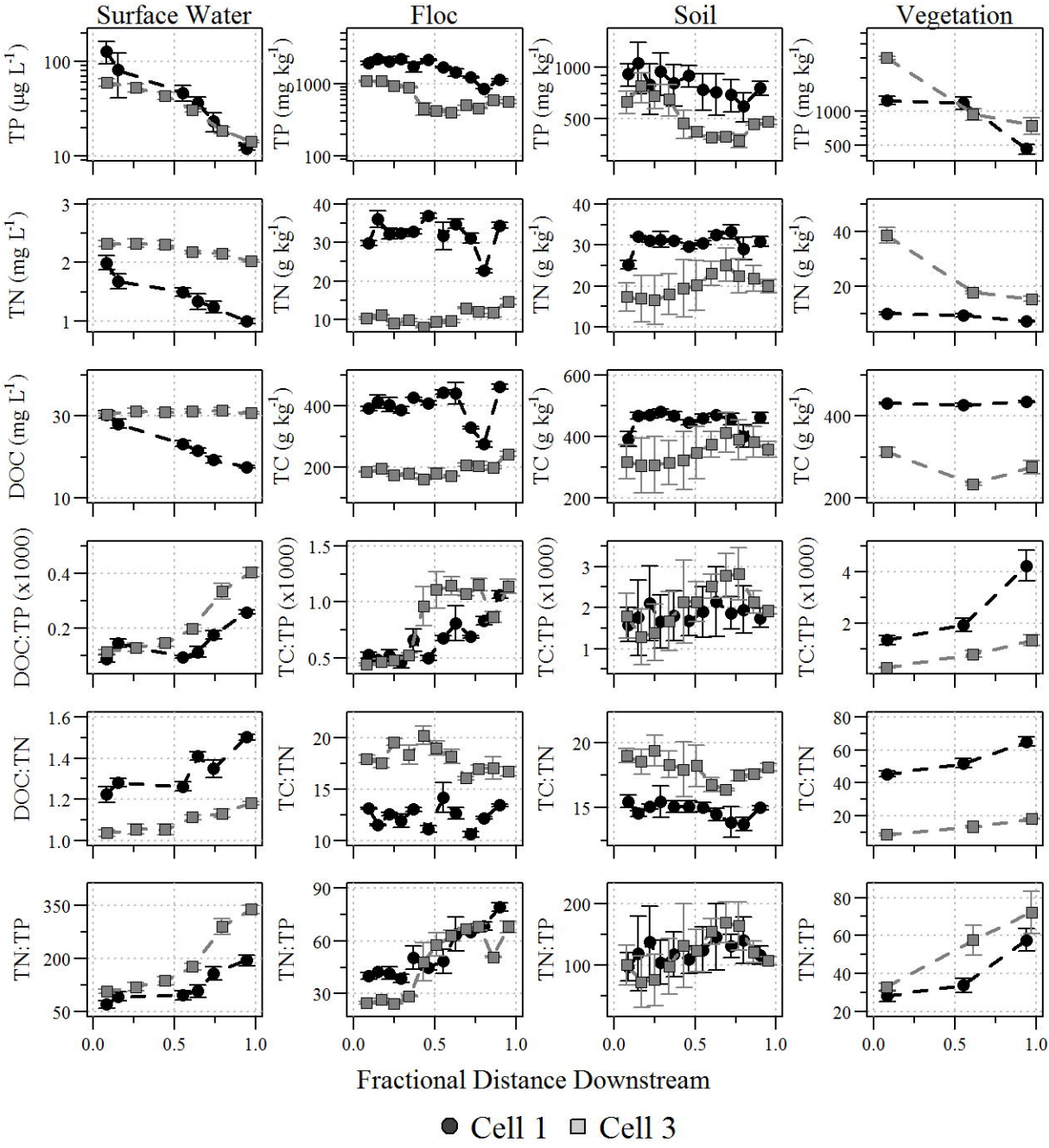
Figure 4. Mean ± SE phosphorus (TP), nitrogen (TN) and associated molar ratios by fractional distance downstream for STA-2 Cells 1 and 3. Carbon is expressed as dissolved organic carbon (DOC) for surface water and total carbon (TC) for soil flocculent material (floc), recently accreted soil and vegetation living 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 path 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 (g kg-1)
TN (mg L-1)
TN (g kg-1)
TP (µg L-1)
TP (mg kg-1)
Cell 1
Cell 3


Surface Water
Floc
Soil
Vegetation
DOC:TP
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
Cell 1
Cell 3
STA-2 Treatment Cells


Surface Water
Floc
Soil
Vegetation
TP (µg L-1)
TP (mg kg-1)
TN (mg L-1)
TN (g kg-1)
DOC (mg L-1)
TC (g kg-1)
DOC:TP (x1000)
TC:TP (x1000)
DOC:TN
TC:TN
TN:TP
Fractional Distance Downstream
Cell 1
Cell 3

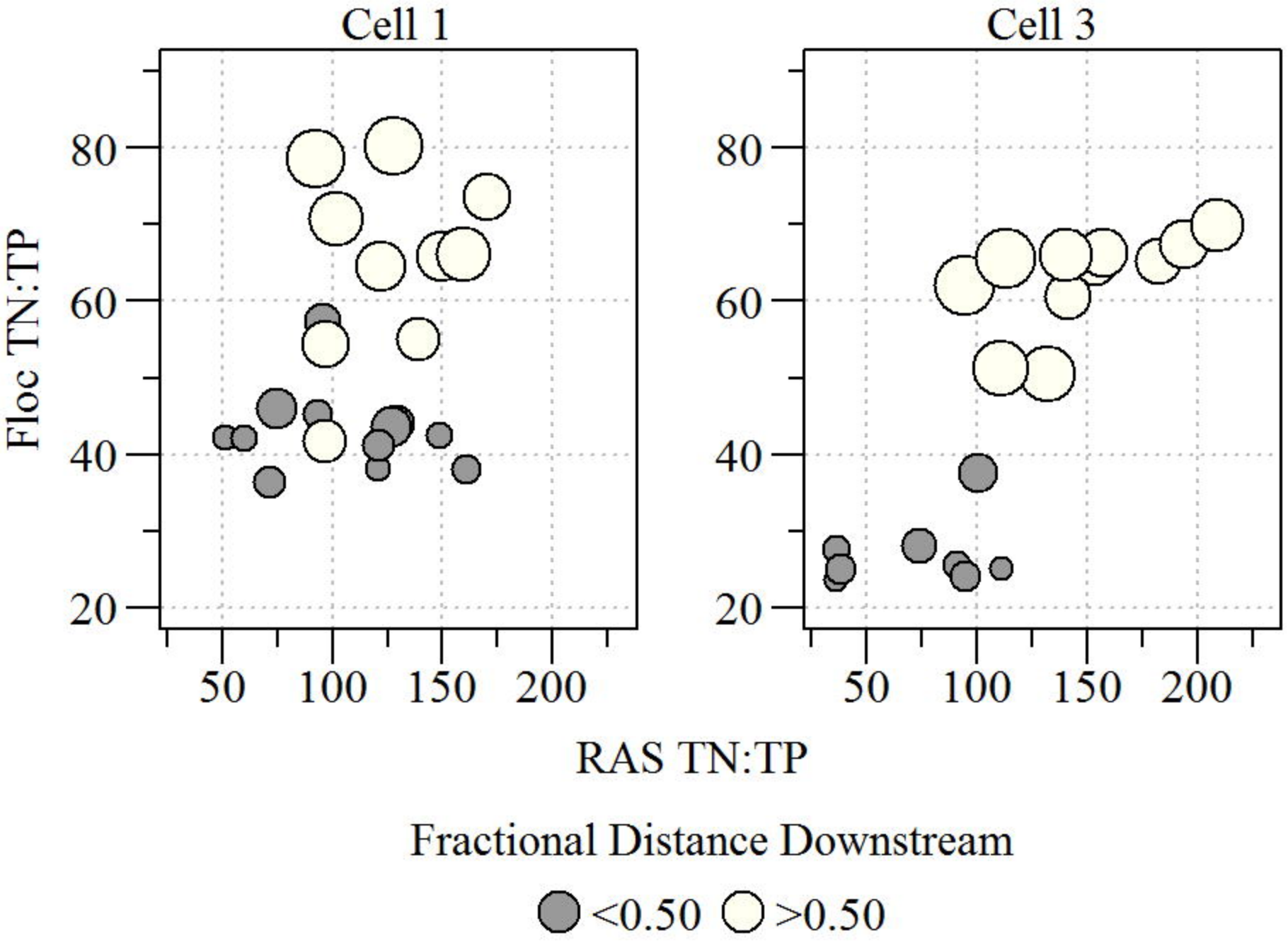

Cell 1
Cell 3
Floc TN:TP
80
60
40
20
50
100
150
200
RAS TN:TP
Fractional Distance Downstream
<0.50
>0.50

Table 1. Summary of parameters, matrices and analytical methods used for this study. Additional parameters were collected 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**	**Method**
Surface Water	Total Phosphorus	TP	SM4500PF	Clesceri et al. (1998)
	Total Nitrogen	TN	SM4500NC	Clesceri et al. (1998)
	Dissolved Organic Carbon	DOC	SM5310B	Clesceri et al. (1998)
Soil and Vegetation	Loss-on-ignition[1]	LOI	SFWMD 1610	SFWMD (2015)
	Total Phosphorus	TP	SM4500PF	Clesceri et al. (1998)
	Total Nitrogen	TN	SFWMD 3200	SFWMD (2015)
	Total Carbon	TC	SFWMD 3200	SFWMD (2015)
	Total Calcium[1]	TCa	EPA 6010C	US EPA (2007)

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

Table 2. Summary statistics for parameters and matrices used in this study of samples collected along the Cell 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.

		Cell 1			**Cell 3**		
Matrix	**Parameter (Units)**	**Mean ± SE**	**Range**	**CV**	**Mean ± SE**	**Range**	**CV**
Surface Water	Dissolved Organic Carbon (mg L^{-1})	23.2 ± 0.7	15.1 - 35.6	23.6	30.9 ± 0.2	18.2 - 40.2	10.7
	Total Phosphorus (µg L^{-1})	54.8 ± 9.6	9.0 – 378.0	145.1	35.1 ± 1.8	6.0 – 293.0	82.9
	Total Nitrogen (mg L^{-1})	1.5 ± 0.1	0.8 - 3.1	32.4	2.2 ± 0.02	1.5 - 4.1	13.8
	DOC:TP (molar)	151.4 ± 9.3	18.7 - 301.8	51.1	237.4 ± 9.7	19.4 - 974.3	66.3
	DOC:TN (molar)	1.3 ± 0.02	0.9 - 1.6	10.8	1.1 ± 0.007	0.6 - 1.3	10.4
	TN:TP (molar)	118.8 ± 7.8	16.3 - 342.8	54.4	206.9 ± 7.7	31.2 - 788.7	60.4
	Chlorophyll-A (µg L^{-1})	5.3 ± 0.5	1.4 – 24	81.8	11.7 ± 0.9	0.03 - 68.8	125.2
Floc	LOI (%)	23.6 ± 2.1	8.6 - 61.2	51.0	77.3 ± 1.2	59.5 - 87.9	9.4
	Total Phosphorus (mg kg^{-1})	1698 ± 81.8	844.0 – 2436.0	28.1	642.1 ± 43.8	307 - 1118	40.3
	Total Nitrogen (g kg^{-1})	32.8 ± 0.7	22.0 - 39.4	11.9	11.4 ± 0.4	7.8 - 17.4	22.5
	Total Carbon (g kg^{-1})	406.1 ± 8.6	265.0 – 488.0	12.3	199.6 ± 5.4	160.0 – 283.0	15.9
	TC:TP (molar)	671.2 ± 38.6	410.9 - 1165.3	33.5	919.1 ± 55.0	426.7 - 1369.2	35.4
	TC:TN (molar)	14.5 ± 0.2	11.9 – 18.2	9.3	20.7 ± 0.3	17.4 – 24.6	8.6
	TN:TP (molar)	46.0 ± 2.3	27.1 -74.9	29.5	44.9 ± 2.8	20.4 -69.2	36.3
	Calcium (g kg^{-1})	80.4 ± 8.2	24.5 – 236	59.4	286.0 ± 4.9	225.4 - 329.2	10.2
Soil	LOI (%)	18.8 ± 1.4	9.0 - 51.4.0	51.8	45.8 ± 3.4	14.7 - 83.5	52.5
	Total Phosphorus (mg kg^{-1})	826.1 ± 48.7	318.0 – 1449.0	42.1	509.3 ± 25.6	312.0 – 947.0	35.9
	Total Nitrogen (g kg^{-1})	29.9 ± 0.5	19.9 - 36.2	11.7	20.2 ± 1.1	7.7 - 31.8	39.4
	Total Carbon (g kg^{-1})	445.3 ± 7.1	290.0 – 504.0	11.4	350.8 ± 16.4	171.0 – 495.0	33.4
	TC:TP (molar)	1777.6 ± 141.5	671.3 - 3916.9	56.8	2069.0 ± 145.0	534.3 - 4091.4	50.0
	TC:TN (molar)	17.4 ± 0.2	13.6 - 21.1	7.8	20.9 ± 0.3	17.9 – 25.8	9.5
	TN:TP (molar)	100.3 ± 7.5	39.5 – 217.0	53.2	103.3 ± 8.0	22.3 – 225.4	55.1
	Calcium (g kg^{-1})	63.2 ± 5.2	26.0 – 179.0	59.0	160.9 ± 13.0	39.2 - 313.9	57.9
Vegetation	Total Phosphorus (mg kg^{-1})	988.6 ± 76	87.2 - 4693.5	83.1	1434.9 ± 181.6	210.0 - 3378.0	72.7
	Total Nitrogen (g kg^{-1})	431.0 ± 1.7	329.0 - 464.0	4.3	271.2 ± 8.5	186.0 - 359.5	18.1
	Total Carbon (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 (molar)	2456.5 ± 242.6	205.4 - 13012.5	106.8	845.3 ± 116.4	237.4 - 2486.7	79.1
	TC:TN (molar)	63.0 ± 1.9	19.3 – 116.6	31.9	16.1 ± 0.8	7.5 – 23.7	30.1
	TN:TP (molar)	33.7 ± 2.3	3.9 – 125.5	74.0	48.1 ± 4.8	12.3 – 120.4	57.4

Table 3. Siegel repeated median's linear model results for stoichiometric relationships within each ecosystem compartment along cell 1 and 3 flow-paths within Stormwater Treatment Area-2. Bold ρ-values indicate models where slope was not significantly different than one.

Y	X	Compartment	Flow Path	N	Slope	Intercept	V-Value	ρ-value
DOC	TP	Surface Water	Cell 1	69	0.21	2.39	990	<0.01
			Cell 3	265	-0.03	3.52	8911	<0.01
TC	TP	Floc	Cell 1	34	-0.13	7.06	205	**0.12**
			Cell 3	35	0.07	4.68	427	<0.05
		Soil	Cell 1	51	-0.05	6.49	77	<0.01
			Cell 3	51	-0.78	10.69	94	<0.01
		Vegetation	Cell 1	117	-0.03	10.58	335	<0.01
			Cell 3	33	0.12	9.55	543	<0.01
DOC	TN	Surface Water	Cell 1	69	0.73	2.91	820	<0.01
			Cell 3	265	0.46	3.10	28251	<0.01
TC	TN	Floc	Cell 1	34	0.77	3.37	580	<0.01
			Cell 3	35	0.61	3.80	630	<0.01
		Soil	Cell 1	51	0.37	4.87	1113	<0.01
			Cell 3	51	0.82	3.41	1326	<0.01
		Vegetation	Cell 1	117	-0.06	10.89	1141	<0.01
			Cell 3	33	0.29	7.83	554	<0.01
TN	TP	Surface Water	Cell 1	69	0.36	-0.94	561	<0.01
			Cell 3	265	0.08	0.50	25901	<0.01
		Floc	Cell 1	34	0.09	2.76	468	<0.01
			Cell 3	35	0.14	1.65	490	<0.01
		Soil	Cell 1	51	0.01	3.36	676	**0.71**
			Cell 3	51	-1.01	9.24	101	<0.01
		Vegetation	Cell 1	117	0.27	5.55	6723	<0.01
			Cell 3	33	0.32	6.02	561	<0.01

Table 4. Comparison by Kruskal-Wallis rank sum test of stoichiometric ratios between cell 1 and 3 for surface water, soil flocculent material (floc) and recently accreted soil ecosystem compartments. Statistically significant comparison are 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 total phosphorous (TP), total nitrogen (TN) and carbon (dissolved organic carbon [DOC] for surface water and total carbon [TC] for floc, soil and vegetation) along cells 1 and 3 of STA-2. Spearman's rank sum correlation and Thiel-Sen Slope estimate results summarized by flow way and ecosystem compartment.

		Cell 1			Cell 3		
Compartment	**Parameter**	**Spearman's ρ**	**ρ-value**	**Theil-Sen Slope**	**Spearman's ρ**	**ρ-value**	**Theil-Sen Slope**
Surface Water	TP	-1.0	<0.01	-113.4	-1.0	<0.01	-55.0
	TN	-1.0	<0.01	-1.1	-0.9	<0.01	-0.3
	DOC	-1.0	<0.01	-14.8	0.3	0.66	0.4
	DOC:TP	0.8	0.06	197.8	1.0	<0.01	326.3
	DOC:TN	0.9	<0.01	0.3	0.9	<0.05	0.2
	TN:TP	1.0	<0.05	132.6	1.0	<0.01	260.4
Floc	TP	-0.8	<0.01	-1531.0	-0.5	0.09	-594.1
	TN	-0.1	0.67	-1.7	0.6	0.07	4.5
	TC	0.1	0.82	50.0	0.5	0.09	42.6
	TC:TP	0.8	<0.01	535.8	0.8	<0.01	798.5
	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	TP	-0.8	<0.01	-358.2	-0.7	<0.05	-353.7
	TN	0.1	0.88	0.8	0.7	<0.05	7.4
	TC	-0.1	0.69	-9.8	0.8	<0.01	95.7
	TC:TP	0.4	0.25	225.2	0.6	<0.05	1388.5
	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
Vegetation	TP	-0.7	<0.01	-1231.2	-0.7	<0.01	-2445.2
	TN	-0.5	<0.01	-2.2	-0.8	<0.01	-20.0
	TC	0.6	<0.01	14.5	-0.8	0.16	-47.2
	TC:TP	0.7	<0.01	1201.0	0.8	<0.01	724.1
	TC:TN	0.5	<0.01	14.3	0.9	<0.01	11.3
	TN:TP	0.7	<0.01	19.0	0.6	<0.01	23.4