Title: Nutrient homeostasis and mechanisms related to nutrient retention by wetland macrophytes in a subtropical wetland.

Running Header: Wetland homeostasis

Paul Julian II[1], Stefan Gerber[2], Jill King[3], 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

Abstract

Central to ecological stoichiometry, nutrient homeostasis relates ambient stoichiometric conditions to a species stoichiometric composition. In wetland ecosystems, vegetation is a large, highly variable and dynamic sink of nutrients. This study investigated wetland plant stoichiometric homeostasis of dominant emergent and submerged aquatic vegetation (EAV and SAV, respectively) within two treatment cells of the Everglades Stormwater Treatment Areas (STAs). The hypotheses of this study assumed wetland vegetation will be non-homeostatic relative to ambient available nutrients and that due to changes in nutrient availability, phosphorus (P) resorption by EAV will vary along the water flow path through the wetland. This study confirmed the hypothesis that wetland vegetation is non-homeostatic along different vegetation communities with homeostasis coefficients of 0.67 ± 0.04 and 0.78 ± 0.03 for EAV and SAV respectively. Furthermore, the study rejected the concept of variable nutrient resorption with EAV resorption remaining relatively constant along the treatment cell relative to changes in nutrient availability as indicated by high EAV TP resorption efficiencies of 74.2 ± 2.3 %. These combined results suggest that vegetation within STAs provides a strong nutrient sink with relatively constant uptake pressure suggesting that vegetation, along with other factors, influence ambient nutrient conditions within a given treatment cell.

Keywords: Phosphorus, Everglades, resorption, sink strength, Stormwater Treatment Area

Introduction

A tenant of ecological stoichiometry is that abundance of carbon (C), nitrogen (N), phosphorus (P) or other elements is regulated by reciprocal interaction between organisms and their environment (Redfield 1958). This reciprocation of organism and environmental nutrients is the central theme of stoichiometric homeostasis. Stoichiometric homeostasis was originally developed in the context of ecosystem disturbances and perturbations to assess whether organisms and ecosystems respond to changing conditions. This concept is of particular interest with respect to nutrient enrichment processes since nutrient homeostatic regulation has the potential to decouple nutrient transport by selective or variable uptake (Sterner and Elser 2002; Small et al 2009). Stoichiometric homeostasis is demonstrated by a nutrient sink (plant, microbes soil, etc.) attaining a constant stoichiometry with respect to its ambient environment while a non-homeostatic system would reflect the ambient environment to a greater degree. The contrast between non-homeostasis and homeostasis is that for a homeostatic system (or organism), nutrient stoichiometry is relatively fixed while non-homeostatic systems (or organisms) impart some flexibility with their respective nutrient stoichiometry.

Stoichiometric flexibility or homeostatic plasticity can manifest across different spatial scales from organism to ecosystem. At the organismal level, stoichiometric flexibility can occur through changes in nutrient allocation to tissue types or biochemical changes in cellular composition (Sistla and Schimel 2012; Rivas-Ubach et al 2012). At the ecosystem level, changes in stoichiometry can occur through changes in ecosystem composition from invasive species or enrichment (Sistla et al 2015). As such, stoichiometric flexibility is driven by species-specific nutrient biomass storage, physiological plasticity, interspecific competition and the variability in nutrient supply versus demand (Elser et al 2010; Sardans et al 2012; Sistla et al 2015). A

specific mechanism that allows plant species to be stoichiometrically flexible is internal nutrient recycling or resorption.

Internal nutrient recycling is a process in which nutrients are withdrawn from senescing leaves before abscission to be used for new growth or stored for later use (Van Heerwaarden et al 2003). Nutrient resorption has been assumed to be one of the most important strategies used by plants to conserve nutrients. Ultimately resorption is the result of several biochemical processes including enzymatic hydrolysis of N- and P-containing compounds, internal redistribution and protein and lipid synthesis. A plants degree of nutrient resorption has the potential to affect litter quality, decomposition and nutrient availability all factors which relate to organic matter decomposition and nutrient cycling (Rejmánková 2005; Corstanje et al 2007). Most nutrient resorption studies focus on terrestrial (upland) evergreen, deciduous, forb and graminoid species (Killingbeck 1996; Aerts and Chapin 1999; Van Heerwaarden et al 2003; Peng et al 2016) with few studies investigating wetland plant species (Rejmánková 2005; Rejmánková and Snyder 2008).

Prior studies of plant species suggest that they range in their degree of homeostasis from very weak to non-homeostatic (Sterner and Elser 2002; Yu et al 2011; Gu et al 2017). However, these studies did not exclusively include wetland plant species. Furthermore, wetland biogeochemistry can be influenced by biotic (i.e. plant, periphyton, algae, etc.) interactions (Reddy and DeLaune 2008; Kadlec and Wallace 2009). Therefore, the primary objective of this study was to evaluate stoichiometric homeostasis of dominant wetland species along two water flow ways within the Everglades Stormwater Treatment Areas (STAs). The primary concept of utilizing vegetation in treatment wetlands is to remove nutrients through biotic uptake, so we assumed that vegetation will be non-homeostatic relative to ambient available nutrients. Very few studies have evaluated

nutrient resorption in emergent aquatic vegetation (EAV) with the few studies determining that EAV species use both “conservation of use” and “enhanced acquisition” of P to fulfill nutrient demand in P-limited systems. These studies have demonstrated that EAV utilize the “conservation of use” strategy and nutrient resorption is dependent upon nutrient availability (Rejmánková 2005; Rejmánková and Snyder 2008) The second objective of this study is to evaluate nutrient resorption dynamics of EAV species within a treatment wetland with the hypothesis that P-resorption will vary due to changes in nutrient availability.

Methods

Study Area

Large freshwater treatment wetlands, known as the Everglades STAs, were constructed and are in current operation to reduce P loading entering the Everglades ecosystem thereby being an integral part of the state of Florida and the federal government’s efforts to preserve and protect the remaining Everglades ecosystem (Chimney 2017). A total of six STAs with an approximate area of 18,000 ha (180 km^2) are located south of Lake Okeechobee in the southern portion of the Everglades Agricultural Area (EAA; Fig 1). Prior land uses within the current STA boundary include pre-existing natural wetlands and agriculture dominated by sugarcane. The primary source of inflow water to the STAs is agricultural runoff from the EAA, an area of approximately 284,000 ha.

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

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). 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

Foliar TN:TP molar ratios for EAV along the cell 1 flow way transect were categorized into potential N-limitation (TN:TP<14), potential N and P co-limitation (14<TN:TP<16) and P limitation (TN:TP>16) for cell 1. Meanwhile foliar TN:TP molar ratios for SAV along the cell 3 flow way transect were categorized as were categorized into potential N-limitation (TN:TP <27) and potential P-limitation (TN:TP>27). These potential nutrient limiting categories were consistent with prior studies for EAV (Koerselman and Meuleman 1996; Rejmánková 2005) and SAV (Duarte 1992; Fernández-Aláez et al 1999) communities. Chi-squared (RxC) analysis compared nutrient limitation categories (described above) and cell region identified as Inflow, Mid and Outflow. Furthermore, foliar TC:TP and TC:TN molar ratios were compared to absolute foliar nutrient concentrations (TN and TP) by spearman's rank sign correlation for each flow with transect separately.

Phosphorus Resorption efficiency (PRE) was evaluated along the STA-2 Cell 1 transect by comparing living (X_{GR}) to dead (X_{SEN}) EAV AGB TP tissue concentrations. Resorption efficiency was estimated using Eq 1 consistent with prior studies (Killingbeck 1996; Reed et al 2012).

$$RE_x = \frac{X_{GR} - X_{SEN}}{X_{GR}} \times 100 \qquad (1)$$

RE_x = Resorption efficiency for a particular nutrient (Percent)
X_{GR} = P concentration of living foliage (mmol kg^{-1})
X_{SEN} = P concentration of dead foliage (mmol kg^{-1})

Phosphorus RE was compared with P-limiting categories identified above for foliar TN:TP, molar ratios by Kruskal-Wallias rank sum test. Mean PRE-values were compared to fractional distance downstream by spearman's rank correlation. Furthermore, RE values were compared between stations using the Dunn's multiple comparison rank sum test ('dunn.test' R package; Dinno 2015).

The strength of vegetation TN:TP stoichiometric homeostasis was characterized using the homeostasis coefficient $H_{N:P}$ (Eq 2). Homeostasis coefficient is also useful to determine if the observed variation in the consumer stoichiometry is the result of physiological adjustments or reflects species specific turnover (Elser et al 2010).

$$\frac{1}{H_{N:P}} = \frac{\log(y) - \log(c)}{\log(x)} \qquad (2)$$

$H_{N:P}$ = homeostasis coefficient (unitless)
y = resource nutrient N:P molar ratio (unitless)
x = consumer nutrient N:P molar ratio (unitless)
c = constant, derived from log-log regression (unitless)

To assess the vegetation homeostatic relationship, resource nutrient N:P pools differ based on the dominant nutrient uptake pathway. Submerged aquatic vegetation primarily assimilates nutrients through the water column with has species dependent limited P storage capabilities. Furthermore, SAV can alter the physio-chemical environment of water resulting in chemical precipitation

(Dierberg et al 2002). Emergent aquatic vegetation are effectively P sinks, however they assimilate little P directly from the water column. Rather, emergent macrophytes mine P from the soils via soil solution (Richardson and Marshall 1986; Reddy et al 1999). To determine vegetative community specific $H_{N:P}$-values where x is the resource TN:TP molar ratio water column TN:TP for SAV and soil TN:TP for EAV, y is community specific living-AGB TN:TP molar ratio and c is a constant. To determine the constant c log-transformed resource TN:TP and living AGB TN:TP were regressed using a Theil-Sen single median linear model ('mblm' package). The c constant is estimated from the intercept of the log-log regression between resource and consumer pools.

Values of $1/ H_{N:P}$ range between 0 and 1 with values approaching 1 indicating a non-homeostatic relationship and values approaching 0 signifying consumer-resource homeostatic. Degree of homeostatic was defined consistent with prior studies (Persson et al 1999; Makino et al 2003; Feijoó et al 2014) as the following:

(1) $0 < 1/ H_{N:P} < 0.25$: Homeostatic

(2) $0.25 < 1/ H_{N:P} < 0.50$; Weakly homeostatic

(3) $0.50 < 1/ H_{N:P} < 0.75$; Weakly non-homeostatic

(4) $0.75 < 1/ H_{N:P} < 1.00$; Non-homeostatic

To evaluate changes in homeostatic conditions amongst vegetative communities along the flow gradient, $1/ H_{N:P}$ values were compared to distance downstream by Spearman's rank correlation within each cell. To evaluate general homeostatic versus non-homeostatic status among vegetation within along the flow path, Wilcoxon signed rank test was used to test if $1/ H_{N:P}$ was significantly different than 0.50. Degree of homeostasis was compared between vegetation by

Kruskal-Wallis rank sum test. Spearman's correlation was used to compare distance from inflow and 1/ $H_{N:P}$ for each flow path.

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

During this study, water column DOC concentrations ranged from 15.1 to 40.2 mg C L^{-1}, TP concentrations ranged from 6 to 378 µg P L^{-1}, and TN concentrations ranged from 0.78 to 4.14 mg N L^{-1} between the two study cells. Generally, TP concentrations were higher in cell 1 while TN and DOC concentrations were greater in cell 3 (Table 2). 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).

Recently accreted soil percent organic matter as indicated by loss-on-ignition (LOI) ranged from 9.0 to 83.5% across the study cells with cell 3 having a higher mean LOI value (45.8 ± 3.4%; Table 2). Soil composition within cell 3 was more mineral in nature with greater TCa concentrations relative to cell 1 soils (Table 2). Soil TC concentration ranged 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 between and within sites, as coefficients of variance reached 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).

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. Living AGB 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 and Fig 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%.

Both SAV and EAV communities spanned their respective potential N-limiting and P-limiting conditions (Table 2). Foliar TN:TP molar ratios were greatest in cell 3 with most of the data observed above the P-limiting categories identified for EAV and SAV communities (Table 2). Furthermore, foliar TC:TN values were greatest in cell 1 ranging from 19 to 116 while cell 3 ranged from 8 to 24 suggesting the potential for some N-limiting conditions. Nutrient limiting conditions were significantly different along the cell 1 transect (χ^2=19.8, df=4, ρ<0.01) with

several values being observed below the TN:TP value of 14 most of which occurred at the front of the cell (i.e. Inflow and Mid regions) and all values above the P-limiting criteria at the Outflow region. Meanwhile, nutrient limiting conditions were not significantly different along the cell 3 flow path transect (χ^2=5.6, df=2, ρ=0.06) with the majority of the TN:TP values above 27 with a total of four values being below 27 occurring in Inflow and Mid regions. Foliar TC:TP and TP concentration was negatively correlated along both the cell 1 (r=-1.00, ρ<0.01) and cell 3 (r=-0.96, ρ<0.01) flow way transect. Additionally, foliar TC:TP and TP concentration was negatively correlated along both the cell 1 (r=-0.99, ρ<0.01) and cell 3 (r=-0.81, ρ<0.01) flow way transect.

Resorption efficiency of vegetation within cell 1 ranged from 23.6 to 90.9 percent along the flow transect. A total of 38 plant tissue samples were collected during the vegetation sampling event where both living and dead aboveground biomass were available, with cattail comprising most of the species collected (N=36). Estimated mean RE_{TP} values for cattails was 74.2 ± 2.5% (N=36), with the data collected single estimates of RE_{TP} for water lily and sawgrass was 69.7% and 77.5%, respectively (Table 3). Resorption efficiency did not significantly different between TN:TP categories (χ^2 = 0.82, df=2, ρ=0.66). Along the cell 1 flow path, RE_{TP} was not significantly correlated with fractional distance downstream (r=0.36, ρ=0.49) or between sites (χ^2 = 3.3, df=2, ρ=0.19).

The degree of stoichiometric homeostasis as indicated by the homeostasis coefficient ($1/H_{N:P}$) ranged from 0.50 to 0.93 between the cell 1 and 3 flow paths with $1/H_{N:P}$ being significantly greater than 0.50 (V-value=66, ρ<0.05) indicating a relative non-homeostatic relationship between vegetation and its respective resource. Fractional distance downstream and $1/H_{N:P}$ was statistically significant for cell 1 (r=0.71, ρ=0.12) and cell 3 (r=-0.21, ρ=0.73) flow paths

suggesting no significant changes in homeostasis along the cell (Fig 3). Furthermore, $1/H_{N:P}$ values between SAV and EAV were significantly different (χ^2=7.5, df=1, ρ<0.05) suggesting a divergent stoichiometric homeostasis which could be related to physiological and biochemical mechanisms associated with nutrient retention and uptake.

Discussion

All organisms are faced with the challenges of acquiring sufficient energy and nutrients, with most organisms dealing with an imbalanced mixture of energy and nutrients. Constraints of energy or nutrients can alter population dynamics, inter-specific competition for resources and even key ecosystem processes (Frost et al 2005). Stoichiometric homeostasis is largely controlled by a set of key physiological processes that regulate uptake, incorporation and eventual release of nutrients (Sterner and Elser 2002). In the context of treatment wetlands, these challenges and physiological processes are exploited and managed to facilitate removal of particular nutrient(s). As in the case of the Everglades STAs, they are optimized to remove P from the water column via biological uptake through vegetative communities.

Several studies have reported extreme variation in the C:N, C:P and N:P ratios of terrestrial plants or freshwater (algal) biomass with C:N ranging from 5 to >100, C:P ranging from <250 to >3500 and N:P ranging from <5 to >65 from terrestrial and aquatic environments (Elser et al 2000; Demars and Edwards 2007; Elser et al 2010). These reported ranges are consistent with the values observed in this study with differences in living-AGB vegetation between flow paths (Table 2 and Fig 2). The difference in C:nutrient stoichiometry amongst vegetation types could reflect ecophysiological processes and species specific characteristics associated with nutrient

uptake and storage with higher C:nutrient values indicating reduced allocation to low-nutrient structural material. In our study, generally cell 3 vegetation (SAV) had higher nutrient concentrations and lower C concentrations in living-AGB relative to cell 1 vegetation (EAV) resulting in higher C:nutrient ratios observed in cell 1 (Table 2, Fig 2). This relationship of live-AGB is consistent with data reported from other studies evaluating plant tissue nutrient concentrations within the STAs and Everglades ecosystem (Chimney and Pietro 2006; DeBusk et al 2011; Miao and Zou 2012).

The amount and composition of nutrients in macrophyte tissues depends upon the physiological capacity for nutrient uptake and storage by a particular species (Fernández-Aláez et al 1999). As suggested by Tilman (1982) nutrient composition in the tissues can be an important feature to identify ecological strategy of species relative to biogeochemical conditions which is the basis of the treatment wetland strategy. Generally, EAV species invest a significant quantity of C to their biomass associated with structural components of different plant parts and generally have higher net primary production than SAV species (Reddy and DeLaune 2008; De Deyn et al 2008). Therefore the tissue composition as indicated by the ratio of lignin and cellulose can differ between plant species and is related to C quality and nutrient availability both internally during metabolism and after senesce during decomposition (DeBusk and Reddy 1998). Furthermore, plant species respond to changes in nutrient available through the storage and recycling of nutrients ultimately shifting absolute and relative tissue nutrient concentrations (Güsewell and Koerselman 2002; Güsewell et al 2003; Rejmánková and Snyder 2008).

Early in the development of stoichiometric theory it was hypothesized that photoautotrophs (i.e. algae and plants) were considered to have very weak stoichiometric homeostasis ($0.25 < 1/H < 0.50$) suggesting that plants phototrophs can regulate biomass stoichiometry relative to the

ambient environment. However, since its development, several studies have adjusted this hypothesis by suggesting that plants can range from weakly homeostatic to non-homeostatic (1/H >0.50; Sterner et al. 1998; Elser et al. 2000; McGroddy et al. 2004; Güsewell 2004). Demars and Edwards (2007) suggested that the relationship between external nutrient concentrations (i.e. soil and water column) and foliar tissue nutrient concentrations was weak for aquatic macrophytes suggesting a weak to non-homeostatic relationship as the plant does not exploit the ambient environment. Furthermore, in the same study C:nutrient ratios were inversely related to nutrient concentrations indicating that ambient nutrient availability and plant metabolism was not coupled. Consistent with the studies discusses above, vegetation in along the Everglades STA flow paths are typically non-homeostatic suggesting that ambient nutrients do not correspond with living-AGB (Fig 3). Furthermore, foliar C:nutrient ratios were generally inversely correlated with foliar nutrient concentration indicating a decoupling of nutrient tissue nutrients and plant metabolism. Vegetation homeostatic and metabolic dynamics relative to ambient and foliar nutrient availability could be the result of two dynamic processes, 1) nutrient uptake is regulated to achieve or maintain closed mass balance of nutrient within plant biochemistry or; 2) nutrient uptake is set a constant level and physiological processes such as nutrient resorption augment internal nutrient.

Nutrient resorption is an important physiological process utilized by terrestrial plants to retain labile nutrients. Prior studies have demonstrated that plants are generally less dependent on current ambient nutrient concentrations, thereby exhibiting some physiological plasticity in response to nutrient availability at an individual species level (Elser et al 2000; Demars and Edwards 2007). Some studies have suggested that nutrient resorption is higher in low fertility ecosystems (Chapin III et al 1990; Aerts 1996; Aerts and Chapin 1999), meanwhile others (Reed

et al 2012) suggest that plants in comparatively N-rich and P-poor areas such as highly weather tropical soils should be under selective pressure to resorb P at a greater rate (i.e. high P-RE). This study has demonstrated that wetland macrophytes within the Everglades STAs are less dependent upon ambient nutrient concentrations because of physiological adaptation as indicated by lack of stoichiometric homeostasis relative to TN:TP (discussed above and Fig 3). For EAV species this lack of homeostasis can be due to several mechanisms including, morphological adaptations (i.e. root morphology) facilitating exploitation of recalcitrant nutrient pools (Lorenzen et al 2001), symbiotic association with mycorrhizae (Cornwell et al 2001; Abel et al 2002) and biochemical adaptations such as hydrolytic enzyme secretion (Ticconi and Abel 2004). Often considered a conservation strategy in nutrient limiting conditions is the resorption of nutrients from senescing tissue where resorption has the most important strategy used by plants to conserve nutrients (Aerts and Chapin 1999; Rejmánková and Snyder 2008).

Phosphorous resorption efficiency of EAV, primarily cattails within the Everglades STAs (Table 3) is consistent with RE_{TP} observed for aquatic macrophytes by other studies elsewhere. Rejmánková (2005) evaluated resorption of several wetland macrophyte species from several wetlands across a broad spatial distribution and observed RE_{TP} for cattail achieved a maximum RE_{TP} of approximately 80% while the study average was 70%. Rejmánková (2005) determined that RE_{TP} was significantly greater in P-limiting conditions. In this study, RE_{TP} was not significantly different along the flow way despite some potential shifts in P-limitations relative as indicated by foliar TN:TP values and nutrient stoichiometry of other compartments (Julian et al. *In Prep*). However, qualitatively mean RE_{TP} values observed at the inflow region were slightly lower with more variability than mid and outflow regions (Fig 4) potentially suggesting differential nutrient sink strength. Despite this variability, RE_{TP} did not differ along the flow way

suggesting that sink strength is not a function of distance downstream or ambient nutrient availability. Southwest Florida has an extended growing season due to the tropical/sub-tropical climate therefore there may be a relatively constant demand for nutrients during growth. This nutrient demand facilitates nutrient resorption of nutrients suggesting a strong sink as observed in studies elsewhere (Carrera et al 2000; Rejmánková 2005; Lambers et al 2008).

Conclusions

This study suggests that vegetation within treatment wetlands has a relatively constant uptake pressure as indicated by the non-homeostatic relationship between foliar and active reservoirs indicating that tissue nutrient concentrations and nutrient demand are independent of the ambient environment. A mechanism which facilitates this non-homeostatic relationship is nutrient resorption from senescent tissue in EAV. The relatively high nutrient resorption efficiency of EAV species along the flow way points to the ability of EAV to be a strong nutrient sink. More detailed analysis is needed to evaluate N-resorption of EAV species and evaluation of resorption dynamics in SAV species to gain more complete information in nutrient retention of wetland macrophytes. Finally, additional study is needed to evaluate these species in the Everglades marsh ecosystem to completely understand ecosystem nutrient dynamics.

Acknowledgements

We would like to SFWMD and UF Wetland Biogeochemistry Laboratory staff members for field and analytical support. We would also like to thank 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).

References

Abel S, Ticconi CA, Delatorre CA (eds) (2002) Phosphate sensing in higher plants. Physiologia Plantarum 115:1–8.

Aerts R (1996) Nutrient Resorption from Senescing Leaves of Perennials: Are there General Patterns? Journal of Ecology 84:597–608. doi: 10.2307/2261481

Aerts R, Chapin FS (1999) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in ecological research 30:1–67.

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

Carrera AL, Sain CL, Bertiller MB (2000) Patterns of nitrogen conservation in shrubs and grasses in the Patagonian Monte, Argentina. Plant and Soil 224:185–193. doi: 10.1023/A:1004841917272

Chapin III FS, Schulze E-D, Mooney HA (1990) The Ecology and Economics of Storage in Plants. Annual Review of Ecology and Systematics 21:423–447.

Chimney M (2017) Performance of the Everglades Stormwater Treatment Areas. 2017 South Fla. Environ. Rep. 1:

Chimney MJ, Pietro KC (2006) Decomposition of macrophyte litter in a subtropical constructed wetland in south Florida (USA). Ecological Engineering 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

Cornwell WK, Bedford BL, Chapin CT (2001) Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. American Journal of Botany 88:1824–1829.

Corstanje R, Reddy KR, Prenger JP, et al (2007) Soil microbial eco-physiological response to nutrient enrichment in a sub-tropical wetland. Ecological Indicators 7:277–289. doi: 10.1016/j.ecolind.2006.02.002

De Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11:516–531. doi: 10.1111/j.1461-0248.2008.01164.x

DeBusk TA, Kharbanda M, Jackson SD, et al (2011) Water, vegetation and sediment gradients in submerged aquatic vegetation mesocosms used for low-level phosphorus removal. Science of The Total Environment 409:5046–5056. doi: 10.1016/j.scitotenv.2011.08.038

DeBusk WF, Reddy KR (1998) Turnover of detrital organic carbon in a nutrient-impacted Everglades marsh. Soil Science Society of America Journal 62:1460–1468.

Demars BOL, Edwards AC (2007) Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshwater Biology 52:2073–2086. doi: 10.1111/j.1365-2427.2007.01817.x

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 research 36:1409–1422.

Dinno A (2015) Dunn's test of multiple comparisons using rank sums. CRAN R-Project

Duarte CM (1992) Nutrient concentration of aquatic plants: Patterns across species. Limnology and Oceanography 37:882–889. doi: 10.4319/lo.1992.37.4.0882

Elser JJ, Fagan WF, Denno RF, et al (2000) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578.

Elser JJ, Fagan WF, Kerkhoff AJ, et al (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytologist 186:593–608. doi: 10.1111/j.1469-8137.2010.03214.x

Feijoó C, Leggieri L, Ocón C, et al (2014) Stoichiometric homeostasis in the food web of a chronically nutrient-rich stream. Freshwater Science 33:820–831. doi: 10.1086/677056

Fernández-Aláez M, Fernández-Aláez C, Bécares E (1999) Nutrient content in macrophytes in Spanish shallow lakes. Hydrobiologia 408:317–326.

Frost PC, Evans-White MA, Finkel ZV, et al (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. Oikos 109:18–28. doi: 10.1111/j.0030-1299.2005.14049.x

Gu Q, Zamin TJ, Grogan P (2017) Stoichiometric homeostasis: a test to predict tundra vascular plant species and community-level responses to climate change. Arctic Science 1–14. doi: 10.1139/as-2016-0032

Güsewell S (2004) N: P ratios in terrestrial plants: variation and functional significance. New Phytologist 164:243–266. doi: 10.1111/j.1469-8137.2004.01192.x

Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. Perspectives in Plant Ecology, Evolution and Systematics 5:37–61. doi: 10.1078/1433-8319-0000022

Güsewell S, Koerselman W, Verhoeven JTA (2003) Biomass n:p ratios as indicators of nutrient limitation for plant populations in wetlands. Ecological Applications 13:372–384. doi: 10.1890/1051-0761(2003)013[0372:BNRAIO]2.0.CO;2

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

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

Killingbeck KT (1996) Nutrients in Senesced Leaves: Keys to the Search for Potential Resorption and Resorption Proficiency. Ecology 77:1716–1727. doi: 10.2307/2265777

Koerselman W, Meuleman AFM (1996) The Vegetation N:P Ratio: a New Tool to Detect the Nature of Nutrient Limitation. The Journal of Applied Ecology 33:1441. doi: 10.2307/2404783

Lambers H, III FSC, Pons TL (2008) Plant Physiological Ecology. Springer Science & Business Media

Lorenzen B, Brix H, Mendelssohn IA, et al (2001) Growth, biomass allocation and nutrient use efficiency in Cladium jamaicense and Typha domingensis as affected by phosphorus and oxygen availability. Aquatic Botany 70:117–133. doi: 10.1016/S0304-3770(01)00155-3

Makino W, Cotner JB, Sterner RW, Elser JJ (2003) Are Bacteria More Like Plants or Animals? Growth Rate and Resource Dependence of Bacterial C: N: P Stoichiometry. Functional Ecology 17:121–130.

McGroddy ME, Daufresne T, Hedin LO (2004) Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. Ecology 85:2390–2401. doi: 10.1890/03-0351

Miao SL, Zou CB (2012) Effects of inundation on growth and nutrient allocation of six major macrophytes in the Florida Everglades. Ecological Engineering 42:10–18. doi: 10.1016/j.ecoleng.2012.01.009

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. Ecological Monographs. doi: 10.1002/ecm.1269

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

Peng H, Chen Y, Yan Z, Han W (2016) Stage-dependent stoichiometric homeostasis and responses of nutrient resorption in Amaranthus mangostanus to nitrogen and phosphorus addition. Scientific Reports. doi: 10.1038/srep37219

Persson J, Somes NLG, Wong THF (1999) Hydraulics efficiency of constructed wetlands and ponds. Water science and technology 40:291–300.

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

Reddy KR, Kadlec RH, Flaig E, Gale PM (1999) Phosphorus Retention in Streams and Wetlands: A Review. Critical Reviews in Environmental Science and Technology 29:83–146. doi: 10.1080/10643389991259182

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

Reed SC, Townsend AR, Davidson EA, Cleveland CC (2012) Stoichiometric patterns in foliar nutrient resorption across multiple scales. New Phytologist 196:173–180. doi: 10.1111/j.1469-8137.2012.04249.x

Rejmánková E (2005) Nutrient resorption in wetland macrophytes: comparison across several regions of different nutrient status. New Phytologist 167:471–482. doi: 10.1111/j.1469-8137.2005.01449.x

Rejmánková E, Snyder JM (2008) Emergent macrophytes in phosphorus limited marshes: do phosphorus usage strategies change after nutrient addition? Plant and Soil 313:141–153. doi: 10.1007/s11104-008-9687-0

Richardson CJ, Marshall PE (1986) Processes Controlling Movement, Storage, and Export of Phosphorus in a Fen Peatland. Ecological Monographs 56:280–302. doi: 10.2307/1942548

Rivas-Ubach A, Sardans J, Pérez-Trujillo M, et al (2012) Strong relationship between elemental stoichiometry and metabolome in plants. Proceedings of the National Academy of Sciences 109:4181–4186. doi: 10.1073/pnas.1116092109

Sardans J, Rivas-Ubach A, Peñuelas J (2012) The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics 14:33–47. doi: 10.1016/j.ppees.2011.08.002

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

Sistla SA, Appling AP, Lewandowska AM, et al (2015) Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. Oikos 124:949–959. doi: 10.1111/oik.02385

Sistla SA, Schimel JP (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. The New Phytologist 196:68–78.

Small GE, Helton AM, Kazanci C (2009) Can consumer stoichiometric regulation control nutrient spiraling in streams? Journal of the North American Benthological Society 28:747–765. doi: 10.1899/08-099.1

Sterner, Clasen, Lampert, Weisse (1998) Carbon:phosphorus stoichiometry and food chain production. Ecology Letters 1:146–150. doi: 10.1046/j.1461-0248.1998.00030.x

Sterner RW, Elser JJ (2002) Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press

Ticconi CA, Abel S (2004) Short on phosphate: plant surveillance and countermeasures. Trends in Plant Science 9:548–555. doi: 10.1016/j.tplants.2004.09.003

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press

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

Van Heerwaarden LM, Toet S, Aerts R (2003) Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. Oikos 101:664–669. doi: 10.1034/j.1600-0706.2003.12351.x

Yu Q, Elser JJ, He N, et al (2011) Stoichiometric homeostasis of vascular plants in the Inner Mongolia grassland. Oecologia 166:1–10. doi: 10.1007/s00442-010-1902-z

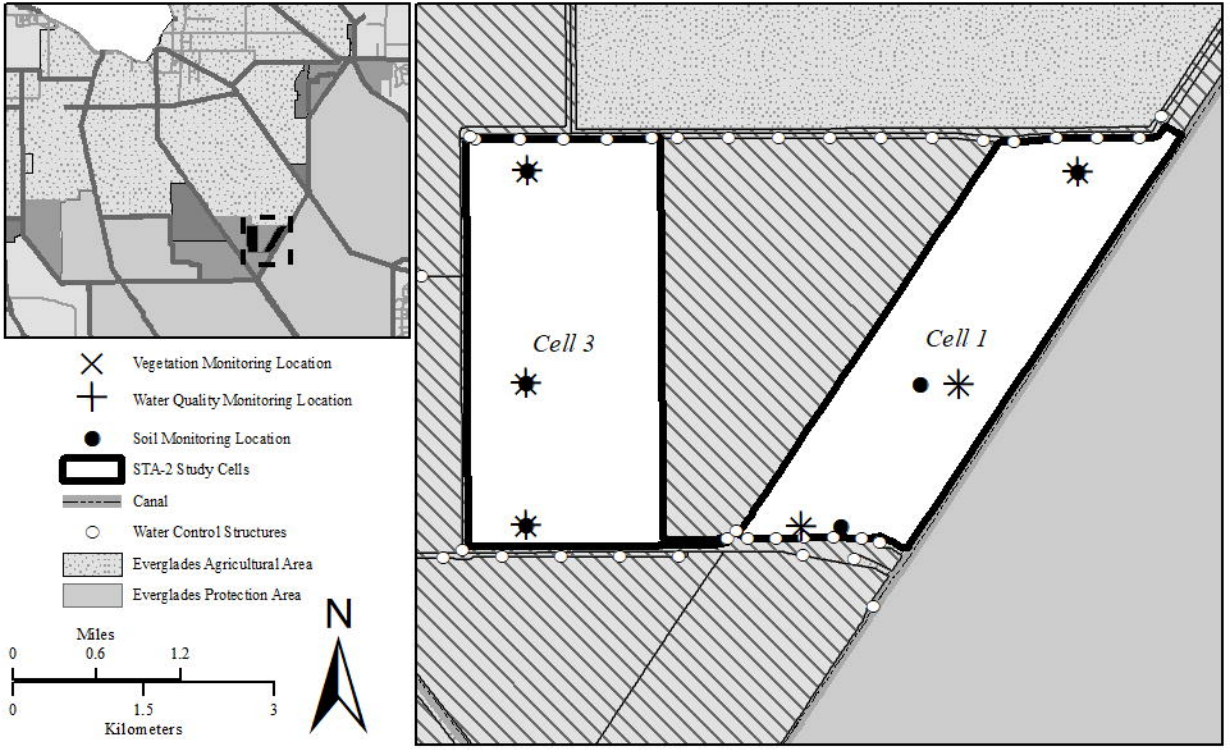
Figures and Tables

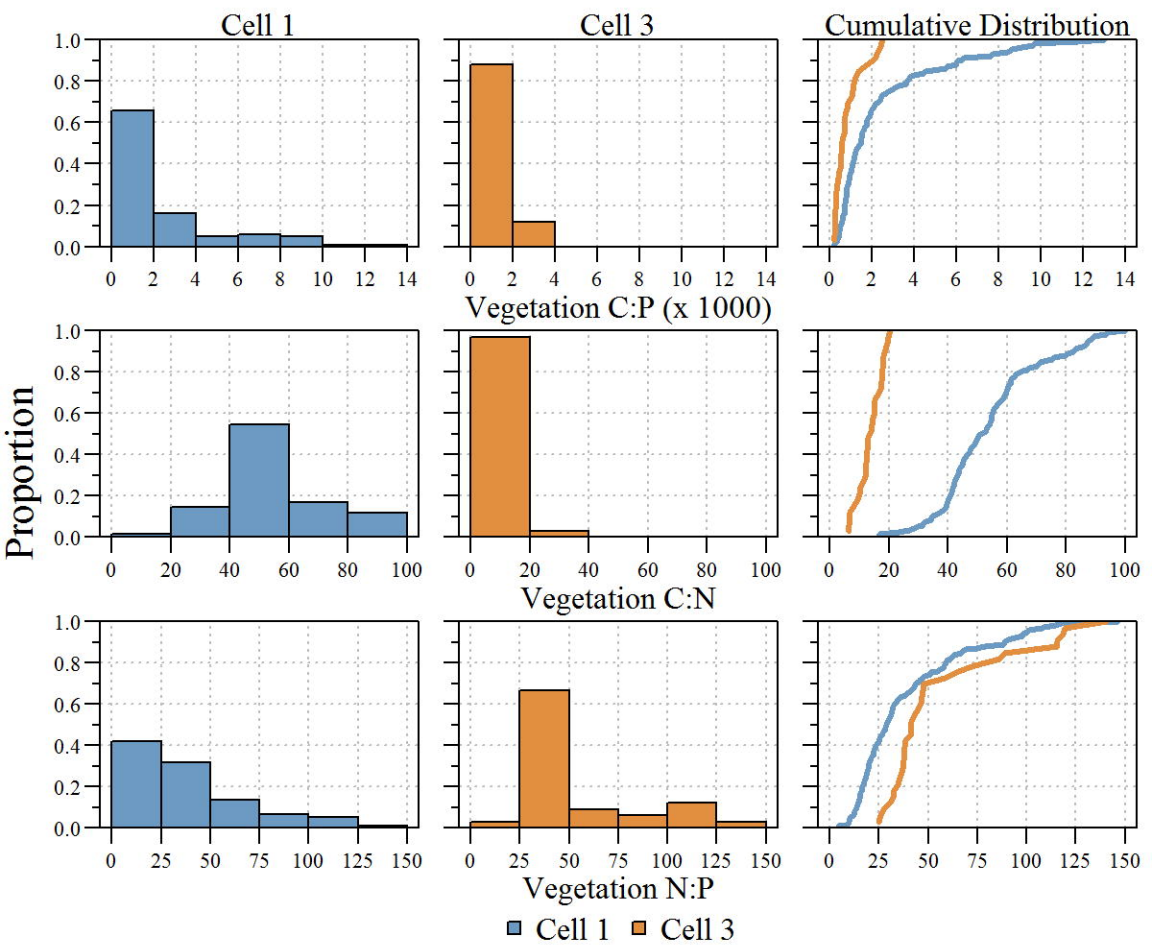
Figure 1. Surface water, soil and vegetation monitoring locations within Everglades Stormwater Treatment Area-2 Cells 1 (right) and 3 (left). Cell 1 is predominately emergent aquatic vegetation (EAV) and Cell 3 is predominately submerged aquatic vegetation (SAV).

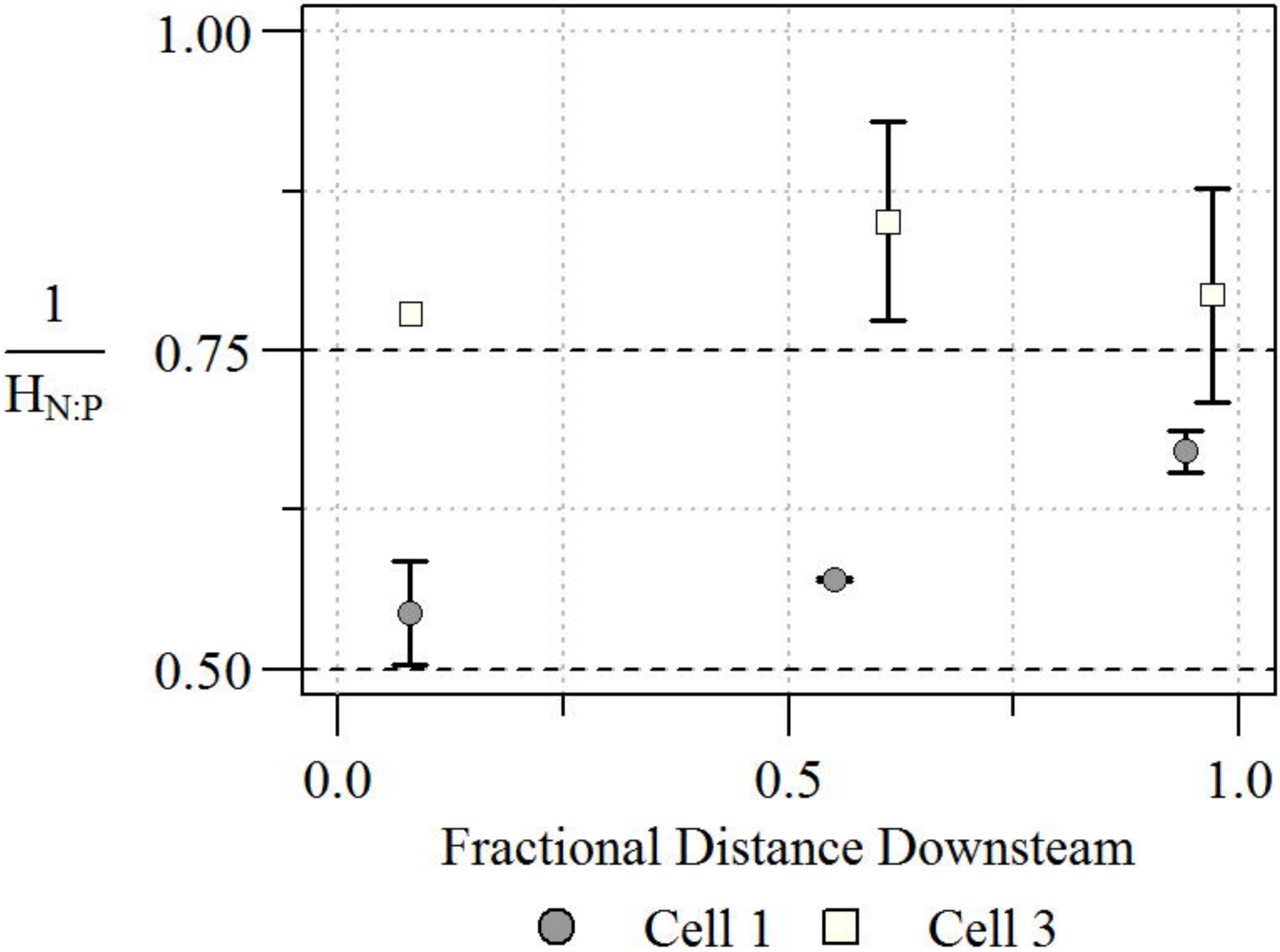
Figure 2. Distribution of plant foliar stoichiometric relationships within Cell 1 and Cell 3 of Stormwater Treatment Area-2. Left and middle panels are histogram representations of each respective stoichiometric relationship. The right panel is a cumulative distribution of stoichiometric relationship for each cell.

Figure 3. Mean ± standard error TN:TP molar ratio degree of stoichiometric homeostasis for emergent and submerged aquatic vegetation of cells 1 and 3, respectively in Stormwater Treatment Area-2. Dashed horizontal lines indicate boundaries of homeostatic categories.

Figure 4. Strip plot of total phosphorus resorption efficiency (RE_{TP}) of cattail within Stormwater Treatment Area-2 Cell 1. Raw data are represented by grey points, while mean ± standard error are represented by black points.


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


Cell 1
Cell 3
Cumulative Distribution
Proportion
Vegetation C:P (x 1000)
Vegetation C:N
Vegetation N:P
Cell 1
Cell 3


1.00
0.75
0.50
1
$H_{N:P}$
0.0
0.5
1.0
Fractional Distance Downsteam
Cell 1
Cell 3

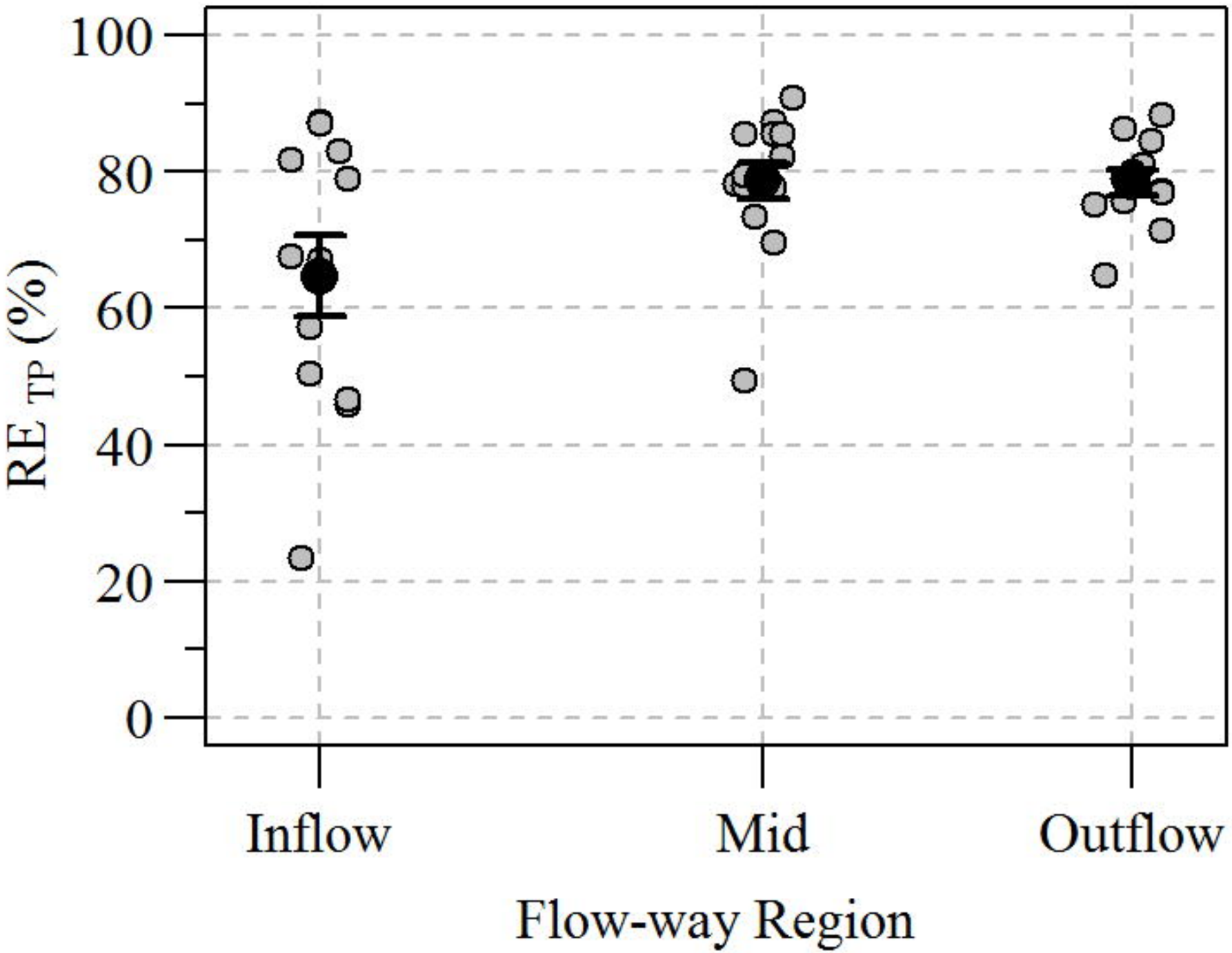

100
80
60
40
20
0
RE TP (%)
Inflow
Mid
Outflow
Flow-way Region

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 (CV). Matrices include surface water, 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
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. Total phosphorus resorption efficiency summary statistics for species collected within Stormwater Treatment Area 2, Cell 1. Total Phosphorus Resorption efficiency calculated consistent with equation 1, using both living and senescent aboveground living biomass. Summary statistics include mean, standard error, range, coefficient of variance and sample size (N).

Species	**Mean ± SE (%)**	**Range (%)**	**Coefficient of Variation**	**N**
Typha domingensis Pers. (cattail)	74.2 ± 2.5	23.6 – 90.9	20.1	36
Nymphaea odorata Aiton (water lily)	69.7	---	---	1
Cladium jamaicense Crantz (sawgrass)	77.5	---	---	1