Seal Tightly and Store in a Cool Dry Place: Exploring Soil Phosphorus Storage in a Subtropical Treatment Wetland.

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Abstract

Oligotrophic wetlands of the Everglades are often the final recipients of nutrients from adjacent ecosystems and tend to accumulate phosphorus (P) in their soils. Understanding P source and sink dynamics in wetlands are critical for managing wetland ecosystems and protecting downstream resources. In this study, soil P storage capacity (SPSC) was evaluated within two treatment flow-ways of the Everglades Stormwater Treatment Areas (STAs). This study hypothesized that SPSC will vary between flow-ways, soil depth, and spatially along the inflow-to-outflow gradient. The P storage capacity in the STAs depend on the proportion of iron, aluminum, calcium, and magnesium (Fe, Al, Ca, and Mg, respectively) to P with floc and recently accreted soils (RAS) being associated more with Ca and Mg and pre-STA soils being associated more with Fe and Al. Phosphorus loss, as indicated from SPSC values would vary between systems and soil depths suggesting a variable condition of P sink and source within and along flow-ways. This result, while limited, demonstrates the applicability of SPSC to wetlands systems and provides information that will aid operational or management decisions associated with improving P retention of the Everglades STAs.

Introduction

Soils and sediments within wetland ecosystems store an appreciable amount of phosphorus (P) relative to other ecosystem components. This dynamic is driven largely by ecosystem position along the terrestrial-aquatic continuum where wetlands exist at the interface between terrestrial and aquatic ecosystems and receive inputs from adjacent upland ecosystems (Reddy and DeLaune 2008; Badiou et al 2018). As such, P accumulated within wetlands from upstream land uses can be both reactive or stable pools of P reflecting historical conditions within the watershed (Nair et al 2015). The storage and retention of P in wetlands are driven by interrelated abiotic and biotic processes. More specifically, in mineral soils, P storage and retention can be dependent on the pH and concentrations of iron (Fe), aluminum (Al), calcium (Ca), and magnesium (Mg; Reddy et al 1999; Reddy et al 2005; Dierberg et al 2017).

While P storage is a benefit of wetland ecosystems, the potential to release stored P can put downstream ecosystems at risk. Therefore reliable techniques of predicting P storage and release have been developed taking into consideration organic and inorganic P, Fe, and Al as reviewed by Reddy et al (1998), Nair et al (2015), and others (Nair et al 2004; Nair and Harris 2004; Nair and Reddy 2013). The Degree of P saturation (DPS) or P saturation ratio (PSR) have been used as indicators of P loss from soils in agricultural applications (Maguire and Sims 2002; Haygarth et al 2005; Kleinman et al 2011). Meanwhile, Nair and Harris (2004) developed the concept of Soil P Storage Capacity (SPSC) where the P sorption capacity and PSR of a given soil are used to determine source/sink conditions relative to the capacity of soil to store P. Later, Nair et al (2015) applied the PSR and SPSC concepts to wetlands, demonstrating that heavily P impacted wetlands could be a potential P source ultimately signifying the value of the PSR-SPSC concept as a valuable tool for evaluating legacy P.

Treatment wetlands leverage biogeochemical processes to retain nutrients, sediments, and contaminants as a strategy to protect downstream ecosystems (Kadlec and Wallace 2009). The most notable are the Everglades stormwater treatment areas (STAs); these treatment wetlands in south Florida were constructed to improve the quality of agricultural runoff water originating in the Everglades Agricultural Area (EAA) before entering the downstream Everglades ecosystem (Chen et al 2015). The Everglades ecosystem is a P-growth limited system with flora and fauna adapted to nutrient-poor (i.e. oligotrophic) conditions (Noe et al 2001). To be protective of these sensitive downstream ecosystems the STAs have a regulatory water quality requirement, outlined in the Everglades Forever Act (§ 373 and § 403 Florida Statutes and 62-302.540 Florida Administrative Code; Florida Department of Environmental Protection 2005) to ensure that discharges entering the Everglades do not cause or contribute to the degradation of water quality, flora, and fauna. Therefore understanding the source and sink dynamics of the Everglades STAs is important for the protection of this essential downstream ecosystem. Prior studies have remarked on notable soil P concentrations and storage gradients spatially stretching from inflow to outflow and along the soil profile (Bhomia et al 2015; Zamorano et al 2018). However, limited information has been synthesized to evaluate SPSC of soils within the STAs. The overall objective of this study is to evaluate soil P sink/source dynamics within the Everglades STAs using the SPSC index. It is expected that as a result of biogeochemical cycling and burial dynamics, SPSC will vary between soil depths and spatially moving from inflow to outflow.

Methods

Study Area

Stormwater treatment areas (STAs) of the Everglades are constructed and managed wetlands that provide a natural reduction in water column pollutants and ultimately improve water quality conditions for downstream ecosystems. In South Florida, treatment wetlands were constructed to improve water quality and restore the biological integrity of the Everglades ecosystem by reducing nutrient concentrations of stormwater run-off from upstream land-uses before discharge to more pristine Everglades wetlands. A total of five STAs with an approximate area of 231 km² are located south of Lake Okeechobee on the southern edge of the Everglades Agricultural Area (Fig. 1). The Everglades STAs are established on land formerly dominated by natural wetlands and/or agricultural farmlands under sugarcane production. The primary source of inflow water to the STAs is agricultural runoff originating from approximately 284 km² of farmland. Everglades STA treatment cells or flow-ways are comprised of a mixture of emergent aquatic vegetation (EAV) and submerged aquatic vegetation (SAV) communities in several configurations including EAV and SAV treatment cells arranged in parallel or in-series (Chen et al 2015).

Stormwater Treatment Area-2 (STA-2) has been in operation since June 1999 with an effective treatment area of approximately 63 km² divided into eight treatment cells/flow-ways. This study was conducted in two flow-ways: flow-ways 1 and 3. Both flow-ways receive the same source water from the same inflow

canal. The vegetative community of flow-way 1 comprises predominately EAV including *Typha domingensis* Pers. (cattail) and *Cladium jamaicense* Crantz (sawgrass), while flow-way 3 is dominated by SAV, including *Chara* spp. (muskgrass), *Potamogeton* spp. (pondweed), and *Najas guadalupensis* Spreng (southern naiad), with approximately a quarter of the flow-way occupied by EAV species. Furthermore, prior to STA-2 construction, flow-way 1 was a historic natural wetland while approximately three-quarters of flow-way 3 was previously farmed and is now managed as an SAV system (Juston and DeBusk 2006).

Soil Sampling

Flocculent (floc) and soil samples were collected from transect locations in STA-2 flow-ways 1 and 3 extending from water inflow points to the wetland interior. Each transect in STA-2 consisted of 11 stations, and three stations along each transect (inflow, midflow, and outflow) were identified as benchmark stations where triplicate soil samples were collected. Soils were sampled using the push-core method consistent with methods used for prior wetland soil studies (Bruland et al 2007; Osborne et al 2011; Newman et al 2017). A 10-cm diameter polycarbonate core tube was pushed into the soil. Samples were extruded from the soil core tube and partitioned into floc and soil. Floc was characterized as the suspended unconsolidated material present on top of the consolidated soil and was segregated into a secondary sampling container, allowed to settle for 4 hours, and supernatant water was removed via aspiration with the remaining floc material being collected. The consolidated soil underneath the floc layer was sectioned into recently accreted soils (RAS) and pre-STA (sectioned into 0–5 and 5 to a maximum of 15 cm) based upon soil density and visual color differences. Sectioned soil samples were placed in heavy-duty air-tight plastic bags and stored at 4°C until analysis. For purposes of this study, floc, RAS, and the 0–5 cm pre-STA layer were used for comparison.

Soil metal and P fractionation

Total phosphorus was determined by a combination of ignition at 550° C and acid digestion to convert organic P into inorganic P, followed by analysis for inorganic P in digestates by ascorbic acid techniques (US EPA 1993). Total inorganic P in soil was extracted with 1 M HCl (soil to solution ratio = 1:50; 3 hours extraction time), filtered using 0.45 µm filter, and analyzed for P (US EPA 1993). Water extractable P in soil was extracted with deionized water using soil to water ratio of 1:10, filtered using 0.45 µm filters, and analyzed for P (US EPA 1993). Total and HCl extractable aluminum (Al), iron (Fe), calcium (Ca), and magnesium (Mg) were extracted with 1 M HCl and analyzed using inductively coupled argon plasma spectroscopy (US EPA 1994). Oxalate-extractable Al, Fe, and P were determined by extraction with 0.1 M oxalic acid and 0.175 M ammonium oxalate (McKeague and Day 1966). The suspension was equilibrated for 4 hours in the dark with continuous shaking, centrifuged, filtered through 0.45 µm filters, and analyzed using inductively coupled argon plasma spectroscopy. All chemical analyses were based on wet weight and corrected for moisture content.

The bulk density of the different soil core sections (i.e., floc, RAS, and pre-STA soils) was determined by taking the dry weight of each material divided by the volume of the material (section depth or thickness ×

area of the core). Soil pH was determined using 1:2 soil and water suspension and loss-on-ignition (LOI) was calculated using percent ash.

Data Analysis

For purposes of this study, the phosphorus saturation ratio (PSR) was broken into two forms, PSR that considers Fe and Al (PSR_{FeAl} ; Eq. 1a) and a PSR that considers Ca and Mg (PSR_{CaMg} ; Eq. 1b). Therefore the total PSR (TPSR) was calculated as the sum of the molar ratio (mmol kg^{-1}) of HCl extracted P to HCl extracted Fe and Al and the ratio of HCl extracted P to HCl extracted Ca and Mg consistent with Eq. 1c (Nair and Reddy 2013; Nair et al. 2015) where HCl extracted P, Fe, Al, Ca and Mg (P_{HCl} , Fe_{HCl} , Al_{HCl} , Ca_{HCl} , and Mg_{HCl} , respectively) are in units of mg kg^{-1} . Similarly, soil phosphorus storage capacity (SPSC) expressed as mg kg^{-1} was decomposed into its two dominant mineral associations, Eq. 2a, and 2b. Soil phosphorus storage capacity ($SPSC_x$) was calculated consistent with prior studies where PSR values compared to a PSR threshold ($PSR_{thres,x}$) corresponding to the critical P solution concentration, estimated from segmented regression ("segmented" R-package; Muggeo 2008) between PSR_x and water extracted total dissolved P consistent prior studies (Nair and Harris 2004; Nair and Reddy 2013). A PSR_{FeAl} threshold value of 0.57 (from segmented regression; Fig. 2) was used to estimate $SPSC_{FeAl}$. Soil phosphorus storage capacity associated with Ca and Mg ($SPSC_{CaMg}$) was calculated similarly to $SPSC_{FeAl}$ (Eq. 2b) where the threshold PSR ($PSR_{thres,CaMg}$) with a value of 0.003 was used. The $PSR_{thres,CaMg}$ value was determined based on the segmented regression analysis of water-soluble extracted P and PSR_{CaMg} (Fig. 2). Total SPSC was estimated as the sum of $SPSC_{FeAl}$ and $SPSC_{CaMg}$ for each sample. While $SPSC_{FeAl}$ relates to both positive and negative storage, the SPSC associated with Ca and Mg will likely be primarily releasable P.

$PSR_{FeAl} = \frac{\frac{P_{HCl}}{31}}{\frac{Fe_{HCl}}{56} + \frac{Al_{HCl}}{27}}$	Equation 1a
$PSR_{CaMg} = \frac{\frac{P_{HCl}}{31}}{\frac{Ca_{HCl}}{40} + \frac{Mg_{HCl}}{24}}$	Equation 1b
$TPSR = PSR_{FeAl} + PSR_{CaMg}$	Equation 1c

$SPSC_{FeAl} = \left(PSR_{thres, FeAl} - PSR_{FeAl} \right) \times \left(\frac{Fe_{HCl}}{56} + \frac{Al_{HCl}}{27} \right) \times 31$	Equation 2a
$SPSC_{CaMg} = \left(PSR_{thres, CaMg} - PSR_{CaMg} \right) \times \left(\frac{Ca_{HCl}}{40} + \frac{Mg_{HCl}}{24} \right) \times 31$	Equation 2b
$TSPSC = SPSC_{FeAl} + SPSC_{CaMg}$	Equation 2c

In addition to calculating PSR_{FeAl} using HCl extractable fractions, PSR_{FeAl} was also calculated using the oxalate extractable fraction consistent with Eq. 1a to evaluate the inter-compatibility between HCl and oxalate PSR_{FeAl} values. Oxalate extractable $SPSC_{FeAl}$ was also calculated with oxalate extractable PSR_{FeAl} threshold value of 0.77 (from segmented regression; Fig. S1). Oxalate and HCl extractable PSR and SPSC were compared using spearman's rank-sum correlation and an SPSC oxalate-HCl conversion equation was developed using median-based linear model (Komsta 2013). Median-based linear model was used rather than least-squares methods due to the violation of linear model assumptions. All other comparisons were made using HCl extractable PSR_{FeAl} and $SPSC_{FeAl}$.

Total SPSC was compared to biogeochemical (oxalate extractable P, total P, N, C, Ca, Mg, Fe, and Al) and physical characteristics (pH, bulk density, % OM and distance downstream) using principal component analysis (PCA, "vegan" R-package; Oksanen and Guillaume 2018). Parameters used to calculate TSPSC (i.e., HCl extracted P, Fe, Al, Ca, and Mg) were excluded from the PCA analysis to reduce co-linearity. Prior to PCA analysis, data were evaluated using Kaiser-Meyer-Olkin measure of sampling adequacy ("REdaS" R-package; Maier 2015) and Bartlett's Test of Sphericity ("REdaS" R-package; Maier 2015).

Soil phosphorus storage capacity was compared across soil depth category and flow-way using Dunn's pairwise comparison. Phosphorus source/sink potential was evaluated by determining if SPSC values were significantly different from zero using the One-Sample Wilcoxon rank sign test. To evaluate how SPSC and soil TP change along each STA flow-way, SPSC and TP were compared to fractional distance downstream using spearman's rank-sum correlation. Finally, SPSC was compared to soil TP for each STA flow-way using spearman's rank-sum correlation.

All plots were generated in base R except for the CaMg, FeAl, and P ternary diagram which used the "ggtern" R-package (Hamilton and Ferry 2018). All tables were formatted using "flextable" R-package (Gohel 2021). All statistical operations were performed using R (Ver 4.0.4, R Foundation for Statistical Computing, Vienna Austria). Unless otherwise stated, all statistical operations were performed using the base R library. The critical level of significance was set at $\alpha = 0.05$.

Results

As reported above, the segmented regression of PSR and water extracted total dissolved phosphorus (TDP) determined PSR thresholds of 0.003 ± 0.001 and 0.57 ± 0.19 (estimate $\pm$ standard error) for HCl extractable $PSR_{\text{thres, CaMg}}$ and $PSR_{\text{thres, FeAl}}$, respectively. The upper and lower 95% confidence interval of the threshold for PSR_{CaMg} was 0.001 and 0.004, respectively with an overall model adjusted R^2 value of 0.48. The upper and lower 95% confidence interval of the threshold for PSR_{FeAl} was 0.20 and 0.94, respectively with an overall model adjusted R^2 value of 0.24. It is expected, given the distribution of the data (Fig. 2) that the overall model adjusted R^2 values would be low, however for purposes of this study the break-point where the slope of the line changes is of particular interest. Likewise, oxalate extractable $PSR_{\text{thres, FeAl}}$ was 0.77 ± 0.63 with an upper and lower 95% confidence interval of -0.48 and 2.02 and an overall R^2 value of 0.44. Segmented regression method is an iterative approach and for the PSR_{CaMg} threshold, convergence was attained after one iteration (relative change 2.42×10^{-7}), two iterations (relative change 2.38×10^{-7}) for the $PSR_{\text{FeAl, HCl}}$ threshold, and five iterations (relative change 1.40×10^{-7}) for $PSR_{\text{FeAl, Ox}}$.

Oxalate and HCl extractable PSR and SPSC were both positively correlated ($r = 0.89$, $p < 0.01$ and $r = 0.88$, $p < 0.01$) for all soil depths (Fig. 3). The relationship between the two calculated PSR indices appears to be non-linear with higher PSR values deviating from the 1:1 line. However, limited data is available in this region. Based on the median-based linear model, the conversion of oxalate and HCl extractable SPSC for the system in this study is nearly a 1:1 relationship with an equation of

$$SPSC_{\text{HCl}} = (0.81 \times SPSC_{\text{Ox}}) + 34.00 \text{ and an } R^2 \text{ of } 0.79 \text{ (Fig. 3).}$$

Overall, Ca_{HCl} and Mg_{HCl} account for a greater proportion by an order of magnitude of mineral-based elements relative to Fe_{HCl} and Al_{HCl} (Fig. 4 and Table 1). While Ca_{HCl} and Mg_{HCl} are in greater proportion across samples, STA2 flow-way 1 soils contain a relatively greater range of Fe_{HCl} and Al_{HCl} when compared to STA2 flow-way 3. Moreover, STA2 flow-way 3 soils (floc and RAS) are predominately Ca_{HCl} and Mg_{HCl} (Fig. 4 and Table 1). Therefore, given the relative proportion of Ca_{HCl} and Mg_{HCl} to P_{HCl} , PSR_{CaMg} values are relatively low ranging from 0.0003 to 0.012 (Table S1). The proportion of Fe_{HCl} and Al_{HCl} was greater with PSR_{FeAl} values range from 0.02 to 5.01 (Table S1). Across the two flow-ways, TSPSC values ranged from - 1090 to 1636 mg kg^{-1} (Table 1) with all soil depth categories being significantly different than zero except for STA2 flow-way 1 RAS (Table 2).

Table 1

Summary statistics for parameters used in this study from samples collected along flow-ways 1 and 3 within Stormwater Treatment Area-2. Summary statistics expressed as mean $\pm$ standard error (sample size). Matrices include flocculent material (floc), recently accreted soil (RAS) and pre-STA soil (Pre-STA).

Mean $\pm$ SE (N)				
Soil Depth	Parameter	units	STA2 FW1	STA2 FW3
Floc	TP	mg kg ⁻¹	1719.7 $\pm$ 106.7 (20)	697.3 $\pm$ 67.8 (17)
	P _{HCl}	mg kg ⁻¹	362.0 $\pm$ 30.0 (20)	315.8 $\pm$ 52.2 (17)
	Ca _{HCl}	g kg ⁻¹	92.5 $\pm$ 11.9 (20)	304.1 $\pm$ 16.4 (17)
	Mg _{HCl}	mg kg ⁻¹	4755.0 $\pm$ 175.5 (20)	9090.0 $\pm$ 471.6 (17)
	Al _{HCl}	mg kg ⁻¹	220.2 $\pm$ 33.8 (20)	210.3 $\pm$ 39.6 (17)
	Fe _{HCl}	mg kg ⁻¹	190.4 $\pm$ 16.4 (20)	94.3 $\pm$ 18.7 (17)
	TPSR	ratio	1.27 $\pm$ 0.14 (20)	1.41 $\pm$ 0.26 (17)
	TSPSC	mg kg ⁻¹	-287.3 $\pm$ 56.1 (20)	276.0 $\pm$ 114.5 (17)
RAS	TP	mg kg ⁻¹	1085.6 $\pm$ 43.0 (19)	596.9 $\pm$ 49.4 (17)
	P _{HCl}	mg kg ⁻¹	226.9 $\pm$ 18.5 (19)	308.7 $\pm$ 50.5 (17)
	Ca _{HCl}	g kg ⁻¹	71.1 $\pm$ 7.6 (19)	277.8 $\pm$ 11.2 (17)
	Mg _{HCl}	mg kg ⁻¹	3954.6 $\pm$ 143.5 (19)	8368.8 $\pm$ 161.3 (17)
	Al _{HCl}	mg kg ⁻¹	290.4 $\pm$ 32.5 (19)	442.8 $\pm$ 44.2 (17)
	Fe _{HCl}	mg kg ⁻¹	171.2 $\pm$ 11.5 (19)	124.3 $\pm$ 12.2 (17)
	TPSR	ratio	0.60 $\pm$ 0.05 (19)	0.59 $\pm$ 0.10 (17)
	TSPSC	mg kg ⁻¹	-29.6 $\pm$ 29.7 (19)	388.1 $\pm$ 92.2 (17)
Pre-STA	TP	mg kg ⁻¹	415.1 $\pm$ 18.4 (19)	412.1 $\pm$ 16.8 (17)
	P _{HCl}	mg kg ⁻¹	80.8 $\pm$ 22.3 (19)	114.7 $\pm$ 15.6 (17)
	Ca _{HCl}	g kg ⁻¹	45.9 $\pm$ 1.3 (19)	68.5 $\pm$ 3.0 (17)
	Mg _{HCl}	mg kg ⁻¹	4307.9 $\pm$ 108.5 (19)	5252.4 $\pm$ 157.7 (17)
	Al _{HCl}	mg kg ⁻¹	1019.3 $\pm$ 55.4 (19)	1366.4 $\pm$ 88.4 (17)
	Fe _{HCl}	mg kg ⁻¹	140.5 $\pm$ 7.2 (19)	183.8 $\pm$ 15.2 (17)

Mean ± SE (N)				
	TPSR	ratio	0.08 ± 0.02 (19)	0.07 ± 0.01 (17)
	TSPSC	mg kg ⁻¹	673.1 ± 65.8 (19)	902.5 ± 62.9 (17)

Table 2

Results of one-sided Wilcoxon rank-sum test comparing soil phosphorus storage capacity (SPSC) for each stormwater treatment area (STA) flow-way and soil depth and testing if observed values were significantly different from zero.

STA	Soil	Median	Median	Median	V-statistic	p-value
Flow-way	Depth	SPSC _{FeAl}	SPSC _{CaMg}	TSPSC		
	Category	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)		
STA-2 FW1	Floc	-176.1	-142.9	-301.8	8	< 0.01
	RAS	14.2	-41.4	-52.6	81	0.59
	Pre-STA	693.5	72.5	758.3	187	< 0.01
STA-2 FW3	Floc	-86.6	473.3	413.5	124	< 0.05
	RAS	42.9	401.2	626.2	134	< 0.01
	Pre-STA	786.9	70.4	891.9	153	< 0.01

The KMO criterion of the dataset used in the PCA analysis was 0.75 indicating that the dataset was suitable for factor analysis. Bartlett's Test of Sphericity indicated that the data was significantly different from an identity matrix and suitable for factor analysis ($\chi^2 = 1730$, $df = 45$, $p < 0.01$). The first two components of the PCA accounted for 66% of the variance in the dataset with components 1 and 2 have eigenvalues greater than one (Fig. S2). In the multi-variate space (Fig. 5), soil depth and flow-ways are generally grouped into two distinct groups. Pre-STA would for both flow-ways group together in the upper right quadrant of the multivariate space (positively loading for PC1 and PC2). Based on the distribution in the ordination space, STA2 flow-way 3 is influenced (loaded) by the soil TCa and TMg and pH while flow-way 1 is more influenced by LOI, TC, and TN. This suggests differences in organic versus mineral (precipitation) biogeochemical pathways/mechanisms. Based on the loading of each variable (direction of arrows; Fig. 5), TSPSC is generally (positively) correlated with BD, TAl, TFe, and to some degree, fractional distance downstream and negatively correlated with P_{ox} and TP in the multivariate space (Fig. 5). Meanwhile, TSPSC was generally not correlated with TCa, pH, TN, LOI, or TC. However, these components all correlated with one another to some degree.

Across both flow-ways, TSPSC was significantly different ($\chi^2 = 12.7$, $df = 1$, $p < 0.01$), irrespective of soils depth. Within flow-way 1, TSPSC was significantly different between soil depth categories with floc, RAS, and Pre-STA soils all being significantly different (Fig. 6). Within flow-way 3, TSPSC was also

significantly different between soil depth categories ($\chi^2 = 31.0$, $df = 2$ $p < 0.01$) however, floc and RAS were similar ($Z = -1.05$, $p = 0.15$) but different than Pre-STA (Floc: $Z = -5.2$, $p < 0.01$; RAS: $Z = -4.2$, $p < 0.01$; Fig. 6).

Soil TP concentration and TSPSC varied at different degrees along each flow-way (Fig. 7). Along flow-way 3, TSPSC was positively correlated with distance downstream for floc ($r = 0.58$, $p < 0.01$), RAS ($r = 0.75$, $p < 0.01$) and Pre-STA ($r = 0.48$, $p = 0.05$). However, TSPSC was not correlated with distance downstream within flow-way 1 (Table 3). Soil TP was negatively correlated with distance downstream for floc and RAS in both flow-ways (Table 3). Given the different degrees of correlation with TSPSC and soil TP concentration, TSPSC was negatively correlated with TP across both flow-ways ($r = -0.87$, $p < 0.01$ and $r = -0.77$ $p < 0.01$ for flow-way 1 and 3, respectively) for all soil depth categories.

Table 3
Spearman's rank correlation results comparing total soil phosphorus storage capacity (TSPSC) and soil total phosphorus (TP) concentration to fraction distance downstream for each stormwater treatment area (STA) flow-way and soil depth.

Soil Depth Category	STA Flow-way	N	TSPSC x Frac. Dist.		TP x Frac. Dist.	
			Spearman's r	p-value	Spearman's r	p-value
Floc	STA-2 FW1	20	0.40	0.08	-0.76	< 0.01
	STA-2 FW3	17	0.58	< 0.01	-0.51	< 0.05
RAS	STA-2 FW1	19	-0.02	0.94	-0.85	< 0.01
	STA-2 FW3	17	0.75	< 0.01	-0.63	< 0.01
Pre-STA	STA-2 FW1	19	0.35	0.14	0.42	0.07
	STA-2 FW3	17	0.48	0.05	0.37	0.14

Discussion

Wetland soils have a finite capacity to store P with increasing P loading potentially pushing the system from being a P sink to a source (Reddy et al 1999). This shift from a sink to a source can increase the risk of impacting downstream ecosystems via eutrophication (Nair and Reddy 2013). The mobility of P is balanced between P retention capacity and P buffering intensity of the soil (Reddy et al 1999), with soil physicochemical characteristics such as Fe and Al complexes (including amorphous and poorly crystalline forms), Ca and Mg-based mineral and organic matter being important in regulating P sorption and buffering intensity (Syers et al 1973; Chambers and Odum 1990; Reddy and DeLaune 2008). In general, P can be integrated with Fe and Al complexes while P is typically associated with (not bound) Ca and Mg minerals in wetland soils. This complexation versus association is amplified by physicochemical dynamics within any given system where fluctuations in pH and redox conditions can cause rapid

dissociation (Reddy and DeLaune 2008). Moreover, this complexation versus association is apparent in the PSR value observed in this study with PSR_{CaMg} being orders of magnitude lower than that of PSR_{FeAl} across flow-ways and soil depths indicating that P complexation with Fe and Al is much more stable. This relationship of complexation versus association between Fe + Al and Ca + Mg, is especially true for acidic soils however, Ige et al (2005) have demonstrated the use of a Ca and Mg DPS is more appropriate in neutral to alkaline soils to evaluate P sink/source in calcareous soils. Given the different processes of P retention between acidic (Fe + Al) and calcareous (Ca + Mg) soils, and in wetland soils in this study, easily releasable P from this “stored” P, indices of P saturation and retention (i.e. PSR, DPS, or SPSC) are not equitable between the different mineral groups.

PSR Threshold variability

The change point between PSR and water-soluble/water-extractable P (i.e. PSR_{thres}) is a soil-specific threshold (Bhadha et al 2010). Several studies have used values ranging from 0.10 to 0.20 as a PSR threshold in predominately sandy soils (Nair et al 2004; Nair and Harris 2004; Nair et al 2015). Bhadha et al (2010), used a PSR threshold value of 0.04 for wetlands soils. Fundamentally these thresholds signify a change point at which water-soluble P begins to change relative to PSR. However, several studies have documented the variability in water-soluble P methods (McDowell and Sharpley 2001; Chrysostome et al 2007b) which could lead to variability PSR_{thres} values. In the current study, PSR_{thres} value with respect to Fe and Al (PSR_{FeAl}) was determined as 0.57 (Fig. 2) for HCl extractable and 0.77 (Fig. S1) for oxalate extractable PSR_{FeAl} using estimates of water-extractable TDP on a wet weight basis. Prior studies have evaluated PSR/SPSC estimates comparing oxalate and Mehlich-extractable analytes suggesting that a conversion factor may be needed to evaluate comparability between methods (Nair and Harris 2004; Chrysostome et al 2007a).

Differences in $PSR_{thres, FeAl}$ across studies could also be due to variability in soil composition, prior loading, and hydrologic conditions. As reviewed by Bhadha et al (2010) and Dieter et al (2015) repeated drying and wetting of soils can affect the capacity of soils to sequester P through changes to crystalline structure and oxidation of Fe. Meanwhile, P sorption by other metals such as Al, Ca and Mg does not involve oxidation-reduction reaction and is much more influence by pH and organic matter content (Traina et al 1986; Diaz et al 1994; Staunton and Leprince 1996). It is likely that the hydrologic conditions of the STAs, where dry-out conditions are typically avoided, remain hydrated and contributed to the difference in $PSR_{thres, FeAl}$ relative to other studies discussed above.

Due to the relatively mineral nature of the soils in this study, PSR and SPSC estimates were evaluated with respect to HCl-extractable Ca and Mg. In acidic mineral soils, Fe and Al are more active in P-cycling and soil P buffering capacity tends to be higher relative to circumneutral Ca-rich soils (Richardson 1985; Richardson and Vaithiyanathan 1995). Prior studies have documented the role of Ca in regulating P sorption and accumulation across the Everglades, especially in the flow-way 3 of STA-2 (Reddy et al 1993; Richardson and Vaithiyanathan 1995; Dierberg et al 2017; Julian et al 2019; Julian et al 2021) The

proportion of P to Ca and Mg in this study is relatively low, however, a change point at which water-soluble P starts to increase is apparent (Fig. 2). While PSR_{FeAl} makes up a large percentage of the TSPSR, significant variability between flow-ways is evident (Table 1 and S1) with Ca being an important component in abiotic retention of P in the STAs (Dierberg et al 2002; Gu 2008; Zamorano et al 2018). Moreover, the P associated with Ca and Mg can be easily releasable relative to Fe and Al bound P.

SPSC Conversion

Numerous studies have used either oxalate or Mehlich extractable P fractions to estimate PSR and SPSC (Maguire and Sims 2002; Nair et al 2004; Chrysostome et al 2007a; Bhadha et al 2010). However very few, if any, compared the indices from different extractions. This study compared HCl and oxalate extraction PSR and SPSC indices to i) ensure consistency between other studies, and ii) evaluate the potential for a simpler analysis for PSR and SPSC studies in the future. Both extraction procedures have their limitation and preferentially extract particular P fractions, for instance, HCl extracts inorganic P while oxalate P extracts both inorganic and organic P (Reddy and DeLaune 2008). Furthermore, HCl extracted P can be classified as the reactive P pool including Fe, Al, Ca and Mg bound P (Reddy et al 2020). In the case of this study, HCl and oxalate extractable PSR and SPSC were comparable (Fig. 3) with the potential of a non-linear relationship between oxalate and HCl PSR. This potential non-linear relationship could be due to differences in soil texture between the two flow-ways, the relative proportions of P (i.e., inorganic versus organic), the extraction efficiencies of the method, or a combination of these methods. While the indices are highly correlated caution should be used applying this across different systems, soil types, and/or nutrient conditions.

Sink- Source Dynamics

While PSR and the PSR_{thres} are important metrics for environmental risk assessments, they do not provide an estimate of P storage capacity or P source/sink dynamics. Originally developed and tested for upland sandy soils (Nair et al 2004; Nair and Harris 2004), SPSC has also been applied to wetland soils (Mukherjee et al 2009; Bhadha et al 2010; Nair et al 2015). Nair et al (2015) concluded that soil organic matter in wetland soils did not affect P retention and release below the PSR_{thres} despite the ability of organic matters' ability to complex with most metals and potentially lowering the affinity for P. Moreover, Nair et al (2015) also noted that wetlands that are heavily P impacted could be a P source where vegetation or other ecosystem components may no longer be able to assimilate addition P. These results are congruent with the results presented in this study where TSPSC was not correlated with organic matter (i.e. LOI) and inversely correlated with TP and P_{Ox} (Figs. 5 and 7).

Wetlands soils are not a homogenous column of accreted material but rather layers of reactive biological material, decaying organic matter, and geologic material. The first layer and possibly the most reactive is the floc layer, a complex matrix of microbes, organic matter, and inorganic material, floc is ecologically important in the aquatic ecosystem and is relevant to several biogeochemical processes (Noe et al 2001; Neto et al 2006). The source/sink dynamics of floc can be variable across systems as noted in this study (Fig. 5 and Table 1). As observed in this study, EAV systems have a distinct partitioning of TSPSC

between soil depths while SAV systems are less notable with respect to surface soils. Generally, floc has a relatively low bulk density (Table S1) and therefore a greater pore space providing the opportunity for more surface area for P to flux onto and from mineral and organic particles within the complex floc matrix.

As demonstrated by prior studies (Nair and Reddy 2013; Nair et al 2015) using SPSC, and this study using TSPSC, SPSC provides a potential indicator of P sink/source (Fig. 7 and Table 2). This metric provides a relative risk of P mobilization for a particular area or soil compartment. As soils build, P storage capacity increases, suggesting that mineral components are largely responsible for P retention of the soil (Nair et al 2015). As observed in this study, generally TSPSC values for pre-STA soils were positive (Figs. 5 and 6), with $SPSC_{FeAl}$ making up a greater proportion of the overall TSPSC (Fig. S3, S4, and Table S1) suggesting P-retention via mineral association and a relatively stable P-sink. Floc or RAS P-retention is presumably associated with a combination of biological (i.e., microbial biomass) and mineral (to a lesser degree) processes. Additionally, pre-STA soils are relatively deep and effectively removed from active P-cycling. However, SPSC values reported in this study for pre-STA soils are relatively high compared to prior studies and presumably linked to the relatively high estimated $PSR_{FeAl, thres}$. Regardless of using the estimated $PSR_{FeAl, thres}$ or literature reported values (i.e., 0.1; Fig. S5), pre-STA soils are consistently a sink of P whilst Floc is a source and RAS is variable across the flow-way. Moreover, it is worth noting that TSPSC only provides an index of soil mineral P storage capacity (Nair et al 2015) and does not account for biological storage capacity via microbial biomass, macrophytes, or organic matter.

The formation of P soil gradients in treatment wetlands can have important implications for the cycling of P within wetland ecosystems (Juston and DeBusk 2011; Zamorano et al 2018). Treatment wetlands remove a greater proportion of water column nutrients near the front of a given flow-way with downstream areas receiving comparatively less nutrient loading through biotic and abiotic retention of nutrients (Kadlec and Wallace 2009). In this study floc and RAS TP concentrations declined with distance downstream (Fig. 7 and Table 3). Concurrent with this declining trend in TP concentration, P sink strength in floc and RAS also generally increased with distance downstream especially in flow-way 3 (Fig. 7 and Table 3). These results are consistent with the conclusions by Nair et al. (2015), where regions with high TP concentrations (inflow) were also generally P sources (Fig. 6).

Biotic and abiotic processes regulate the retention and release of P from soils to the overlying water column (Reddy et al 2005). As discussed above abiotic processes such as soil Fe, Al, Ca and Mg, pH, and other factors regulate the retention of P, however, biotic factors also play a critical role in the retention capacity of a wetland to sequester P. The observed, at times, contrasting gradients of soil TP concentration and TSPSC highlight the variable P source and sink dynamic along the study flow-ways. In STA-2 flow-way 3, the shift of P source to P sink (Fig. 7) is also reflected in the aggregated internal load reported in a recent study that demonstrated internal loading can be a substantial contributor to water column P concentrations, especially at inflow regions of the flow-way (Jerauld et al 2020).

Conclusions

Wetlands are dynamic ecosystems that transform and retain nutrients through biotic and abiotic processes. For treatment wetlands, these processes are leveraged to improve water quality and protect downstream ecosystems. In the case of the Everglades, understanding and estimating P storage and release (i.e., source/sink dynamics) is vitally important to ongoing and future restoration efforts. Based on the SPSC, the potential for P loss from the floc could be greater in flow-way 1 while RAS in flow-way 1 is at or near storage equilibrium. The storage equilibrium concept is supported by corroborating P accretions rates reported for flow-way 1 (Bhomia et al 2015) and average annual P load retention rates (SFWMD, unpublished data). However, despite significant declines in soil TP within flow-way 1, TSPSC was not significantly correlated with distance downstream. Based on the results of the principal component analysis, this lack of significant TSPSC change within flow-way 1 could be due to biological retention and source/sink dynamics.

The potential for P loss within the STA should not be evaluated using surface soil samples only. Similar to the behavior of upland and wetlands soils elsewhere, TSPSC varied with soil depth across flow-ways. In addition to changes with soil depth, the percent contribution of Fe, Al, Ca, and Mg change with soil depth as noted by the shift in $SPSC_{FeAl}$ contributing more to the overall storage capacity in Pre-STA soils. The difference of TSPSC between floc and RAS could vary suggesting P loss from floc in one system (flow-way 1) versus P retention in both floc and RAS in another (flow-way 3). While P associated with Ca and Mg is easily releasable, TSPSC only provides an index of temporary soil mineral P storage capacity and does not account for other storage and retention processes. The contribution integrating Fe + Al and Ca + Mg to TSPSC provides some understanding of the storage strength of soil P relative to surface water P concentration at the present time. This is reflective in flow-way 3 where soils are calcitic in nature and surface water outflow total P concentrations are higher and with more variability relative to flow-way 1 (Villapando and King 2017).

Overall, the use of SPSC in a wetlands application can provide a metric of risk of P release (i.e. source/sink status) and provide evidence to inform management decisions. Within treatment wetlands or wetlands that receive high nutrient discharges, eutrophication gradients can develop where soil and other compartments (i.e., vegetation, water column) begin to become enriched with nutrients thereby reducing the overall efficiency of the system to remove and store P shifting from a sink to a source. The efficiency of this storage and retention of P as it relates to SPSC is also dependant on soil composition (i.e., organic versus mineral). Other factors such as soil and P accretion and loading may also play a role. While this was an initial study in the applicability of SPSC to treatment wetlands, additional investigation evaluating soil mineral composition, a finer soil depth profile, and potentially other metrics of P retention is warranted.

Declarations

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Authors' Contributions

PJ performed data analyses including necessary calculations and statistical analyses and wrote the manuscript. VN was involved with writing of the manuscript. TZO was involved with soil sample collection and writing of the manuscript.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Data Availability

Data available upon request.

Code Availability

The R code used during the current study is available from the corresponding author on reasonable request.

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Ethics declarations

Human and Animal Rights and Informed Consent

We did not capture nor handle any animal.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

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Figures

Figure 1

Soil sampling locations within the Everglades Stormwater Treatment Area (STA) flow-ways 1 and 3 (FW 1 and 3, respectively). STA-2 flow-way 1 is predominately emergent vegetation and STA-2 flow-way 3 is predominately submerged aquatic vegetation.

Figure 2

Relationship between water extracted total dissolved phosphorus (TDP) and calculated phosphorus saturation ratio (PSR) for iron and aluminum PSR (PSR_{FeAl}; left) and calcium and magnesium (PSR_{CaMg}; right) for stormwater treatment area 2 flow-ways 1 and 3 (STA-2 FW1 and FW3, respectively). Change-point, as determined by segment regression, are identified by the vertical dashed line.

Figure 3

Comparison of oxalate and HCl extractable iron and aluminum phosphorus saturation ratio (PSR_{FeAl}) and soil phosphorus storage capacity ($SPSC_{FeAl}$) for stormwater treatment area 2 flow-ways 1 and 3 (STA-2 FW1 and FW3, respectively).

Figure 4

Ternary diagram comparing HCl extracted calcium and magnesium, iron and aluminum, and phosphorous (HCl Ca+Mg, HCl Fe+Al, and HCl P, respectively) for each stormwater treatment area (STA) flow-way and soil depth.

Figure 5

Principal Component Analysis (PCA) comparing total soil phosphorus storage capacity (TSPSC) to soil characteristics such as bulk density (BD), total iron, aluminum, calcium, magnesium, phosphorus, nitrogen, and carbon (TFe, TAl, TCa, TMg, TP, TN and TC, respectively), oxalate-extracted P (Pox), loss-on-ignition (LOI) and fractional distance downstream (Frac. Dist.) each stormwater treatment area (STA) flow-way (FW) separately. Each PCA axis identifies the percent variance explained by each respective component. EAV: Emergent aquatic vegetation EAV; SAV: submerged aquatic vegetation.

Figure 6

Total phosphorus storage capacity for compared across soil depth category within each stormwater treatment areas (STA) flow-way (FW). Letters above each boxplot indicate statistical similarity as determined by Dunn's multiple pair-wise comparisons within each STA FW (not between).

Figure 7

Mean ($\pm$ standard error) soil total phosphorus (TP; top), total soil phosphorus storage capacity (TSPSC; middle) by fractional distance downstream and TSPSC compared to soil TP for each soil depth category along stormwater treatment area 2 flow-way 1 (STA2 flow-way 1; bottom left) and STA2 flow-way 3 (bottom right).

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