DELIVERABLE A-7-A: DRAFT MANUSCRIPT

Inter-Agency Agreement to Conduct Scientific Studies Relevant to the Stormwater Treatment Areas

Agreement No. 4600003125

SUBMITTED: September 15, 2015

PREPARED FOR: South Florida Water Management District, and
Everglades Agricultural Area Environmental Protection District

PREPARED BY: DB Environmental, Inc.

The following represents a DRAFT manuscript addressing selected aspects of DB Environmental's recent PSTA research. This will be re-submitted in a final version for SFWMD review in approximately 2 – 3 weeks, once comments/edits from the SFWMD co-author (D. Ivanoff) are received and incorporated into the document.

Macrophyte and Substrate Effects on Internal Phosphorus Loading In Stormwater Treatment Areas for Everglades Restoration

K. Grace, D. Finn, J. Juston, W. DeBusk, T. DeBusk, D. Ivanoff

Abstract

Since implemented over 15 years ago, farmland best management practices (BMPs) followed by constructed wetlands, known as Stormwater Treatment Areas (STAs), have been highly effective at removing phosphorus (P) from inflowing waters to northern Everglades regions. However, there is a need to produce even lower STA discharge concentrations beyond current apparent limits (<~15 ug/L). Thus, ongoing research focuses on understanding and minimizing internal P loading in back-end STA regions, and on different wetland types that might augment existing STA configurations. A 40 ha pilot-scale implementation of one such system has been operating since 2005; this system contains submerged aquatic vegetation (SAV) with associated periphyton assemblages grown on a limerock substrate. Here, we document a sediment core incubation study aimed at understanding the interaction between vegetation and substrate types on internal loading in these systems. Our study utilized a 3x3 factorial design with three vegetation treatments and three substrate treatments. Vegetation treatments included a non-vascular macro-algae (*Chara sp*.), a rooted macrophyte (*Potamogeton illinoensis*.) (both also prevalent in the pilot- and full-scale STA systems), and an unvegetated control. Substrate treatments included bare limerock, limerock with accrued marl sediment, and muck sediment

more typical of existing STA substrates. We monitored substrate TP, water column P dynamics during sequential two-week batch incubations, and before-after vegetation biomass and tissue P. Results indicated that P "mining" from rooted macrophytes might indeed be contributing to higher internal loads, particularly from enriched peat substrates, in comparison to other treatments. Bare limerock substrate appeared relatively inert across vegetation types. Limerock covered with accrued marl appeared intermediate in reactivity, but was still far more inert than peat sediments. These results are relevant for understanding and possibly improving constructed wetland performance at the lower limits of P removal.

Introduction

As part of Everglades restoration, a system of upstream nutrient source controls from agricultural area discharges followed by a network of large constructed wetlands, known as Stormwater Treatment Areas (STAs), has removed over 4500 MT of phosphorus (P) from entering northern Everglades waters since large-scale implementation began in approximately year 2000 (Adorisio and McCafferty, 2015; Chimney, 2015). This equates to an approximate 90% net reduction in inflow load to Everglades regions that were most impacted by previous nutrient overloads (REFERENCES).

The existing Everglades STA network comprises approximately 275-km^2 of surface area and forms a buffer between upstream discharges and the Everglades Protection Area (EvPA). The six individual STAs, which are managed by the South Florida Water Management District (SFWMD), vary in size and configuration but, in general, all are shallow freshwater marshes divided into cells and flow paths by interior levees. Most of these engineered treatment wetlands were constructed on reclaimed farmlands, which were in turn developed on previous wetland peat soils. Vegetation in the STAs is typically classified as either SAV (submerged aquatic vegetation) or EAV (emergent aquatic vegetation). Most cells contain some amount of both SAV and EAV, along with some floating aquatic vegetation (Chimney, 2015). However, cells typically are classified by their dominant vegetation type.

The majority of final treatment in the STAs is accomplished by SAV-dominated cells, as they tend to be more efficient for P removal and achieve lower outflow P concentrations than most EAV cells (Dierberg et al., 2002; Gu and Dreschel, 2006; Juston and DeBusk, 2006). Additionally, the marl sediment produced by SAV systems is considered more stable and less prone to internal loading than from peat sediment produced by EAV wetlands (REFERENCES). As of this writing, the P removal performance of SAV cells determines the discharge P performance for about 95% of the net STA footprint (from data in Chimney, 2015). The best performing SAV cells and thus STA discharges tend to discharge P concentrations on the order of 15-20 µg/L, but not much lower than this on a consistent basis across systems and/or time (Juston and DeBusk, 2011; Chen et al., 2015; Pietro and Ivanoff, 2015). However, current STA regulatory criteria require even lower STA discharge concentrations on a consistent basis. Thus there is

now considerable focus on how to achieve this (SFWMD, 2013), with emphasis on understanding and minimizing internal P loading in the outflow region of STAs, and on different wetland types that might possibly augment existing STA configurations.

In 2004-05, SFWMD initiated a 40-ha pilot-scale system known as the Periphyton-based STA (PSTA). In this PSTA implementation, the extant peat was scraped to expose the underlying limerock. Peat removal was conducted for multiple purposes, i.e., to discourage colonization by EAV that would shade out the periphyton and to remove a potential internal source of phosphorus that would reduce P removal efficiency (Pietro et al., 2007). These principles follow earlier work with mesocosm experiments on PSTA systems (Bays et al. 2001; DeBusk et al., 2004). This field-scale PSTA implementation is one example of a possible wetland system that might augment the current STA configurations. Another example under research is systems dominated by water lily vegetation (Miao, 2015). Other possibilities may still emerge from ongoing research efforts (SFWMD, 2013).

The vegetation in the ongoing PSTA pilot facility has been dominated by two SAV species: *Potamogeton illinoensis* and *Chara* sp. Both *Potamogeton* and *Chara* are also important SAV species in the full-scale STAs. The former is a vascular plant, with true roots and internal translocation of nutrients, whereas the latter is a non-vascular macro-algae, with holdfasts, rather than roots. Without roots, *Chara* relies on direct uptake of nutrients from the surrounding water. Because of these morphological differences, the soil nutrient "mining" potential of *Potamogeton* may theoretically be greater than that of *Chara*. It should be noted that significant uptake of sediment P has been reported for *Potamogeton* and other rooted SAV species in shallow aquatic systems (Barko and Smart, 1981; Bottomley and Bayly, 1984; Carignan and Kalff, 1980; Rattray et al., 1991). Additionally, after several years of operation, a layer of accrued marl sediment has covered the underlying limerock substrate. Thus, the sustainability of P removal processes in a PSTA system remains an important management concern.

In a modeling study, Juston et al. (2013) explored the potential role of soil-P mining and macrophyte biomass turnover in the P removal performance of SAV treatment wetlands. The study was based on the concept that SAV provides effective P removal from the water column, but simultaneously acts as an internal load to the water column via P cycling through the vegetation itself, thus possibly limiting its ability to achieve ultra low water column P concentrations (<16 µg/L). Modeling results indeed suggested that P release to the water column due to soil-P mining and subsequent biomass turnover could be a significant contributor to outflow P in SAV-dominated treatment wetlands, possibly accounting for a third of the remaining 15-20 µg P/L observed as the lower limit in well-performing SAV systems.

The purpose of the present study is to test whether SAV growth on a P-enriched muck substrate would affect water column P concentrations in treatment wetland systems operating at the lowest limits of P removal. Outdoor soil-water microcosms were established to investigate the effects of SAV species composition and underlying substrate characteristics on plant growth

and P removal efficiency. Results of the microcosm study were evaluated in the context of trends in water chemistry and vegetation P content in the STA outflow region wetlands from which soils were collected. The findings are highly relevant for understanding and possibly improving constructed wetland performance at the lower limits of P removal, such as the pilot-scale PSTA system and full-scale SAV systems in the STAs.

Methods

Study sites

The 40 ha pilot-scale facility is situated along a portion of the back end of the 2190 ha Central flow path of STA-3/4. The upstream vegetation communities include emergent macrophytes (primarily *Typha domingensis*) while SAV becomes more dominant in the downstream half of the flow way. The limerock substrate PSTA cell therefore comprises a final back end treatment process for a portion of the flow in the central flow path (Figure 1). Operations of the PSTA cell were initiated in 2007, and by January 2012 an average of 9.2 cm (3.5-19 cm, N = 39) of marl had accumulated above the limerock substrate (Piccone et al. 2013).

STA-2 Cell 3 is a 920 ha SAV-dominated wetland, with *Chara* sp., *Najas guadalupensis* and *Potamogeton illinoensis* comprising the dominant macrophytes. Among Everglades STAs, this wetland is considered a well-performing STA flow way (Juston and DeBusk 2011). On March 27, 2014, water depth was 50 cm at the muck soil core collection site (26.38020 °N, 80.54911 °W) in the outflow region of STA 2 Cell 3. The total soil thickness above the limestone bedrock was 47 cm, and the surrounding marsh vegetation was dominated by *Najas guadalupensis* and *Potamogeton illinoensis*.

Sediment core incubation

A sediment core incubation study was established using *Potamogeton*, *Chara*, and non-vegetated controls as "vegetation" treatments coupled with three separate "substrate" treatments. For the first substrate treatment, intact cores of marl/muck substrate (accrued calcitic sediment overlying previously-cultivated organic soil) were obtained from the outflow region of STA-2 Cell 3. For the second substrate treatment, a bare limerock substrate was used to represent the initial (startup) PSTA Cell condition after removal of the overlying muck soil. Marl sediments that had accrued in the PSTA Cell over more than 7 years of operation were selected for the third substrate treatment. This material was intended to represent the steady-state physico-chemical condition for sediments in a PSTA-type system.

The 3 x 3 factorial design was conducted as a batch-feed study using 15-cm diameter acrylic coring tubes, incubated outdoors in a water bath under partial shade (to minimize water temperature fluctuations). Triplicate cores were set up for each combination of vegetation and substrate. The bare limerock ("limerock") substrate treatments contained limerock cobbles added to a depth of 10 cm. In the "PSTA" treatments, limerock was added to a depth of 10 cm

and covered by an additional 10 cm of accrued sediment from the outflow region of the PSTA Cell. For "Muck" treatments, intact soil cores were collected from STA2 Cell 3 to a depth of 26 ± 1 cm, from an area where *Potamogeton* was the dominant SAV (*Najas guadalupensis* was also present). The accrued sediment layer overlying the muck soil was a greyish marl typical of SAV-dominated STAs, and averaged 11.4 ± 0.4 cm in depth at the time and location of soil core collection. Plants were carefully removed from all cores following collection, leaving the accrued sediment layer intact.

A 30-cm water column depth was maintained during the study for all treatments. Plants were added at 20 g wet weight per vegetated core, consisting of several *Chara* strands or three *Potamogeton* plants with both new root growth and healthy apical leaf tips. Plants, limerock and PSTA sediments were all collected from the PSTA Cell on April 1 2014. Surface water collected from the PSTA cell was added to each of the cores, and exchanged every 2 weeks, taking care to avoid physical disturbance to plants and sediments. Six water exchanges were performed over the 84-day study.

Water samples were collected from the reflood water at Day 0 (the beginning of each cycle), and from each core on Day 7 (mid-point) and Day 14 (end of each exchange cycle). Phosphorus removal performance by the soil-water microcosms was quantified as the average water TP concentration at the end of each of the six 14-day cycles. Soil P content was determined from cores collected and sectioned in the field into the accrued sediment (both sites), and underlying muck layers (0-10 cm and 10-20 cm layers) in STA 2 Cell 3. Plant chemistry was measured at the beginning (initial inoculum) and end of the study.

STA field monitoring

Total P content was determined for SAV and accrued sediments collected during field monitoring surveys conducted in the PSTA Cell and STA 2 Cell 3 within the period 2005-2015 (Figure 1). For this study, we used plant tissue TP data for *Chara* only, in order to compare sediment interactions with a single SAV species occurring at both sites. We restricted the *Chara* tissue TP data for STA 2 Cell 3 to the outflow region ("I" transect) only, in order to approximate the water TP concentration regime found in the PSTA Cell. *Chara* was collected in the PSTA Cell along previously-established monitoring transects during three monitoring events, in August 2012, January-February 2014 and June 2015. In STA 2 Cell 3, *Chara* was collected in July 2004 and January 2010. Samples of the accrued sediment (marl) layer were collected in the PSTA Cell at monitoring transect stations during May 2012 and May 2014, and in the outflow region of STA 2 Cell 3 during September 2005 and January 2010.

Annual flow-weighted mean surface water TP concentrations at the PSTA Cell and STA 2 Cell 3 outflow sites were obtained for water years (WY) 2008 through 2014 from the South Florida Environmental Reports (SFER) published in 2013, 2014 and 2015.

Analytical methods

Water samples were analyzed for TP by the ascorbic acid-molybdenum blue method (EPA 365.1) using an Astoria-Pacific 2 segmented flow analyzer, following a persulfate digestion and neutralization procedure. The method detection limit (MDL) for TP in water samples was 4 µg/L.

Total P and TCa content for plant and sediment were determined by first digesting 50 mg of finely ground dried sample in concentrated nitric acid, followed by perchloric acid digestion at incrementally higher temperatures which ended at 210°C (COE 3-227; Plumb, 1981). Analysis of the digestate for TP was performed using the ascorbic acid-molybdenum blue method (EPA 365.2) on a Spectronics Genesys 5 spectrophotometer. Total Ca was determined by flame atomic absorption (EPA 215.1; EPA, 1979) using a Perkin-Elmer 3110 atomic absorption spectrometer.

[add methods for additional parameters reported in Table 1]

Statistical methods

Substrate and vegetation treatment effects on water and vegetation TP concentrations were determined by a two-factor ANOVA procedure using JMP software (version 4), SAS Institute, Cary NC. Comparison of water and plant tissue TP concentrations among substrate or vegetation treatments was performed using one-way ANOVA and Student's t test in JMP. For all tests an alpha level of 0.05 was used.

Results

Sediment core incubation

Water column phosphorus dynamics

Chemical characteristics of the source water for the core study (reflood water) are shown in Table 1. During the initial flooding cycle, surface water TP concentrations in the reflood water, collected from the PSTA Cell outflow (G388), were quite low (14-15 µg/L). The core study water source subsequently was changed from the PSTA cell outflow to the inflow (G390B structure) for the remaining 5 water exchanges, to provide a TP concentration range more representative of water entering STA outflow regions (17-26 µg/L).

During the batch incubations, significant substrate ($P<0.0001$) and vegetation (**$P=0.0002$**) effects on water column TP concentration were observed (Table 2). The limerock-based cores maintained the lowest TP concentrations, across all vegetation treatments (Figure 2). With the exception of the first flooding cycle, the final (day 14) TP concentration was lower than initial TP for all vegetation treatments with bare limerock substrate. The overall mean day-14 (end of cycle) TP concentration in limerock-based cores was 11 µg/L, where the mean initial (day 0) TP concentration was 19 µg/L. Within the limerock treatment there was a significant vegetation

treatment effect on water column TP, with higher TP concentrations occurring in cores with *Potamogeton* (mean=12.6 µg/L) than in *Chara* or non-vegetated cores. TP concentrations in the *Chara* treatment (mean=10.7 µg/L) were slightly, but not significantly, higher than in the non-vegetated treatment (mean=9.5 µg/L), among limerock-based cores.

Cores with PSTA substrate contained significantly higher water TP concentrations than cores with bare-limerock substrate, but significantly lower concentrations than in cores with muck substrate, for all vegetation treatments (Figure 1). Among all cores with PSTA substrate, the mean TP concentration at day 14 was 20 µg/L. Mean day-14 TP concentrations for the PSTA substrate were significantly higher in cores containing *Potamogeton* (22.4 µg/L) than in either *Chara* (18.3 µg/L) or non-vegetated (19.3 µg/L) cores, for which the difference in water TP was not significant.

In contrast to other substrates, water column TP concentrations in muck-based cores typically increased during each flooding cycle, including an increase in the non-vegetated columns during 5 of the 6 cycles. Among all muck-substrate treatments, day-14 water TP concentrations averaged of 25.5 µg/L, significantly higher than in both PSTA and limerock substrates. Within the muck-based cores, *Chara* (21.2 µg/L) provided significantly lower final water column TP levels than did *Potamogeton* (28.9 µg/L), while neither of those species provided final TP levels significantly different from non-vegetated muck cores (26.3 µg/L). Differences in final TP concentrations between Chara and the other vegetation treatments were greatest during the final flooding cycle.

Vegetation response to sediment conditions

Higher TP concentrations were measured in STA 2 Cell 3 sediments (472 ± 5 mg/kg for the accrued layer, 456 ± 11 mg/kg in upper muck 0-10 cm, and 407 ± 48 mg/kg in the lower 10-20 cm muck layer) than in PSTA sediments (322 ± 3 mg/kg). Phosphorus in the buried muck layer of the STA 3 Cell 3 profile was potentially available for uptake by *Potamogeton*, due to the extension of new root biomass below the accrued sediment layer during the study period. Accordingly, growth and TP content of *Chara* and *Potamogeton* were affected by substrate characteristics.

During the 84-day study, *Chara* and *Potamogeton* exhibited biomass increases in muck substrate treatments, while no change in biomass was observed for PSTA-substrate cores (Figure 3). Biomass of both SAV species exhibited slight declines in biomass in limerock-based cores. However, differences among substrate treatments for mean biomass of *Chara* and *Potamogeton* at the end of the study were not significant, due in part to high within-treatment variability.

Vegetation P content was significantly influenced by substrate type, where plant tissue P increased with substrate P concentration (Figure 3). This trend suggests that low P substrates are important for maintaining low P concentrations in macrophyte tissues. *Chara* and *Potamogeton* tissue P concentrations increased in muck and PSTA substrate treatments during the study, but decreased in bare limerock treatments (Figure 3). This suggests that the

marl/muck soil, rather than the reflood water in the cores, was the principal source of the P that accumulated in the plant tissues. At the end of the study, tissue P concentration in *Potamogeton* was significantly greater in muck substrate than in PSTA and bare limerock substrates. Tissue P content of the non-rooted *Chara*, on the other hand, was significantly greater in muck cores vs. PSTA cores, and in PSTA cores vs. limerock cores. These results support the theory that the sediments represented a significant source of P for both *Chara* and *Potamogeton*, but with different P uptake pathways exploited by the two species. *Chara* apparently obtained sediment P indirectly, via diffusive flux of P to the water column (with relative sediment P flux from muck > PSTA > limerock), while *Potamogeton* was able to tap directly into the substrate, including the buried muck layer (i.e., "P mining") in STA 2 Cell 3 sediment.

Overall, tissue P concentrations were slightly (not significantly) greater in Potamogeton than in Chara within the muck- and PSTA-substrate cores. Tissue P concentrations for both species were relatively low in the limerock-substrate treatment; nevertheless, P content of *Potamogeton* was more than twice the P content of *Chara* in limerock-based cores. Such a large difference in P content may be due to a combination of minimal (or no) P flux from substrate to water column, and the capability of *Potamogeton* to extract tightly-bound P from the limerock substrate. **[Implications for field-scale systems? Do we need to add P content of limerock… don't think we have this for the rock in the study, but we previously have analyzed P content of LR from this source)**

On all soils, SAV tissue Ca content showed minimal change during the study, thus the changes observed in dry weight biomass were likely the result of growth or senescence of plant tissue, rather than gain or loss of calcium carbonate encrustations commonly associated with macrophytes in alkaline waters. *Potamogeton* tissue in PSTA substrate treatments showed the greatest change in Ca content, increasing from 5.5 ± 0.2 % Ca to 8.0 ± 0.6 % Ca over the course of the study. At the end of the study, *Potamogeton* Ca content was lowest in muck substrate (6.4 ± 0.3 %) and intermediate in limerock substrate (7.1 ± 2.5 %). Differences among substrate types were not significant, however. The Ca content of *Chara* was higher initially (23%), then decreased slightly to ca. 21%, with no significant difference among substrates (Figure 3).

STA field monitoring

Annual mean flow-weighted TP concentrations during WY 2008-2014 ranged from 8 to 13 µg/L and averaged 11 µg/L at the PSTA Cell outflow [Note: Water Year (WY) 2014 = May 1 2013 – April 30, 2014]. Corresponding TP concentrations at the STA 2 Cell 3 outflow ranged from 14 to 29 µg/L and averaged 19.3 µg/L. **[is it worthwhile to show a comparative time-series plot of annual FW mean TP?] It should be mentioned that there is little to no bioavailable P (SRP) in the outflow from either system.** Total P concentrations in accrued sediments in the PSTA Cell were also lower than accrued sediment TP in the outflow region of STA 2 Cell 3 (Figure 4). Mean ± SE TP concentrations measured in the PSTA Cell (n=9 per date) during 2012 and 2014 were 272 ± 24 mg/kg and 257 ± 33 mg/kg, respectively. In comparison, accrued sediment TP

near the STA 2 Cell 3 outflow averaged 603 ± 129 mg/kg in 2005 and 729 ± 138 mg/kg in 2010 (n=4).

Tissue P concentrations in *Chara* collected from the two wetlands also reflected the lower TP conditions that prevailed in the PSTA Cell (Figure 5). *Chara* TP concentrations in the PSTA Cell averaged 368 ± 55 mg/kg in 2012 (N=7), 258 ± 20 mg/kg in 2014 (N=3) and 159 ± 18 mg/kg in 2015 (N=7). *Chara* collected from the STA 2 Cell 3 outflow region had tissue TP concentrations of 823 mg/kg in 2004 (one station) and 690 mg/kg in 2010 (composite sample from three stations).

Discussion

The stability of sediment P is important in treatment wetlands, as the sediment represents the long-term storage pool for sequestered nutrients. The ability of rooted vascular macrophytes to use sediment P for tissue growth, then release P into the water column upon senescence, has previously been described as a "nutrient pump" in lakes (Carignan and Kalff, 1980; Howard-Williams and Allanson, 1981; Moore et al., 1984). The consequences of rooted macrophytes accessing the sediment P pool are two-fold. First, an increase in tissue P content is likely to occur in response to increased nutrient supply. Low tissue nutrient contents would minimize internal P loading from senescent vegetation, whereas high tissue P contents imply that the macrophyte community could be an important source of internally recycled P to a water body. Second, an increase in SAV biomass and/or growth rate may occur in response to increased nutrient supply from the substrates. Biomass growth can increase the nutrient storage pool in SAV tissues. However, any such increase is temporary and tissue P will become an internal P source upon senescence.

Evidence of soil-P uptake by macrophytes has also been reported for treatment wetlands. White et al. (2006) found that 47% of P assimilated by SAV (Chara and Hydrilla) was derived from the muck sediments, rather than surface water inputs, in a mesocosm study where inflow P loading was low (0.17 g P/m^2/yr). The portion of biomass P obtained from the soil P pool was even greater (87%) for emergent macrophytes (Typha sp.) in that study. Thus, especially when external P loading is low, wetland macrophytes will obtain available nutrients from the sediment.

The outflow region of treatment wetlands often have very low concentrations of bioavailable P in the water column, as biologically-mediated P removal pathways have reduced this nutrient to very low levels. Despite low water TP concentrations and low external P loading rates to the wetland outflow, newly accrued sediments can be found to contain large stores of phosphorus. A modeling study by Juston et al. (2013) used soil, vegetation and water chemistry data from STA 2 Cell 3 to show that *Potamogeton illinoensis* mined sufficient P from the underlying muck layer (as much as 2.5 g P/m^2 (**time frame?)** to account for the observed P enrichment of the accrued marl layer in that cell.

In the present core study, the *Chara* and non-vegetated control treatments showed similar P removal performance within each substrate treatment or across all substrates, indicating this macroalga had no measurable effect on P exchange between substrate and the water column. However, *Potamogeton* resulted in higher TP concentrations in the water column than *Chara*, regardless of substrate type. The rooted macrophyte had a negative effect on P removal performance even on the low-P LR substrate.

Tissue P content of both *Potamogeton* and *Chara* increased over time on muck and PSTA sediments, and to the greatest extent in the muck soil treatment. Phosphorus enrichment of both plant types would have resulted from P diffusion into the water column, followed by uptake directly into tissues. In addition, *Potamogeton* likely obtained additional P from the underlying muck soil, based on observations of root growth at that depth in the soil cores. This ability to acquire additional P into *Potamogeton* tissues did not improve P removal performance, and in fact, may have impaired performance. Water TP above muck soils (high P supply) was not different between *Potamogeton* and non-vegetated controls, but water in *Potamogeton* vessels was significantly higher than control for both LR and PSTA substrates.

Upon initial construction of a PSTA system, an exposed limerock substrate may provide limited nutritive benefit to colonizing macrophytes. Limerock did not support an increase in tissue P content of either macrophyte type. Biomass growth on limerock was also negligible (for *Potamogeton*) or negative (for *Chara*). By contrast, biomass of *Chara* and *Potamogeton* increased on muck substrates over 84 days in this study. Final biomass values were variable on PSTA sediments and not significantly different from the weight of the initial stocking material.

These findings provide insight into the sustainability of effective P removal by PSTA systems. The limerock substrate, representing the "original condition" of the PSTA cell, provided the best water column P removal performance, as well as lowest tissue P concentrations for the two incubated SAV species. The muck substrate clearly provided greater P enrichment, as manifested both in the water column TP concentrations and macrophyte tissue P levels. The accrued PSTA sediments caused an intermediate response. These data suggest that as sediments have accrued in the PSTA wetland over 7 years of operation, the growth rate and nutrient content of macrophytes may have increased, relative to initial conditions on the exposed limerock substrate. However, each of these substrates (LR and PSTA sediment) provided less P for macrophyte uptake, than the muck soil from the outflow region of a well-performing flow way, STA 2 Cell 3.

Comparison of *Chara* Biomass and Tissue P from Field-scale Systems

The differences in P concentrations between the two wetlands were reflected in surface waters, *Chara* tissues, and accrued sediment composition. At times, *Chara* biomass is likely to serve as an internal source of P to the water column. However, the restricted availability of P from PSTA sediments vs. Cell 3 sediments resulted in attenuated P cycling in the PSTA cell water column.

That is, P release from senescing plant tissue is offset by plant uptake of P. Thus, *Chara* and accrued sediment in the PSTA cell represent a net sink for P).

Possibly add comparison of SAV biomass values from STA 2 Cell 3 and PSTA Cell ?

Since its inception, the STA 3/4 PSTA Cell has produced a sediment that, despite being low in TP content, appears capable of supporting modestly higher macrophyte growth rates and tissue P contents than would be expected on the original, bare limerock substrate. However, to date there has been no indication that the PSTA cell P removal performance is declining over time: annual mean outflow TP levels for each of the five WYs from 2011 through 2015 were 11, 12, 11, 13 and 11 µg/L, respectively. Further research is needed to better clarify the substrate and vegetation (macrophyte/periphyton) relationships in the STA 3/4 PSTA cell, but to date, the accumulating PSTA sediments appear reasonably well-suited for limiting the effects of internal P loading, while supporting modest SAV growth in the treatment wetland.

Reference

Adorisio, C. and McCafferty, R. 2015 Nutrient Source Control Programs, in 2015 South Florida Environmental Report, chap. 4. South Fla. Water Manage. Dist., West Palm Beach.

Barko, J.W., Smart, R.M., 1981. Sediment-based nutrition of submersed macrophytes. Aquatic Biology 10, 339–352.

Bays et al 2001

Burkholder, J. M., and R. G. Wetzel. 1990. Epiphytic alkaline phosphatase on natural and artificial plants in an oligotrophic lake: Re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. Limnology and Oceanography 35(3): 736-747.

Bottomley, E.Z., Bayly, I.L., 1984. A sediment porewater sampler used in root zone studies of the submerged macrophyte *Myriophyllum spicatum*. Limnology and Oceanography 29 (3), 671–673.

Carignan, R., Kalff, J., 1980. Phosphorus sources for aquatic weeds: water or sediment. Science 207, 987–989.

Chen, H., Ivanoff, D., & Pietro, K., 2015. Long-term phosphorus removal in the Everglades stormwater treatment areas of South Florida in the United States.*Ecological Engineering*, *79*, 158-168.

Chimney, M.J. 2015. Performance of the Everglades Stormwater Treatment Areas, in 2015 South Florida Environmental Report, chap. 5B, pp. 5B-1 to 5B-83. South Fla. Water Manage. Dist., West Palm Beach.

Corps of Engineers (COE) Methods reference

Dierberg et al., 2002

Gu, B., Dreschel, T., 2006. Effects of plant community and phosphorus loading rate on constructed wetland performance in Florida, USA. Wetlands 28 (1), 81–91.

Howard-Williams, C., Allanson, B.R., 1981. Phosphorus cycling in a dense *Potamoge- ton pectinatus* L. bed. Oecologia (Berl) 49, 56–66.

Juston, J.M., DeBusk, T.A., 2006. Phosphorus mass load and outflow concentration relationships in stormwater treatment areas for Everglades restoration. Ecolog- ical Engineering 26, 206–223.

Juston, J.M., DeBusk, T.A., Grace, K.A., and Jackson, S.D. 2013. A model of phosphorus cycling to explore the role of biomass turnover in submerged aquatic vegetation wetlands for Everglades restoration. Ecological Modelling 251:135-149.

Miao 2015

Moore, B.C., Gibbons, H.L., Funk, W.H., McKarns, T., Nyznyk, J., Gibbons, M.V., 1984. Enhancement of internal cycling of phosphorus by aquatic macrophytes, with implications for lake management. Lake and Reservoir Management 1 (1), 113–117.

Piccone et al. 2013. STA-3/4 Periphyton-Based Stormwater Treatment Area Project in Chapter 5 SFER 2013 pp. 61-68 (**Need better citation**).

Pietro K., R. Bearzotti, M. Chimney, G. Germain, N. Iricanin, and T. Piccone. 2007. STA performance, compliance, and optimization, in 2007 South Florida Environmental Report, chap. 5, pp. 5-1 to 5-128, South Fla. Water Manage. Dist.,

Pietro, K. C., & Ivanoff, D., 2015. Comparison of long-term phosphorus removal performance of two large-scale constructed wetlands in South Florida, USA. *Ecological Engineering*, *79*, 143-157.

Rattray, M.R., Howard-William, C., Brown, J.M.A., 1991. Sediment and water as sources of nitrogen and phosphorus for submerged rooted aquatic macrophytes. Aquatic Biology 40, 225–237.

SFWMD 2013 **??**

USEPA. 1979. Methods for chemical analysis of water and wastes, 1978. EPA Rep. 600/4-79-020. USEPA, Washington, DC.

White, J.R., Reddy, K.R., Majer-Newman, J., 2006. Hydrologic and vegetation effects on water column phosphorus in wetland mesocosms. Soil Science Society of America Journal 70, 1242–1251.

Table 1. Concentrations of selected nutrient species in the source water for the core incubation study, collected from the Everglades STA 3/4 PSTA Cell at the beginning of each 2-week reflood cycle. Values represent mean and range of concentrations across all reflood cycles (n=6).

Parameter	Units	Mean	Min	Max
TP	µg/L	19	15	26
SRP	µg/L	2	<2	3
DOP	µg/L	7	4	9
PP	µg/L	11	8	16
TKN	mg/L	2.5	2.4	2.5
Ammonia-N	mg/L	0.116	0.069	0.236
NO_x-N	mg/L	0.014	0.008	0.021
DOC	mg/L	35	32	40

Table 2. Mean (± 1 SE) day-14 (final) water column TP concentration across six 2-week water exchange cycles, in cores incubated outdoors with one of three substrates and one of three vegetation treatments. Also shown are combined treatment mean and SE values. For each substrate treatment, vegetation treatment means followed by different letters are significantly different for P≤0.05. For each vegetation treatment, all substrate treatment means were significantly different.

Substrate Treatments	Vegetation Treatments			
	Non-vegetated	*Chara*	*Potamogeton*	Combined Data
	- *TP (µg/L)* -			
Limerock	9.5 ± 0.4a	10.7 ± 0.4a	12.6 ± 0.7b	10.9 ± 0.3a"
PSTA	19.3 ± 0.7a	18.4 ± 0.8a	22.4 ± 1.4b	20.0 ± 0.6b"
Muck	26.4 ± 2.4ab	21.2 ± 1.1a	28.9 ± 2.1b	25.5 ± 1.2c"
Combined Data	18.4 ± 1.3a'b'	16.8 ± 0.8a'	21.3 ± 1.3b'	

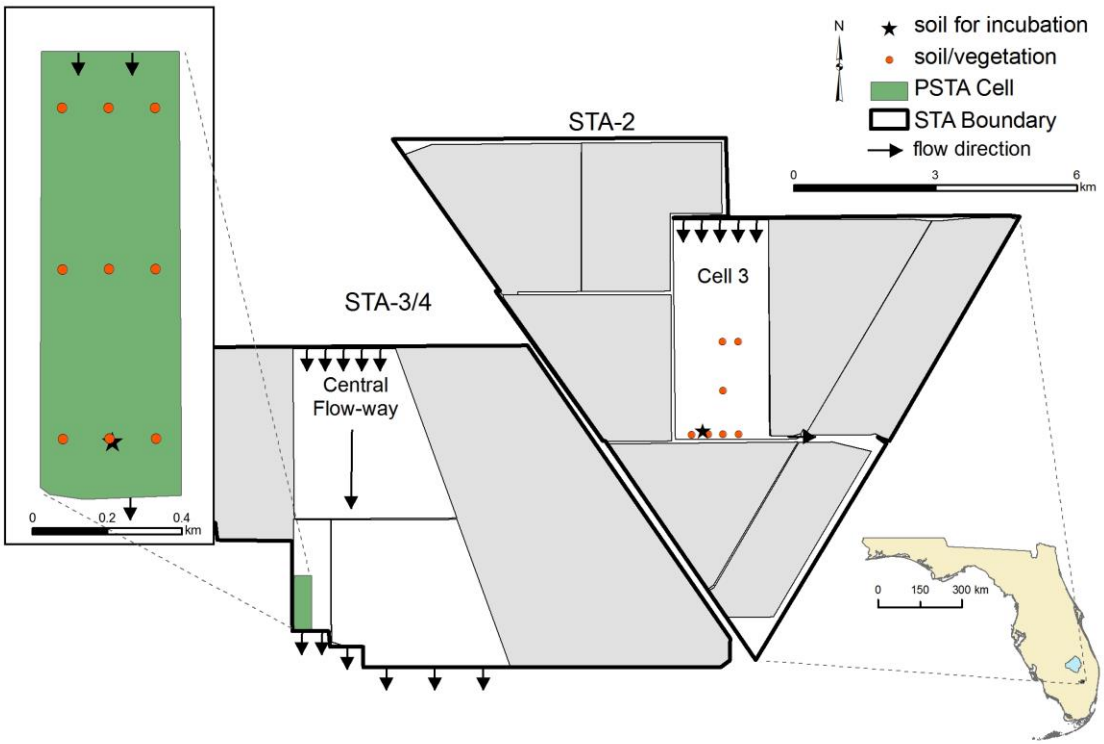

Figure 1. Location of the two soil collection sites for the 84 day core incubation study. Both *Chara* and *Potamogeton* tissues used in the incubation were collected from the PSTA Cell in STA 3/4.

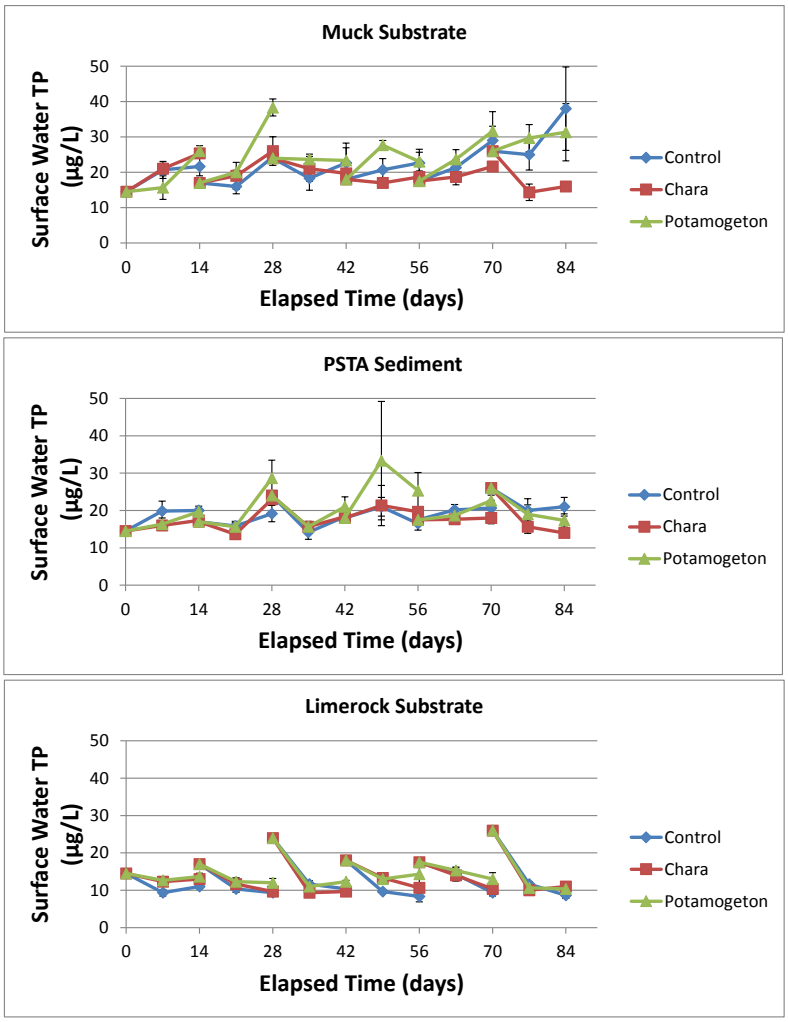

Figure 2. Surface water total phosphorus (TP) concentrations in cores incubated outdoors with one of three substrates (limerock (LR), PSTA sediment, or muck), and one of three vegetation treatments, during a 12-week study period. The control cores were unvegetated. Error bars denote ± 1 SE around the mean of triplicates under each treatment.

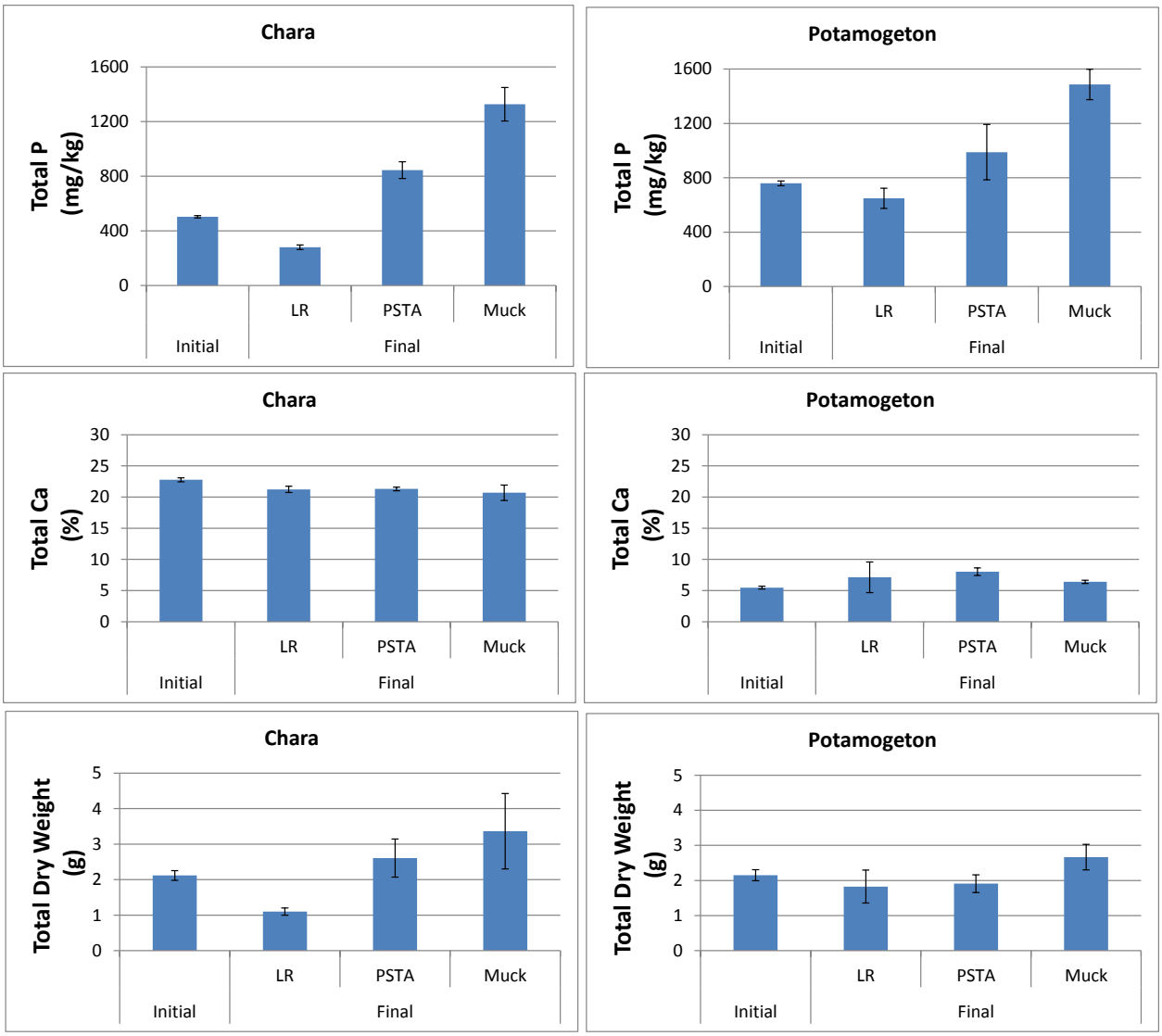

Figure 3. Chemical composition and dry weights of *Chara*, and *Potamogeton* tissues in cores incubated outdoors for 84 days, compared to the initial conditions of the inoculum, as a function of sediment type. Error bars denote ± 1 SE around the mean of triplicates under each treatment.

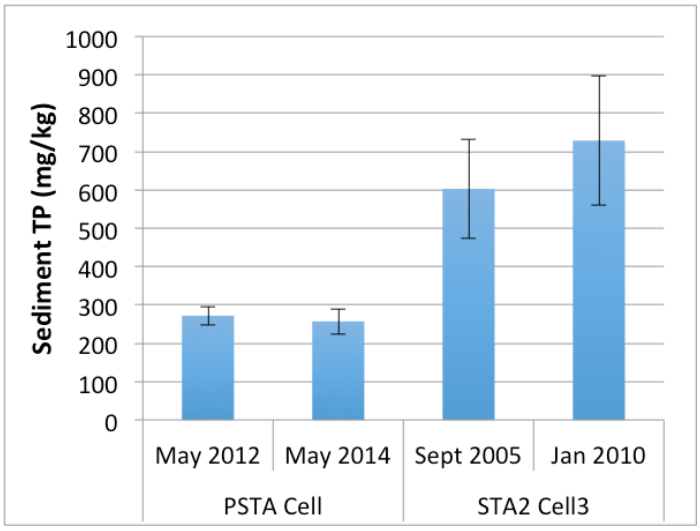

Figure 4. TP content of accrued sediment collected at internal transect stations in the PSTA cell during May 2012 (n=9) and May 2014 (n=9), and in the outflow region of STA 2 Cell 3 (n=4) during September 2005 and January 2010. Error bars denote ± 1 SE around the mean of the replicates for each sampling event.

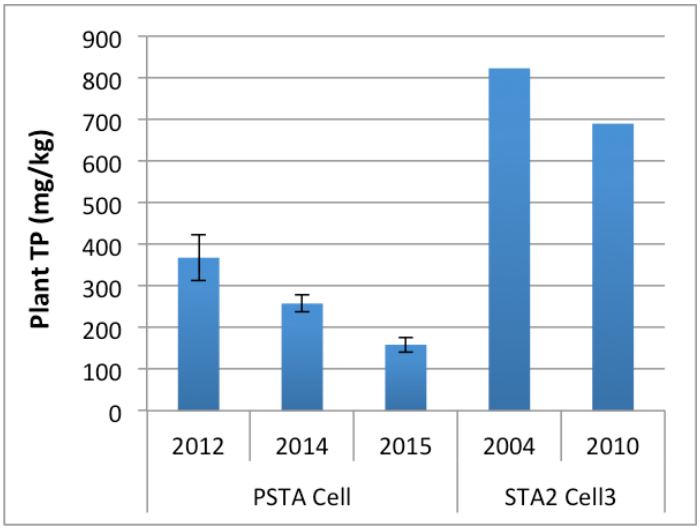

Figure 5. TP content of *Chara* tissue collected at internal transect stations in the PSTA cell during 2012 (n=7), 2014 (n=3) and 2015 (n=7), and in STA 2 Cell 3 during 2004 (n= 1 composite of 3 stations) and 2010 (n=1). Error bars denote ± 1 SE around the mean of the replicates for each sampling event.