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Water, vegetation and sediment gradients in submerged aquatic vegetation mesocosms used for low-level phosphorus removal

T.A. DeBusk *, M. Kharbanda, S.D. Jackson, K.A. Grace, K. Hileman, F.E. Dierberg

DB Environmental, Inc., 365 Gus Hipp Blvd., Rockledge, FL 32955, USA

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ABSTRACT

Gradients in phosphorus (P) removal and storage were investigated over 6 years using mesocosms (each consisting of three tanks in series) containing submerged aquatic vegetation (SAV) grown on muck and limerock (LR) substrates. Mean inflow total P concentrations (TP) of 32 μg L^{-1} were reduced to 15 and 17 μg L^{-1} in the muck and LR mesocosms, respectively. Mesocosm P loading rates (mean = 1.75 g m^{-2} $year^{-1}$) varied widely during the study and were not correlated with outflow TP, which instead varied seasonally with lowest monthly mean values in December and January.

The mesocosms initially were stocked with *Najas guadalupensis*, *Ceratophyllum demersum*, and *Chara zeylanica*, but became dominated by *C. zeylanica*. At the end of the study, highest vegetative biomass (1.1 and 1.4 kg m^{-2} for muck and LR substrates) and tissue P content (1775 and 1160 mg kg^{-1}) occurred in the first tank in series, and lowest biomass (1.0 and 0.2 kg m^{-2}) and tissue P (147 and 120 mg kg^{-1}) in the third tank. Sediment accretion rates (2.5, 1.9 and 0.9 cm yr^{-1} on muck substrates), accrued sediment TP (378, 309 and 272 mg kg^{-1}), and porewater soluble reactive P (SRP) concentrations (40, 6 and 4 μg L^{-1}) in the first, second and third tanks, respectively, exhibited a similar decreasing spatial trend. Plant tissue calcium (Ca) near mesocosm inflow (19–30% dry weight) and outflow (23–26%) were not significantly different, and sediment Ca was also similar (range of 24 to 28%) among sequential tanks.

Well-defined vegetation and sediment enrichment gradients developed in SAV wetlands operated under low TP conditions. While the mesocosm data did not reflect deterioration in treatment performance over 6 years, accumulation of P-enriched sediments near the inflow could eventually compromise hydraulic storage and P removal effectiveness of these shallow systems.

1. Introduction

Wetlands that receive nutrient-enriched drainage waters can develop marked biological and chemical gradients. For example, spatial trends have been documented in Water Conservation Area 2A (WCA-2A), an impounded marsh in the Everglades (Florida, USA), including gradients in macrophyte productivity and community structure (Miao and Sklar, 1998; Newman et al., 1998; Miao and DeBusk, 1999; Vaithiyanathan and Richardson, 1999), periphyton assemblages (McCormick and Stevenson, 1998; McCormick et al., 1998), soil microbial activity (Wright and Reddy, 2001), and microbial community structure (Drake et al., 1996). These gradients in ecosystem characteristics were observed along a ~10-km flow-path extending away from inflow structures, and where surface water TP concentrations decreased from ~200 to 10 μg L^{-1} (McCormick and Laing, 2003). Water column P is depleted with distance from the wetland inflow point, and a nutrient enrichment gradient becomes established (Walker, 1995). The higher nutrient regime of the inflow region of WCA-2A was shown to exhibit a higher sediment TP content, higher sediment accretion rate, and support metabolic conditions that resulted in depressed water column dissolved oxygen (DO) concentrations and smaller diel DO fluctuations than the lower nutrient region of the wetland (Craft and Richardson, 1993; DeBusk and Reddy, 2003; DeBusk et al., 1994; Reddy et al., 1993).

The Everglades Stormwater Treatment Areas (STAs) are six large wetlands, ranging from 363 to 6695 ha, constructed within the Everglades Agricultural Area to remove phosphorus (P) from agricultural drainage waters (ADW) and discharges from Lake Okeechobee, a eutrophic lake, before the surface waters enter the Everglades marsh (Chimney and Goforth, 2001). Water column P is removed by the STA wetlands through a suite of physical, biological and chemical mechanisms, and ultimately the P is sequestered in accrued sediments.

All STAs are comprised of multiple flow-paths, and most of these eventually will be configured with a front-end cell or region containing emergent macrophytes, and a back-end region dominated by submerged aquatic vegetation (SAV) (Burns and McDonnell, 2003). Prior research has shown that SAV is particularly effective for P removal from south Florida ADWs. Submerged macrophytes remove water-column P by direct uptake, and also contribute to chemical removal

* Corresponding author. Tel.: +1 321 639 4896; fax: +1 321 631 3169.
E-mail address: tom@dbenv.com (T.A. DeBusk).

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mechanisms such as P adsorption to, or co-precipitation with, $CaCO_3$ (Dierberg et al., 2002a). The front-end emergent community is utilized to attenuate much of the inflow mass P and solid load prior to reaching the outflow region SAV, since emergent macrophytes are thought to be more robust than SAV to perturbations from high nutrient and/or turbid inflow waters (Horppila and Numinen, 2001). The outflow region SAV community is utilized to reduce P down to the lowest level practicable.

In south Florida wetlands, differences in dominant vegetation types along a nutrient gradient can be pronounced, even for narrow P concentrations ranges (Richardson et al., 2007). For example, a shallow experimental raceway that reduced average TP inflow waters from 18 to 10 μg L^{-1} developed a stand of SAV (the macroalga *Chara zeylanica Klein ex. Wild.*) in the inflow region, while calcareous periphyton dominated the mid- and outflow-regions (DeBusk et al., 2004). Phosphorus dosing experiments have confirmed the P-sensitive nature of the Everglades marsh communities (McCormick et al., 2001). As a result, the target outflow concentration for STA wetlands is 10 μg L^{-1} total P (TP), although to date the best-performing STA flow paths constructed on previously farmed lands have achieved long-term mean outflow TP levels of only 14–18 μg L^{-1} (Juston and DeBusk, 2006).

At present, longitudinal gradients within the SAV-based portions of the STAs are poorly understood. This information, however, is extremely important from an STA management standpoint, since the sediment accumulation rate impacts long-term water storage capability of these wetlands. Furthermore, information on gradients in P concentrations of vegetation, sediments, and sediment porewaters can be useful in evaluating the minimum achievable outflow TP concentrations from such wetlands, as well as defining the ability of the wetland to sustain desired performance over its operational lifetime.

Besides hydraulic and P loading rates, plant communities, and flow distribution, an additional factor that may influence longitudinal gradients, and STA performance, is the type of substrate upon which the wetland is constructed. The existing Everglades STAs have been established primarily on organic, muck soils. Soils in chronically P-enriched areas can exhibit increased return P flux to the water column (Fisher and Reddy, 2001). Some investigators have proposed establishing “back-end” communities on limerock (LR), rock aggregates derived from the limestone deposits that occur throughout south Florida beneath the organic surficial soils. The inorganic LR substrate would presumably lack the organic P stores that potentially contribute to internal P loading in an STA, and could produce lower outflow TP concentrations (Goforth, 2001; Thomas et al., 2002; Gu and Dreschel, 2008).

The objectives of this study were to characterize the water quality, vegetation and sediment P gradients that develop in SAV wetlands used to “polish” drainage waters that previously had been treated in an upstream wetland. For this effort, we established mesocosms with three sequential tanks, so that biogeochemical characteristics of vegetation and accrued sediments could be evaluated periodically as a function of distance from the inflow. Separate SAV mesocosms were established on LR and muck substrates in order to evaluate if sediment type is an important factor that influences P removal performance and lowest achievable surface water TP concentrations for SAV communities.

2. Methods

2.1. Experimental design

This study was conducted at an experimental facility near the outflow of STA-1W, in western Palm Beach County, Florida (Fig. 1). Four wetland mesocosms (2 treatments × 2 replicates) were constructed using open elliptical, polyurethane tanks (0.78 m wide × 1.13 m

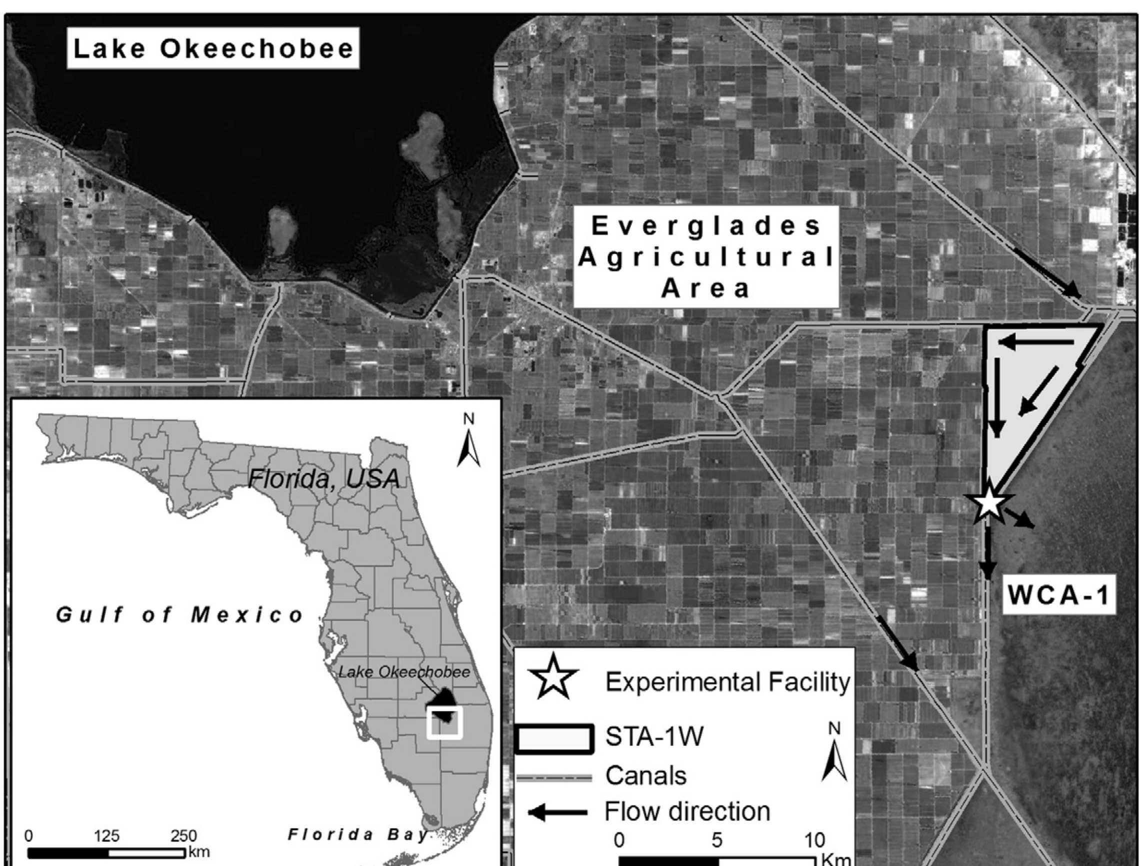

Fig. 1. Location of the experimental mesocosm facility near the outflow of Stormwater Treatment Area (STA) — 1W in Florida. Arrows denote flow direction.

long × 0.61 m deep). Each mesocosm consisted of three tanks in series, providing a total treatment surface area per mesocosm of 2.07 m^2. The three sequential tanks in each mesocosm provided an effective means of compartmentalizing vegetation and accrued sediments along an inflow–outflow gradient.

Outflow water from STA-1W was pumped to an elevated tank, from where it was gravity-fed to all the mesocosms in a semi-continuous manner. Gravity flow also was used to convey water from the first through the third tank in series within each mesocosm. Vertical standpipes maintained a fixed water depth of 40 cm above the substrate, resulting in a nominal volume of 0.90 m^3 for each mesocosm tank. Each tank was established with 15 cm of substrate. One pair of mesocosms contained organic muck (a highly organic Histosol suborder: Saprists), collected from nearby STA-1W Cell 5, whereas the other pair contained LR (~2.5 cm in diameter) obtained from a nearby quarry.

Flow was initiated to the four mesocosms on May 25, 1999 at a hydraulic loading rate (HLR) of 33 cm day^{-1}. After this initial period of higher loading rates, the flows were reduced to induce P limitation and to assess whether outflow TP concentrations are influenced by HLR (Fig. 2). To ensure accuracy of flows, each inflow stream was measured several times per week. Inflow to the mesocosms was stopped for a brief period during late summer 2004 (July–August) in order to test the response of the mesocosms to stagnant conditions. Inflow also was shut down for 2 weeks due to power outages following two hurricanes that passed near the site in September 2004. Due to the varying HLR (0 to 33 cm day^{-1}) during the course of the study, the nominal hydraulic retention times (HRT) within each mesocosm varied as well, ranging from 1.2 to 7.7 days during flowing conditions.

All mesocosms were stocked with three species of SAV, each at a standing crop of 1.3 kg (wet) m^{-2}. *Najas guadalupensis* (Spreng.) Magnus and *Ceratophyllum demersum* L. were stocked on June 18, 1999 and *C. zeylanica* on June 29, 1999. *N. guadalupensis* and *C. demersum* were collected from within the STA-1W western flow-path, and *C. zeylanica* from a pilot-scale wetland adjacent to STA-1W.

2.2. Field methods

Water quality measurements in the mesocosms were initiated on July 8, 1999. Grab water samples were collected from the inflow at the point of entry into the mesocosms and the outflows from the third tank in each mesocosm process train. From July 1999 until February 2004, water samples were collected weekly and analyzed for TP and pH. Water pH was measured with a Corning Model 313 pH meter equipped with a gel-filled "3-in-1" combination electrode. For the final 3 years of the study, we characterized P speciation of both the inflow and outflow of the mesocosms. Soluble reactive P (SRP) and total soluble P (TSP) concentrations were determined on samples that were filtered (0.45 μm polyethersulfone) immediately

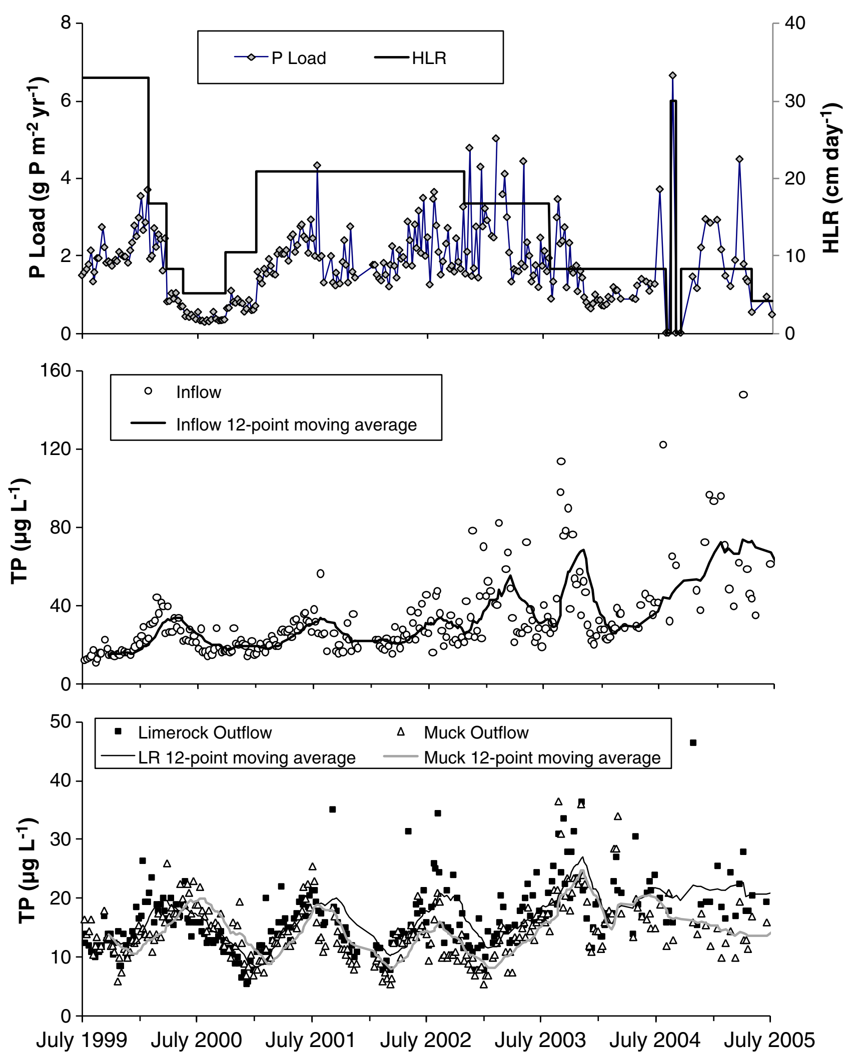

Fig. 2. Hydraulic loading rates (HLR) and phosphorus (P) mass loadings to mesocosms operated for 6 years on muck or limerock (LR) substrates. Inflow total P (TP) concentrations and outflow concentrations are shown as the average of duplicate mesocosms for each substrate. The moving average TP concentrations of 12 previous observations are also shown.

upon collection. Beginning March 2004, the sampling frequency was reduced from weekly to bi-weekly for all parameters for the remainder of the study (through July 2005).

Additional water quality measurements were performed during the study to characterize longitudinal gradients in surface water P and related physicochemical properties. During the first of these efforts, spanning the period August 6–November 5, 2001, water samples were collected weekly at the outflows of each of the three tanks within each mesocosm and analyzed for TP.

The second longitudinal gradient sampling effort characterized the water chemistry within SAV beds under stagnant conditions. Samples from the surface and bottom of the water column in each mesocosm tank were collected before (7/29/04) and after (8/17/2004) a 3-week period of stagnant conditions for SRP and TSP analysis. Total P was measured in surface waters but not in bottom waters, as flocculent particulate matter was abundant in the mesocosms and was easily disturbed during bottom water retrieval, which would lead to overestimates of the particulate P (PP) fraction. Sample collection on these dates occurred during early morning (0800) and afternoon (1600) to determine diel variability in P and DO concentrations along the nutrient gradients established within the mesocosms, and how these gradients might differ under flowing or stagnant conditions. Surface and bottom DO concentrations were measured *in situ*. Dissolved oxygen concentrations were measured using a Yellow Springs Instrument (YSI) 550 field meter.

The vegetation standing crop was characterized within each mesocosm tank on three occasions. During January 2003, a 12-cm diameter pipe was carefully inserted vertically through the water column at the mid-point of each tank. All SAV biomass captured within this pipe was collected and weighed. In July 2004, the same technique was implemented by using a 20-cm diameter sampling pipe, while plant material was collected from a different location, at the tank inflow region. On December 12, 2004 a 20-cm diameter pipe was again utilized to sample biomass near each tank outflow. On each date, a subset of the plant material collected from each tank was transported to the laboratory for analysis of dry matter content and TP, total nitrogen (TN), total carbon (TC) and calcium (Ca) contents.

Accrued sediment was collected on December 15, 2004, using a 10.5-cm diameter aluminum coring device. One core was retrieved from each mesocosm tank just upstream of the outflow standpipe and analyzed for TP, bulk density, Ca, TN, and TC contents. In the muck mesocosms, sediment accretion depth was measured by discerning color and texture differences from the underlying muck. Accretion rate could not be measured with an acceptable level of accuracy in the LR mesocosms, due to the uneven surface of the substrate. Consequently, accretion rates are not presented for the LR mesocosms.

A 2.54-cm diameter PVC porewater well was installed within each muck mesocosm tank on 7/15/04. These wells were located at the mid region of the tanks, with the well screen situated so that porewater could be collected from within the sediments at a depth of 6–10 cm. Porewater was collected from the wells on three occasions (7/23/04, 12/9/04, and 4/19/05) using a hand-operated vacuum pump and analyzed for SRP.

2.3. Laboratory analyses

Water samples for SRP, TSP, and TP were analyzed by the ascorbic acid-molybdenum blue method (EPA 365.1, EPA, 1999) using either a Spectronics Genesys 5 spectrophotometer (before May 2004) or an Astoria-Pacific 2 segmented flow analyzer (after April 2004). Total P and TSP analyses included a persulfate digestion and neutralization.

Total N and TC content of plants and sediments were determined by combusting finely-ground samples (passed through a no. 40 or 100 standard sieve) in a Carbon–Nitrogen–Sulfur elemental analyzer (Fisons NA 1500). Total P and total Ca content for both matrices was determined by first digesting 50 mg of finely ground sample in concentrated nitric acid, followed by perchloric acid digestion at incrementally higher temperatures which ended at 210 °C (COE 3–227 in Plumb, 1981). Analysis of the digestates for TP was performed by the ascorbic acid-molybdenum blue method (EPA 365.2; EPA, 1999) using a Spectronics Genesys 5 spectrophotometer. Sediment bulk density was calculated as dry mass per unit volume, based on an undisturbed sediment core sample.

2.4. Data analyses

The mean outflow TP concentrations for duplicate mesocosms of each substrate (muck and LR) were compared using a paired *t*-test ($n=261$ weekly–biweekly values), after Shapiro–Wilks tests indicated that the distributions were normally distributed at $alpha=0.05$. Following the 3-week stagnant period (no inflow), paired *t*-tests using pooled morning and afternoon data from both substrates were used to determine if surface water TP concentrations changed significantly as a function of position along the gradient. Mean comparisons among mesocosm tanks and substrate type were performed on water and sediment data using analyses of variance (ANOVA) or a *t*-test procedure (at $alpha=0.05$) using JMP software (SAS Institute, Inc., Cary, NC). Vegetation data from three sampling events were incorporated into a repeated measure ANOVA using Statistica software (Statsoft, Inc., Tulsa OK), and significant differences in plant characteristics between substrate types, along the gradient, and over time were identified with Tukey HSD at $p<0.05$.

In order to identify factors that influenced mesocosm outflow TP concentrations, we performed Kendall's Tau correlations to compare monthly mean values of outflow TP concentration to three potential factors: HLR, mass P load, and inflow TP concentration. Seasonality was identified using Kruskal–Wallis test for differences among months across the entire 6-year period of record. Kendall's Tau and Kruskal–Wallis tests were both performed using Statistica, and significant relationships were identified where $p<0.05$.

Mass accretion of P in sediments was calculated as the product of TP concentration, bulk density, and the depth of newly-accreted sediment. Dissolved organic P and PP concentrations in water quality samples were calculated by difference as follows:

$$DOP = TSP - SRP \tag{1}$$

$$PP = TP - TSP. \tag{2}$$

Method detection limits (MDLs) for SRP, TSP and TP in water samples were 2, 4, and 4 μg L^{-1}, respectively. The only parameter that frequently occurred at or below the MDL was SRP. One-half the value of the MDL was used in all calculations when the SRP concentration was less than the MDL.

3. Results

3.1. Water quality, hydraulic and P loading rates

The feedwaters to our mesocosm experiment, originating from the STA-1W outflow, were relatively hard (mean calcium of 73.2 mg L^{-1}, mean alkalinity of 224 mg L^{-1}as $CaCO_3$) with moderately low nutrient concentrations (mean TKN, NOx–N, ammonia–N and TP levels of 2.12, 0.045, 0.106 and 0.035 mg L^{-1}, respectively) during the 6-year study (SFWMD DBHYDRO database; www.sfwmd.gov/dbhydro). A record of weekly–biweekly inflow TP concentrations, inflow HLR and TP loads, and outflow concentrations from the muck and LR mesocosms is provided in Fig. 2. Over the 6-year operational period the mean outflow from the muck mesocosms (15 μg L^{-1}) was significantly lower than the LR mesocosm outflow (17 μg L^{-1}).

Fig. 3 summarizes P loading, HLR, and inflow–outflow TP concentrations for the mesocosms on an annual average basis. Mean annual inflow TP concentrations averaged 32 μg L^{-1}, and increased from 23 to 65 μg L^{-1} during the 6-year study (weekly–biweekly range: 9–150 μg L^{-1}). Inflow TP concentrations increased sharply in response to the reduced effectiveness of the upstream STA in removing an increased external P load. Annual mean HLRs ranged from 8 to 24 cm day^{-1} and averaged 16.6 cm day^{-1} for the 6-year study. Annual P loads ranged from 1.22 to 2.36 g m^{-1} $year^{-1}$ with an average of 1.75 g m^{-1} $year^{-1}$. Variability in P loading resulted from fluctuations in both HLR and inflow P concentrations.

During the first 3 years of the study (summer 1999 through summer 2002), inflow TP concentrations averaged 24 μg L^{-1}, and ranged from 11 to 57 μg L^{-1}. The mean HLR and P loading rate to the mesocosms during this period averaged 19.5 cm day^{-1} and 1.64 g P m^{-2} $year^{-1}$, respectively. Muck and LR mesocosm outflow TP concentrations were comparable, averaging 14 and 15 μg L^{-1}, respectively. Average (±S.D.) daytime water pH values were 7.7 ± 0.2 at the inflow, and increased during passage through both the muck (8.0 ± 0.4) and LR (7.9 ± 0.3) mesocosms.

During the final 3 years of the study, treatment performance of the two mesocosm substrate types diverged, with TP outflow concentrations for muck and LR mesocosms averaging 15 and 19 μg L^{-1}, respectively (Fig. 2). Inflow TP concentration during this period averaged 44 μg L^{-1}, and ranged from 16 to 148 μg L^{-1} (Fig. 2). We generally maintained a lower HLR (mean of 15.0 cm day^{-1}) during this time, which resulted in a mean P loading rate of 1.91 g P m^{-2} $year^{-1}$. Mean daytime pH values for inflow (7.6 ± 0.2), muck outflow (8.1 ± 0.4), and LR outflow (8.0 ± 0.4) during the final 3 years were comparable to those observed during the first 3 years of the study.

3.2. Factors controlling outflow TP concentrations

Inflow TP concentration was significantly correlated to outflow TP in both muck and LR mesocosms, for the 6-year study period and during the first 3 years of the study, prior to the advent of frequent spikes in inflow TP concentration (Table 1). In contrast, during the final 3 years of the study, outflow TP concentration was not significantly related to inflow TP concentration for either muck or LR mesocosms. The relationship between outflow TP concentration and TP loading was less clearly defined and, when viewed over the entire study period, was not significant. Monthly TP loading was correlated to outflow TP concentration for LR mesocosms only during the first 3 years, and for muck mesocosms only during the final 3 years. Outflow TP was significantly related to hydraulic loading rate in the muck mesocosms during the final 3 years of the study in the muck mesocosms, and in both substrate treatments over the 6-year period, but neither substrate during the initial 3-year period (Table 1).

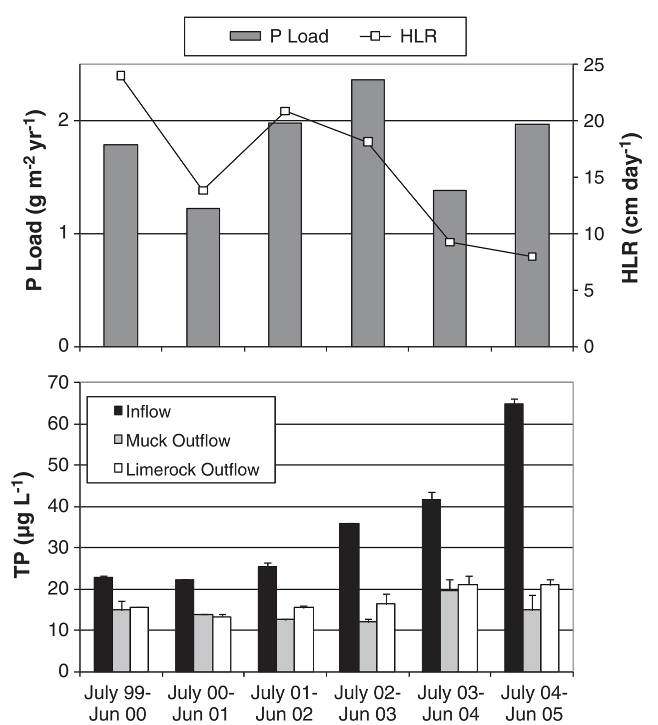

Fig. 3. Annual phosphorus (P) loading, hydraulic loading rate (HLR), and inflow and outflow total P (TP) concentrations of duplicate mesocosms operated on muck or limerock substrates for 6 years. The error bars represent +1 S.D. of the mean of duplicate mesocosms.

3.3. P forms in outflow waters

On average, the inflow waters consisted of equal parts of DOP, PP and SRP (Fig. 7). For both substrate treatments, the outflow waters consisted of equal fractions of DOP and PP with very little SRP present (at or below MDL). The LR mesocosms removed SRP and DOP at a rate comparable to the muck mesocosms, but were less effective than the muck mesocosms at removing PP.

3.4. Longitudinal gradients

Water quality sampling in individual mesocosm tanks provided a basic characterization of longitudinal gradients in water column P along the flow-path. During the first gradient sampling effort (August 2001–November 2001), for which samples were collected weekly, inflow TP concentrations averaged 22 μg L^{-1}, the HLR was 20.9 cm day^{-1} and the average P load was 1.69 g m^{-2} $year^{-1}$. For the muck mesocosms, no TP reduction occurred within the initial tank, but TP levels then declined sequentially through the remaining two tanks (Fig. 4). Mean TP concentration along the muck mesocosm flow-path ranged from 23 μg L^{-1} in the initial tank outflow to 15 and 12 μg L^{-1} in the middle and final tanks outflow, respectively. The LR mesocosms achieved a reduction in TP in the initial tank outflow, followed by no reduction in the second, and a slight reduction in the final tank (Fig. 4). Mean TP concentration in the LR mesocosms was 19 μg L^{-1} in the initial and middle tank outflows, and 17 μg L^{-1} in the final tank outflow.

3.5. Responses to stagnant conditions

The second gradient sampling period (before and during stagnant conditions) provided additional information on internal dynamics of P within the water column. Non-flowing conditions occur frequently during STA operations and in the absence of external loading, the effects of internal P loading can be observed directly. After 3 weeks of stagnant conditions, surface water TP concentrations decreased significantly (initial > middle > final tanks, $p < 0.005$). Phosphorus species concentrations were higher in the initial mesocosm tanks than the

Table 1
Kendall's Tau correlation coefficients for comparison of monthly mean outflow TP concentrations to three potential drivers: hydraulic loading rate (HLR), mass P load, and inflow TP concentration. Values are shown for muck and limerock treatments during the entire period of record (POR) (July 1999–June 2005), the initial 3 years (July 1999–June 2002) and final 3 years (July 2002–June 2005) of the study.

Independent variable	Treatment	Entire POR	Initial 3 years	Final 3 years
Inflow TP	Muck	0.303[a]	0.304[a]	0.143
Inflow TP	Limerock	0.390[a]	0.504[a]	0.073
TP loading	Muck	−0.127	0.113	−0.304[a]
TP loading	Limerock	0.151	0.418[a]	−0.105
HLR	Muck	−0.399[a]	−0.217	−0.534[a]
HLR	Limerock	−0.167[a]	0.050	−0.210

[a] Significant at 0.05 probability level.

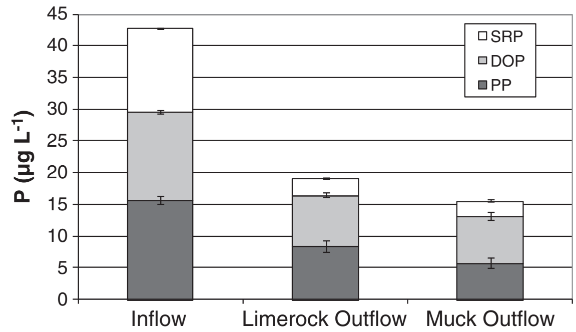

Fig. 4. Mean (±1 S.D.) concentrations of phosphorus (P) species for duplicate mesocosms operated on muck or limerock substrates collected during the final 3 years (June 2002–June 2005) of the study.

final tanks for both muck and LR substrates, with the exceptions of early morning SRP and afternoon PP concentrations in the LR tanks surface water, and the afternoon SRP concentrations in muck tank bottom waters (Fig. 6). This is consistent with the fact that the initial tanks received greater mass P loading during flowing conditions than the final tanks and, consequently, had greater potential for internal P loading from sediments or biomass to the water column.

3.6. Diurnal changes in water P, pH and DO

Hypoxic conditions (≤0.8 mg DO L^{-1}) were observed in all tanks during early morning sampling, both during flow-through operations and during stagnant conditions. Daytime increases in DO were observed in all tanks (Fig. 6). DO levels were consistently lower in the initial tanks of each mesocosm series, as compared to the final tank, regardless of substrate or time of day. Bottom water DO levels were near-zero in the initial tanks of mesocosms on both substrates, even during afternoon sampling, while bottom water in the final tanks in-series experienced oxygenated conditions in the afternoon (Fig. 6).

Significant diurnal changes in surface water TP were observed only in the initial tanks of muck and LR mesocosms. DOP concentrations were lower in the final tank than the initial tank regardless of substrate or time of day. No difference was observed between surface and bottom DOP concentrations within the mesocosms, despite the marked change in oxygen levels (Fig. 6). These data indicated that position along the nutrient gradient was a stronger determinant of DOP concentrations than substrate type, sampling depth, or time of day (Fig. 6). Under stagnant conditions, surface water PP concentrations in the first tanks in series were higher in the early morning samples than afternoon samples for both LR and muck-based systems. A diel effect on SRP concentrations was observed only in the initial tank of the LR systems. In fact, under stagnant conditions the morning SRP concentrations were at the MDL in the surface waters of all LR tanks, whereas the muck tanks exhibited a strong inflow–outflow SRP gradient in the surface samples collected in the early morning.

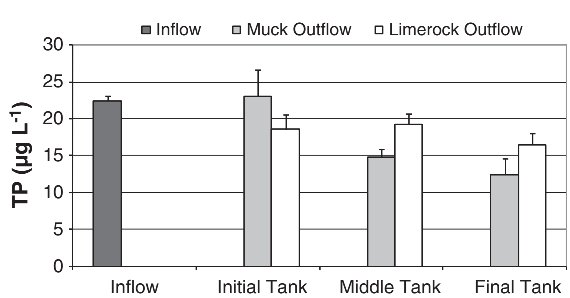

Fig. 5. Mean (+1 S.D.) total phosphorus (TP) concentrations at the inflow to the mesocosm and outflow from each tank-in-series along the flow path for duplicate mesocosms with muck or limerock substrates. Data were collected during a 3-month period from August 2001–November 2001.

3.7. Seasonal gradients in surface water TP

Monthly outflow TP concentrations from the muck mesocosms were highest in June and lowest in December and January (Fig. 7). The seasonal pattern was significant, though not nearly as pronounced as for air temperature, which peaked in August. By contrast, inflow TP concentration and LR outflow concentration showed no significant seasonality across the 6 years of this study.

3.8. Vegetation

By May 2001, all of the mesocosms were dominated by *C. zeylanica*. Plant standing crop measurements and tissue analyses, performed on three occasions during the final 2.5 years of the study, revealed few significant differences in plant biomass and tissue composition between the muck and LR mesocosms and among individual mesocosm tanks (Table 2). Biomass standing crop varied with sampling date, with the lowest levels measured during the final sampling event. Biomass also exhibited a significant decreasing trend along the flow path of the mesocosms, but no difference was observed between LR and muck systems. The biomass in the final LR tanks (0.14 to 0.95 kg dry wt m^{-2}) was low relative to the range observed in the muck final tanks (0.84–1.63 kg m^{-2}), though the difference between substrates was not significant.

Total P concentrations in plant tissues did not vary significantly with sampling date or substrate type (Table 2). However, in both muck and LR mesocosms plant tissue TP decreased significantly along the flow-path from the initial to final tanks. Standing crop of TP (mass of TP per unit area) followed the same trend, decreasing monotonically with increasing distance from the mesocosm inflows. Plant P storage also changed over time, increasing between the first and second sampling events. No difference in P storage between substrate types was observed. Plant tissue TN in the initial tank was higher than in the second and third tanks, but no difference was observed over time or between substrate types. Plant tissue TC and Ca concentrations showed no significant difference between substrate types, over time, or with distance from the inflow in the mesocosms, and averaged 20.9±2.3 and 23.3±2.3% wt, respectively, across all samples.

3.9. Sediments

Sediment accretion in muck mesocosms was significantly higher in the initial tanks, and decreased sequentially in the middle and final tanks, following the downstream TP gradient (Fig. 8). The muck mesocosms accreted sediment at a rate of 2.5 and 0.9 cm $year^{-1}$ for the initial and final tanks, respectively. There were no significant differences in TP concentration of accreted sediment between the muck and LR mesocosms; however, sediment TP decreased significantly along the flow path in the muck mesocosms (Table 3). Mass P accretion during the study period exhibited a significant decreasing trend from the initial to final tanks in the muck mesocosms (Fig. 8). The P accretion rate in the initial tank was 1.16 g P m^{-2} $year^{-1}$, compared with 0.48 g P m^{-2} $year^{-1}$ in the final tank.

For the other parameters analyzed in the newly accreted sediment, there were no differences between the muck and LR mesocosms; however, differences were observed among the initial, middle and final tanks within each mesocosm (Table 3). Sediment bulk density showed a significant increase from inflow to outflow in the muck mesocosms, while no significant trend was observed for the LR mesocosms. Sediment Ca and TN contents did not vary significantly along the flow-path of mesocosms on either substrate, while TC content showed a small but significant downstream increase in mesocosms on both substrate types.

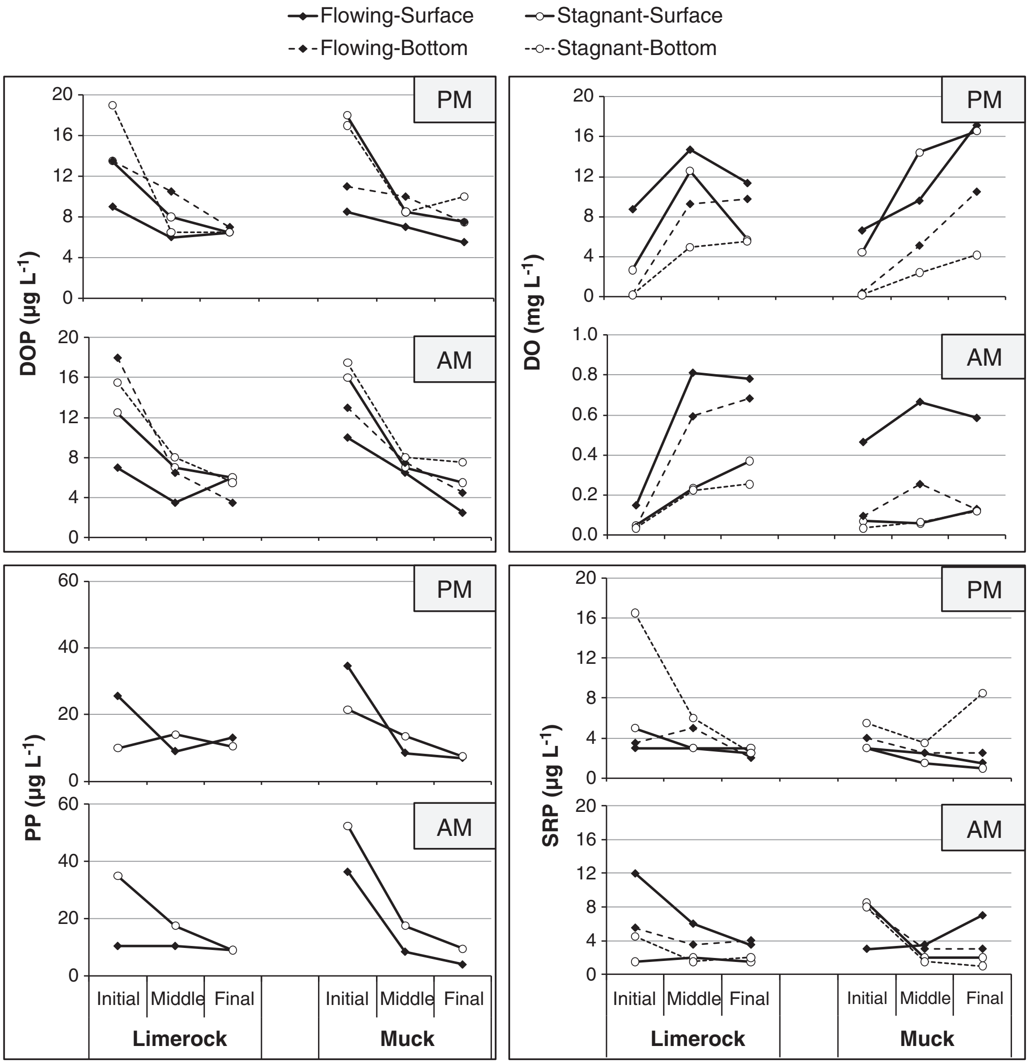

Fig. 6. Diel surface and bottom water dissolved organic P (DOP), particulate P (PP), soluble reactive P (SRP) and dissolved oxygen (DO) concentrations from each mesocosm-in-series along the flow path for duplicate mesocosms operated on muck or limerock substrates. Samples were collected during flowing conditions (7/29/04) and at the end of a three-week stagnant period (8/17/04). Bottom water PP was not measured.

During all three porewater sampling events, the porewater SRP was the highest in the initial tanks containing muck (14, 58, and 49 µg L^{-1} during July and December 2004, and April 2005, respectively), particularly for the December 2004 and April 2005 sampling events (Fig. 9). For all three events, the HLR was 8.3 cm day^{-1}. However, between the July and December events the HLR fluctuated considerably, and the mesocosms were subjected to both stagnant and high-flow conditions (Fig. 2).

3.10. Phosphorus mass balance

Based on HLR and TP analysis of influent and effluent streams, a net removal of 5.0 g P m^{-2} was calculated for the muck mesocosms during the 6-year study period. The majority of P removed was recovered as newly accreted sediment, accounting for 92% of P removal. A net increase of 0.07 g P m^{-2} in standing crop plant biomass accounted for 1.4% of P removal for this treatment. The total recovery of removed P in the muck mesocosms was 93.4%, with the remaining balance likely accounted for by extrapolation of small errors in measuring accreted sediment and plant standing crop, flow rates, and TP concentrations. A mass balance was not constructed for the LR mesocosms due to uncertainty in sediment accretion depth above (and within) the LR substrate. For the muck mesocosms, and likely for the LR mesocosm (based on semi-quantitative mass P accretion data), sediment accretion was the primary P sink during the 6-year study period, while plant standing crop biomass was relatively insignificant.

4. Discussion

Despite the known limitations of micro- and mesocosm studies (Carpenter, 1996; Fraser and Keddy, 1997), the mesocosms employed for the present study achieved water column P removal rates comparable to optimized full-scale STA process trains. For example, Juston and DeBusk (2011) found a central tendency of 16 µg L^{-1} for "background" surface water TP concentration across nine different SAV-dominated STA treatment cells. During the present study, the muck mesocosms reduced TP from 32 to 15 µg L^{-1}, at a P loading rate of 1.75 g P m^{-2} $year^{-1}$.

Also in common with full-scale STA systems, the concentration of biologically active SRP was reduced to the limits of analytical detection, with DOP and PP representing the dominant fractions exported from the mesocosm outflows (Dierberg and DeBusk, 2008). These similar findings are evidence that water chemistry data generated from these experimental mesocosms are transferable to the full-

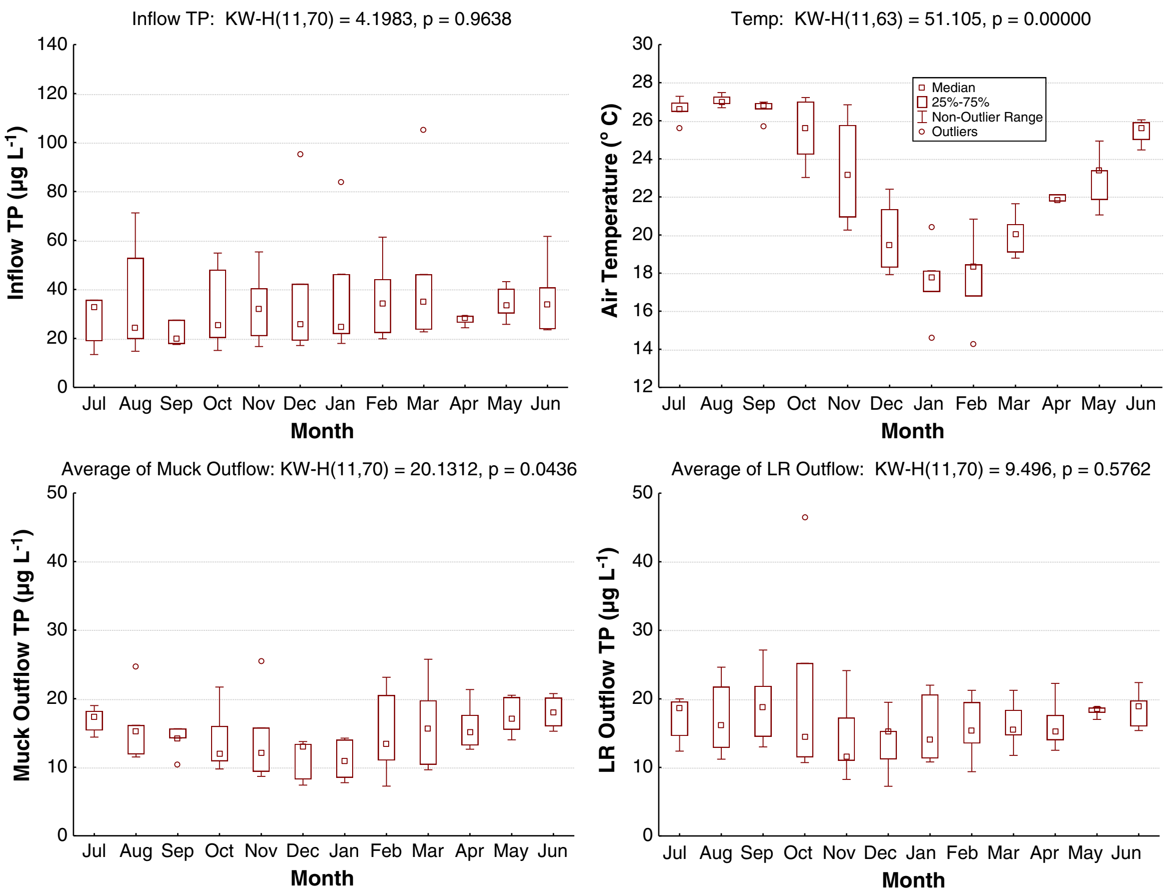

Fig. 7. Seasonal trends in air temperature and total phosphorus (TP) concentrations in mesocosm inflow and outflow surface waters over the period of record. Values are derived from monthly averages across 6 years for duplicate mesocosms on limerock or muck substrates. Air temperature data from the South Florida Water Management District was recorded at station ENR308, ~2.7 km north of the mesocosm facility.

scale STA wetlands, and may be useful in evaluating and predicting the treatment performance of soil management alternatives for the larger systems.

Table 2
Plant biomass and tissue analysis in initial, middle and final mesocosm tanks for three sampling events. Values represent means of two replicates each for muck and limerock substrates, with standard deviation in parentheses. Average values with different letters are significantly different at alpha = 0.05.

	Initial	Middle	Final	Average
Biomass (kg dry m^{-2})				
1/7/03	1.8 (0.4)	1.6 (0.8)	1.0 (0.7)	1.5 (0.7)a
7/7/04	2.7 (0.6)	1.9 (0.7)	1.0 (0.4)	1.9 (0.9)a
12/14/04	1.3 (0.3)	1.1 (0.2)	0.6 (0.5)	1.0 (0.4)b
Average	1.9 (0.8)a	1.5 (0.7)a	0.9 (0.5)b	
Total P (mg kg^{-1})				
1/7/03	960 (117)	273 (55)	167 (59)	466 (374)a
7/7/04	948 (249)	292 (76)	136 (12)	459 (392)a
12/14/04	1468 (545)	346 (138)	133 (29)	649 (678)a
Average	1125 (407)a	304 (93)b	146 (39)b	
Total P (g m^{-2})				
1/7/03	1.74 (0.36)	0.41 (0.17)	0.17 (0.10)	0.77 (0.75)a
7/7/04	2.49 (0.20)	0.58 (0.36)	0.14 (0.06)	1.07 (1.09)b
12/14/04	1.84 (0.76)	0.41 (0.24)	0.08 (0.07)	0.77 (0.90)a
Average	2.0 (0.6)a	0.5 (0.3)b	0.1 (0.1)b	
Total N (%)				
1/7/03	1.1 (0.4)	0.9 (0.2)	1.3 (0.6)	1.1 (0.4)a
7/7/04	1.5 (0.3)	1.0 (0.2)	0.9 (0.1)	1.1 (0.3)a
12/14/04	1.8 (0.2)	1.1 (0.4)	0.9 (0.1)	1.3 (0.5)a
Average	1.5 (0.4)a	1.0 (0.2)b	1.0 (0.4)b	

Mesocosms containing LR produced slightly higher outflow TP concentrations than muck mesocosms (17 vs. 15 µg L^{-1}). These experimental results run counter to the hypothesis that Ca-rich inorganic substrates would provide better TP removal than organic substrates in wetland treatment systems (Goforth, 2001; Thomas et al., 2002; Gu and Dreschel, 2008). It should be noted that the type of inorganic substrate may also be an important determinant in the extent of P removal. That is, Fe- and Al-bearing substrates can have higher P retention capacity than calcareous substrates (Richardson, 1985).

We observed weak relationships between outflow and inflow TP concentrations, and between outflow TP and HLR, over the 6-year period of record. Outflow TP levels were not correlated with P load (annual mean loading rate, 1.22–2.36 g P m^{-2} year^{-1}) over the entire 6-year period of record in the present study. However, there is evidence

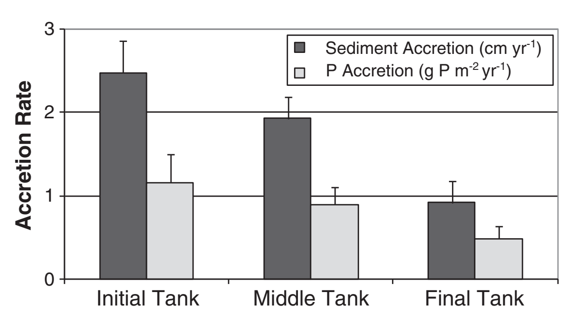

Fig. 8. Mean (+1 S.D.) sediment and P accretion rates from each tank-in-series along the flow-path for duplicate mesocosms operated on muck substrates. Sediment was collected on December 15, 2004, after the mesocosms had been operational for 5.5 years.

Table 3
Physical and chemical characteristics of newly accreted sediment from initial, middle and final tanks in muck and limerock substrate mesocosms. Values represent means of two measurements, with standard deviation in parentheses. Sediment was sampled on December 15, 2004.

	Muck			Limerock		
	Initial	Middle	Final	Initial	Middle	Final
Bulk density	0.123	0.149	0.192	0.134	0.127	0.110
($g\ cm^{-3}$)	*(0.018)*	*(0.011)*	*(0.008)*	*(0.021)*	*(0.052)*	*(0.027)*
TP	378	309	272	358	324	243
($mg\ kg^{-1}$)	*(2.1)*	*(8.5)*	*(21)*	*(28)*	*(51)*	*(44)*
TC	16.2	16.9	17.7	16.2	16.5	18.1
(%)	*(0.12)*	*(0.64)*	*(0.13)*	*(0.39)*	*(0.15)*	*(0.52)*
TN	0.77	0.79	0.88	0.76	0.76	0.84
(%)	*(0.011)*	*(0.104)*	*(0.011)*	*(0.042)*	*(0.013)*	*(0.018)*
Ca	27	27	24	27	26	28
(%)	*(0.0)*	*(0.7)*	*(2.8)*	*(0.7)*	*(0.07)*	*(0.7)*

that low to moderate P loads are necessary to achieve low outflow TP concentrations (DeBusk et al., 2004; Juston and DeBusk, 2006). A shallow (9-cm water depth) periphyton-dominated raceway (44 m long × 0.3 m wide) on LR substrate achieved 10 μg TP L^{-1} over an 18-month period at an average P loading rate of 0.7 g P m^{-2} $year^{-1}$ (DeBusk et al., 2004). That study suggested that a constraint to achieving ultra-low TP outflows appears related to P speciation. The shallow water column of the raceways may have facilitated UV degradation of the DOP, while quiescent conditions of the small raceways minimized internal particle production (DeBusk et al., 2004). In the raceway study and in other studies on wetland treatment of south Florida ADW, wetland outflows consist primarily of potentially recalcitrant P forms, namely PP and DOP (Dierberg et al., 2002b; DeBusk et al., 2004; Dierberg and DeBusk, 2008).

Despite a relatively shallow water column (40 cm) and the presence of a LR substrate, conditions favorable for calcareous periphyton growth in south Florida marshes (Browder et al., 1994), we observed no proliferation of periphyton during the study. Periodic drought may also benefit periphyton development (Browder et al., 1994). Mesocosms in this study were continuously flooded, and it is possible that differences in P retention between sediment types may be more pronounced in systems that experience dry-out and reflooding events.

Development of a downstream gradient in TP concentration occurred on both muck and LR substrates, as SAV biomass and TP concentration in surface water and sediments each decreased along the flow-path. An exception was the similarity in TP concentration between the feed water (inflow) and the outflow of the initial muck mesocosm tanks, indicating that little or no P removal was occurring in the front end of those mesocosms, even during the third year of the study (Fig. 5). This could indicate P saturation of the initial tank in the muck mesocosm, which, in a non-segmented wetland system, would be manifested as a P saturation "front" (Howard-Williams, 1985; Smith and McCormick, 2001). Elevated porewater SRP concentrations in the initial tanks of the muck mesocosms may have contributed to internal loading at that position along the gradient, but porewater SRP

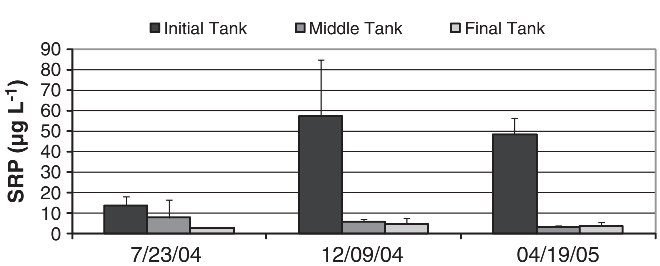

Fig. 9. Mean (+1 S.D.) sediment porewater concentration in initial, middle and final tanks of duplicate muck-substrate mesocosms, for three different sampling dates.

remained low in the middle and final tanks (Fig. 9). It is uncertain whether P-saturation would have continued to propagate through the system beyond the 6-year study period at the P loading rate applied in this experiment.

In contrast to the muck mesocosms, the LR mesocosms did not exhibit a steep horizontal gradient of water column TP concentration (Fig. 4), despite the development of a gradient in sediment TP (Table 3). The lack of a distinct gradient, and lower front-end water column TP concentrations relative to feed water TP, suggest that the assimilative capacity of the initial tanks in the LR mesocosms had not been reached at the conclusion of the study.

Diel cycling between oxic and anoxic conditions in dense SAV of the initial tanks was coincident with changes in water column TP and PP concentrations, but not with DOP (Fig. 5). Since essentially all SRP was removed during passage through the mesocosm tanks, and mean outflow DOP concentrations were equivalent (~8 μg L^{-1}) across treatments, the difference in outflow TP concentrations between muck and LR mesocosms during the final 3 years of the study could be accounted for by PP. Internal generation of PP was demonstrated by diel measurements of water column P during stagnant conditions, but this single sampling event is not necessarily representative of PP dynamics over the entire study period. However, the elevated early morning P levels indicate that sequestered P mobilized as PP is a plausible mechanism that would contribute to a "moving front" of P-enrichment. For a 3-year period at the end of this study, the LR mesocosms produced higher average daytime PP levels (8–9 μg L^{-1}) than the muck mesocosms (5–6 μg L^{-1}), which may be indicative of either greater phytoplankton growth or increased sloughing of macrophyte detritus in the LR mesocosms. Decreased standing crop biomass in the final tank, especially on LR, may have allowed phytoplankton growth to a greater extent than in the densely vegetated muck mesocosms, via reduced competition for light, space, and bio-available nutrients.

Removal of P from Everglades surface waters by co-precipitation of orthophosphate with calcium carbonate can result from photosynthesis-driven pH increases during daytime hours (Diaz et al., 1994). The degree to which dissolution of co-precipitated P occurs at night, when pH returns to circumneutral values, is unknown. A lack of discernable difference between morning and afternoon P concentrations in the waters of the middle and final tanks of the mesocosms suggests that diel activities, such as algal P uptake, zooplankton feeding behavior, and dissolution of Fe-hydroxides during night-time anoxia, did not result in a pronounced diel pattern in water column P concentrations under stagnant conditions at that position along the gradient.

The apparent seasonal pattern observed in muck mesocosm outflow TP concentrations could be a consequence of seasonal changes in the quality of mesocosm inflow water, or changes to internal P cycling from sediments and vegetation within the mesocosms themselves. Mesocosms were operated with water previously treated by STA-1W, and either the recalcitrance of P compounds in the mesocosm inflow water or other qualities may have changed as a result of STA operations. Seasonal trends in STA-1W outflow waters were previously reported for several parameters (e.g., Ca, DOC, conductivity), but not for TP or SRP (Gu et al., 2006).

Performance data from multiple treatment wetland systems suggested that P removal mechanisms are not influenced by temperature to the same degree as nitrogen removal processes (Kadlec and Reddy, 2001). However, the lowest mesocosm outflow TP concentrations coincided with colder months (Fig. 7). These data support the hypothesis that temperature affects water column P at low levels by regulating microbial mineralization of sediment P and internal P loading (Jensen and Andersen, 1992). Another possible reason for relatively higher outflow TP concentrations in the warm summer months is that high temperatures can stress aquatic plants and increase nutrient loss from macrophyte tissues to the surrounding surface waters. Maximum surface water temperatures in our mesocosms (38.6 °C on August 4, 2003) and in natural SAV beds within STA-1W (38.5 °C; Chimney et al., 2006) were above thermal optimum conditions (28–32 °C) for growth of many SAV species, a condition which can increase respiration and advance the onset of senescence (Barko and Smart, 1981).

Three submerged macrophytes (*N. guadalupensis*, *C. demersum* and *C. zeylanica*) were initially stocked to our mesocosms, but each tank quickly became dominated by the latter species. Several investigators have noted that in lakes *Chara* can be quite competitive with submerged vascular plants such as *Najas*, through mechanisms such as efficient carbon utilization and allelopathy (Van den Berg et al., 2001; Van Donk and Van de Bund, 2002). The mechanism for *C. zeylanica* dominance over the vascular plants and calcareous periphyton in this study is unknown, but may be related to the low P content of the sediments relative to other wetland systems (described below).

The biomass of *C. zeylanica* in the final muck tank was higher than in the final LR tank throughout the study, despite relatively comparable tissue N and P contents between treatments. While *C. zeylanica* has no true roots, it may be possible that sediment-to-water column diffusion of macro- or micro-nutrients from the muck substrate enhanced standing crop development over that in the LR substrate systems. Such an effect may have been masked in the initial and mid-region tanks by the presence of a complete complement of nutrients that accompanied the inflowing waters. Chimney et al. (2006) found strong vertical DO gradients and thermal stratification in SAV-dominated areas of STA-1W. Dense *C. zeylanica* growth within the mesocosms, especially in the initial tanks, may have reinforced DO gradients observed from oxygenated surface waters to hypoxic bottom waters and anoxic sediments, as has been suggested for *Chara* in lakes (Kufel and Kufel, 2002).

Biomass in natural stands of charophytes in lakes reportedly ranges from 0.042 to 0.500 dry kg m^{-2}, and P content from 200 to 2900 mg kg^{-1} (Kufel and Kufel, 2002). In the present study, biomass in all but the final LR tanks was above that range. By contrast, the tissue P levels of *C. zeylanica* in this study were within the reported range, with the exception of the plants in the final tanks, which exhibited tissue P levels as low as 120 mg kg^{-1}. Natural *Chara* beds have been shown to sequester an average of 0.3 g P m^{-2} (Kufel and Kufel, 2002), a storage most closely approximated by the vegetation in our mid-tanks.

The P content of inflow and outflow region vegetation in STA-1W Cell 4 (where the dominant species was *N. guadalupensis*), at 1946 and 1675 mg kg^{-1} (DB Environmental, unpublished data), was higher than that of *C. zeylanica* in our initial and final muck mesocosms (1249 and 143 mg kg^{-1}, respectively). Other investigators have noted that *Chara* species typically contain lower TP levels than submerged angiosperms, in part because of high tissue Ca levels (Kufel and Kufel, 2002; Siong et al., 2006).

While P loading and removal rates, outflow P concentrations and tissue P contents were all similar between mesocosms in this study and full-scale STAs, sediment TP concentrations in this study were not typical of sediment TP in the inflow regions of downstream cells of STA-1W. After 8 years of operation, inflow region sediments of STA-1W Cell 4 exhibited a TP content of 1301 mg kg^{-1}, with 19.8% Ca and 0.082 g cm^{-3} bulk density (DB Environmental, 2002), substantially higher than TP in sediments recovered from the initial mesocosms (358–378 mg kg^{-1}). Sediment TP content in the final tanks (243–272 mg kg^{-1}) was also lower than the 631 mg kg^{-1} in STA-1W Cell 4 outflow sediments (23% Ca and 0.155 g cm^{-3} bulk density) observed in that earlier study.

There are potential constraints to scale-up of the LR-substrate treatment system, such as the costs of importing LR materials or removing organic soils to expose buried LR substrates. Findings from this mesocosm study do not support the hypothesis that a LR substrate will provide improved P removal relative to a muck substrate. It is possible, however, that superior performance by LR substrate systems, relative to muck, may be achieved under lower P loading conditions than evaluated in the present study, although the accrual of new sediments above the initial substrate could ultimately limit the potential benefits of LR for long-term P removal performance.

5. Summary and conclusions

Gradients in P removal and storage were investigated over 6 years using mesocosms containing SAV grown on muck and LR substrates. Average water column P removal was slightly greater in the muck than LR mesocosms, with mean inflow TP levels of 32 μg L^{-1} reduced to 15 and 17 μg L^{-1} in these respective treatments. Mesocosm P loading rates (average of 1.75 g m^{-2} $year^{-1}$) varied widely during the study, but did not exhibit a pronounced influence on outflow total P concentrations. A seasonal pattern of lowest outflow P concentrations during December and January prevailed throughout the study, but exact mechanisms for this phenomenon were not determined.

The preponderance of DOP and PP in the outflows of both the muck and LR substrate systems suggests that large-scale systems will be challenged in achieving ultra-low (<15 μg L^{-1}) outflow concentrations. Stagnant conditions favored increased water column DOP levels, while PP varied diurnally, and average concentrations of both forms were strongly influenced by position along the nutrient gradient.

Our study results demonstrated that well-defined vegetation and sediment enrichment gradients developed in SAV-dominated wetlands operated under low P concentration ranges. While the mesocosm data did not reflect significant deterioration in treatment performance during the six-year study period, the gradual accumulation of P-enriched sediments near the wetland inflow could eventually compromise hydraulic storage and P removal effectiveness of these treatment wetlands.

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