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

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Everglades Agricultural Area Environmental Protection District

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Introduction

Presence of a periphyton community adapted to low water column P concentrations is considered the central element that leads to superior P removal performance by the periphyton-based stormwater treatment area (PSTA) technology. Periphyton communities are complex assemblages of cyanobacteria, eubacteria, diatoms and eukaryotic algae and are found in lakes, streams and wetlands, including the marshes of the Everglades (McCormick and O'Dell 1996). Several characteristics of periphyton communities make them well suited for biological treatment of surface waters in wetlands (Dodds 2003). Periphyton typically have a high affinity for P and respond to P inputs more rapidly than other wetland components (macrophytes, soils) and thus are important in the uptake and storage of P (McCormick et al. 1996). Typically, periphyton growth in PSTA systems is encouraged by several design features, including a benthic surface dominated by calcitic minerals, typically limerock; limited densities of emergent macrophytes, to reduce shading; and shallow water depths, to support light penetration.

It is believed that excessive inputs to a wetland of readily available P (such as SRP), either through inflow waters or via sediment-to-water column flux from enriched sediments, can constrain the development of periphyton adapted to low-P conditions, as well as the production of phosphatase enzymes. Hence, the presence of a limerock substrate in PSTA systems, which is thought to curtail substrate P transport to the water column via diffusive flux or macrophyte "mining", is expected to facilitate the development and enhanced P removal function of periphyton communities.

Periphyton communities vary across the Everglades landscape, and have been previously reviewed by Browder, Gleason and Swift (1994) and more recently by Gaiser et al. (2011) and Hagerthey et al. (2011). Based on previous work with Everglades periphyton, low-P communities are dominated by diatoms and blue green algae, with P-sensitive species such as *Cymbella minuta* and *Mastogloia smithii* present (See table 3-2 in McCormick et al. 2000). In an

STA setting, less is known about the species composition of periphyton. Moreover, the removal of muck soils as an internal nutrient source (as in a PSTA cell) may promote the development of specific periphyton communities that differ from those in typical muck-based STA flow paths. Hence, the STA-3/4 PSTA Cell offers a unique platform in which to evaluate a well-established periphyton community.

Periphyton-based systems appear able to provide lower particulate P (PP) and dissolved organic P (DOP) concentrations, relative to muck-based EAV and SAV marshes. Phosphorus in those pools is less available for biological uptake or chemical sorption than soluble reactive P (SRP), and so more difficult to remove in treatment wetlands. Under certain conditions, however, PP and DOP can be broken down through hydrolysis reactions into more bioavailable forms of P. Phosphatase enzymes are naturally-produced proteins that catalyze these hydrolysis reactions, effectively increasing the transformation of selected DOP and PP molecules into more bioavailable P forms. Phosphatases are found internally within organisms and as extracellular moieties within the aquatic environment (Wetzel 1991). In ultra-oligotrophic south Florida marshes, periphyton mats are thought to be a key locus for production of these enzymes.

PSTA systems also are thought to require relatively shallow water conditions for optimum performance. Shallow conditions facilitate light penetration to the sediment (benthic) surface. In highly colored surface waters typical of the STAs, light is rapidly attenuated, so small increases in water depth can dramatically reduce light penetration to the benthic surface. McCormick et al. (2006) noted that nearly all photosynthetically active radiation (λ = 400-700 nm) was attenuated within the upper 30 cm (1 ft) of the water column in STA-1W Cell 4. Shallow water depths may confer additional benefits to PSTA systems. Theoretically, lower depths increase the ratio of benthic (periphyton) surface area to water volume, increasing treatment contact. Also, shallow conditions may promote the breakdown of dissolved organic compounds (including DOP) by UV radiation (Wetzel et al. 1995).

Through the mechanisms described above, there clearly are potential benefits to shallow water depths in PSTA systems. Shallow depths may be difficult to establish and maintain in full-scale STA flow paths, however, and the effects of water depth on PSTA performance must be better defined. Prior research at 30 or 60 cm water depths showed no difference in P performance, though static depth treatments outperformed variable depth treatments (CH2M-Hill 2003). The best performing experimental PSTA platforms were operated at 30 cm (0.2 ha test cell and mesocosms) and 9 cm (DB Raceways, DeBusk et al. 2004). The STA-3/4 PSTA Cell typically has operated at a depth of 30-45 cm, with high flow events increasing average water depths to just over 60 cm.

The ability of these prior PSTA research efforts to define suitable water depth ranges has been limited in some cases by a lack of replication, and in other instances by the inability to produce ultra-low outflow TP concentrations. To overcome these limitations and provide insights into the effects of water depth on surface water TP concentrations, periphyton communities and P

removal mechanisms in PSTA systems, we currently are operating a replicated outdoor mesocosm study using periphyton and macrophytes collected from the STA-3/4 PSTA Cell.

The relative densities of macrophytes and periphyton in these PSTA mesocosms, as well as the enzyme activity of the benthic periphyton layer were presented in an earlier report (DBE 2015c). Phosphorus removal performance, TP concentrations and other water quality characteristics of these mesocosms were also previously described (DBE 2015b). The current report focuses on water depth effects on macrophyte and periphyton communities across a range of P loading rates and along nutrient gradients. We examine the relationship between phosphorus loading rate and outflow TP concentrations for mesocosms operated at a range of water depths from 23 to 92 cm. Nutrient gradients that have been established within this research platform facilitate an assessment of the effects of P loading rate on outflow P. To verify that the mesocosms are representative of field-scale conditions, mesocosm periphyton communities in this study are being compared to communities in the PSTA Cell. To address whether outflow waters from both the PSTA Cell and our mesocosms are capable of supporting the same P-sensitive periphyton communities found in the natural Everglades marshes, the periphyton community composition also is being examined at each of these locations.

Methods

Experimental Design

Operational requirements of a PSTA system are being investigated in mesocosms situated at the experimental facility near the outflow of STA-1W. Triplicate flow ways with a local limerock substrate were established under each of four water depth treatments. The first two treatments are static in depth. Shallow treatments (23 cm) and slightly deeper treatments (46 cm) consist of 4 tanks (each 1.8 m^2) plumbed in series. These tanks were initially established in September 2013, under constant flows that provide a hydraulic retention time (HRT) of 5 and 10 days for the shallow (23 cm) and slightly deeper (46 cm) flow ways, respectively. Delivery of a constant flow rate to both shallow and deep tanks insures equal P mass loading rate (PLR) to those treatments on an area basis.

In January 2014, additional mesocosms were established to test PSTA performance at greater water depths. Six new flow ways were constructed using larger tanks (2.8 m^2 per tank) plumbed two in series (Figure 1). These systems were initially established at 46 cm depth, and flows are being delivered to provide equivalent HRT and PLR conditions to the existing mesocosms operating with 4 tanks-in-series at 46 cm depth. The first tanks in series of the new flow ways receive an equivalent PLR to the first half (first 2 tanks) of the 4-in-series systems. This approach enables a comparison of "midpoint" and "outflow" positions with equivalent HLR and P loading across static and variable-depth treatments (Figure 2). Key operational parameters of these systems are outlined in Table 1.

After an initial phase of comparable operations, the newer mesocosms were assigned to two new variable water depth treatments (46-69 cm and 46-92 cm). The transition to deeper conditions began after the water sampling effort on 5/29/2014 (Figure 3). On 9/15/2014, water depths were lowered to 46 cm in the variable depth treatments, and the "shallow" conditions were maintained until January 15, 2015. Since January, water depths have been maintained at 69 and 92 cm in the respective variable-depth treatments to examine the P removal performance and periphyton community response to longer-duration deep water conditions.

Figure 1. Mesocosms were established at a range of water depths to explore the effects of operating conditions on P removal effectiveness and biological community response. The shorter tanks (to the lower left) were plumbed 4 in-series at water depths of 23 or 46 cm. The larger mesocosms were plumbed 2 in-series and were also established at 46 cm before transitioning to 69 cm or 92 cm water depths.

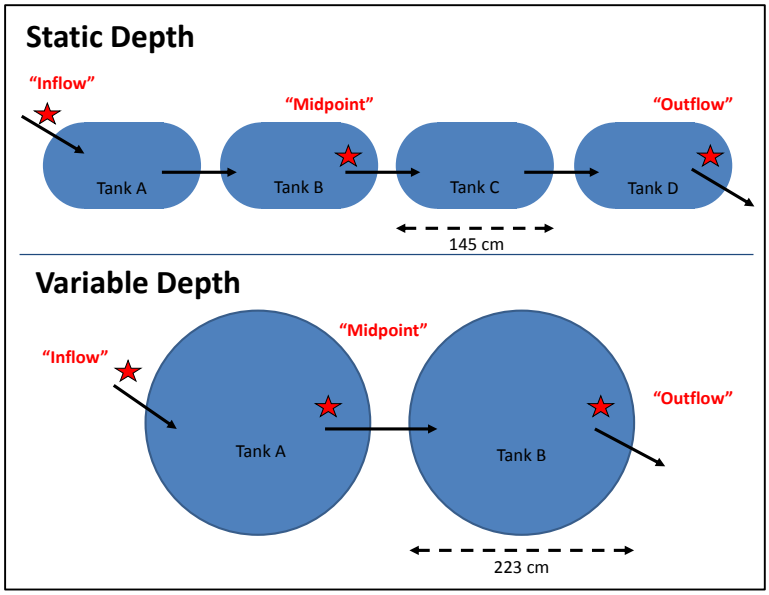

Figure 2. Water sampling locations in the static depth and variable depth mesocosms.

Table 1. Operational targets for experimental flow ways assigned to one of four depth treatments.

Static Depth Treatments

	Tank			
	A	B	C	D
HLR (m/day)	0.182	0.091	0.061	0.046
PLR at 20 ppb (g P/m^2/yr)	1.33	0.67	0.44	0.33

Water Depth	HRT (days)			
23 cm	1.3	2.5	3.8	5.0
46 cm	2.5	5.0	7.5	10.0

Variable Depth Treatments

	First Tank	Last Tank
HLR (m/day)	0.091	0.046
PLR at 20 ppb (g P/m^2/yr)	0.67	0.33

Water Depth	HRT (days)	
46 cm	5	10
69 cm	7.5	15
92 cm	10	20

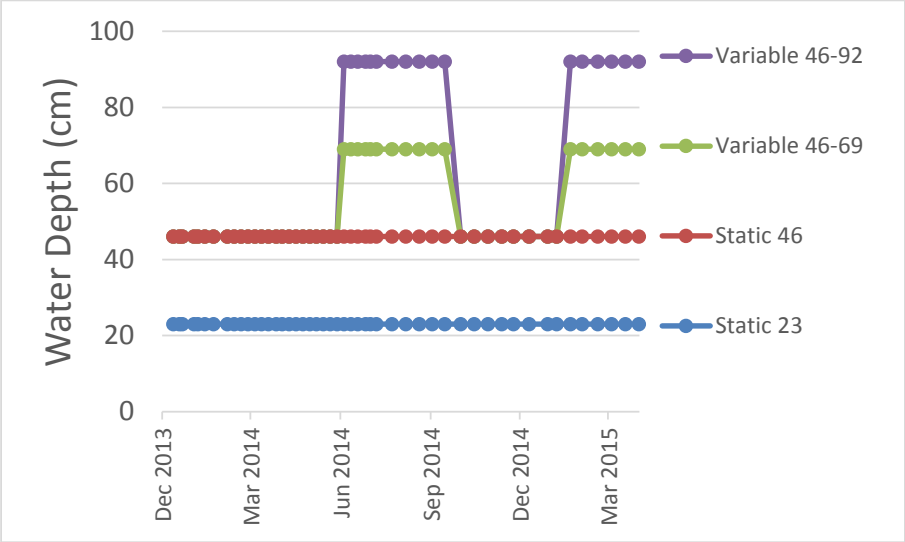

Figure 3. Temporal changes in water depths in each of four depth treatments during the monitoring period (December 12, 2013 – April 1, 2015).

Measurement of Light at the Benthic Surface

Algal growth is strongly controlled by the amount of light available for photosynthesis. The intensity of photosynthetically active radiation (PAR) at the benthic surface was determined by placing a spherical underwater quantum sensor (LiCOR, Lincoln, NE) in a recess within the outflow region of each process train, so that the sensor was even with the benthic surface (Figure 4). The PAR available at the benthic surface was determined as a percent of ambient levels, as measured above each tank. Measurements were made twice monthly from March 20, 2014 through June 18, 2014, then once monthly beginning in July 2014. The period of record in this report includes all measurements through May 27, 2015 (N = 18 events).

While PAR is able to penetrate into the water column to depths of several feet, ultraviolet wavelengths are more rapidly attenuated by DOM. This form of naturally occurring solar radiation is thought to be responsible for photo-oxidation of organic compounds into more bioavailable forms (Lindell et al., 1995; Wetzel et al., 1995). Further, UV photolysis of DOM can release previously complexed phosphatases (Wetzel et al., 1995). For these reasons, we have monitored the absorbance properties of surface waters in the PSTA Cell, as well as experimental waters exposed to different substrates and water depth conditions (DBE 2015d for details). UV254 provides a measure of absorbance of shortwave radiation by dissolved organic matter in the water column, and was measured periodically during this study. For further details, refer to the water quality monitoring results in DBE 2015b.

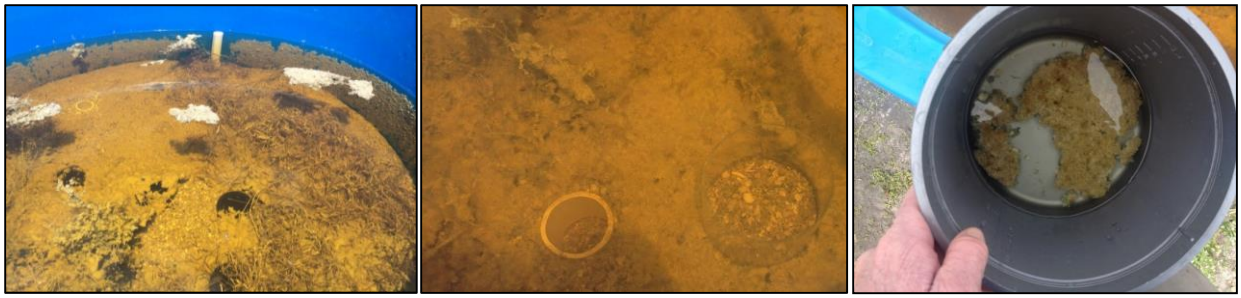

Figure 4. Periphyton in mesocosms has developed on the benthic surface, as well as tank walls, plant surfaces, and in some areas, benthic mats have separated to form floating mats (left image). The middle image shows a sampling collar used to define the area for benthic periphyton sampling, and a recess for measuring photosynthetically active radiation (PAR) at the benthic surface. The benthic periphyton mat shown in the left image was removed from the outflow region of a mesocosm on May 29, 2014.

Benthic periphyton sampling in outdoor mesocosms

Following initiation of water flows, benthic periphyton mats developed readily on the limerock mesocosm substrates. In order to compare biomass growth across depth treatments, we measured areal biomass (dry mass of periphyton per unit area of benthic surface) on three sampling dates: May 29, 2014 (prior to first deep phase); September 16, 2014 (end of 1st deep phase); and January 15, 2015 (prior to 2nd deep phase). Benthic samples were collected from each tank on each of the three sampling dates, with the following exception: static depth "B" and "C" tanks were only sampled from one replicate in May 2014.

A clean plastic bucket was submerged into the mesocosm water column next to the target sampling area. A sampling collar (14.6 cm inside diameter) was inserted into the limerock substrate. Periphyton was carefully transferred from the benthic surface by hand into the submerged bucket. Limerock gravel was also transferred, as necessary, to ensure that all periphyton was included in the sample. For each location, two grab samples were collected and composited into one sample representing a total benthic surface area of 335 cm^2. In contrast to water sampling efforts (see Figure 2), benthic samples were collected throughout the tanks to avoid resampling areas affected by earlier sampling efforts.

Periphyton Processing

In the laboratory, excess site water from each settled sample was decanted. Gravel was rinsed free of periphyton, using DI water as rinsate in a large beaker. The sample was stirred so that periphyton fragments suspended, while the gravel settled to the bottom of the beaker. Then, the suspension was transferred into the mixer (leaving the gravel behind in the beaker). Once the gravel was isolated from the bulk of the algal material, DI was added to the beaker and swirled to dislodge any remaining algal material from the gravel. While the algae was in suspension,

the liquid and algae was poured off while holding back the gravel. The gravel was inspected for any remaining periphyton fragments, then discarded. Once the gravel was removed, the algal suspension was settled, then overlying water was decanted, taking care to retain all particles.

The sample was gently stirred with a metal spoon, then "unhomogenized" samples (i.e., not blended) were transferred into 2 clean scintillation vials. The samples were then thoroughly homogenized in a food mixer. The homogenized slurry was again sub-sampled for enzyme assay (20 mL) and "homogenized" taxonomy samples (2 vials, each 20 mL). Another subsample of the remaining slurry (150 mL) was transferred into a pre-weighed plastic cup and dried to constant weight at 65°C. The remaining sample was transferred to a graduated cylinder to record the remaining volume. Periphyton samples were preserved in 4% formalin buffered with sodium borate for taxonomic identification and biovolume determination.

Enzyme Assay

Periphyton samples were diluted by dispensing a known volume of the enzyme subsample into a pre-weighed centrifuge tube, and adding DI as necessary, to achieve appropriate hydrolysis rates for the assays. The remainder of the diluted sample, once the enzyme assay was complete, was then dried to constant weight to calculate the enzyme assay bulk density. Results of the assay were normalized to dry weight of periphyton in the slurry as assayed. Benthic periphyton enzyme activity was converted to μmol MUF released/cm^2/hr, using the APA per unit weight, and the dry weight biomass of periphyton per unit area ("areal biomass").

Periphyton Community Composition

An aliquot from each benthic periphyton sample was preserved with formalin, archived and will be prioritized for identification at the conclusion of the study. Epiphytic periphyton and the host macrophyte tissues within each mesocosm will be sampled at the conclusion of the study. A subset of the benthic periphyton samples collected from these PSTA mesocosms was examined in FY2015 for preliminary community comparisons.

Periphyton Taxonomy

Periphyton community composition was classified by Dr. Klara Rehakova (Laboratory of Phytoplankton Ecology, Biological Centre of the Academy of Sciences of the Czech Republic). Each sample was examined to determine dominant species by biovolume. Relative dominance within the two major groups (diatoms and cyanobacteria) was also calculated separately. The results presented in this report focus on presence/absence comparisons at the genus level within three common classes of periphyton: blue-green algae, diatoms and green algae.

Community comparisons were made between water depth treatments across two dates selected to represent conditions at the end of the first deep water phase (9/16/2014) and the conditions following a period of recovery after a return to shallow conditions (1/15/2015) in the variable depth treatments. Samples from the static depth treatments were also examined for comparison

to the variable-depth treatments. The above comparisons (across dates and across depth treatments) were made using periphyton collected from the midpoint of each process train, as this location showed the greatest differences in water TP concentration between depth treatments. Another taxonomic comparison was made using samples from the 9/16/2014 event, whereby midpoint samples were compared to outflow-region periphyton samples. A final comparison looked at the taxa present in mesocosm samples, as well as periphyton collected from the PSTA Cell and un-enriched areas of the natural everglades marshes in WCA2A and WCA3A.

Periphyton Sampling in the PSTA Cell, Cell 3B and Natural Everglades Marshes

Our mesocosm platform successfully established periphyton communities within the range of water depths and P loading rates anticipated by a full-scale PSTA treatment cell, and produced very low outflow TP concentrations. This platform was established using limerock as a substrate, and periphyton and macrophytes collected from the 100-acre PSTA-based treatment cell in STA-3/4. There is still little known about whether the periphyton community within these mesocosms is similar to the larger PSTA Cell from which the inoculants were collected. Further, it is unknown how well the periphyton community in the PSTA Cell resembles the communities found in unenriched regions of the natural Everglades marshes.

As noted above, the Everglades environments which support periphyton growth are vast and varied, but typically are restricted to low nutrient environments. The PSTA Cell and STAs in general are alkaline freshwater environments, and would be expected to more closely resemble hard-water marshes of WCA2A and WCA3A than soft-water water marshes (such as the interior of WCA-1) or brackish environments found in some Southern Everglades locations. For a comparison to naturally-occurring low-nutrient periphyton communities, we conducted a synoptic sampling of epiphytic periphyton found in the PSTA Cell, in WCA 2A (near site U3) and to WCA 3A (near site DB15).

Macrophyte vegetation was collected from the PSTA Cell on several occasions, including August 2013, January 2014, July 2014, February 2015, and June 2015. On each occasion, periphyton was separated from the host tissue and analyzed for nutrient contents and enzyme activity. On three occasions, in addition to these periphyton isolates, the host macrophyte tissues were examined for nutrient contents. In January 2014, the macrophyte-periphyton tissue complex was analyzed together. To examine the potential for different responses by the periphyton and macrophyte components, macrophyte tissues collected in July 2014 and June 2015 were analyzed for nutrient contents after periphyton removal.

Preliminary periphyton sampling efforts in the PSTA Cell in August 2013 and January 2014 targeted available substrates for periphytic growth, and have been previously reported (DBE 2015d). More recent sampling efforts in July 2014, February 2015 and June 2015 were conducted using a replicated sampling approach. Three stations along inflow, middle and outflow transects were sampled during each event. At each station, a representative grab sample of SAV

tissues and associated epiphytes was collected in a re-sealable plastic bag, and stored on ice for transport to the lab. At the lab, epiphytes were removed from the host tissue using a rinsing procedure that resulted in an algal slurry (periphyton) and a rinsed host plant tissue sample. These components were analyzed separately for TP content, and the periphyton was assayed for enzyme activity.

Results

Light Conditions as a Function of Water Depth

The available PAR at the benthic surface was highest in the shallow treatments, and lowest in the depth-variable treatments (Figure 5). During shallow-phase operations, PAR levels were similar between the two variable depth treatments operating at 46 cm, while under deep-phased operations, the increased water depth in the 46-92 cm water depth treatment reduced PAR to 33% of incident levels, as compared to 65-70% in the shallow 23 cm treatment.

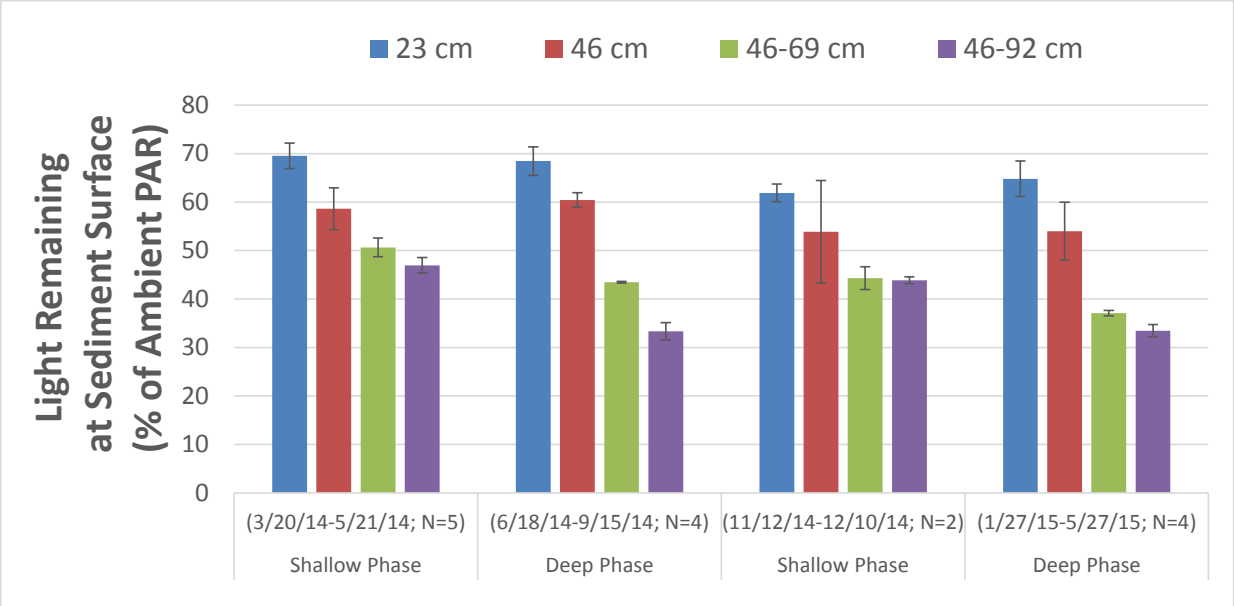

Figure 5. Light remaining at the sediment surface, as a fraction of the ambient light available above the water column, in the outflow region of mesocosms operated under four depth treatments. Error bars denote ± SE around period averages for triplicate flow ways under each treatment. Light was measured in the photosynthetically-active range (400-700 nm) during midday.

For comparison of the light available to benthic periphyton in these mesocosms to that available to the periphyton communities in the PSTA Cell in STA-3/4, the PAR available above and below the water surface on April 1, 2014 is shown in Figure 6. Ambient PAR was reduced to

~70% just beneath the surface, due to reflection of incoming light by the water surface. Further reductions occurred such that < 40% of ambient PAR remained at a depth of 50 cm. Our mesocosm benthic surfaces were exposed to 33-70% of ambient light, across all depth treatments (Figure 5). This reduction is not as dramatic as the example for STA-1W described in this report's Introduction, although it is clear from this assessment that shallow waters provide enhanced light for benthic periphyton development.

The range of UV254 absorbance values in the mesocosms was wider than the range observed during PSTA internal water quality surveys (Figure 7). The minimum absorbance value (0.376 m^{-1}) occurred in the outflow waters of the shallow (23 cm) depth treatment, when inflow waters were also at a minimum (.0.594 m^{-1}). At this absorbance level, > 2% of incident UV radiation remains at a water depth of 10 cm. At the other extreme, < 0.0002 % of UV radiation remained at 10 cm when absorbance was at its maximum value of 1.331 m^{-1} in the mesocosms. The maximum UV absorbance occurred during a dry season sampling event (April 2014) when the inflow waters to the mesocosms would have been affected by long contact times with the muck soil and vegetation in STA 1W. By contrast, our minimum UV254 value was observed during the wet season event of September 2014, when STA waters likely had shorter residence times. These seasonal differences in light quality imparted by the DOM pool must be considered when interpreting the results of short-term studies.

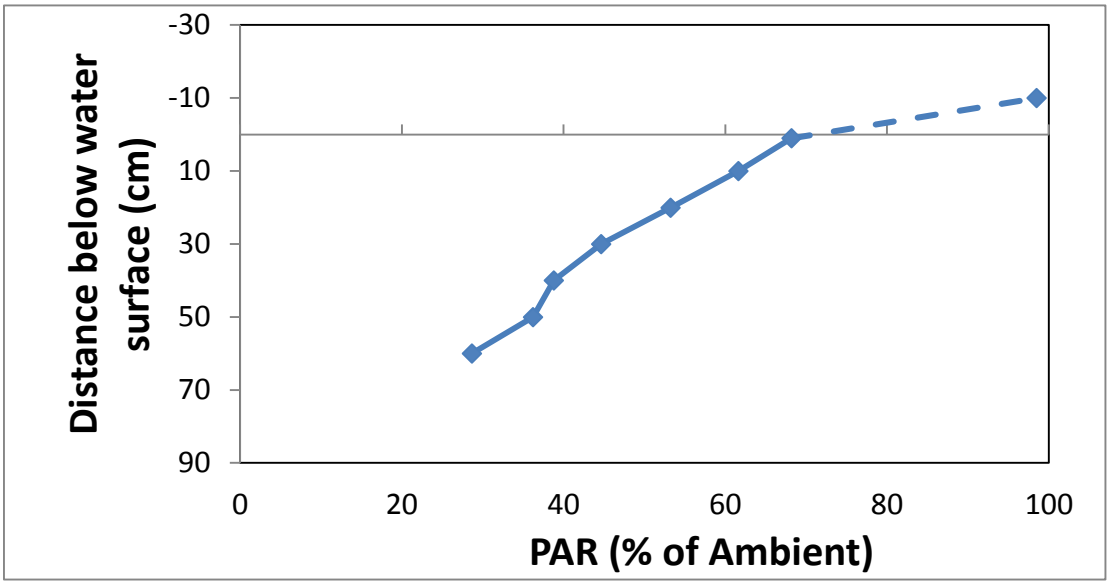

Figure 6. Example of the vertical profile in photosynthetically-active radiation within the water column, as measured in the PSTA Cell between 11:00 and 11:15 am on April 1, 2014.

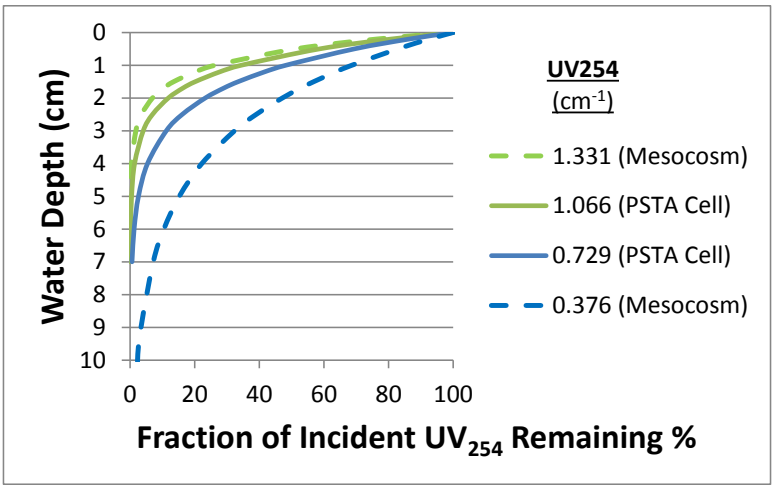

Figure 7. Fraction of incident ultraviolet (UV) radiation remaining with depth. Values were calculated from the minimum and maximum UV254 (absorbance of UV light at 254 nm as determined on 0.45 µm filtered samples), and measured either in the PSTA Cell outflow surface waters (N=14 events between May 2012 and June 2015, details in DBE 2015a), or on mesocosm surface waters (N = 18 sampling events between February 2014 and April 2015, details in DBE 2015b).

Effect of Water Depth on P Removal by PSTA Mesocosms

Phosphorus removal performance, TP concentrations and other water quality characteristics of these mesocosms were previously described (DBE 2015b). We therefore briefly summarize the long-term average TP concentrations for mesocosms operated at static or variable depths, for the period between December 2013 and April 2015 (Figure 8). For the period of record, slightly greater TP concentration reductions were observed in the static shallow treatments than the variable treatments, but only at the midpoint of the process trains. All depth treatments were equivalent at the outflow monitoring location, and produced an average outflow TP concentration of 8 µg/L from an inflow of 21 µg/L.

Periphyton Community Composition

The periphyton community composition at the midpoint and outflow of each process train were compared for samples collected on September 16, 2015. Across the four depth treatments, 15 of the 17 genera of blue green algae found in the midpoint samples were also found in the outflow region (Table 2). Diatom genera were also similar between the two locations on this date, and P-sensitive species such as *Mastogloia smithii* and *Cymbella minuta* were observed in samples from both midpoint and outflow mesocosm locations. There were fewer green algae genera represented in the outflow region than in the midpoint (Table 2).

Periphyton communities in each water depth treatment are compared in Table 3 using midpoint sample data, pooled for two events (9/16/2014 and 1/15/2015). We focused this preliminary

comparison on the midpoint, as the location where differences in P removal performance between water depth treatments were most apparent. The green algae in the deepest treatment (46-92 cm) were limited to a single taxon (*Tetraedron incus*), which contrasts the 5-6 genera observed in the shallower treatments. *Scytonema* sp is a blue-green alga that is commonly reported as a major component in Everglades periphyton (Gleason and Spackman 1974), but was observed only in the shallowest treatment (23 cm). The P-sensitive diatoms *Mastogloia smithii* and *Cymbella minuta* were observed in all depth treatments (Table 3) and on both dates (Table 4) at the midpoint, which underscores the ultra-low P conditions established in these PSTA systems. Interestingly, despite the low P levels observed at the process train midpoints, the PSTA systems were able to provide additional P removal between midpoint and outflows, regardless of depth regime (Figure 8).

There were differences in the benthic communities present on the two dates, though 23 genera were common to both dates (Table 4). Six genera, including three blue green taxa and three green algae were present in September but not in January samples, while nine taxa were observed in January that had not been observed in September. Analysis of additional samples from replicate mesocosms may revise these preliminary observations, but it is clear that a diverse assemblage of blue green algae, diatoms, and green algae taxa have become established, suggesting that the mesocosms are providing a suitable, replicated platform for examining the response of these communities to a variety of depth and loading conditions.

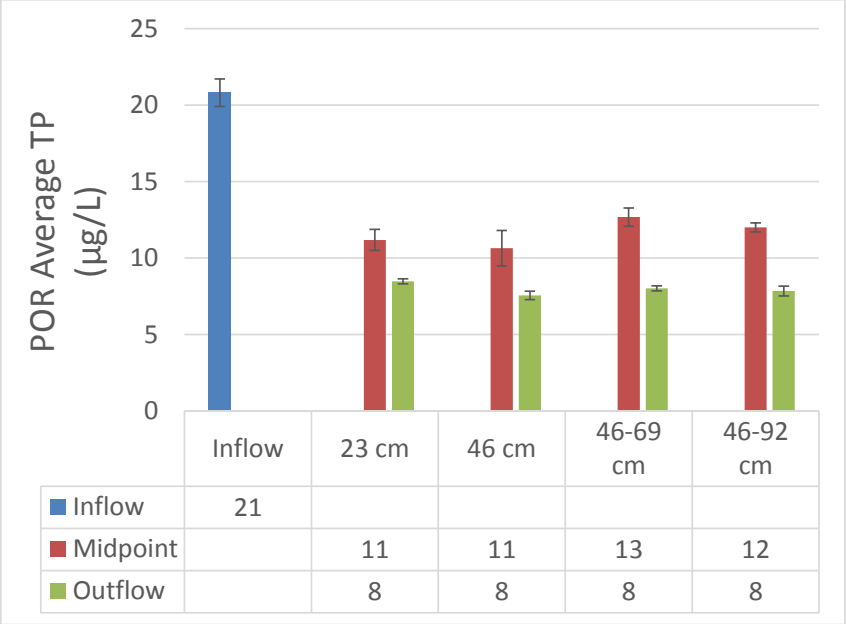

Figure 8. Inflow, midpoint and outflow concentrations of total phosphorus (TP) for mesocosm flow ways operated at either static depths (23 cm or 46 cm) or variable depths (46-69 cm or 46-92 cm). Values represent the average ± standard error for triplicate flow ways under each depth treatment, for the period of record (December 2013 – April 2015).

Table 2. Comparison of periphyton taxa observed in midpoint and outflow samples collected on 9/16/2014, using the pooled results from each of four depth treatments.

Common Class	Genus	Location	
		Midpoint	Outflow
Bluegreen Algae	*Aphanocapsa*	X	X
	Aphanotece	X	
	Aphanothece	X	X
	Bacullaria	X	
	Chroococcus	X	X
	Cyanothece	X	X
	Gloeothece	X	X
	Halomicronema	X	X
	Hassalia	X	X
	Johannesbaptistia	X	X
	Konvophoron	X	X
	Leptolyngbya	X	X
	Phormidesmis	X	X
	Phormidium	X	X
	Scytonema	X	X
	Spirulina	X	X
	Synechocystis	X	X
Diatoms	*Cocconeis*		X
	Cymbella	X	X
	Denticula	X	X
	Mastogloia	X	X
	Navicula	X	X
	Nitzschia	X	X
	Rhopalodia	X	X
Green Algae	*Cosmarium*	X	X
	Euastrum	X	
	Monoraphidium	X	
	Oedogonium	X	X
	Oscillatoria	X	
	Scenedesmus	X	X

Table 3. Comparison of periphyton taxa observed in each of four water depth treatments, using the pooled results from two sampling dates (9/16/14 and 1/15/2015).

Common Class	Genus	Depth Treatment			
		23 cm	46 cm	46-69 cm	46-92 cm
Bluegreen Algae	*Anabaena*	X	X		X
	Aphanocapsa	X	X	X	X
	Aphanotece	X	X	X	
	Aphanothece	X	X	X	X
	Bacullaria		X		
	Chroococcus	X	X	X	
	Cyanothece	X		X	
	Gloeothece	X	X	X	
	Halomicronema	X	X	X	
	Hassalia	X	X		
	Johannesbaptistia	X			
	Konvophoron	X	X	X	X
	Lemmermanniella			X	
	Leptolyngbya	X	X	X	X
	Phormidesmis	X	X	X	
	Phormidium	X	X	X	X
	Scytonema	X			
	Spirulina	X	X	X	
	Synechocystis	X	X	X	X
Diatoms	*Achnanthes*				X
	Cymbella	X	X	X	X
	Denticula			X	
	Eunotia				X
	Gomphonema			X	X
	Mastogloia	X	X	X	X
	Navicula	X	X	X	X
	Nitzschia	X	X	X	X
	Pinnularia				X
	Rhopalodia	X	X	X	
Green Algae	*Cosmarium*	X	X	X	
	Euastrum		X		
	Monoraphidium	X		X	
	Mougeotia			X	
	Oedogonium	X	X	X	
	Oocystis	X	X		
	Oscillatoria	X			
	Scenedesmus	X	X	X	
	Tetraedron		X		X

Table 4. Comparison of periphyton taxa on two sampling dates, from the midpoint of mesocosm flow ways, using pooled data from across the four water depth treatments.

Common Class	Genus	Sampling Date	
		9/16/2014	1/15/2015
Bluegreen Algae	*Anabaena*		X
	Aphanocapsa	X	X
	Aphanotece	X	X
	Aphanothece	X	X
	Bacullaria	X	
	Chroococcus	X	X
	Cyanothece	X	X
	Gloeothece	X	X
	Halomicronema	X	X
	Hassalia	X	X
	Johannesbaptistia	X	X
	Konvophoron	X	X
	Lemmermanniella		X
	Leptolyngbya	X	X
	Phormidesmis	X	
	Phormidium	X	X
	Scytonema	X	
	Spirulina	X	X
	Synechocystis	X	X
Diatoms	*Achnanthes*		X
	Cymbella	X	X
	Denticula	X	X
	Eunotia		X
	Gomphonema		X
	Mastogloia	X	X
	Navicula	X	X
	Nitzschia	X	X
	Pinnularia		X
	Rhopalodia	X	X
Green Algae	*Cosmarium*	X	X
	Euastrum	X	
	Monoraphidium	X	
	Mougeotia		X
	Oedogonium	X	X
	Oocystis		X
	Oscillatoria	X	
	Scenedesmus	X	X
	Tetraedron		X

Benthic Periphyton Response to Phosphorus Loading Gradients in Mesocosms

As of May 29, 2014, the static depth mesocosms had been operational for 9 months. At this time, the biomass of benthic periphyton remained low (1.3 ± 0.4 g/m^2) in the inflow region of mesocosms at both 23 cm and 46 cm depths. However, downstream tanks supported higher biomass levels, especially at the 23 cm depth (Figure 9). Repeated sampling of these communities in September 2014 and January 2015 showed increasing biomass over time for a given position along the gradient. One exception was the second tank in series for the shallow (23 cm depth) treatment, which showed no change or a slight decline in biomass over time. Areal biomass was equal or lower in May 2015 than it was for a given position along the gradient in January 2015. The temporal declines in biomass were most dramatic in the fourth tank in series of 23-cm treatments and the third tank in series of the 46 cm depth treatment (Figure 9).

Benthic periphyton TP contents in static depth treatments decreased along the four tanks in series from inflow to outflow (Figures 10 and 11). No change in TP content was observed for these treatments across the first three sampling events, while slightly higher TP was observed in the outflow region in May 2015. By contrast, the benthic periphyton APA showed consistently increasing levels over time for both 23 cm and 46 cm static depth treatments.

The trend of increasing biomass over the first three sampling events was also apparent in the variable-depth mesocosms (Figure 12). Gains in biomass were greater in the outflow region than at the midpoint. It is noteworthy that benthic periphyton biomass did increase in the outflow region between May and September 2014, when the variable depth mesocosms were operated at water depths of either 69 or 92 cm. By contrast, the midpoint sampling indicated that low biomass conditions persisted through this period in those treatments. During the subsequent shallow period (September 2014 – January 2015) areal biomass values increased in the midpoint of variable-depth treatments, but to a much smaller degree than the biomass gains over the same period in the outflow region. No change in the areal biomass was observed between January and May 2015 at the midpoint of each flow way. By contrast, the benthic periphyton biomass in the outflow region decreased over the same time period for all treatments.

Enzyme assays of the periphyton from static and variable depth treatments showed increased activity (per gram dry weight of epiphyte) over time in nearly every position sampled, the one exception being the 46-92 cm treatment in January 2015, where a decrease from a relatively high average value (95 ± 34 µmol MUF released/g epiphyte/hr), to 54 ± 13 µmol MUF released/g epiphyte/hr was observed (Figure 12). By combining the APA rates (dry weight basis) with areal biomass estimates of periphyton biomass per unit area of benthic surface, the data indicate that shallower conditions provided the highest enzyme activities (per unit area) among the four depth treatments (Figure 12).

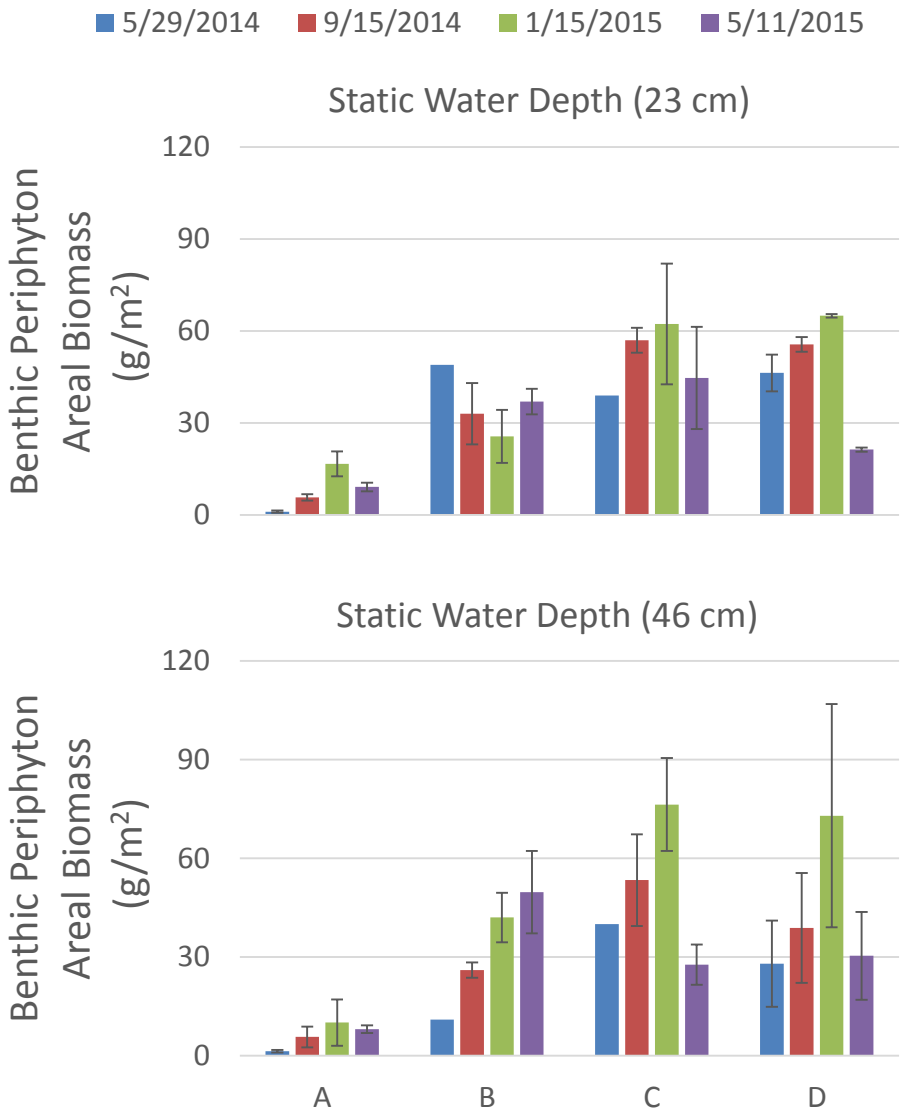

Figure 9. Areal biomass development on the benthic surface of mesocosms operated at either 23 cm or 46 cm water depths. The process trains consisted of four tanks in series, with inflow into "A" tanks and outflow from "D" tanks. The error bars denote the standard error around the mean values from triplicate process trains under each depth treatment.

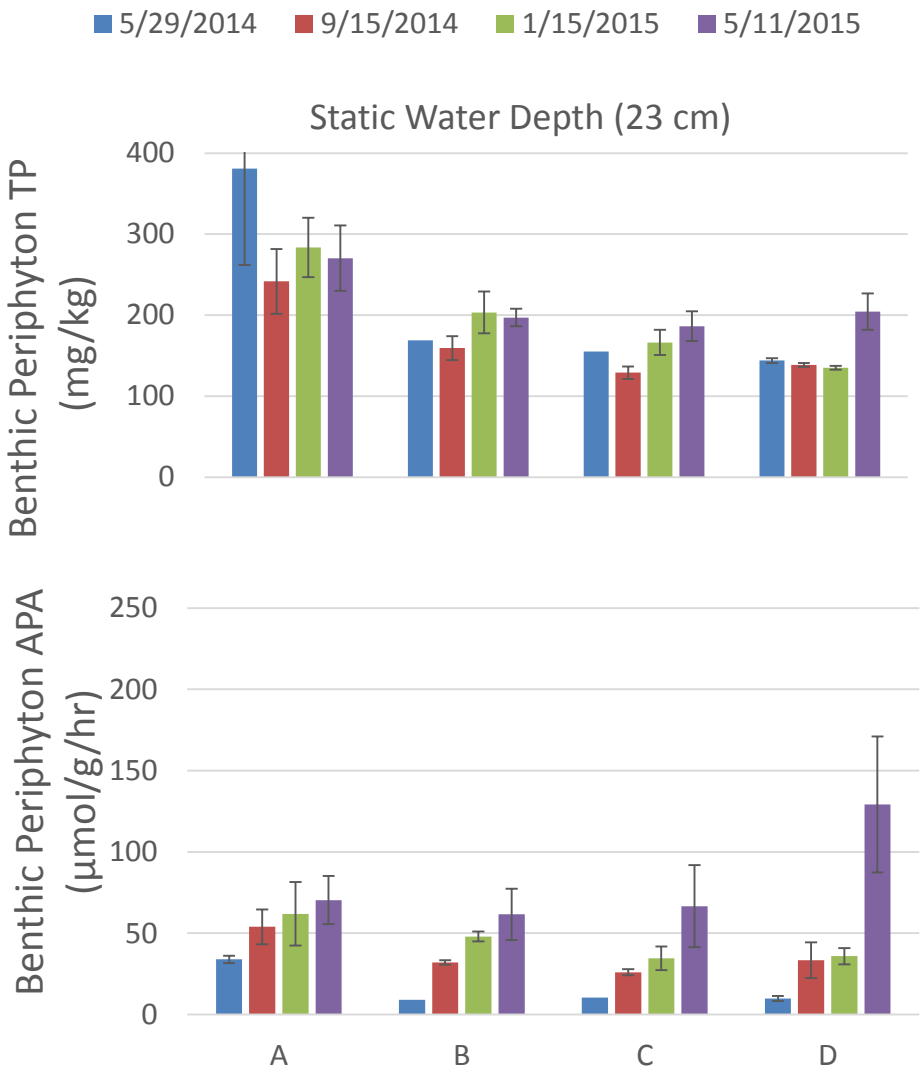

Figure 10. Total phosphorus (TP) content (top panel) and alkaline phosphatase activity (APA) of the benthic periphyton in mesocosms operated at a constant water depth of 23 cm. The process trains consisted of four tanks in series, with inflow into "A" tanks and outflow from "D" tanks. The error bars denote the standard error around the mean values from triplicate process trains under each depth treatment.

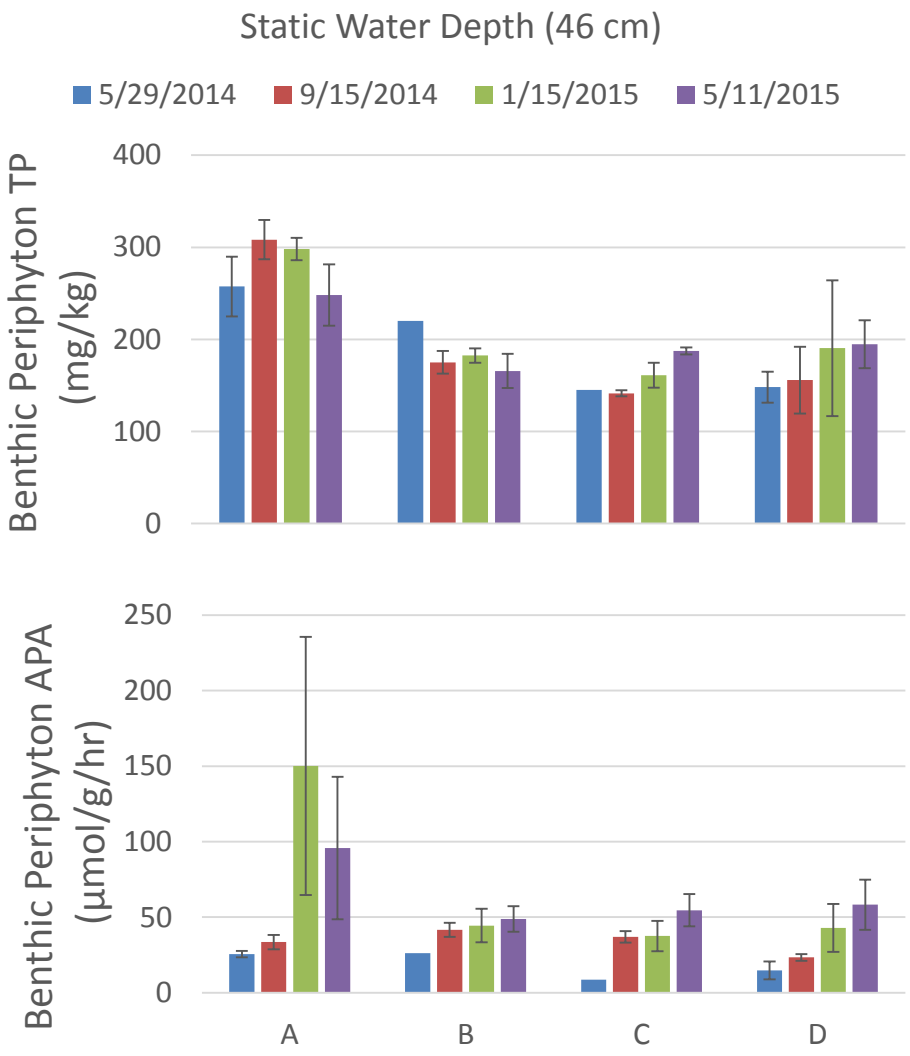

Figure 11. Total phosphorus (TP) content (top panel) and alkaline phosphatase activity (APA) of the benthic periphyton in mesocosms operated at a constant water depth of 46 cm. The process trains consisted of four tanks in series, with inflow into "A" tanks and outflow from "D" tanks. The error bars denote the standard error around the mean values from triplicate process trains under each depth treatment.

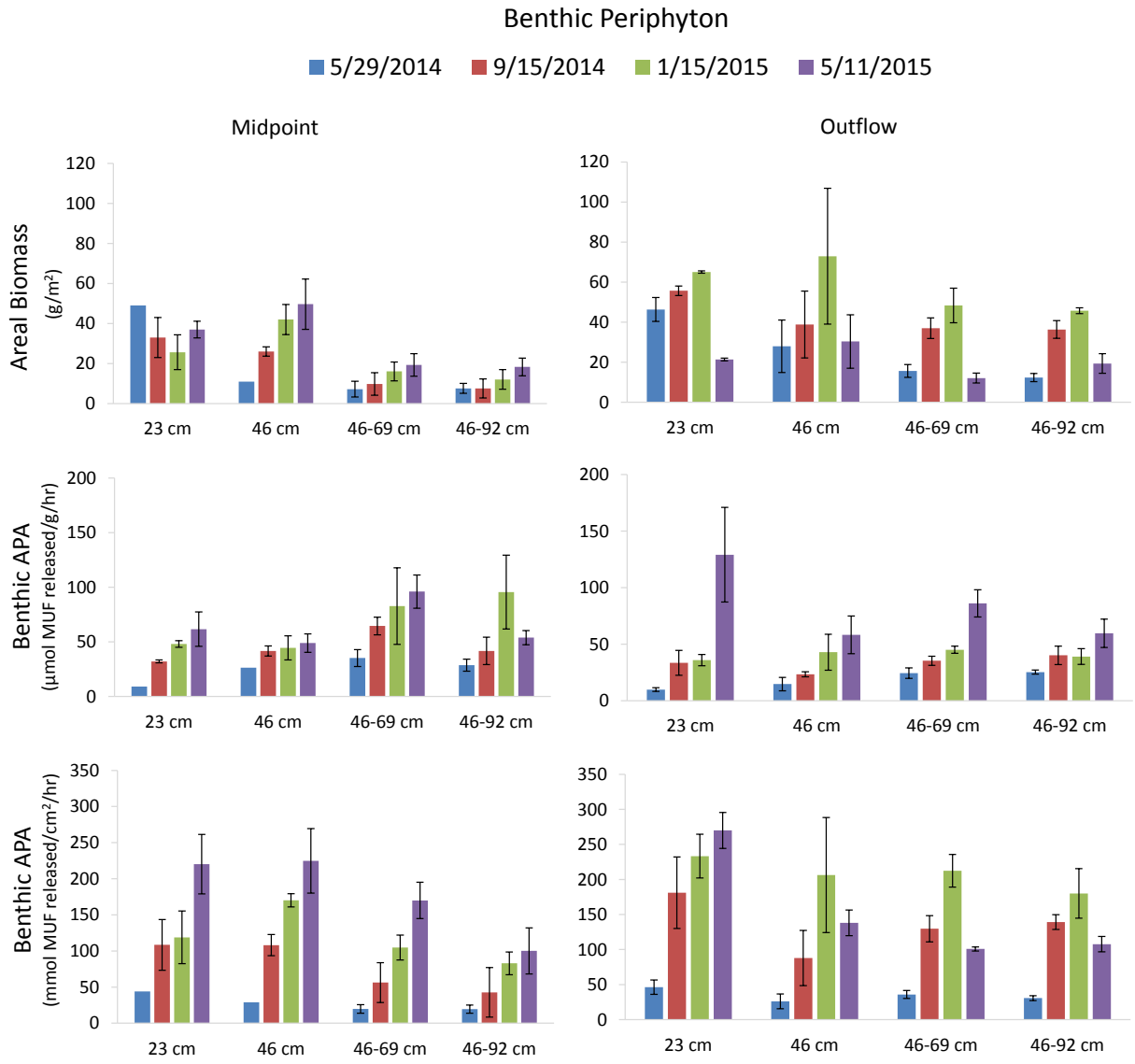

Figure 12. Areal biomass of benthic periphyton grown in mesocosms operated at either static water depth (23 or 46 cm) or variable water depth, on four sampling dates (top panels). The midpoints and outflows of each flow way were sampled. Benthic periphyton was assayed for alkaline phosphatase activity (APA) and normalized to the dry weight of the periphyton (middle panels) and the benthic surface area supporting periphyton growth (bottom panels). The error bars denote standard error around the mean of values from three replicates under each treatment.

Nutrient Content of Benthic Periphyton in Mesocosms

Phosphorus content of periphyton was elevated in the variable depth treatments relative to the static depth treatments, but only at the midpoint of each flow way (Figure 13). In the outflow region of variable-depth treatments, TP content appears to have increased over the period from September 2014 through May 2015. These increased TP contents remained below the levels observed for the midpoint of the same treatments. Overall, outflow TP concentrations ranged from 135 – 231 mg/kg across the four depth treatments and over four sampling events. Tissue nitrogen (N) levels indicated enrichment during the period between May and September 2014, which coincided with the first deep phase in the variable-depth treatments. However, this pattern was observed both at the midpoint and outflow and across both static and variable-depth treatments, and therefore was likely related to factors other than the change in water depth. Subsequent sampling events showed inconsistent patterns in TN across treatments, with no difference between midpoint and outflow samples (Figure 13). The changes in N and P contents observed between May and September 2014 resulted in increased N:P ratios for periphyton in the outflow of the four depth treatments, as well as for the midpoints of the two static depth treatments. A decreased N:P ratio was observed in the midpoint samples from the deepest (46-92 cm) flow ways between May and September, as a result of P accumulation in those tissues.

Carbon and calcium contents of the periphyton indicated fairly stable concentrations across depth treatments (Figure 14). A slight increasing trend in TC over time was observed in all treatments for both midpoint samples and outflow-region samples. Calcium content in the deepest treatment (23.2 – 26.5%) was higher than the remaining treatments (19.0 – 24.8%), perhaps due to the longer hydraulic retention time (HRT) in those systems.

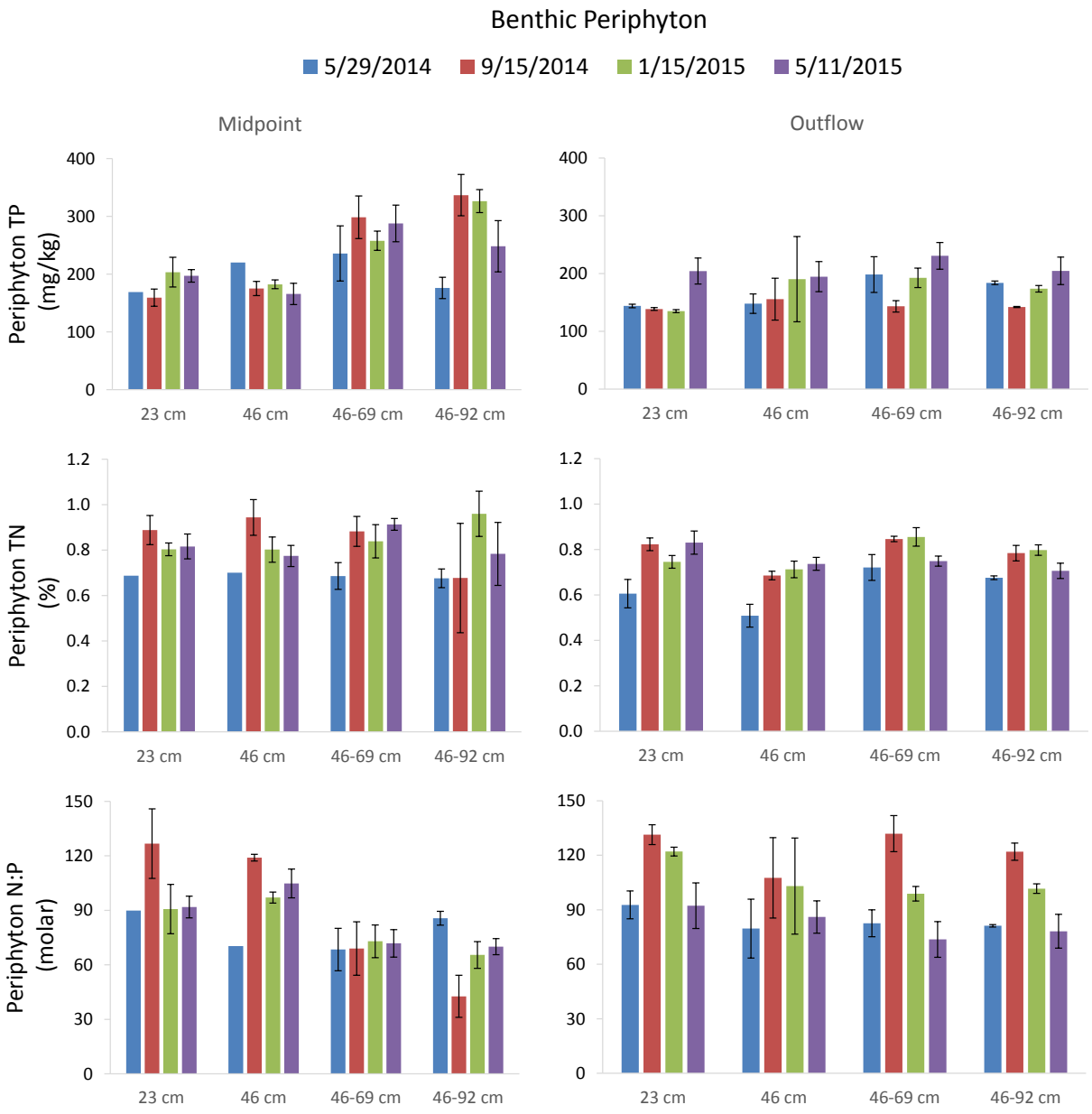

Figure 13. Benthic periphyton phosphorus and nitrogen contents and nutrient ratios on four sampling dates between May 2014 and May 2015, in mesocosms operated under different water depth regimes. Values represent the mean (± SE) of triplicate process trains under each treatment. Samples were collected at the midpoint and outflow region of each process train on each sampling date.

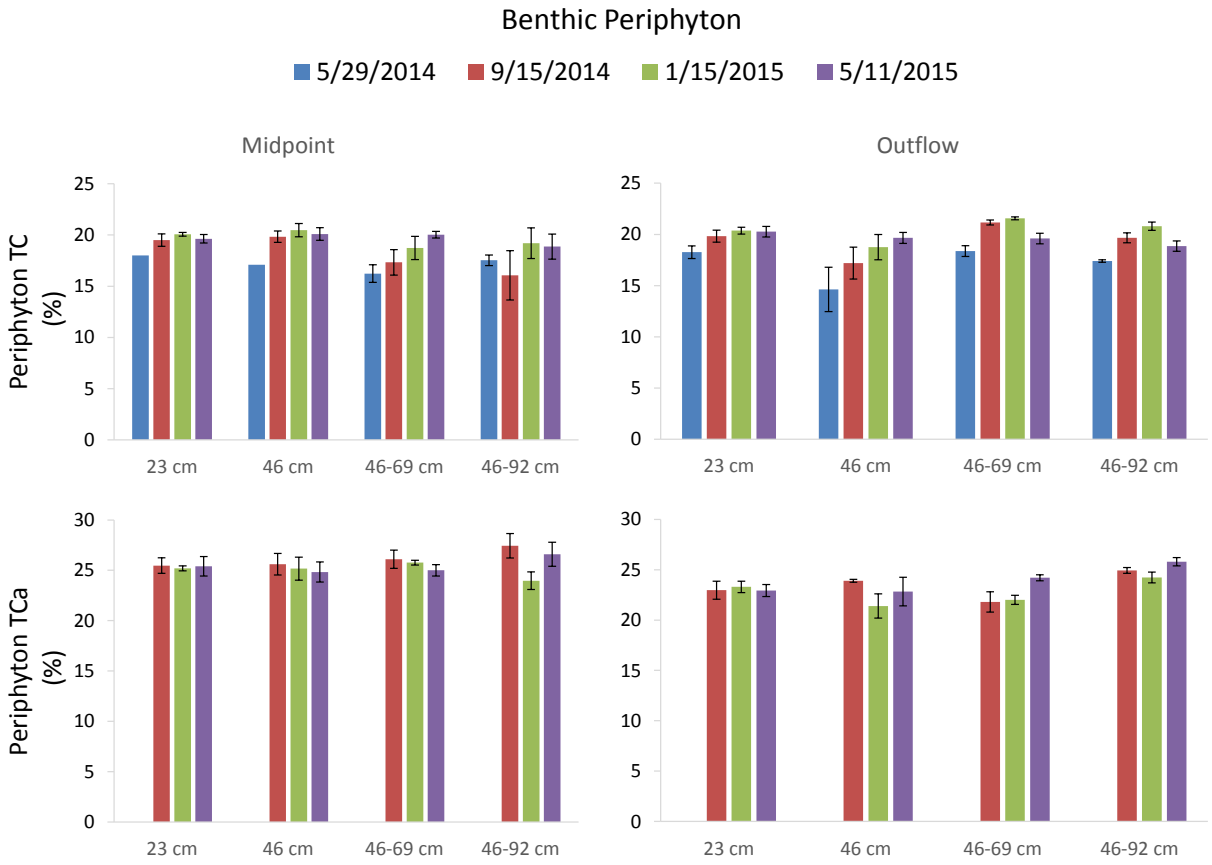

Figure 14. Average carbon and calcium contents of benthic periphyton sampled on four and three dates, respectively, between May 2014 and May 2015, in mesocosms operated under different water depth regimes. Values represent the mean (± SE) of triplicate process trains under each treatment. Samples were collected at the midpoint and outflow region of each process train on each sampling date.

Effects of Hydraulic Retention Time (HRT) and P Loading on Surface Water TP

To date, the STA-1W PSTA mesocosms have demonstrated effective P reductions to very low levels. Using mesocosm data, treatment area and water depth effects were explored by comparing relationships between HRT and surface water TP concentrations during both shallow and deep water conditions. The HRT was calculated for each position along the process trains for both four tanks in series (static water depth treatments operated at 23 cm or 46 cm) as well as for those with two tanks in series (variable water depth treatments operated at 46-69 cm or 46-92 cm), using nominal inflow flow rates and water depths. The surface water TP concentrations at the outflow of each tank were then averaged for four periods, corresponding to the water depth changes in variable depth treatments.

Nominal residence times ranged from 1.3 days in the first tank of the shallowest static-depth treatment (23 cm), to 20 days in the second tank of the variable-depth treatment (46-92 cm). During the initial phase of shallow water conditions, variable-depth treatments were operated at 46 cm, and produced similar outflow TP concentrations to the static depth treatment at that same depth, for both 5 and 10 day HRTs (Figure 15). During that same period, the 23-cm depth treatment produced lower TP concentrations for a given HRT.

During the subsequent deeper phase between June and September 2014, the increased depths in variable-depth treatments resulted in increased HRTs, but without a resultant decrease in TP concentrations. During that period, mean outflow TP concentrations of the four treatments were 9, 7, 8, and 8 µg/L for the 23, 46, 46-69, and 46-92 cm depth treatments, respectively.

The second shallow phase occurred after water levels were returned to 46 cm in the two variable-depth treatments on 9/16/2014, and the results shown in Figure 15 reflect outflow TP concentrations for the period between October 1, 2014 and January 7, 2015. During this period, the static depth treatments outperformed the variable depth treatments for a given HRT, and the shallowest treatment produced a very low outflow TP concentration of 6 µg/L for the period.

The relationship between outflow TP and HRT during the period of the second deep water phase was similar to the first deep-water period, with essentially no benefit to P removal gained by increasing the HRT from 5 to 20 days. The deeper conditions have been maintained in the variable depth treatments since the beginning of this period (January 2015) and a further evaluation of these trends will be provided in a future report. However, the findings of this preliminary analysis clearly show that longer HRT gained by increasing water depth was not as beneficial to P removal as HRT gained by adding treatment area under a given water depth. To date, a 5-day nominal HRT, at a 23 cm water depth, appears to be sufficient to produce the "lowest achievable P concentration" for this experimental platform, at the inflow TP concentrations and loads provided during the study.

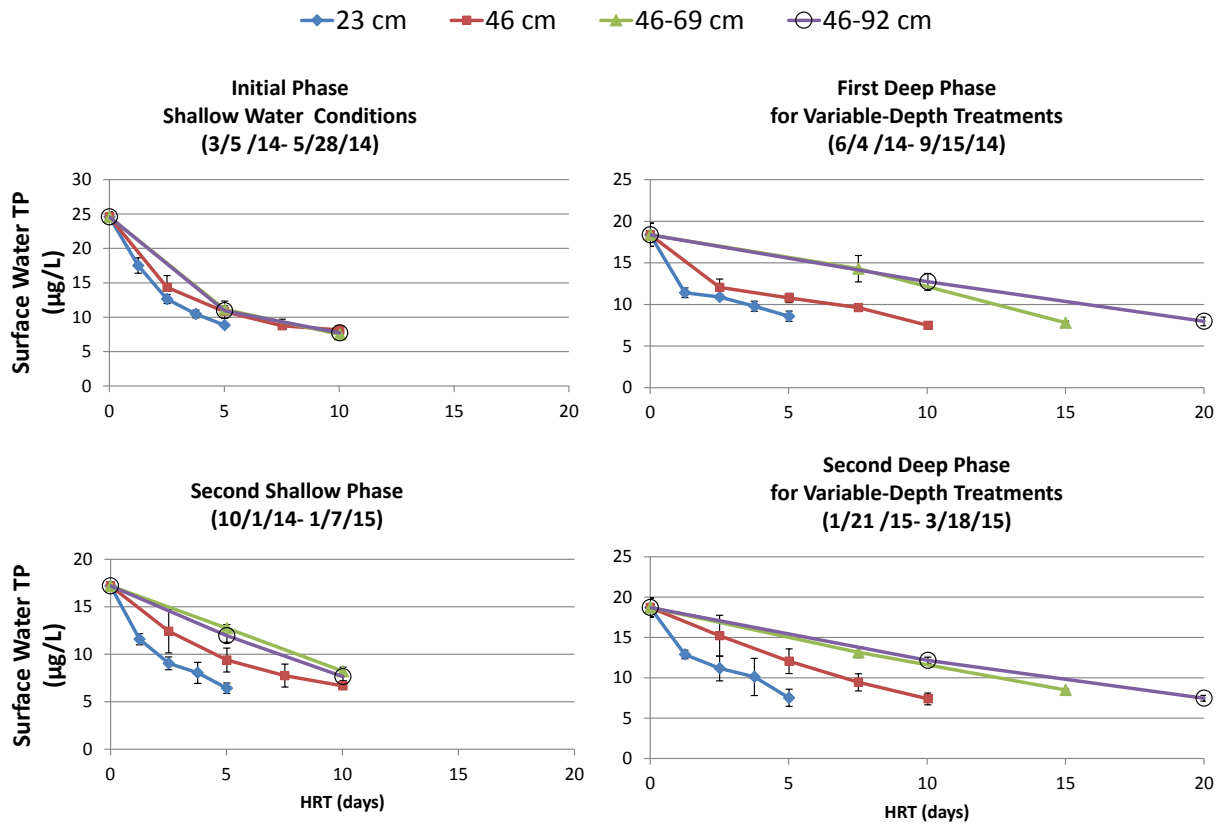

Figure 15. Relationships between hydraulic retention time and surface water total phosphorus (TP) concentrations in the surface water from mesocosms established under static or variable depths. Each value represents the average of triplicate flow ways sampled weekly during four periods between March 2014 and March 2015. The values at HRT = 0 indicate the average inflow TP concentration for each period. Error bars denote the standard error around the mean value from triplicate process trains under each water depth treatment.

Similar to the above analysis of HRT, P loading rates were calculated for each tank by taking the P concentration of either the inflow water (for the first tank in series) or the upstream tank (for 2nd, 3rd, or 4th tanks in series), and multiplying by the hydraulic loading rate to each individual tank. This product gives an annualized P loading rate on an area basis (g P/m^2/yr). The outflow TP concentration data for each replicate tank were then used to calculate the percent of measurements over the entire period of record (December 12, 2013 – April 1, 2015) that fell at or below a threshold TP value. These percentages were then plotted as a function of P loading rate for each replicate tank, using ≤ 13 µg/L as the threshold value (Figure 16).

The range of P loads to individual tanks ranged from 0.4 to 1.4 g P/m^2/yr. Across all depth treatments, a general pattern emerges of increasing occurrence of low TP concentrations with lower P loading rates. Both static and variable depth treatments showed low frequencies of low outflow TP concentrations at the higher end of the P loading spectrum (first tanks in series,

closest to the inflow), but very high (94-100%) occurrence of low TP concentrations where P loading was < 0.6 g P/m²/yr. From this perspective, there did not appear to be an inherent risk of deeper water conditions to P removal, so long as P loading rates were sufficiently low. Above a loading rate of about 0.7 g P/m²/yr, however, the deeper systems were the first to exhibit lower probabilities of achieving the target value.

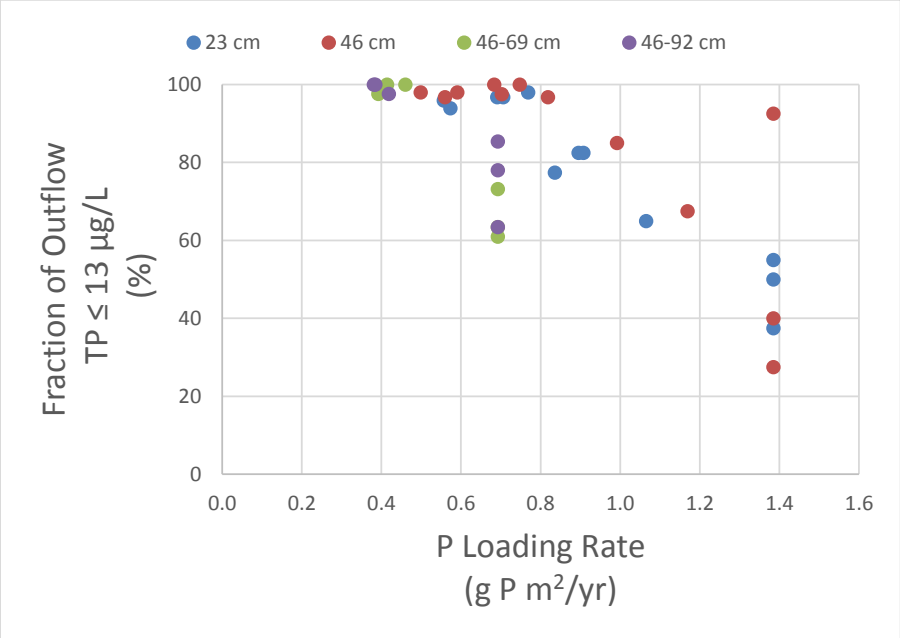

Figure 16. Relationship between P loading rates to each mesocosm and the fraction of surface water samples with TP concentrations ≤ 13 µg/L.

Community composition of Benthic Periphyton in the Mesocosms, the PSTA Cell and unenriched regions of the Everglades

Phosphorus concentrations in the mesocosm periphyton samples averaged 242 ± 19 mg/kg at the midpoint of process trains, and decreased to an average 173 ± 18 mg/kg in the outflow region. The outflow-region values are similar to what was observed in the PSTA Cell inflow region samples collected at the same time of year, while WCA samples were found to be even lower in TP content (Table 5). Literature values indicate these low TP contents for WCA periphyton are within the ranges previously reported for interior marsh sites (Table 6). However, our periphyton tissue N values for mesocosms, the PSTA Cell, and WCA samples were low, relative to the reported values from previous work in the Everglades. Carbon contents were slightly lower in the mesocosms and PSTA Cell than was observed for the mesocosms, while the reverse was true for calcium contents (Table 5).

Table 5. Summary of nutrient contents for periphyton communities in mesocosms, in the STA-3/4 PSTA Cell, and at two natural Everglades marsh sites (WCA2A U3 and WCA3A DB15), all sampled in January and February 2015.

	P	N	C	Ca
	mg/kg	----------*% wt.*----------		
Mesocosms				
Midpoint	242 ± 19	0.85 ± 0.04	19.6 ± 0.5	25.0 ± 0.4
Outflow	173 ± 18	0.78 ± 0.02	20.4 ± 0.4	22.7 ± 0.5
PSTA Cell	178 ± 28	0.68 ± 0.03	19.5 ± 0.2	n.d.
WCA 2A U3 *	73 ± 3	0.97 ± 0.01	23.0 ± 0.1	21.3 ± 0.3
WCA3A DB15 **	98 ± 11	1.08 ± 0.08	22.0 ± 0.1	21.7 ± 0.5

* WCA2A U3 located at 26.283167 °N, 80.407250 °W

** WCA3A DB15 located at 26.042627 °N, 80.726220 °W

Table 6. Phosphorus and nitrogen contents of periphyton reported for the interior region of Water Conservation Area 2A.

Location	Phosphorus (mg/kg)	Nitrogen (% wt)	Source
Interior WCA 2A	30 - 454	1.23 - 2.72	Vymazal and Richardson 1995
	60 - 490	1.12 - 3.01	Swift 1981, 1984
	100 - 940	0.99 - 3.57	Swift and Nicholas 1987
	104 - 289	1.1 - 2.3	Scinto and Reddy 2003

Tissue P contents of periphyton within the PSTA Cell have varied over time, more so than with distance through the cell (Figure 17). The tissue concentrations presented in Table 5 reflect the February 2015 sampling event in the PSTA Cell, at a time of moderate to low P contents. In July 2014 and June 2015, P contents were higher, as much as 584 mg/kg for periphyton growing on *Chara*.

Community comparisons between periphyton in the mesocosms, PSTA Cell and the WCAs highlight the absence of green algae in the WCA samples, with the exception of *Cosmarium*, an alga also observed in the mesocosms (Table 7). Three green algae taxa were identified in the PSTA Cell samples. Mesocosm samples contained many more (9) green algae taxa, though as was mentioned previously, these were often affiliated with the process train midpoint and less with the outflow region samples. Three blue-green algae taxa were unique to the mesocosm platform, while 1 blue-green alga was only observed in the PSTA Cell samples. All blue-green taxa observed in the WCA samples were represented within both the mesocosm samples and

PSTA Cell samples collected during this effort. *Leptolyngbya* and *Phormidium* were reported to be the dominant blue green algae in oligotrophic sites within WCA-2A (Vymazal et al., 2008), and these genera were observed in samples from all locations (Table 7), dates (Table 4), depths (Table 3), and locations within the mesocosm platforms (Table 2).

Diatoms were also more diverse in the mesocosm samples (N=12) than in the PSTA Cell samples (N=3) or WCA samples (N=4), though these differences may be influenced, in part, by low sample numbers. Replicate samples are available in our archives, and upon analysis may better define community composition changes that have resulted from experimental treatments. However, at this stage of preliminary analysis, it appears that the mesocosm platform was able to support the periphyton communities that represent the oligotrophic, alkaline, freshwater marshes of the interior WCAs in the Northern Everglades.

The enzyme activity of periphyton from these different locations was surveyed on several dates, and together with TP content, APA results are summarized in Figures 18 through 22. Enzyme activity was higher in recent samples of the PSTA Cell (June 2015) than sampling events in 2013 and 2014, but the recent data from PSTA Cell surveys was closer to the rates observed in the periphyton from interior WCA sites. Rates of APA in the mesocosms were low initially, but over time have approached the high levels observed in the low P environments in the interior of the WCAs.

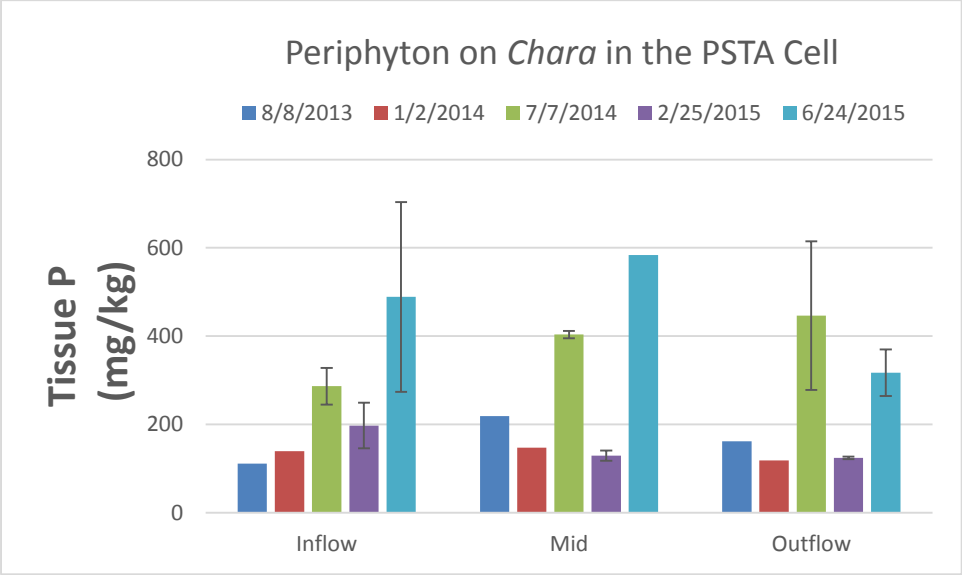

Figure 17. Phosphorus concentrations of periphyton associated with *Chara* along inflow, mid and outflow transects within the PSTA Cell, on five dates between August 2013 and June 2015.

Table 7. Presence of periphyton taxa in samples collected from three environments: the Everglades WCAs (N=4 in Jan-Feb 2015), the PSTA Cell (N=3 in Feb 2015) and in the PSTA mesocosms (N=8 on 9/16/2014 and N=4 on 1/16/2015).

Common Class	Genus	WCA2A, WCA3A	PSTA Cell	Mesocosms
Bluegreen Algae	*Anabaena*			X
	Aphanocapsa	X	X	X
	Aphanotece		X	X
	Aphanothece	X	X	X
	Bacullaria			X
	Chroococcus	X	X	X
	Cyanosarcina	X		
	Cyanothece	X		X
	Gloeothece	X		X
	Halomicronema	X	X	X
	Hassalia	X		X
	Johannesbaptistia			X
	Konvophoron	X	X	X
	Lemmermanniella			X
	Leptolyngbya	X	X	X
	Phormidesmis	X		X
	Phormidium	X	X	X
	Scytonema	X		X
	Snowella		X	
	Spirulina	X	X	X
	Synechocystis	X	X	X
Diatoms	*Achnanthes*		X	X
	Amphora	X		
	Cocconeis			X
	Cymbella	X	X	X
	Denticula		X	X
	Eunotia			X
	Gomphonema		X	X
	Mastogloia	X	X	X
	Navicula	X	X	X
	Nitzschia	X	X	X
	Pinnularia			X
	Rhopalodia	X	X	X
Green Algae	*Closterium*		X	
	Cosmarium	X		X
	Euastrum		X	X
	Monoraphidium			X
	Mougeotia			X
	Oedogonium		X	X
	Oocystis			X
	Oscillatoria			X
	Scenedesmus			X
	Tetraedron			X

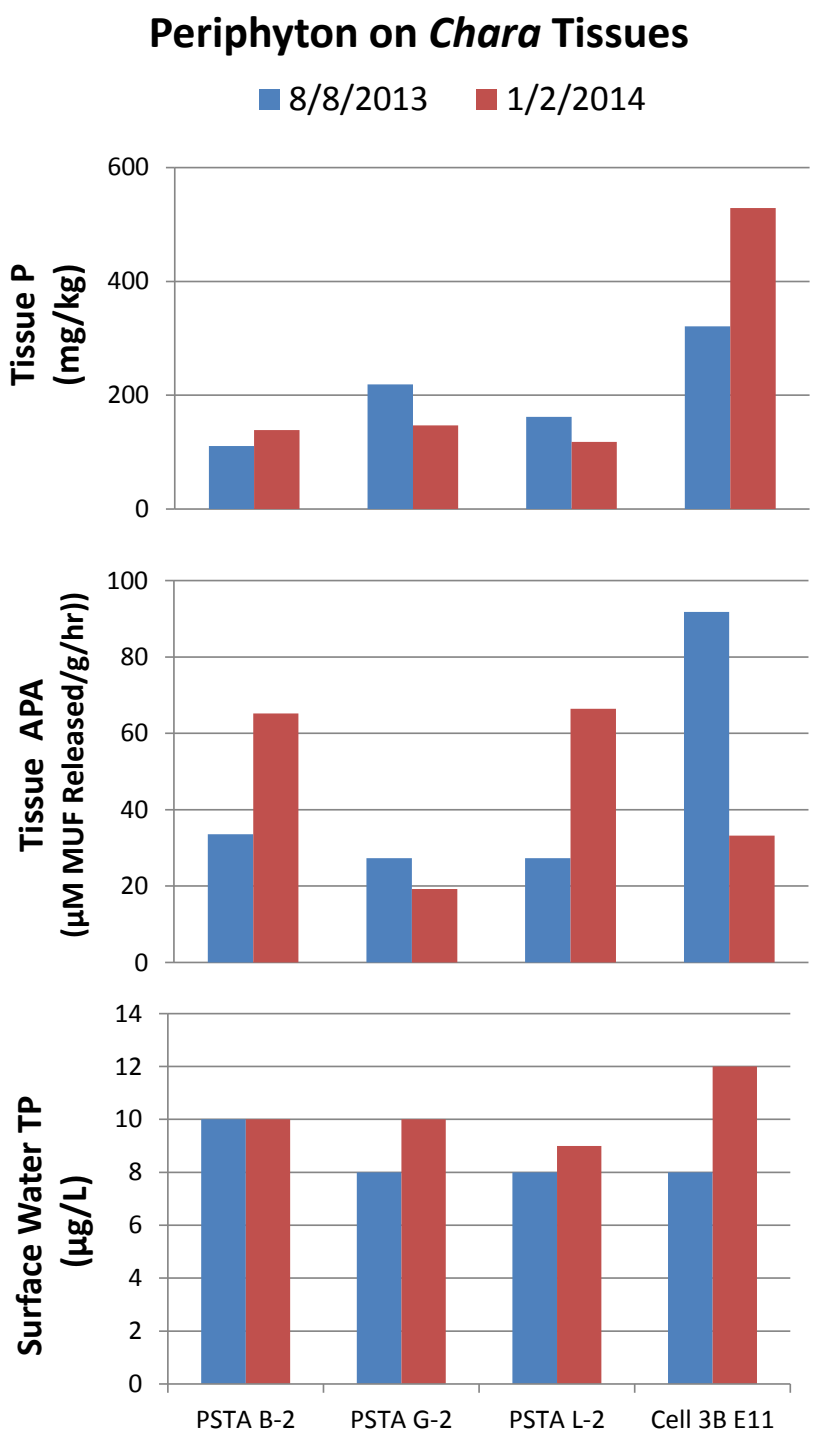

Figure 18. Periphyton tissues removed from Chara growing in the PSTA Cell and the outflow region of a neighboring muck-based wetland (Cell 3B, station E11) on two dates, were assayed for alkaline phosphatase activity (APA) and total phosphorus content. Surface water TP concentrations in grab samples at the time of collection are also shown.

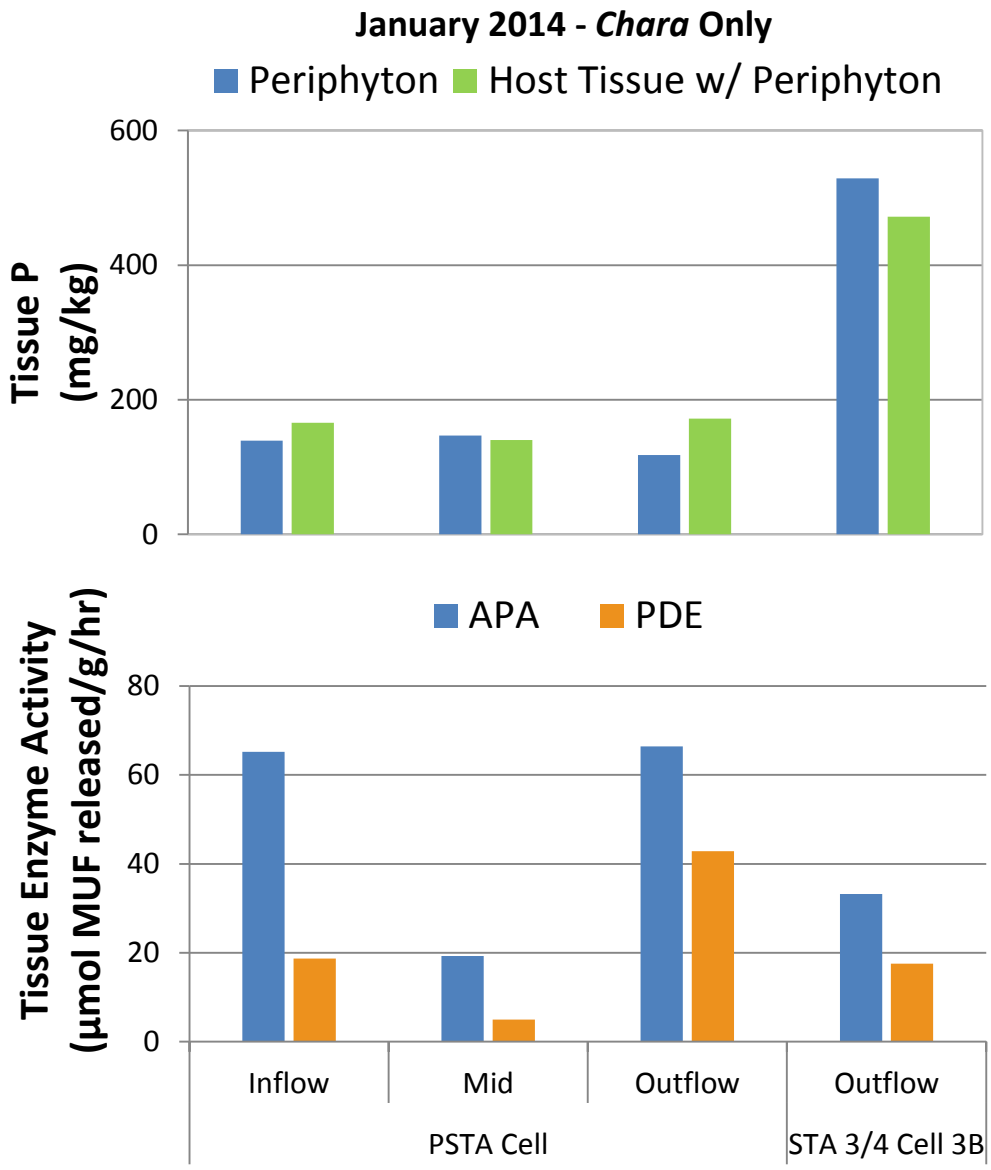

Figure 19. Periphyton phosphorus contents, along with the enzyme activity of monoesterases (APA) and phosphodiesterases (PDE) of alkaline phosphatase, collected in January 2014. Values represent single samples. The host tissue/periphyton assemblage was also analyzed for TP content.

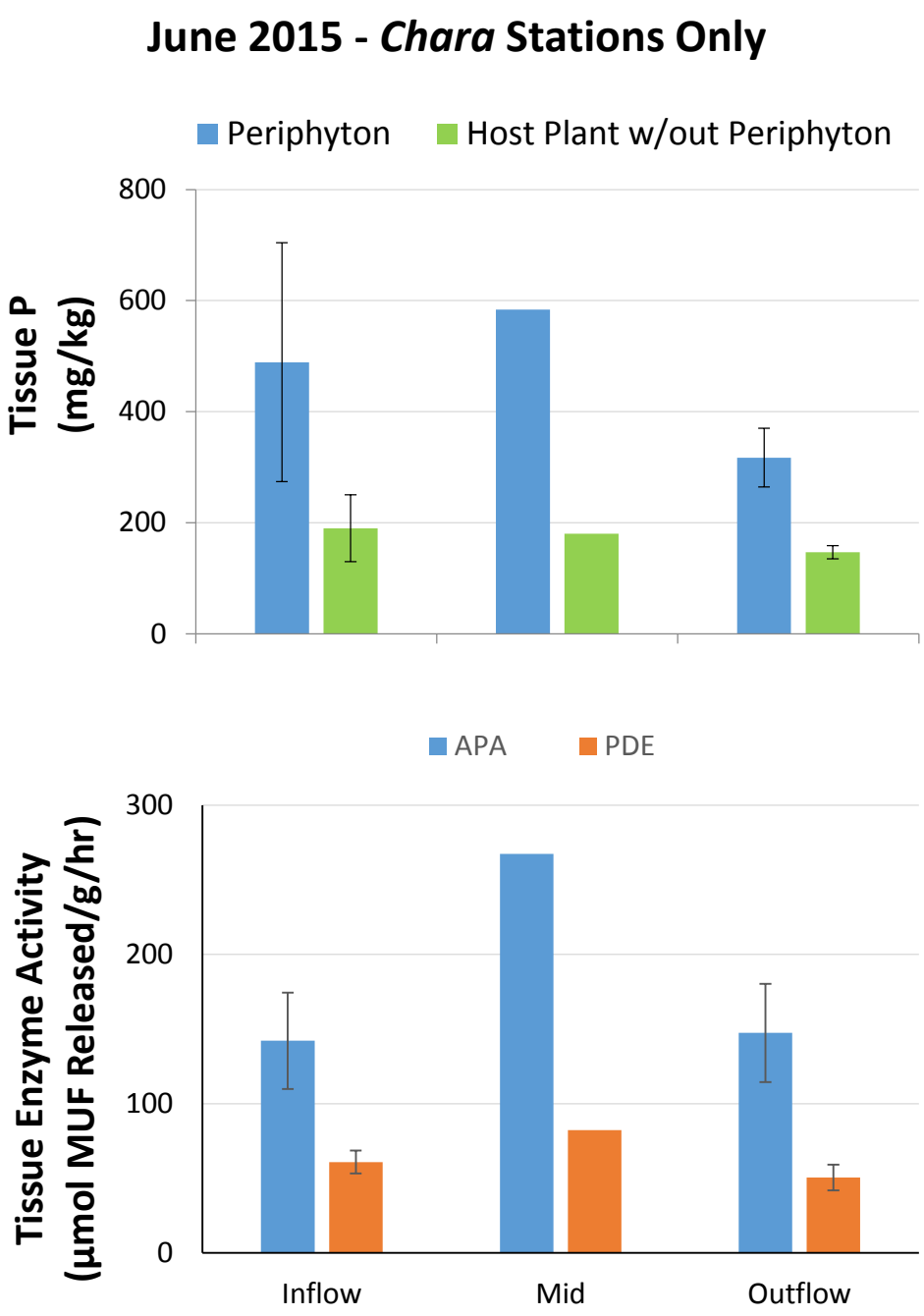

Figure 20. Average (±SE) total phosphorus (TP) contents and enzyme activity (alkaline phosphatase, APA and phosphodiesterase, PDE) in epiphytes on five host plants in the PSTA Cell on June 24, 2015.

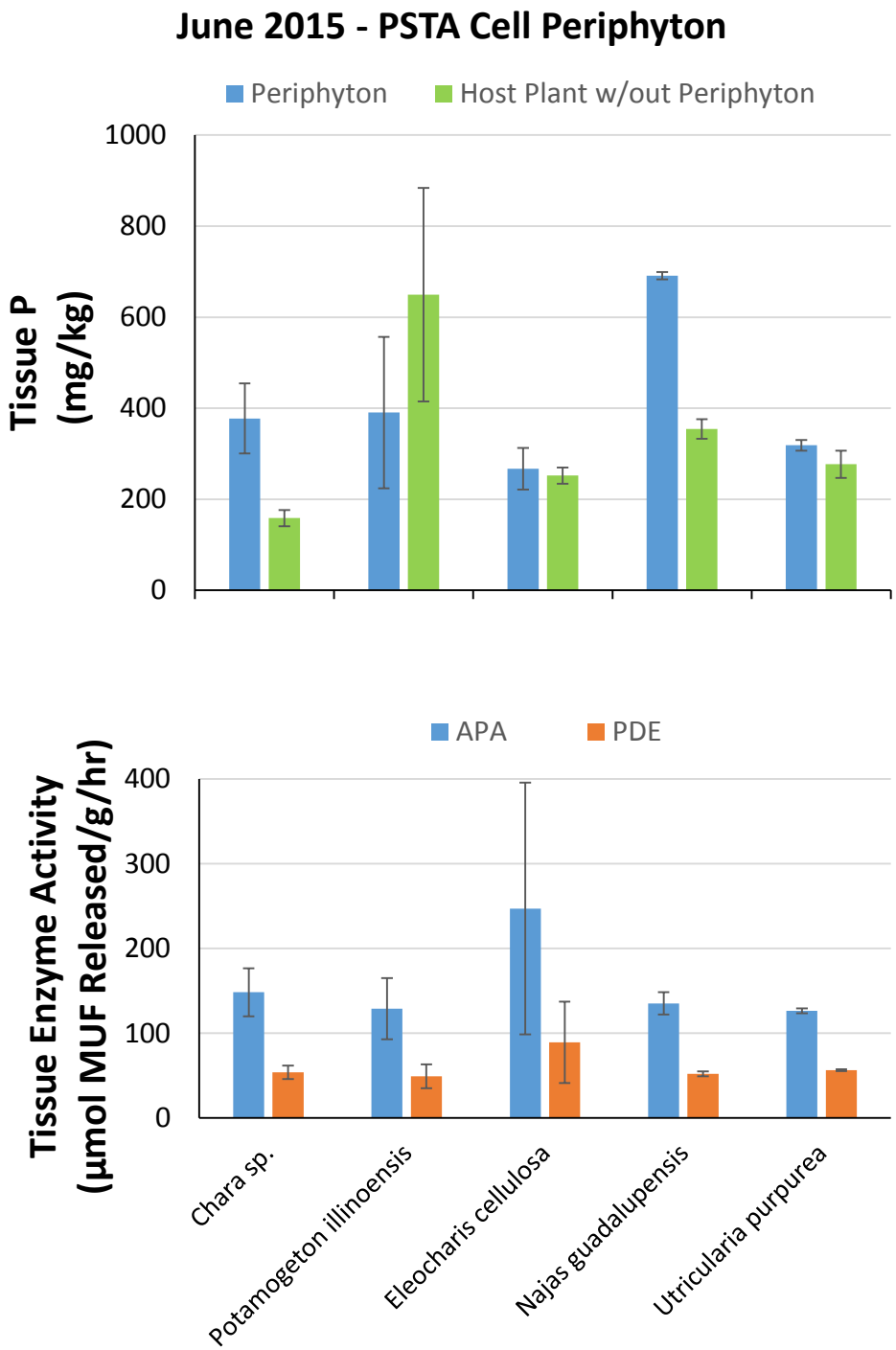

Figure 21. Average (±SE) total phosphorus (TP) contents and enzyme activity (alkaline phosphatase, APA and phosphodiesterase, PDE) in epiphytes on five host plants in the PSTA Cell on June 24, 2015.

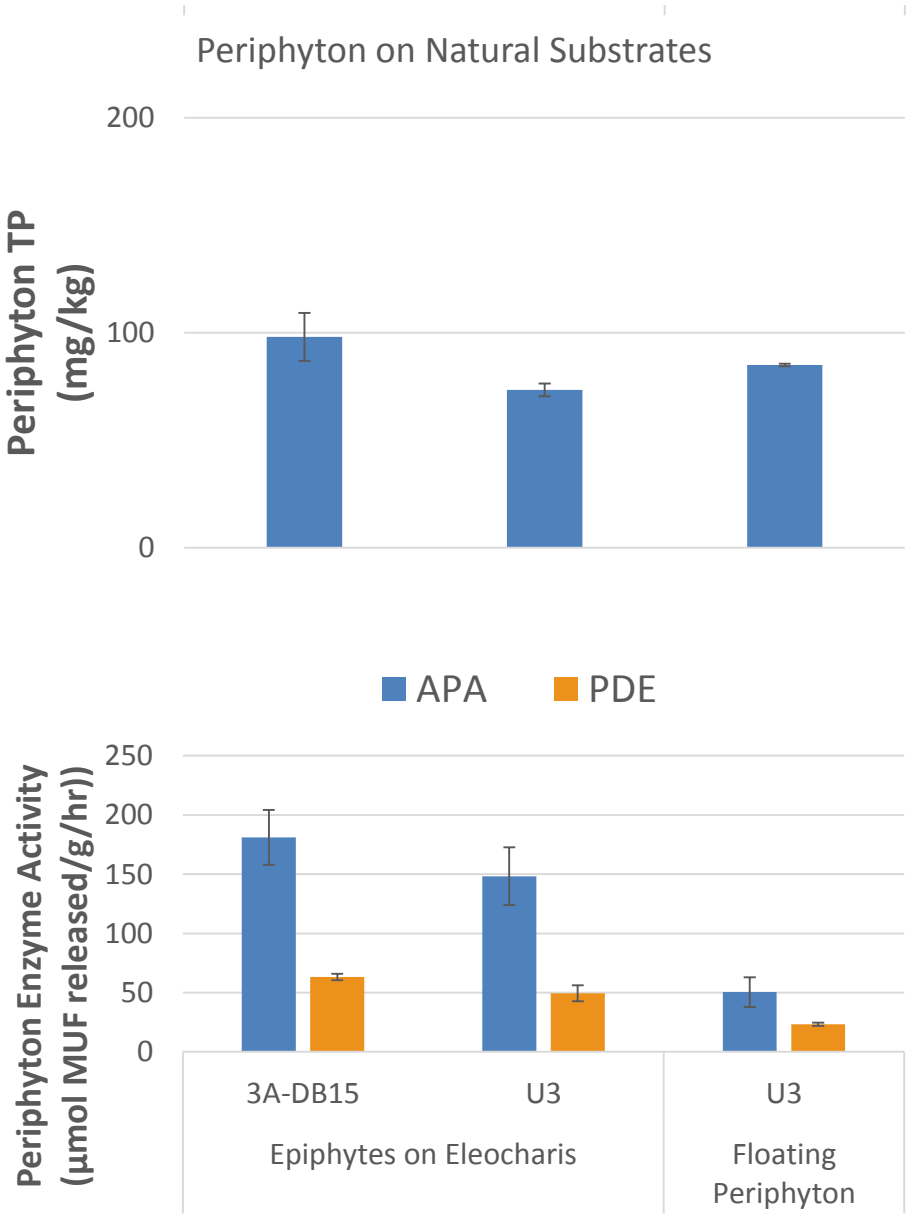

Figure 22. Periphyton sampled in two low-nutrient areas of the natural marshes downstream of the STAs: site 3A-DB15 located in WCA-3A (collection date: 1/28/2015), and site U3 in Water Conservation Area 2A (collection date: 2/5/2015).

Synopsis of Findings

Successful low-level treatment of P-laden waters was demonstrated at a range of water depths (23 - 92 cm) in PSTA mesocosms receiving STA outflow waters. The mesocosms reduced TP concentrations from an average inflow of 21 µg/L to an average outflow concentration of 8 µg/L across all depth treatments. Water and periphyton TP concentrations at the process train midpoints were more elevated in the deeper, variable-depth treatments than in the shallower, static treatments.

Benthic periphyton was more P-enriched in the inflow region of the mesocosms than the outflow region, and P contents in the outflow-region periphyton were similar to levels in periphyton growing in the PSTA Cell. Enzyme activities of the periphyton in our mesocosms increased over time, approaching levels observed for periphyton from the interior WCAs. Water depths up to 92 cm did not appear to disrupt the potential for enzyme activity in periphyton directly, but there was evidence of lower periphyton biomass in deeper treatments, which reduced the potential for enzyme hydrolysis on an area basis.

A diverse assemblage of blue green algae, diatoms, and green algae taxa has become established in the mesocosms. Similarities between taxa observed in the mesocosms and the PSTA Cell indicate that this former platform is suitable for examining the response of these communities to a variety of depth and loading conditions. Preliminary comparisons among the mesocosms, PSTA Cell, and WCA periphyton communities suggest that a community transition towards increasing similarity with the assemblages of low-nutrient regions of the interior WCAs may be occurring within the mesocosm platform.

Both static and variable depth treatments exhibited low frequencies for outflow TP concentrations ≤ 13 µg/L at the higher end of the P loading spectrum (first tanks in series, closest to the inflow), but very high (94-100%) occurrence of such low TP concentrations when P loading was < 0.6 g $P/m^2/yr$.

The relationship between outflow TP and HRT during periods of deep water operations showed no benefit to P removal by increasing the HRT from 5 to 20 days through increased water depth. By contrast, gradients in water TP concentrations indicated that an increase in treatment area, which effectively increased HRT and lowered areal P loading rates, resulted in decreased water column P concentrations. This finding is in contrast to observations in muck-based SAV flow paths, where TP concentrations tend to "plateau" around 16 µg/L at the outflow region of the STA. Additional analyses of depth, loading and outflow TP relationships therefore will be required upon completion of this mesocosm study to confirm these preliminary findings.

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