

Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs)

Work Order #: 4600003031-WO01

PREPARED FOR: South Florida Water Management District
3301 Gun Club Road
West Palm Beach, FL 33406

PREPARED BY: Wetland Biogeochemistry Laboratory
Soil and Water Science Department - IFAS
University of Florida
Gainesville, FL 32611

This quarterly progress report summarizes the activities performed during the period of June – September 2015, as per tasks described in the Science Plan project - Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs). This period covered the first quarter of the project and included various activities that were initiated to meet the objectives laid out under multiple tasks. Following sections discuss the progress made in this quarter.

1 Introduction

The primary objectives of this project are to: (1) determine relative storages of non-mobile and mobile phosphorus (P) in the EAV and SAV treatment trains; (2) quantify the interactions between mobile P and non-mobile P in the soil and water column; (3) enhance the understanding of biotic and abiotic mechanisms and factors regulating P dynamics, especially in the lower reaches of treatment trains, and (4) document current soil conditions in the STAs and provide process-level information on P uptake and release, and transport of mobile P across the soil/water interface, as well as movement of P within the soil profile. These broad objectives will be accomplished by conducting specific studies in STA-2 and STA-3/4. In addition, similar studies will be conducted at select sites along soil P and vegetation gradient in WCA-2A for comparisons. Please refer to the Project Work Plan (UF WBL, 2015) for details on specific objectives and tasks.

This quarterly report describes activities related to the following tasks:

- Task 1. Project kick off meeting and site visit
- Task 2. Project work plan
- Task 3. Soil sample collection
- Task 7a. Transect study: Surface water quality monitoring
- Task 7b. Transect study: Enzyme assays

2 Kick-off Meeting, Project Orientation and Field Reconnaissance [Task 1]

The project kick-off meeting was held on June 29, 2015 at the South Florida Water Management District (SFWMD or District) in West Palm Beach, FL. This meeting was attended by the University and District Scientists (see attached list of attendees). A field trip was conducted on June 30, 2015 to visit study sites in STA-2 and STA-3/4. A summary of kick-off meeting minutes was submitted to the District on July 14, 2015 (Appendix 1).

The University's assigned personnel participated in project orientation and field reconnaissance. The orientation and field reconnaissance comprised of discussions and field visits to further refine the scope, identify sampling locations, training on laboratory sample submission, and District's field sampling and safety protocols.

A follow-up meeting with the District laboratory and field staff was conducted on July 15, 2015. The meeting was attended by University of Florida (UF) investigators Reddy (PI) and Wright (Co-PI). A discussion of laboratory and field protocols was the primary objective of the meeting. District staff provided SOPs for the determination of key physico-chemical parameters in water, floc and soils.

3 Project Work Plan [Task 2]

This task represents the preparation of a Project Work Plan that details the project scope, activities, tasks and associated time lines. The draft work plan was submitted to the District on July 20, 2015. Final work plan was submitted on September 18, 2015.

4 Soil Sample Collection [Task 3]

The purpose of Task 3 is to document baseline soil conditions in the STAs and capture temporal dynamics of floc/soil biogeochemical parameters that can potentially regulate surface water quality. The activities under this task include: a) spatial sampling, b) transect sampling, and c) in depth soil characterization at benchmark sites.

4.1 Spatial Soil Sampling (Year 1)

The objective of this task is to document the current baseline soil conditions in the STAs and obtain an accurate estimate of nutrient storages in floc and soils in each cell which can be used to determine long-term spatial patterns. Soil sampling locations were selected based on the results of a previous data mining contract (Tetra Tech, 2014), field reconnaissance, and recommendations of District Scientists. The grid pattern of sampling sites chosen for this work

ensures equal representation of all areas within the sampling cells, avoiding under or oversampling of areas. This strategy maximizes logistical and analytical resources and reduces chances of future problems with accurate resampling of sites. It also results in co-location of several other ongoing studies such that all studies using these sites benefit from additional information collected by this work. The first phase of sampling is planned for STA-2 Cell 3 and STA 3/4 Cell 3B during the second quarter of the project.

4.1.1 STA-2 – Cell 3

An existing soil sampling grid provided by the District was utilized to identify sampling stations in STA-2 Cell 3. A detailed list of sampling stations and GPS coordinates is provided in Appendix 2. A total of 55 stations were identified, with 11 stations falling along a linear transect from inflow to outflow. The number of sampling stations along the transect depended on the configuration of STA cell. Where STA cell shape and orientation had an elongated treatment flow path, the number of sampling stations in transect was greater than in cells which had relatively shorter flow paths. Embedded within this transect are three benchmark sites where triplicate samples are to be collected on a recurring basis throughout the study period (**Figure 1**). Surface water samples will be collected from these 11 stations, including three benchmark sites near inflow, center and outflow of the cell. These surface water samples will be submitted to the District lab for determination of TP, TDP, SRP, NH_4^+ , NO_3^- , DON, TN, DOC, TOC, calcium, magnesium and sulfate. Field parameters (e.g. pH, Cond, Temp. and DO) will be measured at each sampling location with a handheld water quality sonde/probe. Soil and water depths will also be measured at each sampling location.

Intact soil cores will be collected at all 55 stations, including triplicate soil cores from benchmark sites using thick walled, 10-cm diameter clear polycarbonate tubes inserted into the soil to a minimum depth of 30 cm. While soil sampling is routinely conducted in Everglades studies with a similarly sized stainless steel corer, polycarbonate core tubes allow for visual identification of the different soil layers (horizons). Soil cores will be stored in a cold room ($\sim 4^\circ\text{C}$) until separated into plant litter (when present), floc, recently accreted soil (RAS), and pre-STA soil. An additional 17 soil cores will be collected from the transect (including triplicate cores at benchmark sites) for microbial P and C biomass analysis at Wetland Biogeochemistry Laboratory, University of Florida.

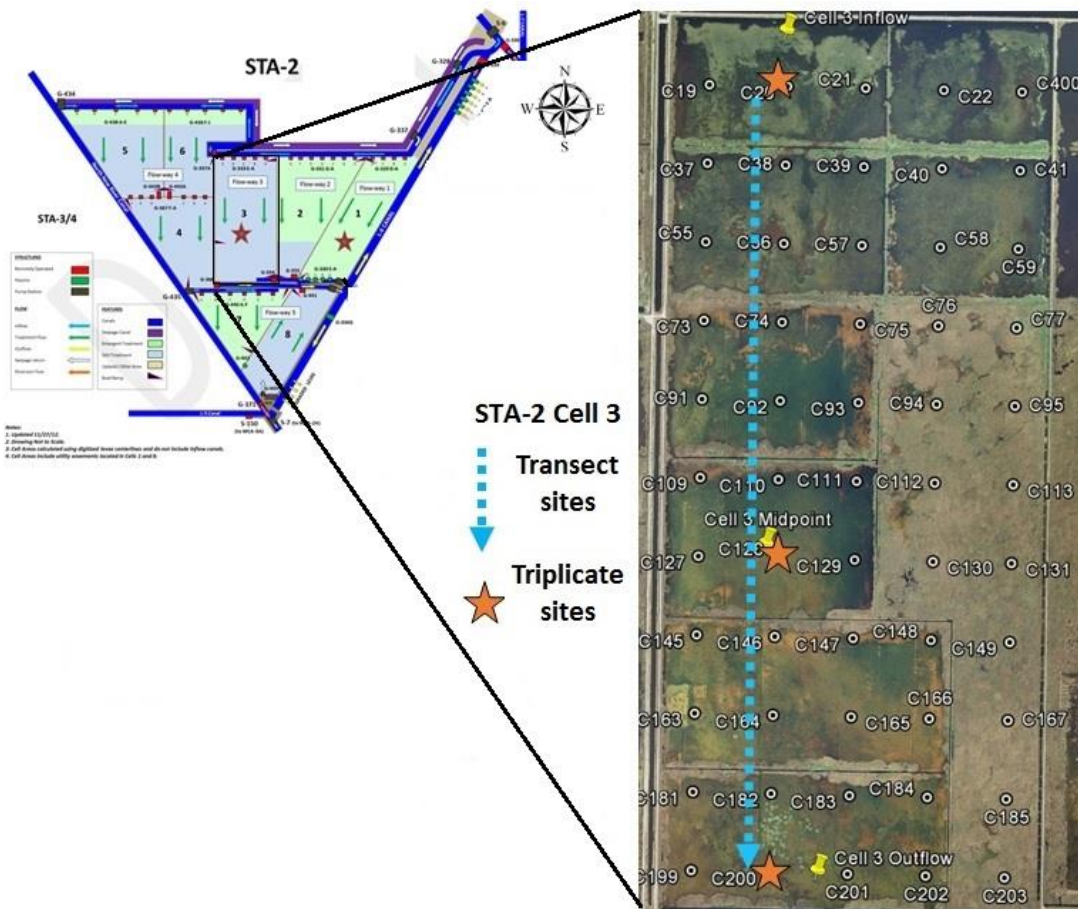


Figure 1. Soil and surface water sampling locations – STA-2 Cell 3.

4.1.2 STA-3/4 - Cell 3B

For STA-3/4, Cell 3B, the grid sampling locations from DB Environmental Lab's existing vegetation survey map will be used (**Figure 2**). Surface water samples will be collected from four stations, including two benchmark sites near inflow and outflow of the cell and triplicate samples from benchmark sites. A detailed list of sampling stations and GPS coordinates is provided in Appendix 2. All surface water samples will be submitted to the District lab for the analysis of TP, TDP, SRP, NH_4^+ , NO_3^- , DON, TN, DOC, TOC, calcium, magnesium and sulfate). Field parameters (e.g. pH, Cond, Temp., and DO) will be measured at each sampling location with a handheld water quality sonde/probe. Soil and water depth will also be measured at each sampling location. Intact soil cores will be collected at all 56 stations, including triplicate soil cores from benchmark sites using thick walled, 10-cm diameter clear polycarbonate tubes inserted into the soil to a minimum depth of 30 cm. Soil cores will be stored in a cold room ($\sim 4^\circ\text{C}$) until separated into plant litter (when present), floc, recently accreted soil (RAS), and pre-STA soil. Additional eight soil cores will be collected from the transect (including triplicate cores at benchmark sites) for microbial P and C biomass analysis at Wetland Biogeochemistry Laboratory, University of Florida.

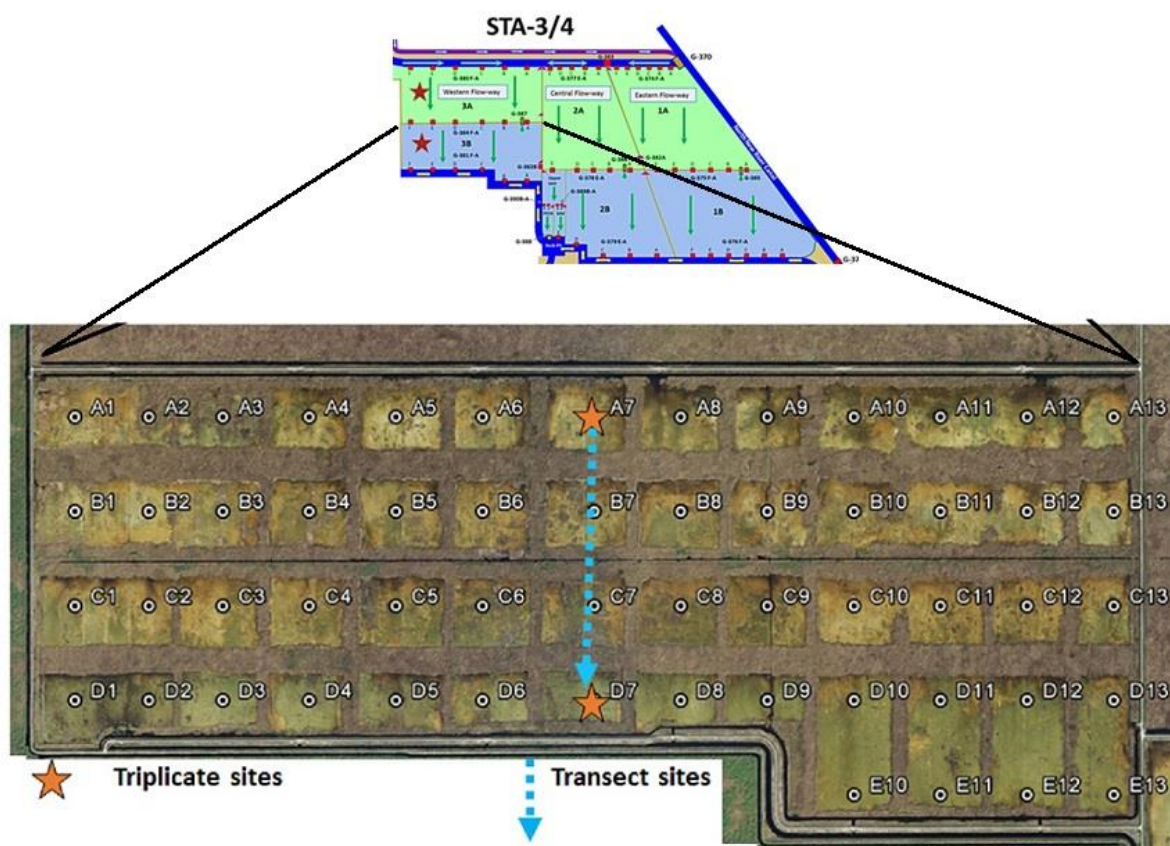


Figure 2. Soil and surface water sampling locations – STA-3/4 Cell 3B.

5 Transect Study- Surface Water Quality Monitoring [Task 7a]

The objective of this task is to investigate changes in water column nutrients under different flow scenarios and understand changes in P concentrations, and movement of P and P forms within a flow way. The approach is to collect high spatial-temporal resolution data using auto-samplers. This section of the report describes preliminary results from the first flow event – Stagnant flow that took place on 17-31 August 2015, along with a preceding pre-flow period on 10-16 August and a post-flow period on 1-14 September, 2014.

5.1 Work Completed During This Quarter

The first flow scenario in STA-2 Cell 1 - Stagnant flow event was conducted from August 10 to September 14, 2015 where inflows to the cell were strictly controlled/manipulated and surface water quality was monitored. This event consisted of a pre-flow period from August 10 to August 16, 2015, followed by a stagnant flow period from August 17 to August 31, 2015, and then a post-flow period from September 1 to September 14, 2015. Cell 1 received normal flows during ‘pre-flow’ conditions, no inflows during ‘stagnant’ conditions and resumed normal inflows during ‘post-flow’ conditions. Inflow and outflow water volumes and nutrient

concentrations are monitored at inflow stations – G-329 (A-D) and outflow stations – G-330 (A-E), respectively, to measure STA performance. This information was utilized to examine the effect of flow conditions on TP concentrations in the water column experienced by the cell during the first flow event.

Auto-samplers were deployed along STA-2, Cell 1 flow way at six locations (**Figure 3**). Water samples were collected every 4 hours (2 am, 6 am, 10 am, 2 pm, 6 pm and 10 pm) and analyzed for TN and TP. Weekly surface water grab samples were also collected from these sites. Water samples were analyzed for TP, TN, SRP, Ca, Mg, NH_4^+ , NO_x , DOC, Fe, sulfate, chloride, alkalinity, color and chlorophyll by the District lab.

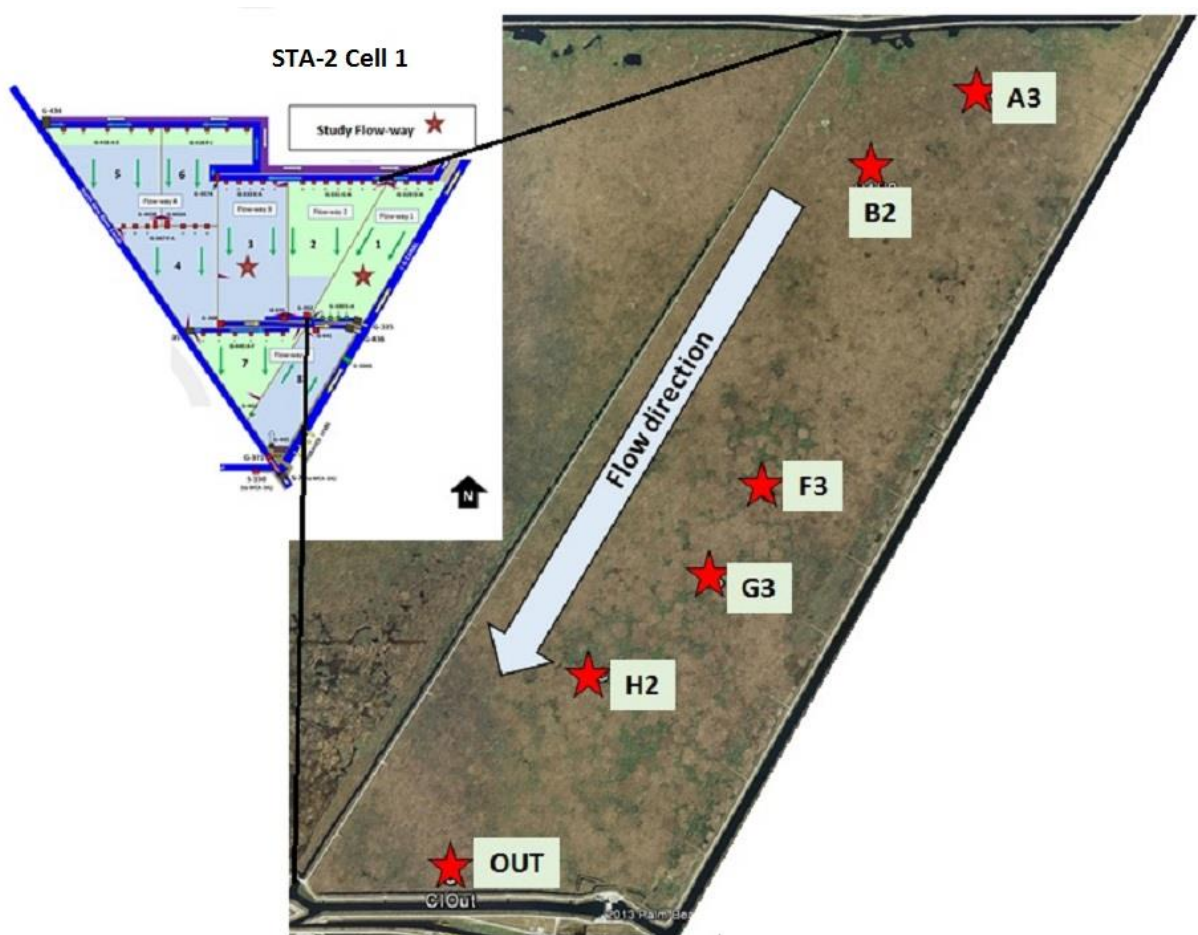


Figure 3. Surface water sampling locations – STA-2 Cell 1. Six stations where auto-samplers were deployed for continuous monitoring and weekly grab samples collection.

5.2 Results

Data analysis – Total phosphorus

A clear reduction in total P concentrations was observed along the treatment flow way at all phases of the flow event (**Figure 4**). The surface water near inflow stations (A3 and B2) had elevated total P concentrations during the stagnant phase of the flow event. TP concentrations measured during “stagnant” and “post-flow phases of this event were not markedly different, however data are currently being examined statistically to determine whether or not the observed differences were significant. Grab samples also showed similar trend of reduction in TP concentrations along the flow way, however elevated TP concentrations were observed at A3 station during pre-flow and stagnant phases of the flow event (**Figure 5**). Soluble reactive P (SRP) showed a similar pattern as TP that declined steadily from inflow to outflow (**Figure 6**). At the outflow station, SRP was practically non-detectable. Total calcium (Ca) concentrations declined from inflow to outflow stations, and a slight reduction was also observed following the stagnant flow event (**Figure 7**). Decreases in Ca concentrations from inflow to outflow stations may be due to abiotic retention as calcium carbonate and co-precipitation with P, most likely via periphyton mats in the lower reaches of the cell.

Uncommonly high TP values were observed at Station A3 on two occasions, however, surface water had relatively low SRP at that time (**Figure 6**). It is likely that the water samples at this station had more particulate matter that resulted in elevated TP. Total dissolved P (TDP) in the grab water samples is necessary in order to quantify the fraction of TP coming from the particulate matter.

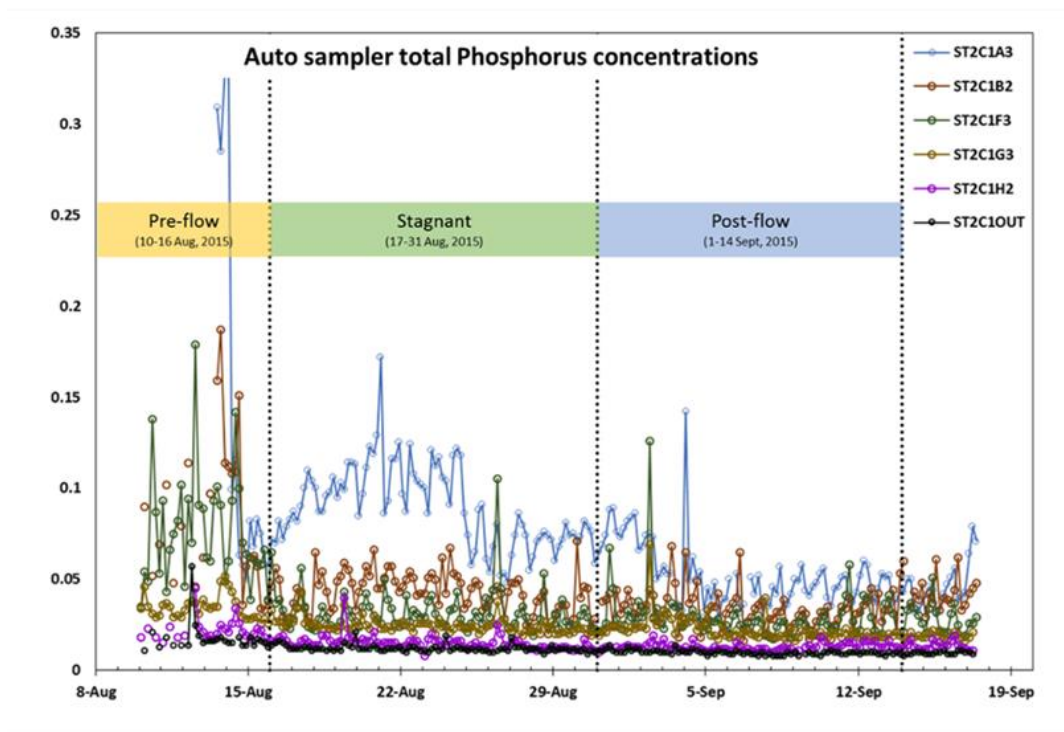


Figure 4. STA-2 Cell 1: Total phosphorus concentrations in surface water at six sampling locations during stagnant flow event.

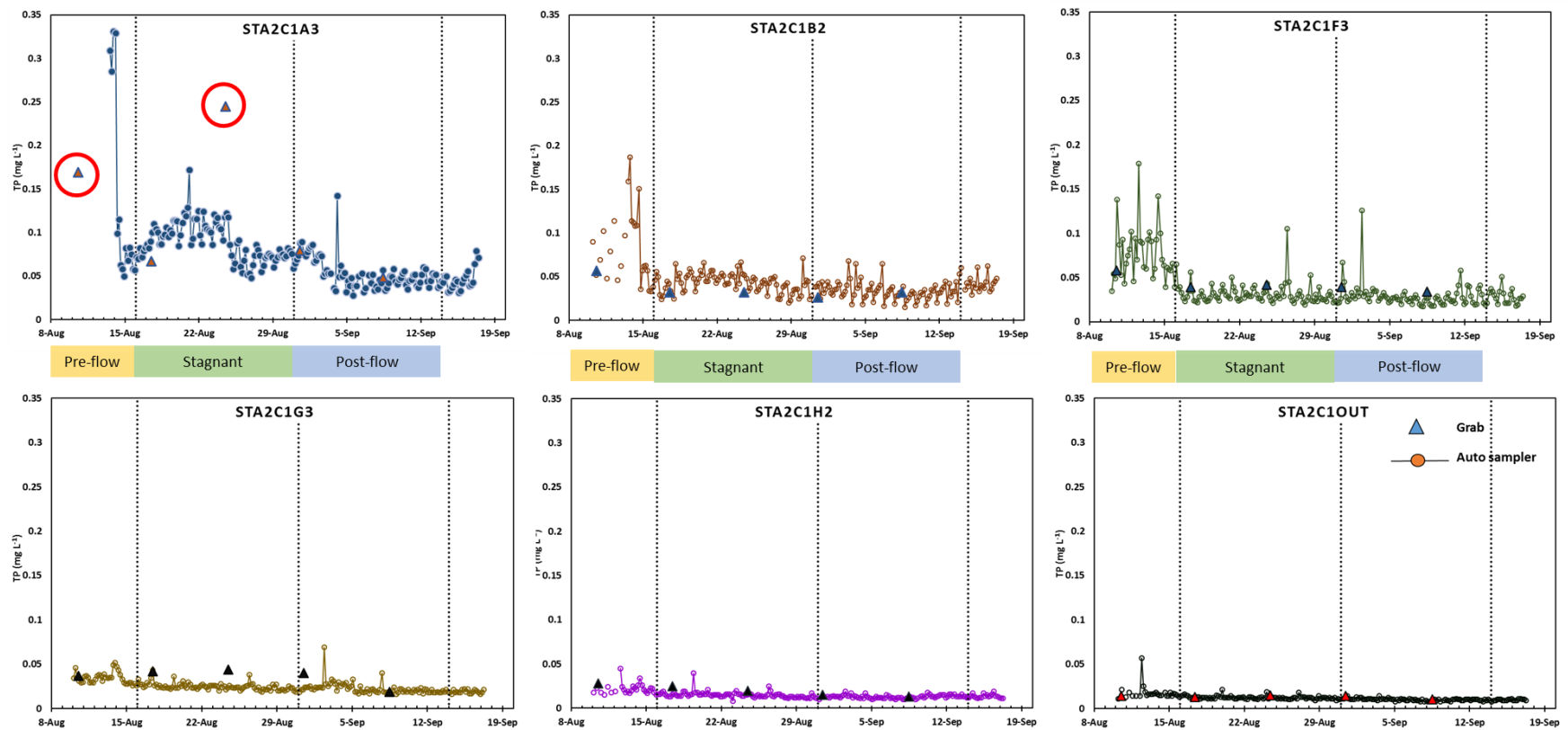


Figure 5. STA-2 Cell 1: Auto-sampler and grab total phosphorus concentrations in the surface water.

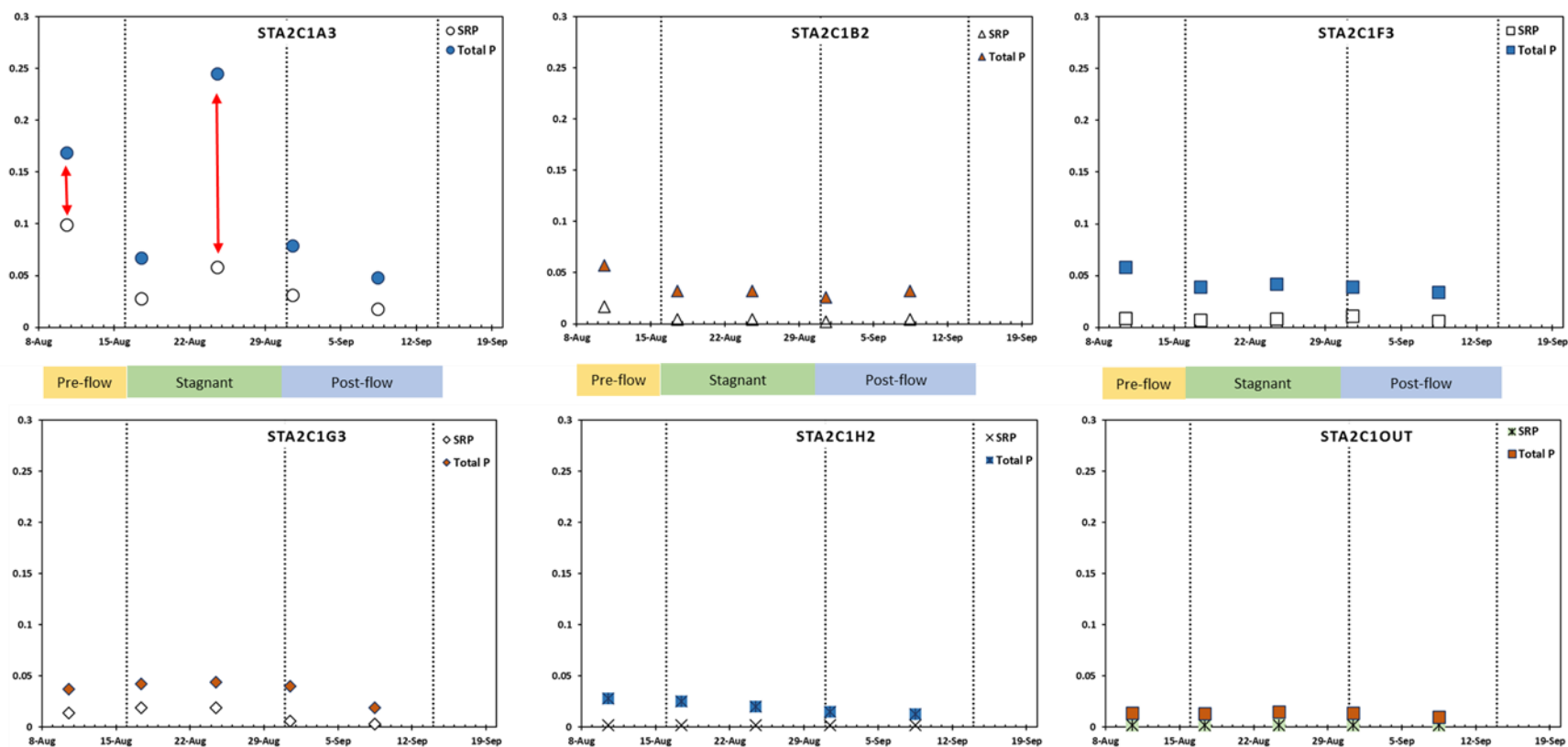


Figure 6. STA-2 Cell 1: Weekly grab soluble reactive phosphorus and total phosphorus in surface water at six sampling locations during stagnant flow.

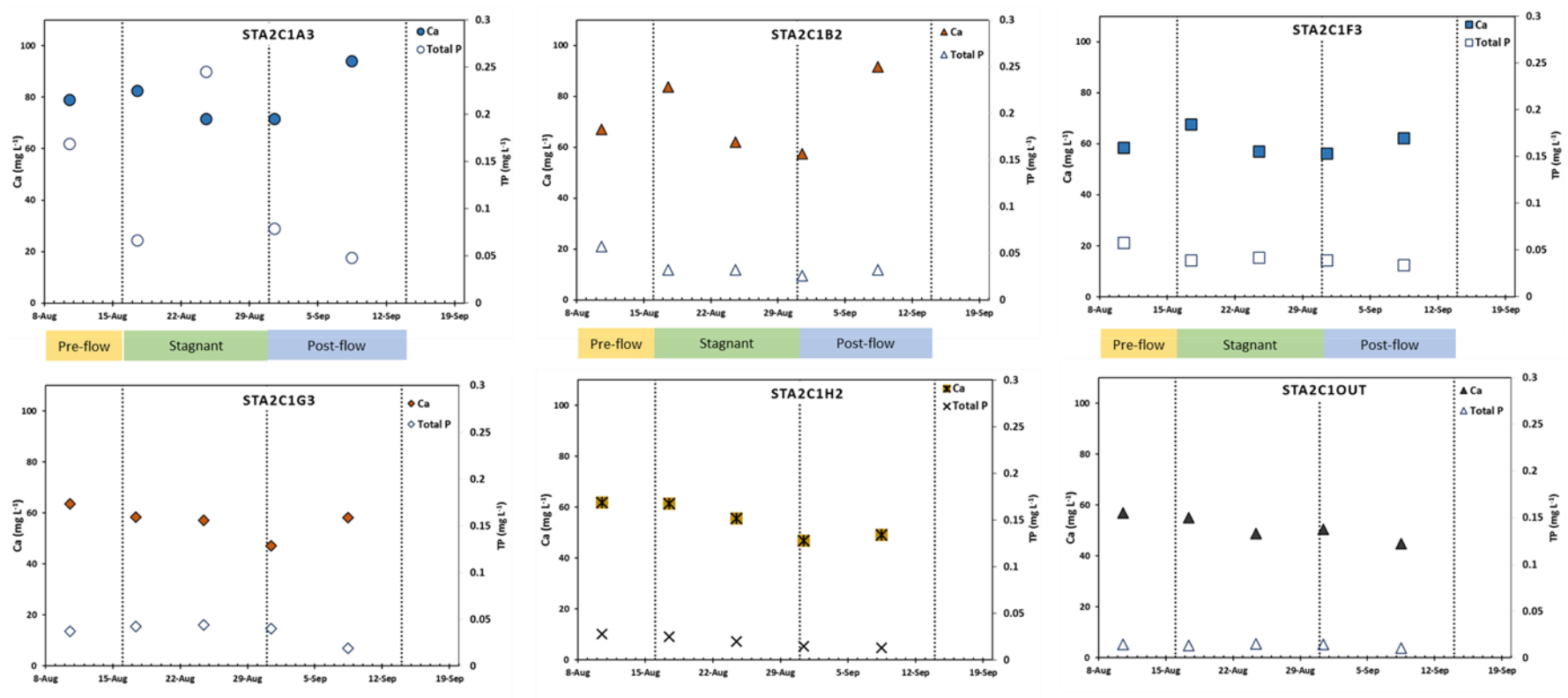


Figure 7. STA-2 Cell 1: Total phosphorus and calcium concentrations in weekly surface grab water samples at six sampling locations during stagnant flow.

Data analysis – Total nitrogen

A reduction in total N concentrations was observed along the treatment flow way at all phases of the flow event (**Figure 8**). The surface water near inflow stations (A3 and B2) had higher total N concentrations during the stagnant phase of the flow event. TN concentrations did not vary widely between "stagnant" and "post flow" phases of the flow event.

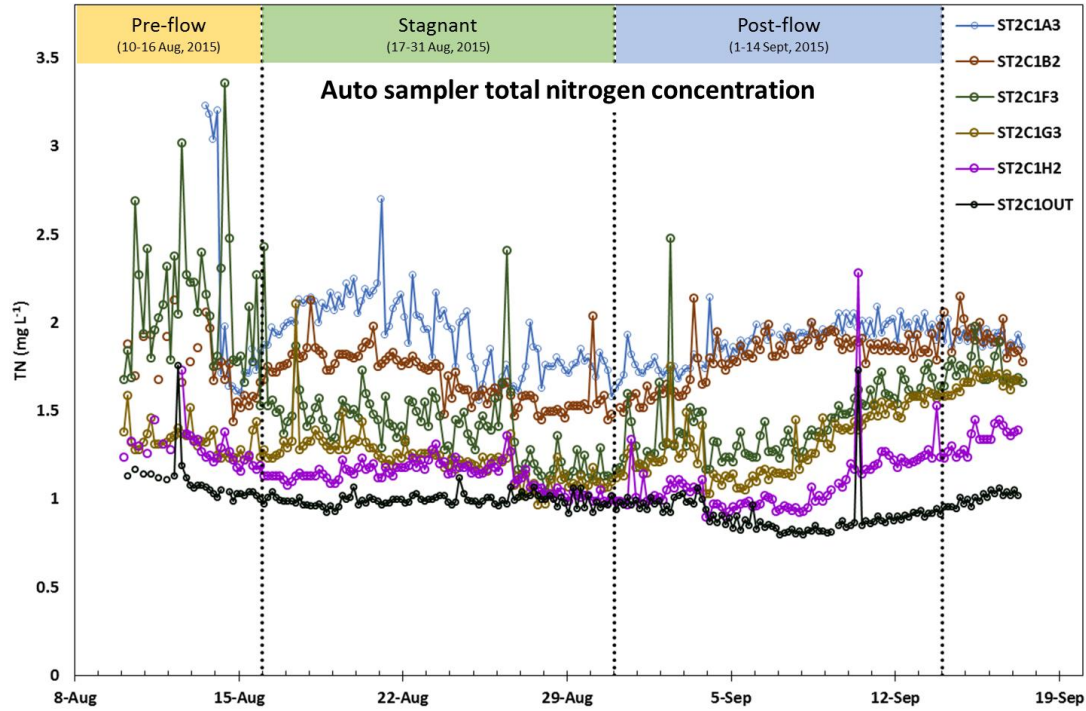


Figure 8. STA-2 Cell 1: Total nitrogen concentrations in surface water at six sampling locations during stagnant flow event.

Impact of Airboat presence on surface water TP

In order to determine the impact of air-boat traffic on surface water quality, auto-sampler data were screened for the data points associated with the presence of air boat/field crew in the vicinity of the auto-sampler (**Figure 9**). Total P concentrations in the sample collected after an air boat visit at a station were identified to determine if the presence of air boat/ field crew impacted P concentrations in the water column.

A minor spike in TP values which coincided with the field activity was noted, however this spiked signal dampened immediately, and the TP concentration in the water sample collected after four hours were similar to TP values recorded before arrival of an airboat. It appears that the impacts of such events /activities were not long lasting and did not necessarily affect overall treatment performance of this cell. The TP data are being analyzed statistically to ascertain the impacts of airboat presence. The results will be reported in the second quarterly report.

Summary of findings

A clear reduction in water column TP concentration from inflow to outflow was observed during the duration of the flow event. Auto-sampler data near the inflow station showed elevated TP concentrations during the stagnant event which may be a delayed effect of inflows as the pre-flow phase concluded (**Figure 10**). An examination of inflow and outflow water volumes in the cell showed that water did not stagnate during the 'stagnant' phase of the flow event, and outflows from the cell continued (**Figure 11** and **Figure 12**). The movement of water parcels could also be affecting data collected at various sampling locations within the cell. Previous water quality monitoring data from within Cell 1 showed internal P profiles where the majority of treatment occurs between 'B' and 'H' stations in the lower end of the cell [STA-2 Performance and activities section, Chapter 5B, SFER 2016 Draft (page 5B-46)].

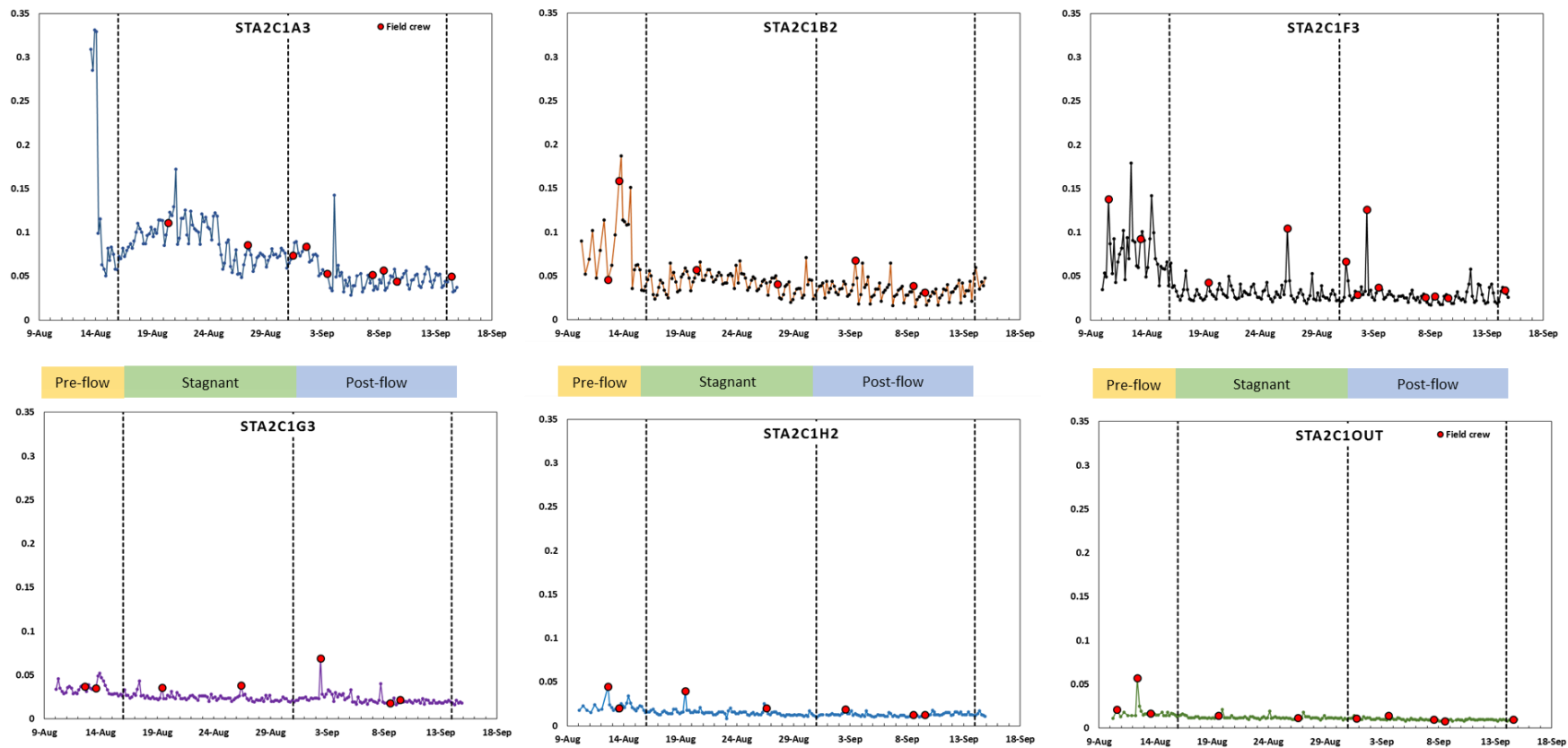


Figure 9. STA-2 Cell 1: Impact of air boat presence on total phosphorus concentrations at various internal water quality monitoring stations. Red dots indicate water sampled after an air boat has passed through a station.

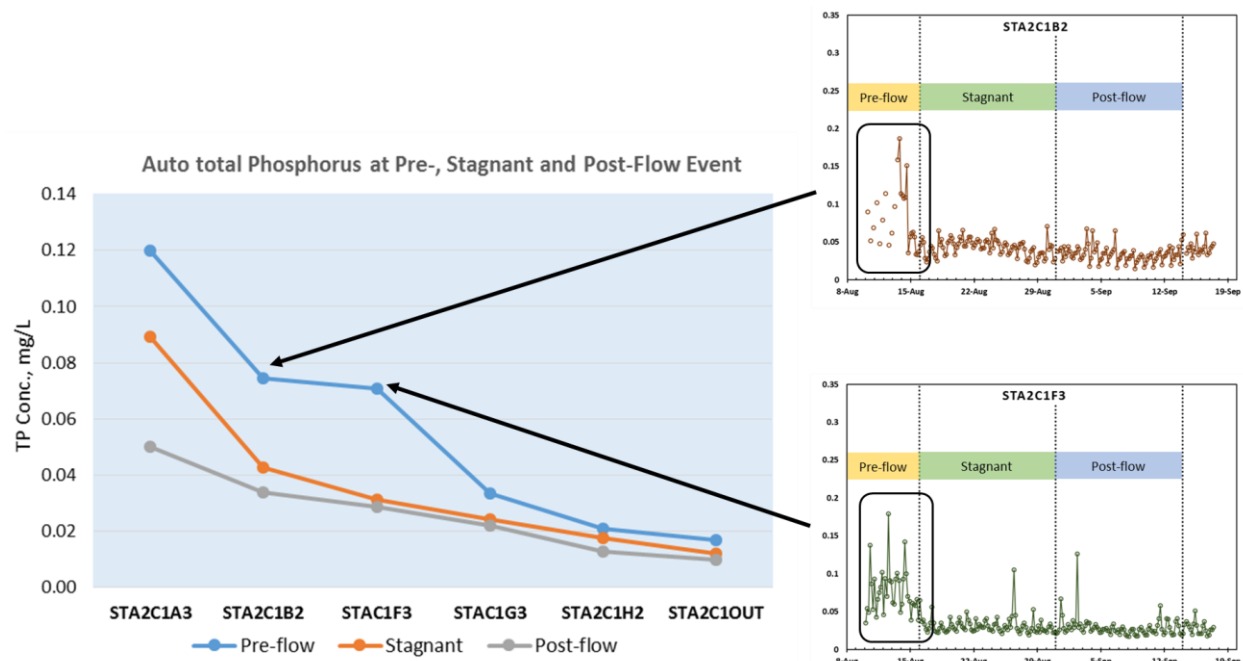


Figure 10. STA-2 Cell 1: Mean TP concentrations at various sampling stations during three stages of flow event. Two plots on the right indicate higher concentration at stations – C1B2 and C1F3 during pre-flow stage.

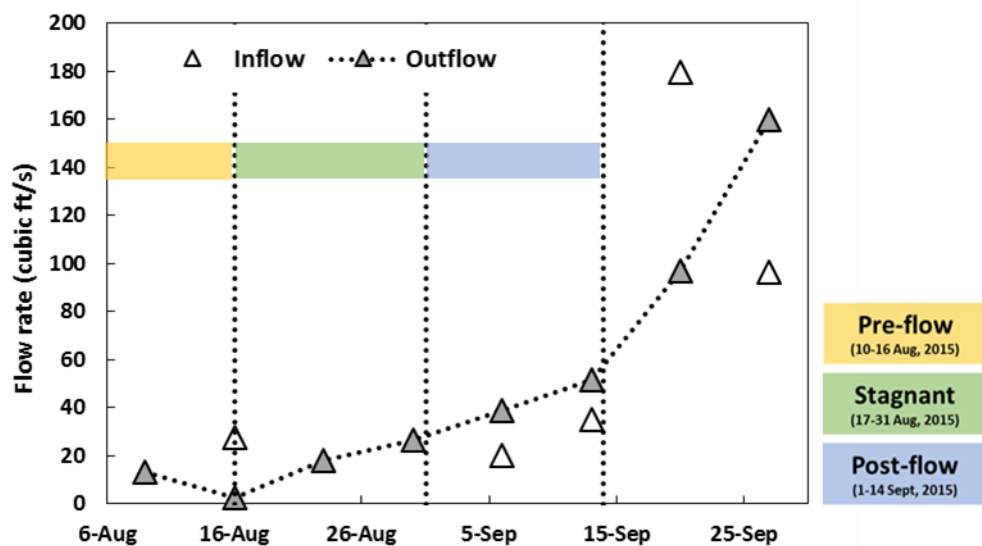


Figure 11. STA-2 Cell 1: Inflow and outflow rates (cfs) during different flow regimes. Data represent weekly average from inflow stations G-329 (A-D) and outflow stations – G-330 (A-E).

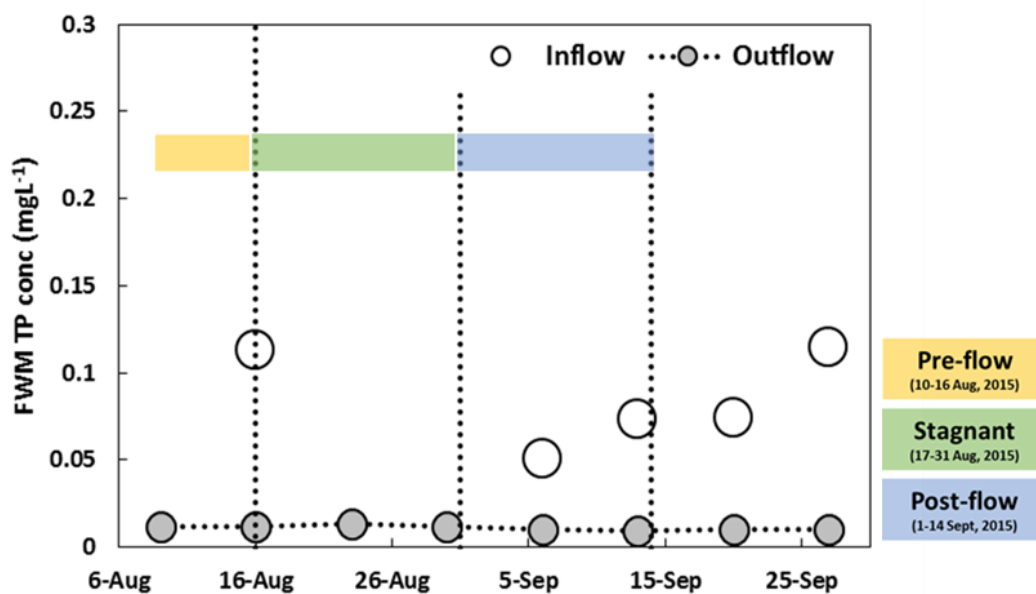


Figure 12. STA-2, Cell 1: flow weighted mean total phosphorus concentration (mg P L⁻¹) within inflow and outflow. Data represent weekly average from inflow stations G-329 (A-D) and outflow stations – G-330 (A-E). [FWM TP conc. = Flow weighted mean total phosphorus concentration].

6 Transect Study- Enzyme Assays [Task 7b]

The objectives of Task 7b are to assess patterns of phosphatase expression in the major STA communities (SAV and EAV) and to determine the most reactive components within each system (floc, periphyton, water). This task will be accomplished in two parts. In addition to phosphatase, other key enzymes involved in microbial element cycling (e.g., C- and N-related) will be measured to better describe the overall enzyme activity (i.e., nutrient demand/limitation) of the STA microbial communities. Part 1: The first part of this task will involve assessment of enzyme activities in the water, periphyton, detritus and floc under various hydrologic flow regimes. The second part of this task involves spatial assessment of enzyme activities in litter, floc, and surface soils from the transect stations in each of the STA-2 and STA-3/4 study areas.

During this quarter, phone meetings were conducted to devise sample handling and analysis protocols. In these discussions with District personnel, we formalized the protocol to be followed by both labs for the enzyme activity analyses. During this period, test samples were collected on September 21, 2015 from the inflow (A3) and outflow (OUT) locations of STA-2 Cell 1. Cores were processed by the District and split for comparison between UF and SFWMD protocols. Samples were received by UF on September 23, 2015 and processed on September 24, 2015 for analysis of phosphatase (monoesterase and diesterase), b-glucosidase, and leucine aminopeptidase.

The basic protocol which was developed is a modified version of the approach we routinely use (Inglett et al 2011; Liao et al, 2014). Such high throughput procedures not only facilitate comparisons between spatially separate sites or matrices, but also substantially reduce the cost of such assays by reducing overall reagent volumes needed per sample. The adapted version is as follows (**Figures 13-15**):

Core sectioning occurs either in the field (preferable) or lab. Floc material is collected by pouring from the core tube while litter is picked by hand from the pourable fraction using forceps. Materials are kept at 4°C until analyzed. Both litter and floc components are homogenized with a hand blender/tissue macerator. Subsamples of the homogenized suspension are taken for dilution and enzyme/fluorescence measurements and dry: wet ratio determination (lyophilization for 2 days). Aliquots (1 mL) for enzyme analysis are diluted (1:100 sample:water).

Kinetic measurements using several substrate concentrations are tested to ensure rates are near maximum (i.e., at V_{max}). Enzyme activity rates are recorded as linear increases in fluorescence with time during the first 2 hours using a Biotek, Gen5.0 (Winooski, VT, USA) fluorometric plate reader (Inglett and Inglett, 2013; Bell et al, 2013; Marx et al., 2001). Measured fluorescence readings are compared with known MUF standards prepared in sample matrix and final rates are expressed as moles of substrate hydrolyzed per time, per gram of floc or litter material ($\text{mol g}^{-1} \text{hr}^{-1}$).

Based on these procedures an annotated Powerpoint presentation was prepared to illustrate the process. Currently we are still processing the data from the test samples and investigating potential correlations with other biogeochemical parameters determined during this period.

Subsequent meetings are scheduled to discuss results as well as the calculations involved in the enzyme activity assays.

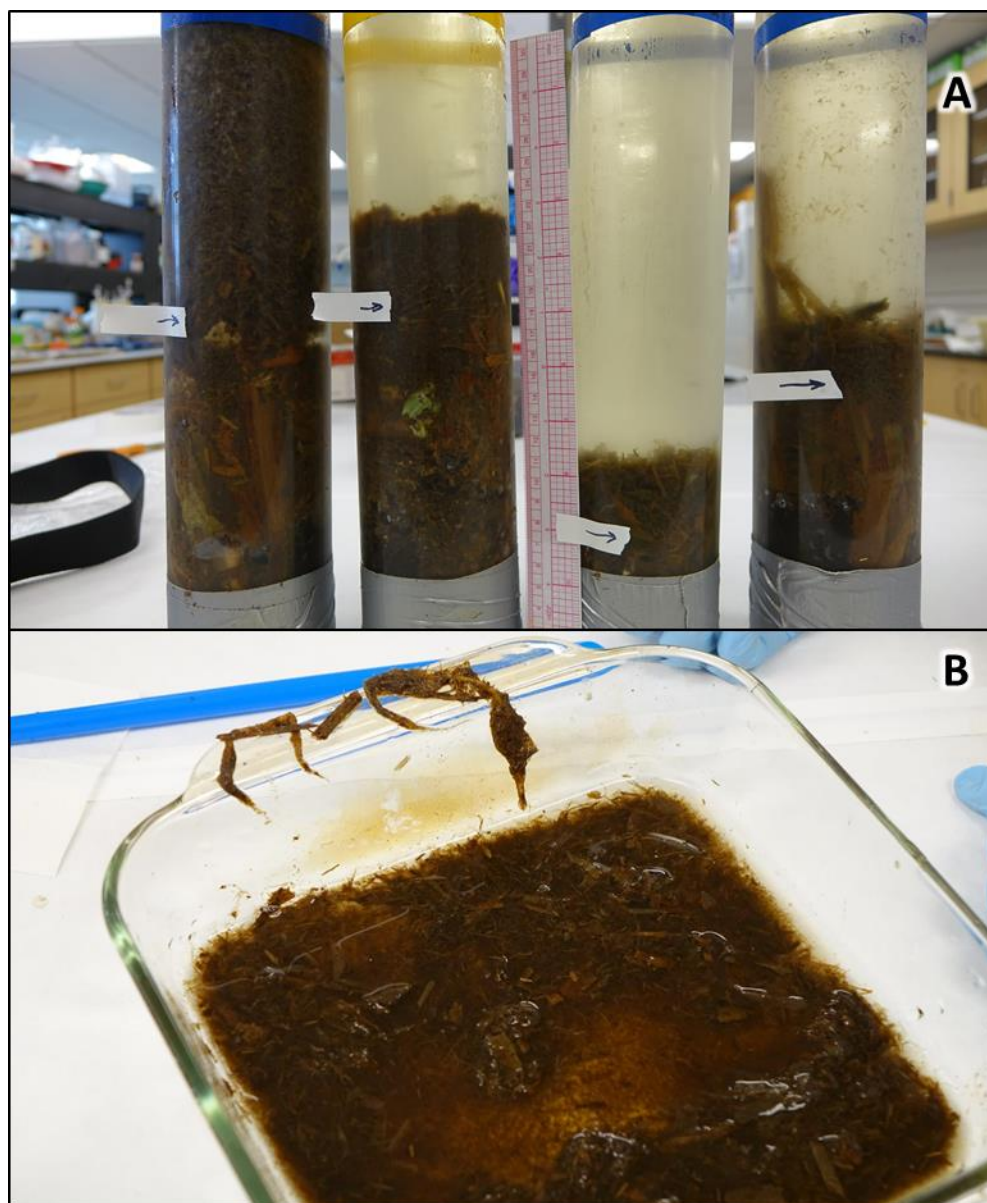


Figure 13: Images depicting key steps in the preparation of floc and litter samples for enzyme activity analysis. A) sample core with depth of floc layer indicated by an arrow B) initial sample of floc and litter poured from core.



Figure 14: Images depicting key steps in the preparation of floc and litter samples for enzyme activity analysis. A) hand selection of litter pieces and bagged litter sample, B) homogenization of litter and floc materials using tissue blender.

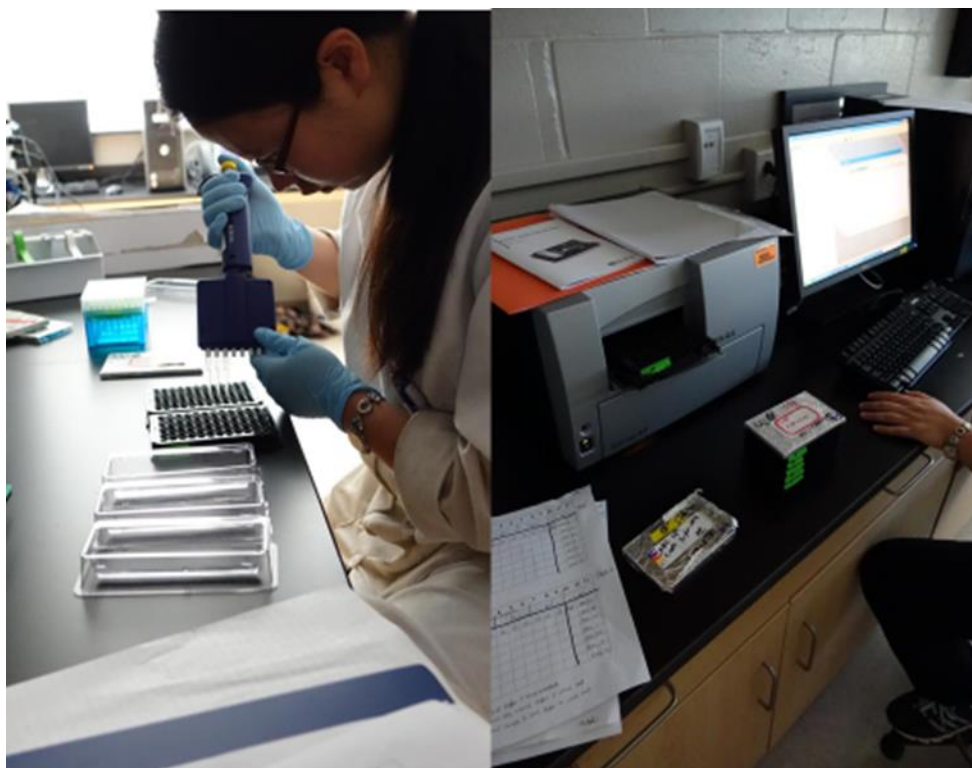


Figure 15: Images depicting the analysis of floc and litter samples for enzyme activity analysis using 96-well plates (left) and the fluorometric plate reader (right).

7 Action items for the next quarter

- Soil and water sample collections in STA-2 Cell 3 and STA-3/4 Cell 3B during mid-October and November.
- Enzyme activity analyses from STA-2 Cell 1 transect study.

References

- Inglett K S., Inglett P. W., Reddy K. R. 2011. Soil Microbial Community Composition in a Restored Calcareous Subtropical Wetland Soil Sci. Soc. Am. J. 75:1731–1740
- Liao, X., Inglett, P. W., and Inglett, K. S. 2014. Vegetation and microbial indicators of nutrient status: Testing their consistency and sufficiency in restored calcareous wetlands. *Ecological Indicators*, 46:358-366.
- Tetra Tech, 2014. Final report - Data Mining and Comprehensive Data Analysis to Determine the Usability of Existing Information and Uncertainties in Evaluating the Forms, Sources, Cycling, and movement of Phosphorus in Stormwater Treatment Areas. Tetra Tech, Inc. and Cranfield University.
- UF –WBL. 2015. Project work plan - Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs). Work Order # 4600003031-WO01, South Florida Water Management Dist. West Palm Beach, FL.

Preliminary Data Disclaimer: *The water quality data from the transect study are provisional and as such, are subject to change. Given this, and the limited amount of available data (i.e., a single event from a single flow-way), the discussion of results from the first flow event is considered preliminary. A more comprehensive analysis will be performed on the final, validated water quality data, and perhaps with a larger dataset including other STA cells selected for this project.*

Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas

Collaborative Research Initiative Science Plan for the Everglades Stormwater Treatment Areas [CRESTA]

Project Kickoff Meeting Minutes June 29-30, 2015

Kickoff meeting was attended by the following: Dawn Sierer Finn (DBE); Luis Cañedo (SFWMD); Delia Ivanoff (SFWMD); Jill King (SFWMD); John Moorman (via phone, SFWMD); Sue Newman (SFWMD); Ceyda Polatel (SFWMD); Kathy Pietro (SFWMD); Colin Sanders (via phone, SFWMD); Larry Schwartz (SFWMD); Odi Villapando (SFWMD), Richard Walker (SFWMD); Stefan Gerber (UF); Patrick Inglett (UF); Todd Osborne (UF); Alan Wright (UF); and K. Ramesh Reddy (UF).

Delia Ivanoff briefly mentioned the overall scope of the STA Science Plan and goals and objectives of the program. She discussed linkages among all projects.

Odi Villapando presented an overview and indicated that the current UF project is a component of the “P sources, forms, flux and transformation processes” main study of the Science Plan (see attached presentation). The UF study will focus on soil factors and processes influencing P flux in the STAs. Other sub-studies mentioned included: field measurements of P flux and flow-way assessments, in-situ sediment dynamics, hydraulic and hydrologic measurements, microbial assays, vegetation assessments and aquatic faunal surveys. Collectively, these studies are designed to generate quantitative information pertinent to STA function and performance optimization. The District team members involved on various P-flux study are listed below.

- P-flux Science Plan – Delia Ivanoff
- Soil Biogeochemical Properties/Processes - Odi Villapando, UF contract
- In-situ P Flux/Flow-way Assessments – Jill King, DBE contract
- Identification/Quantification of Organic P Forms – Sue Newman
- In-Situ Sediment Dynamics - Colin
- Hydrologic and Hydraulic Measurements - Ceyda/Colin
- Microbial Activity – Kathy Pietro
- Vegetation Assessments – Christa
- Faunal Assemblages and Excretion – Mark
- Conceptual Model Development/Refinement - Naiming/Colin

K. Ramesh Reddy, UF project PI, introduced the UF team. Stefan Gerber (UF); Patrick Inglett (UF); Todd Osborne (UF); Alan Wright (UF). Responsibilities of each Co-PI is listed in the attached copy of the presentation. Reddy also mentioned that Rupesh Bhomia was hired as Post-doctoral Associate to work on this project and he will join the team on September 7, 2015. Rupesh will be based at the District office and will play a key role in the project by closely working with UF team and District scientists.

Reddy presented the conceptual framework, experimental approaches, sampling strategies, and expected outcomes of the project. Presentation included the following topics: Introduction/Background; Rationale and Project Objectives; Project Tasks; Discussion of Project Tasks; Integration and Synthesis; Field Resources from the District; Laboratory; Resources from the District; and Data Resources from the District (see attached copy of the presentation).

There was general agreement from all UF and District investigators that the SOPs used or biogeochemical properties measured should be same and agreed upon by both groups. There was some discussion on SOPs related to identification of floc depth. Osborne agreed to write an SOP for soil coring and floc depth identification and floc bulk density measurements

Enzyme assays are by both UF (Kanika Inglett and Patrick Inglett) and District (Pietro and Newman) scientists. It was agreed that these groups will share SOPs with each other and make modifications to SOP as needed.

Wright and Reddy agreed to District SOPs used for select biogeochemical properties and compare with UF-WBL methods and suggest any modifications or improvements. Wright and Reddy are planning to meet with Odi Villapando and District Lab staff on July 15, 2015 to discuss laboratory and field protocols.

Reddy will work with Newman and identify sampling locations in WCA-2A transect.

Wright and Reddy will work with Villapando to design soil core P-flux method and identify locations to conduct the study.

Reddy identified several project resources to be provided by the District. Bhomia and Wright will work with Villapando to obtain these resources.

UF added Stefan Gerber as Co-PI on the project. Gerber is planning to work primarily on “Integration and Synthesis” part of the project.

Field trip was attended by Gerber, Inglett, Wright, and Reddy, along with District scientists including: Ivanoff, Villapando, Canedo, King and Zamorano. The group visited STA-2 and STA-3/4. The field trip was excellent and useful to understand the complexity and challenges of treating water using STAs.

Action Items

- SOP for soil coring and floc layer identification, and sampling identifiable litter (Osborne)
- Meeting with District laboratory staff to SOPs related to the project (Reddy)
- Revised Project Work Plan will be submitted to the District by July 19, 2015 (Reddy)
- Reddy to develop Gant Chart for project activities (Reddy)
- Soil sampling schedule (Osborne)

Appendix 2. Table of sampling locations in STA-2 Cell 3.

STA-2 Cell 3 Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
1	C19	26.41726	-80.5530		
2	C20	26.41725	-80.5489	✓	✓✓✓
3	C21	26.41724	-80.5449		
4	C22	26.41722	-80.5408		
5	C23	26.41721	-80.5367		
6	C37	26.4136	-80.553		
7	C38	26.41358	-80.5489	✓	
8	C39	26.41357	-80.5449		
9	C40	26.41356	-80.5408		
10	C41	26.41354	-80.5367		
11	C55	26.40993	-80.553		
12	C56	26.40992	-80.5489	✓	
13	C57	26.4099	-80.5449		
14	C58	26.40989	-80.5408		
15	C59	26.40988	-80.5367		
16	C73	26.40626	-80.553		
17	C74	26.40625	-80.549	✓	
18	C75	26.40624	-80.5449		
19	C76	26.40622	-80.5408		
20	C77	26.4062	-80.5367		
21	C91	26.40259	-80.553		
22	C92	26.40258	-80.549	✓	
23	C93	26.40257	-80.5449		
24	C94	26.40256	-80.5408		
25	C95	26.40255	-80.5368		
26	C109	26.39893	-80.5531		
27	C110	26.39891	-80.549	✓	
28	C111	26.3989	-80.5449		
29	C112	26.39889	-80.5409		
30	C113	26.39888	-80.5368		
31	C127	26.39526	-80.5531		
32	C128	26.39525	-80.549	✓	✓✓✓
33	C129	26.39523	-80.5449		
34	C130	26.39522	-80.5409		
35	C131	26.39521	-80.5368		

STA-2 Cell 3 Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
36	C145	26.39159	-80.5531		
37	C146	26.39158	-80.549	✓	
38	C147	26.39157	-80.5449		
39	C148	26.39155	-80.5409		
40	C149	26.39153	-80.5368		
41	C163	26.38793	-80.5531		
42	C164	26.38791	-80.549	✓	
43	C165	26.3879	-80.545		
44	C166	26.38789	-80.5409		
45	C167	26.38788	-80.5368		
46	C181	26.38426	-80.5531		
47	C182	26.38425	-80.5491	✓	
48	C183	26.38423	-80.545		
49	C184	26.38422	-80.5409		
50	C185	26.38421	-80.5368		
51	C199	26.38059	-80.5531		
52	C200	26.38058	-80.5491	✓	✓✓✓
53	C201	26.38057	-80.545		
54	C202	26.38055	-80.5409		
55	C203	26.38053	-80.5368		

Appendix 3. Table of sampling locations in STA-3/4 Cell 3B.

STA-3/4 Cell 3B Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
1	A1	26.37603	-80.6831		
2	A2	26.37603	-80.6798		
3	A3	26.37603	-80.6766		
4	A4	26.37603	-80.6728		
5	A5	26.37603	-80.6689		
6	A6	26.37603	-80.6651		
7	A7	26.37603	-80.6602	✓	✓✓✓
8	A8	26.37603	-80.6564		
9	A9	26.37603	-80.6526		
10	A10	26.37603	-80.6487		
11	A11	26.37603	-80.6449		
12	A12	26.37603	-80.6411		
13	A13	26.37603	-80.6373		
14	B1	26.3723	-80.6831		
15	B2	26.3723	-80.6798		
16	B3	26.3723	-80.6766		
17	B4	26.3723	-80.6728		
18	B5	26.3723	-80.6689		
19	B6	26.3723	-80.6651		
20	B7	26.3723	-80.6602	✓	
21	B8	26.3723	-80.6564		
22	B9	26.3723	-80.6526		
23	B10	26.3723	-80.6487		
24	B11	26.3723	-80.6449		
25	B12	26.3723	-80.6411		
26	B13	26.3723	-80.6373		
27	C1	26.36857	-80.6831		
28	C2	26.36857	-80.6798		
29	C3	26.36857	-80.6766		
30	C4	26.36857	-80.6728		
31	C5	26.36857	-80.6689		
32	C6	26.36857	-80.6651		
33	C7	26.36857	-80.6602	✓	

STA-3/4 Cell 3B Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
34	C8	26.36857	-80.6564		
35	C9	26.36857	-80.6526		
36	C10	26.36857	-80.6487		
37	C11	26.36857	-80.6449		
38	C12	26.36857	-80.6411		
39	C13	26.36857	-80.6373		
40	D1	26.36484	-80.6831		
41	D2	26.36484	-80.6798		
42	D3	26.36484	-80.6766		
43	D4	26.36484	-80.6728		
44	D5	26.36484	-80.6689		
45	D6	26.36484	-80.6651		
46	D7	26.36484	-80.6602	✓	✓✓✓
47	D8	26.36484	-80.6564		
48	D9	26.36484	-80.6526		
49	D10	26.36484	-80.6487		
50	D11	26.36484	-80.6449		
51	D12	26.36484	-80.6411		
52	D13	26.36484	-80.6373		
53	E10	26.36111	-80.6487		
54	E11	26.36111	-80.6449		
55	E12	26.36111	-80.6411		
56	E13	26.36111	-80.6373		