DECEMBER 2015 QUARTERLY REPORT - 2

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

Work Order #: 4600003031-WO01

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This quarterly progress report summarizes the activities performed during the period of October – December 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 second 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

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 comparison. Project Work Plan (Work Order # 4600003031-WO01) includes details on specific objectives and tasks.

This quarterly report describes activities related to the following tasks:

- Task 3. Spatial soil sampling
- Task 7b. Transect study: Enzyme assays

2 Soil Sample Collection [Task 3]

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 from the existing grid established by the District scientists or contractors for the purpose of soil and vegetation monitoring and survey. 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. During this quarter, the first phase of sampling was conducted for STA-2 Cell 3 and STA-3/4 Cell 3B.

2.1 Work Completed During This Quarter

Coordination of field sampling between UF and District project personnel resulted in completion of spatial sampling in STA-2 Cell 3 and STA-3/4 Cell 3B. Field samples were submitted to the District laboratory and UF Wetland Biogeochemistry laboratory for analysis.

2.2 STA-2 – Cell 3 (Year 1)

Spatial soil sampling was first conducted in STA-2 Cell 3 during October 13 - 15, 2015. A quarter mile sampling grid was utilized to identify sampling stations. Soil cores and surface water samples were collected from the cell and field parameters (e.g. pH, Cond, Temp., DO, soil and water depth) were recorded at each sampling location. A transect consisting of 11 stations (station ids – 20, 38, 56, 74, 92, 110, 128, 146, 164, 182 and 200) was established along the hydrologic flow path. Three stations, one each at inflow, center, and outflow of the cell were demarcated as benchmark sites (20, 128, and 200) for future sampling. Three soil cores and three surface water samples were collected at the benchmark sites. **(Figure 1** and **Figure 2**) [*For GPS coordinates of sampling locations, refer to Appendices of 1st quarterly progress report*].

Although STA-2 Cell 3 is categorized as a submerged aquatic vegetation (SAV) cell, approximately 20% of the treatment area within south - eastern region of the cell consists of predominantly emergent vegetation (EAV; primarily *Typha domingensis,* with some patches of *Cladium jamaicense)* **(Figure 1**). The samples collected from stations within the EAV dominant zone will be treated separately for data analysis and interpretation, while the rest of the stations will be considered as representative of SAV cell.

Surface water samples were collected from 11 stations along a transect parallel to flow direction (**Figure 1**). Three replicate water samples were collected from the benchmark sites including: near inflow, center, and outflow. A total of 17 water samples were submitted to the District lab for chemical analysis on October 13, 2015.

Intact soil cores were collected at 55 stations, including three benchmark sites using 10 cm diameter polycarbonate core tubes. A total of 61 intact soil cores were collected from this cell.

Soil cores were stored in a cold room (~4^{o}C) and subsequently separated into plant litter (when present), floc, recently accreted soil (RAS), and pre-STA soil.

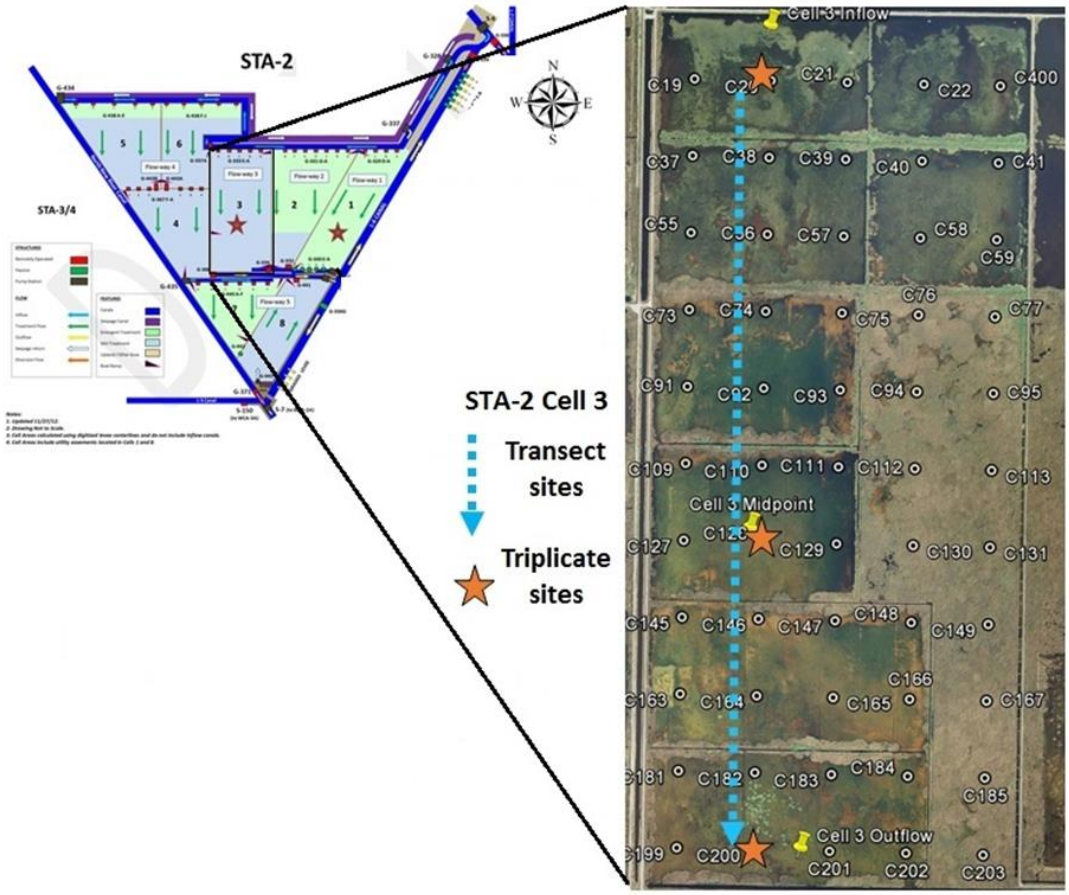
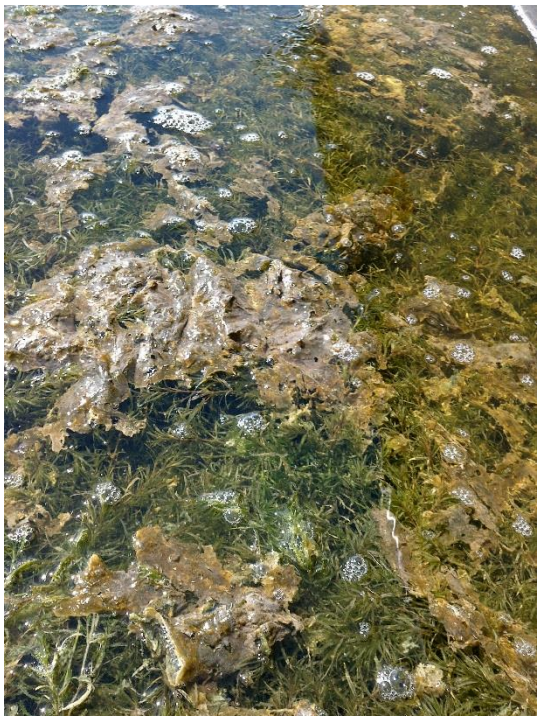


Figure 1. STA-2 Cell 3 – Soil and surface water sampling locations.

(a) Illinois pondweed – *Potamogeton illinoensis* and Southern naiad – *Najas guadalupensis*

(b) Intact soil core, water lilies – *Nymphaea spp.* and southern naiad – *Najas guadalupensis*



(c) Southern naiad – *Najas guadalupensis* and Periphyton

(d) Intact soil core and southern naiad – *Najas guadalupensis*

Figure 2. STA-2, Cell 3 – Representative soil cores and commonly observed vegetation at sampling stations (photos taken during October 13-15, 2015).

Floc was characterized as the suspended unconsolidated material on top of consolidated RAS. It was poured into a plastic bag and the underlying RAS layer was sectioned based on its thickness. Floc depth was measured by incorporating an extra step that allowed settling of the suspended flocculent material before determination of floc depth. Floc was poured into empty plastic tubes (same dimension as the soil core tubes), and allowed to settle for 4 hours. The supernatant water was syringed off, and the thickness of the settled floc was measured (**Figure 3**, steps 1 through 3). The floc material was transferred in ziploc bags and submitted to the District lab for analysis.

RAS was divided into two sections: 0-5 cm and 5 cm to remaining RAS depth. We did not encounter any station with RAS depth > 10 cm. Pre-STA soil, which was separated from the overlying RAS based on color, texture and consistency. The thickness (depth) of pre-STA soil varied from one location to another depending on the total depth of the soil core, but it was > 10 cm for all locations (**Table 1**). A total of 192 samples were submitted to the District lab for chemical analysis on October 23, 2015.

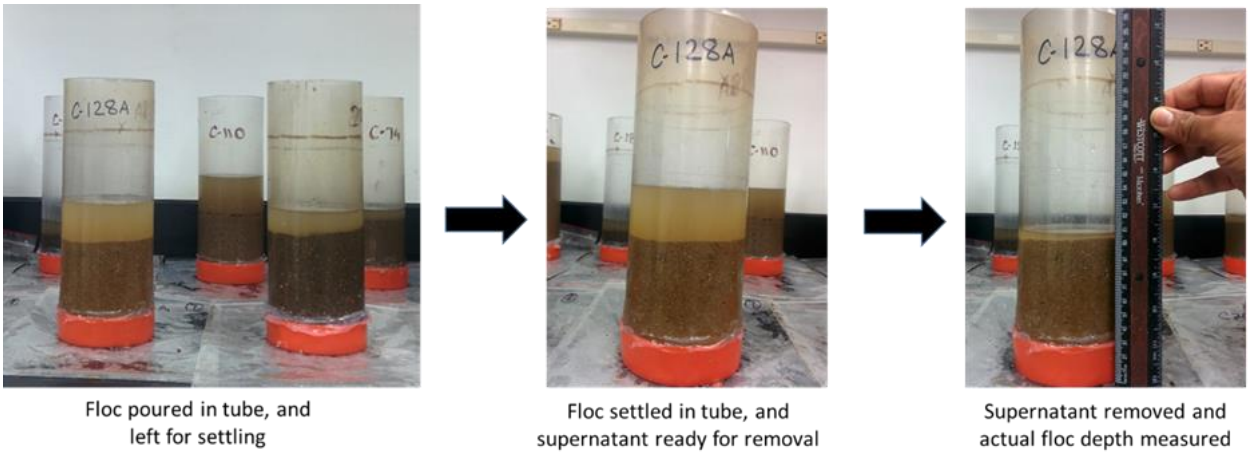


Figure 3. Steps for determination of floc depth.

Significant observations from STA-2 Cell 3 sampling effort included:

1. Soil break point between pre-STA soil and RAS is very clear given the change from dark organic soil color (10YR 3/1) in pre-STA soil to lighter calcite dominated soil matrix (10YR5/3) of the RAS.
2. RAS material had lower bulk density than pre-STA soil, which has been substantially compacted due to previous agricultural operations as compared to undisturbed Everglades peat soils of WCAs.
3. Eastern portions of STA-2 Cell 3 is dominated by dense cattail (*Typha domingensis*) with very little or no SAV.

Additional soil cores were collected on November 17, 2015 from the transect locations (station ids –38, 56, 74, 92, 110, 128, 146, 164, 182) including benchmark sites (station ids –20, 128 and 200) to determine enzyme activity and microbial P and C biomass. Soil cores were separated into floc, RAS and pre-STA soils and stored in cold room at 4^{o}C until transported to the UF's Wetland Biogeochemistry Lab on December 14, 2015 for analysis.

Table 1. STA-2 Cell 3: Thickness of various core sections.

STA-2 Cell 3 Site #	Station id	Thickness of section (cm)			
		Floc	RAS (0-5 cm)	RAS (>5 cm)	Pre-STA
1	C-19	5	3	--	7.5
2	C-20A	7.5	2.5	--	15
3	C-20B	8	3	--	17
4	C-20C	8.3	1	--	17.5
5	C-21	7.2	3	--	9
6	C-22	9	5	1.5	12
7	C-23	12.5	5	--	19
8	C-37	12	2.5	--	9
9	C-38	4	2	--	13
10	C-39	8.6	3	--	17
11	C-40	16.2	3	--	10
12	C-41	4	1	--	12
13	C-55	13.5	5	--	15
14	C-56	7.1	1.5	--	20
15	C-57	11.5	2	--	11
16	C-58	8.5	1	--	12
17	C-59	19	2.5	--	12
18	C-73	13	1.5	--	21
19	C-74	9.7	5	2	20
20	C-75	12	2	--	19
21	C-76	4.5	4.5	--	20
22	C-77	25	2.5	--	20
23	C-91	12.5	5	--	14
24	C-92	9.5	3	--	17.5
25	C-93	17.3	3	--	12.5
26	C-94	9	3	--	20
27	C-95	19.5	1.5	--	20
28	C-109	9.6	5	2	20
29	C-110	8.6	1.5	--	18
30	C-111	16.5	3	--	13
31	C-112	5.5	3	--	20
32	C-113	7	3	--	13
33	C-127	13.1	2.5	--	12.5
34	C-128A	10.5	1.5	--	16.5

Table 1 continued…

STA-2 Cell 3 Site #	Station id	Thickness of section (cm)			
		Floc	RAS (0-5 cm)	RAS (>5 cm)	Pre-STA
35	C-128B	7.6	2	--	16.5
36	C-128C	18.4	3	--	13
37	C-129	13.6	3	--	13.5
38	C-130	4	3.5	--	20
39	C-131	6.6	5	--	20
40	C-145	10.5	5	3	20
41	C-146	11	5	--	18
42	C-147	16	2	--	19
43	C-148	12.8	2	--	20
44	C-149	6	1.5	--	20
45	C-163	12	--	--	--
46	C-164	14	4	--	16.5
47	C-165	5.4	1	--	22
48	C-166	10.3	3	--	20
49	C-167	9.4	1	--	20
50	C-181	14.4	3	--	20
51	C-182	5.6	5	1.5	23.5
52	C-183	15	2	--	23.5
53	C-184	10	2	--	20
54	C-185	9	2	--	20
55	C-199	7.5	2	--	20
56	C-200A	11	3	--	20
57	C-200B	7	3	--	20
58	C-200C	6.5	4	--	20
59	C-201	9.8	4.5	--	25
60	C-202	11.3	5	--	20
61	C-203	19	2	--	8
Total number of samples from STA-2 Cell 3		**61**	**60**	**5**	**60**

2.4 STA-3/4 – Cell 3B

The second baseline sampling was carried out in STA-3/4 Cell 3B on November 17-20, 2015. Sampling stations were identified using DB Environmental's existing vegetation survey map points (**Figure 4** and **Figure 5**). This cell is categorized as an SAV cell, however approximately 30 to 40% of the cell is covered with EAV (primarily *Typha domingensis*). [*For GPS coordinates of sampling locations, refer to appendices of 1st quarterly progress report*].

Intact soil cores were collected at 56 stations. Three replicate soil cores were collected from benchmark sites resulting in total 60 intact soil cores from this cell. Soil cores were stored in a cold room (~4^{o}C) before separating into plant litter (when present), floc, RAS and pre-STA soil fractions (**Table 2**). Floc depth was measured for all soil cores using the procedure outlined in Section 2.2 (**Figure 3**) of this report. Additional 8 soil cores were collected from transect locations (station ids - A7, B7, C7 and D7), including three cores at benchmark sites) to determine P enzyme activity and microbial P and C biomass. Samples were transported to the UF-Wetland Biogeochemistry Lab on November 20, 2015, for analysis.

Surface water samples were collected from four stations, including benchmark sites near inflow and outflow of the cell. Three replicate water samples were collected from benchmark sites, resulting in eight water samples that were submitted to the District lab for analysis on November 17, 2015. Field parameters (e.g. pH, Cond, Temp., DO, soil and water depth) were measured at each sampling location.

RAS was divided into two sections: 0-5 cm and 5 cm to remaining RAS depth. We did not encounter any station with RAS depth > 5cm. Pre-STA soil, which was discernible from the overlying RAS was separated based on color, texture and consistency. The thickness (depth) of pre-STA soil varied from one location to another depending on total depth of the soil core **(Table 2)**. A total 182 samples were submitted to the District lab for analysis on November 25, 2015.

The following observations were made during soil sampling in STA-3/4 Cell 3B:

1) Cell 3B was converted from a cattail (*Typha domingensis)* dominant cell to an SAV cell, however a large proportion of the cell (~30-40 %) is still covered by EAVs.
2) Soil break point between pre-STA soil and RAS is very clear given the change from dark organic soil color (10YR 3/1) in historic soil to lighter calcite dominated soil matrix (10YR5/3) of the RAS.
3) RAS material has much lower bulk density than historic soil, but the pre-STA soils were not as compacted as in STA-2 Cell 3.

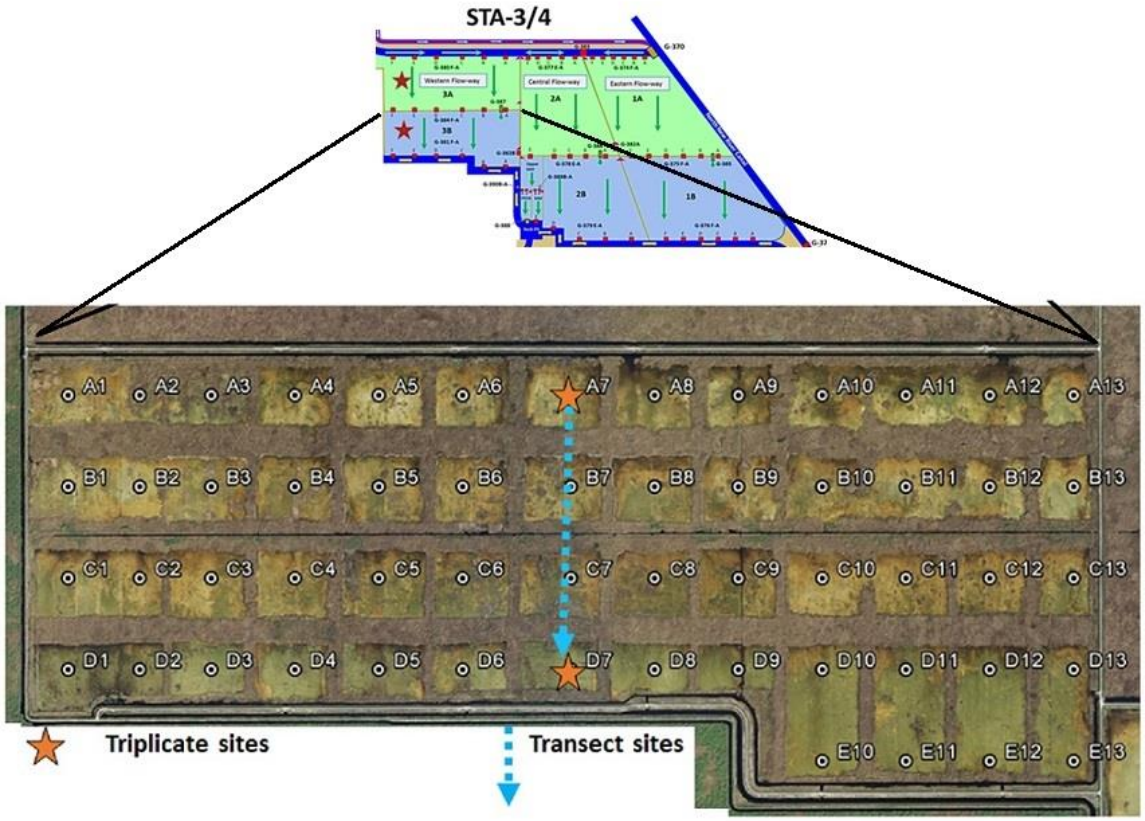


Figure 4. STA-3/4 Cell 3B – Soil and surface water sampling locations.

(a) Intact soil core and few cattail plants – *Typha domingensis*

(b) Sparse cattail – *Typha domingensis* patch

(c) Thick cattail – *Typha domingensis*

(d) Open water with suspended floc

Figure 5. STA-3/4, Cell 3B – Representative sampling locations within the cell (photos taken during November 17-20, 2015).

Table 2. STA-3/4 Cell 3B: Thickness of different core sections.

STA-3/4 Cell 3B Site #	Station id	Thickness of section (cm)			
		Floc	RAS (0-5 cm)	RAS (>5 cm)	Pre-STA
1	A1	13	3	--	20
2	A2	--	5	--	18
3	A3	12.5	2	--	16
4	A4	9	2	--	20
5	A5	14	2	--	13
6	A6	11	3	--	20
7	A7A	12	1	--	11
8	A7B	13.5	2	--	13
9	A7C	8.5	2	--	18
10	A8	9	2	--	15
11	A9	4	1	--	20
12	A10	12	3	--	13
13	A11	8.5	4	--	13
14	A12	7.5	2.5	--	12
15	A13	13.5	3	--	14
16	B1	13.5	1.5	--	20
17	B2	12.5	3	--	20
18	B3	10	1	--	15
19	B4	14	2	--	18
20	B5	9	2.5	--	14
21	B6	13.5	3	--	20
22	B7	9	1	--	14
23	B8	6.5	1	--	20
24	B9	8	2.5	--	20
25	B10	5	--	--	--
26	B11	15.5	1.5	--	12
27	B12	8.5	3	--	15
28	B13	10.5	5	--	17.5
29	C1	10	1.5	--	18
30	C2	5.5	1	--	20
31	C3	3.5	1.5	--	20
32	C4	12	5	--	20
33	C5	8.5	2.5	--	12
34	C6	8	2	--	17

Table 2 continued…

STA-3/4 Cell 3B Site #	Station id	Thickness of section (cm)			
		Floc	RAS (0-5 cm)	RAS (>5 cm)	Pre-STA
35	C7	12	3	--	18
36	C8	8.5	1	--	15
37	C9	6.5	2.5	--	20
38	C10	7	3	--	12
39	C11	10	2	--	16
40	C12	9	2	--	19
41	C13	12	3	--	13
42	D1	7	4.5	--	20
43	D2	2	1.5	--	15
44	D3	7.5	2.5	--	17
45	D4	4	2.5	--	20
46	D5	4	2	--	20
47	D6	5	3	--	13
48	D7A	11.5	3	--	7
49	D7B	7.5	2	--	4
50	D7C	12.5	3	--	8
51	D8	11	1	--	19
52	D9	7	2	--	14
53	D10	5.5	2.5	--	19
54	D11	9	1.5	--	20
55	D12	13	1	--	18
56	D13	7.5	2	--	13
57	E10	4	3	--	20
58	E11	4	3	--	18
59	E12	8	2	--	20
60	E13	6.5	2	--	14
Total number of samples from STA-3/4 Cell 3B		**60**	**61**	**--**	**61**

3 Transect Study- 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 within a flow way. Data collected during the first flow event - stagnant flow that took place during 17-31 Aug, 2015, was organized for statistical analysis after preliminary trend analysis (reported in first quarterly report). High temporal resolution data on field parameters from EXO-sondes was processed for further analysis. Further data analysis from the second flow event – impact of Lake Okeechobee water release to STAs will be reported in the next quarterly report.

4 Transect Study- Enzyme Activity [Task 7b]

The objectives of Task 7b are to assess patterns of enzyme activity in the water, periphyton, detritus and floc under various hydrologic flow regimes.

During this quarter, three phone meetings were conducted to discuss sampling events surrounding the flow experiment, the results of the preliminary enzyme activity analyses, as well as to assist District personnel with calculations involved in the determination of enzyme activity. The bulk of effort for this quarter has been analysis of core samples collected from STA-2 Cell 1 (**Figure 6**). Two of these sets of samples were retrieved during quarter 1 of the project with the analyses now being completed for preliminary presentation.

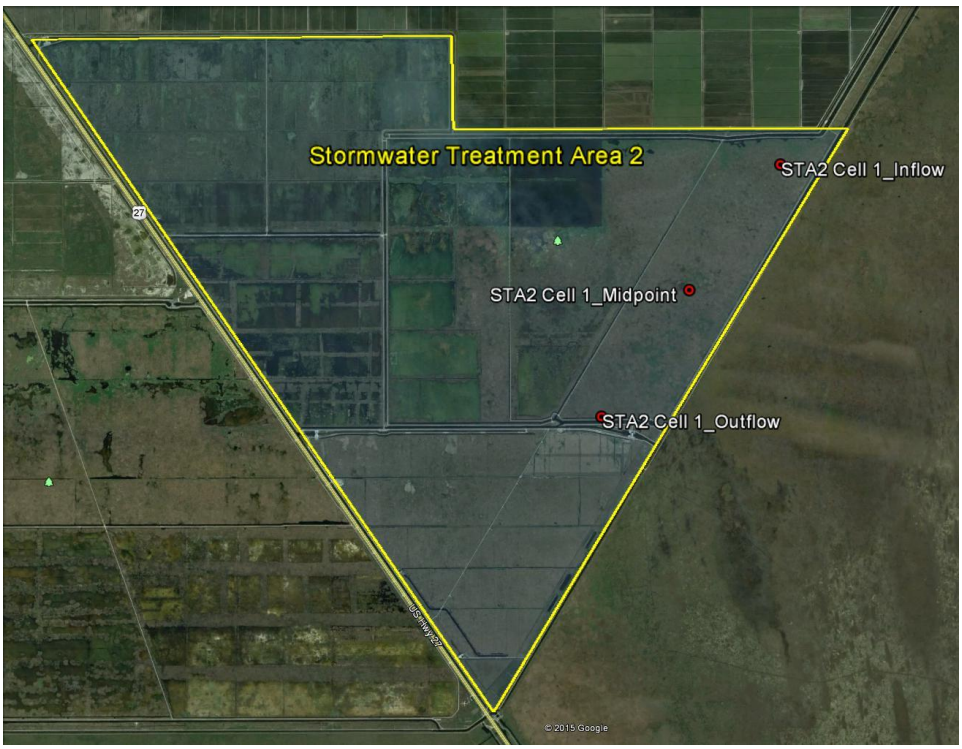


Figure 6. Map showing locations of sampling stations within STA-2 Cell 1.

4.1 Methods

Sample set 1: UF received the first set of test samples during the previous quarter on August 12 2015. These samples consisted of two replicate cores from STA-2 Cell 1 sites A3 and OUT. These cores were extruded at UF and split into floc and water components. Floc samples were homogenized and analyzed for activities of four enzymes including phosphatase (mono- and di-esterase), beta-glucosidase, and aminopeptidase. The activity in two sample volumes was determined to ensure that the volume size was not greatly affecting the samples.

Sample set 2: During the previous period, floc samples were collected on 9/21/2015 from the inflow (A3) and outflow (OUT) locations of STA-2 Cell 1. These cores were processed by the District and split for comparison between UF and SFWMD protocols. Analyses of phosphatase (mono- and di-esterase), beta-glucosidase, and leucine aminopeptidase activity were completed during the second quarter.

Sample set 3: A third set of cores were collected on October 5 and intended to serve as the initial characterization of the flow experiment in STA-2 Cell 1. In this sampling, three replicate cores were taken at STA-2 Cell 1 Inflow (A3), Midflow, and Outflow sites. The cores were delivered to UF where they were processed into floc and litter sections according to the protocols developed in the previous quarter. On October 6-7, samples were analyzed for phosphatase (mono- and di-esterase), beta-glucosidase, and leucine aminopeptidase activity.

The basic protocol which was developed is a modified version of the approach routinely used in the UF laboratory (Inglett et al 2011; Liao et al, 2014). Such a 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.

Floc material was collected by pouring from the core tube while litter was picked by hand from the pourable fraction using forceps. Materials were kept at 4 deg C until analyzed. Both litter and floc components were homogenized with hand blender/tissue macerator. Subsamples of the homogenized suspension were taken for dilution and enzyme/fluorescence measurements and dry: wet ratio determination (lyophilization for 2 days). Aliquots (1 mL) for enzyme activity analyses were diluted (1:100 sample:water).

Kinetic measurements using several substrate concentrations were tested to ensure rates are near maximum (i.e., at Vmax). Enzyme rates were 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; Marx et al., 2001). Measured fluorescence readers were 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 (mol g^{-1} $time^{-1}$).

4.2 Preliminary Results

Though the results obtained thus far are preliminary they indicate that there is an apparent substrate dependency (i.e., difference between floc and litter) for certain enzymes. This difference is undoubtedly due to different abundances of microbial biomass in relation to stages of decomposition in the case of litter, or the abundance of algal biomass in the floc. An important difference was also noted in the levels of phosphatase activity in the water column versus floc in the August 2015 sampling **(Figure 7)** where patterns of phosphatase activity in the floc material were not reflected in those of the water column above. These observations highlight the important distinction between the microbial communities operating in various ecosystem components.

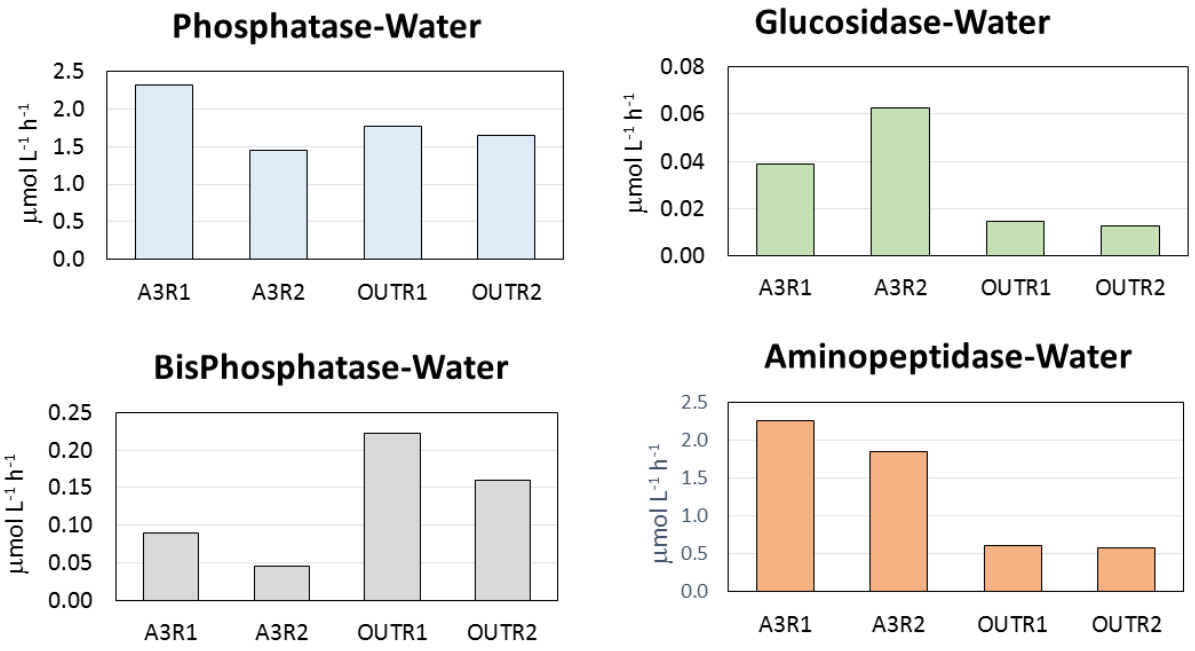


Figure 7. Potential enzyme activities of duplicate water samples (floodwater of soil cores) collected in August 2015 from STA -2 Cell 1 at locations near inflow (A3R1 and R2) and outflow (OUT R1 and R2).

Another important observation from these preliminary results in STA-2 is the presence of spatial patterns within the microbial communities reflected in the enzyme activities. In general, the inflow areas exhibited higher rates of both C and N enzyme activity, while the outflow areas exhibited higher rates of P enzyme activity (**Figures 7- 9**). This was not always the case, however and the results from the October 2015 sampling showed no overall inflow to outflow trend in beta-glucosidase or aminopeptidase activities in either the floc or litter (**Figures 10-13**). Patterns of phosphatase activity were consistent in all three samplings.

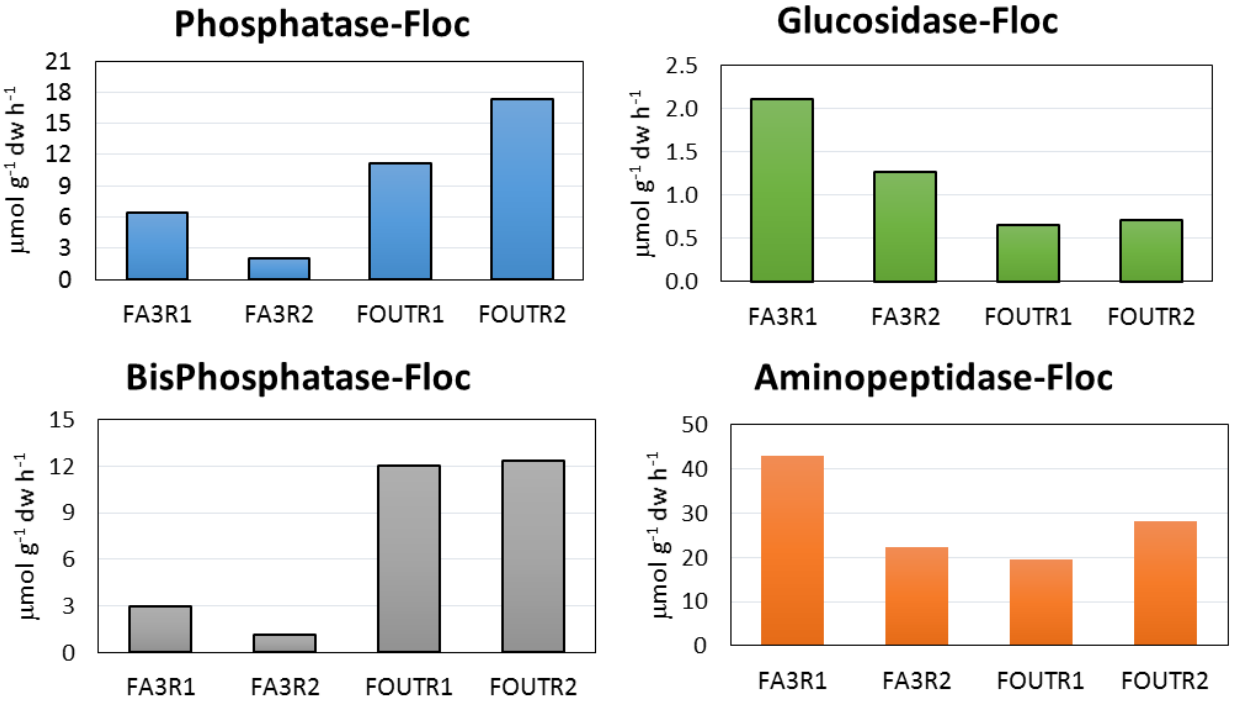


Figure 8. Potential enzyme activities of duplicate floc samples collected in August 2015 from STA -2 Cell 1 at two locations near inflow (A3R1 and R2) and outflow (OUT R1 and R2).

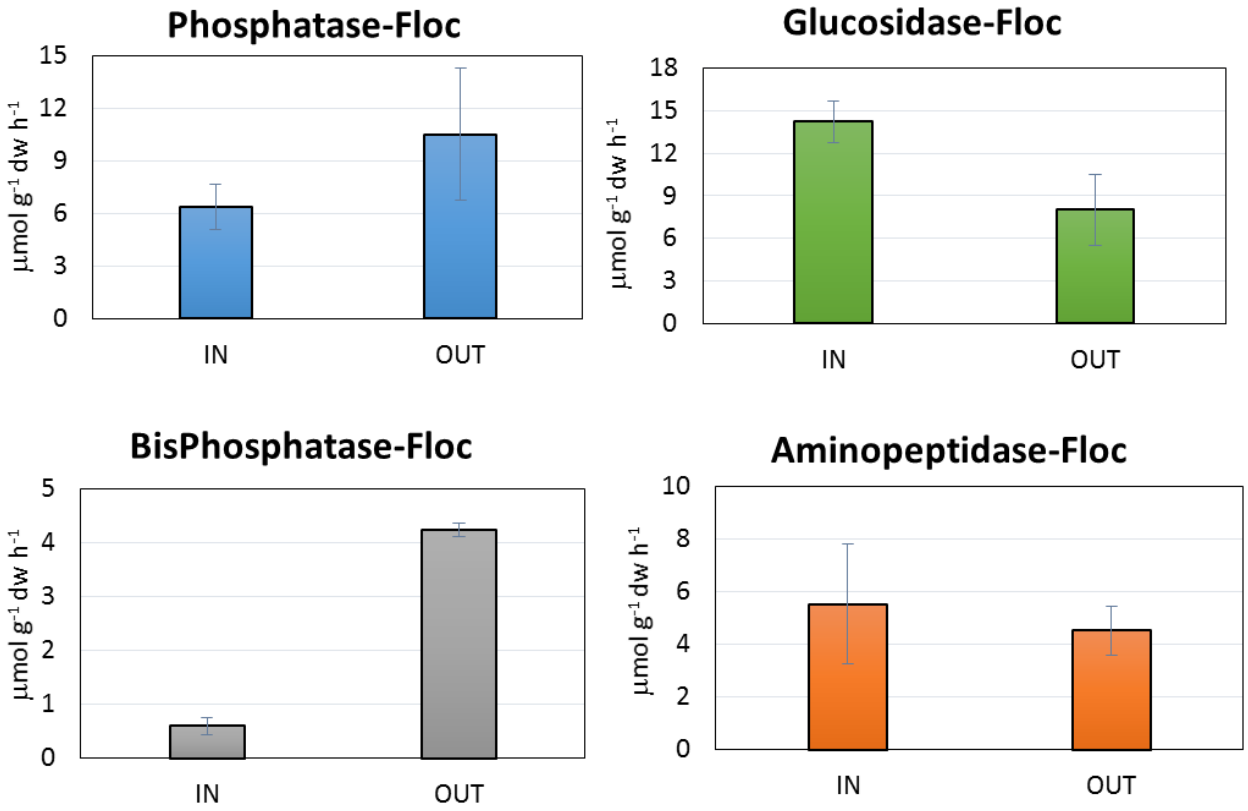


Figure 9. UF results for enzyme activity analyses of split floc samples collected in September 2015 from STA-2 Cell 1 inflow (In) and outflow (Out) locations. Each bar represents the mean ± SE of two floc samples.

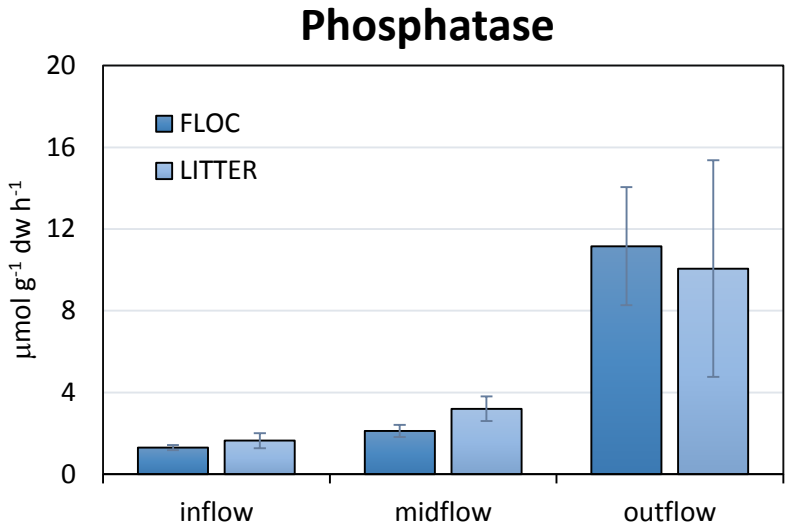


Figure 10. Potential phosphatase (monoesterase) activity measured in floc and litter samples collected in October 2015 from 3 STA-2 Cell 1 sampling locations. Each bar represents the mean ± SE of three cores.

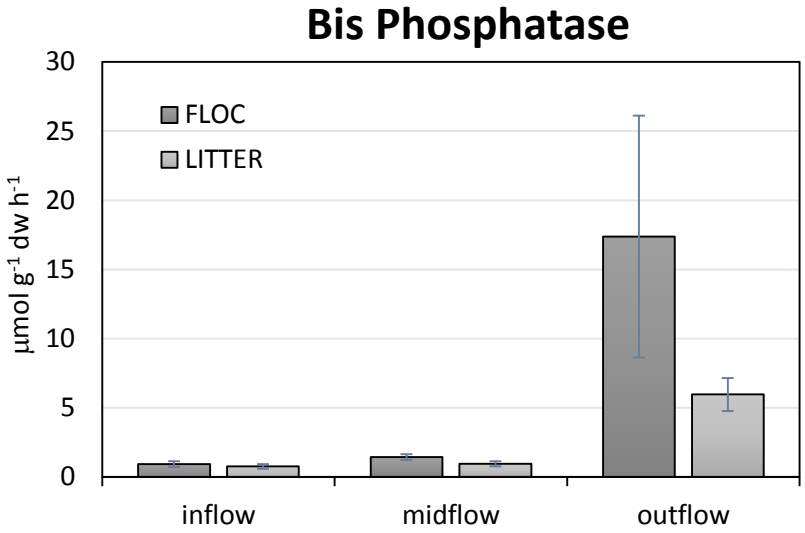


Figure 11. Potential phosphatase (diesterase) and (Bis-phosphatase) activity measured in floc and litter samples collected in October 2015 from 3 STA-2 Cell 1 sampling locations. Each bar represents the mean ± SE of three cores.

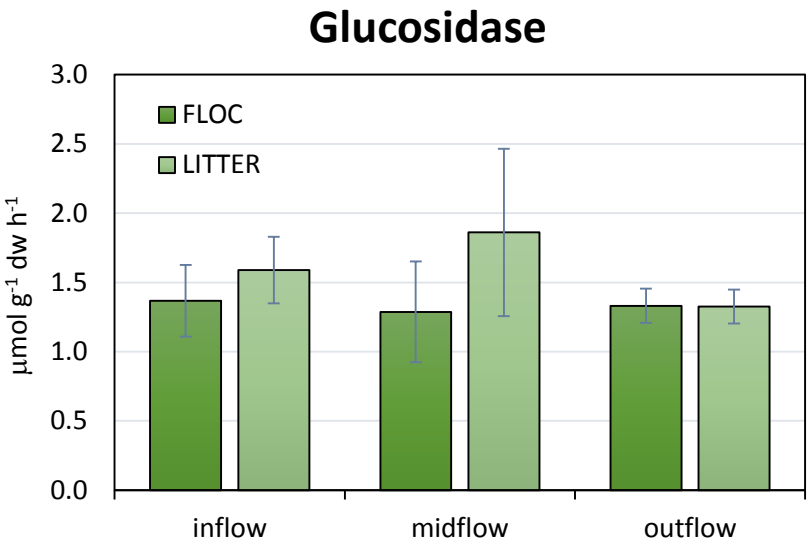


Figure 12. Potential β-glucosidase activity measured in floc and litter samples collected in October 2015 from 3 STA-2 Cell 1 sampling locations. Each bar represents the mean ± SE of three cores.

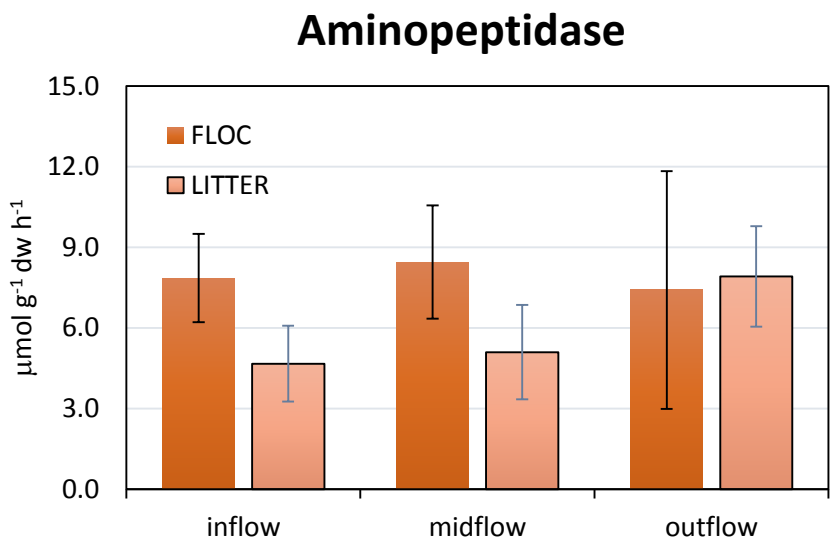


Figure 13. Potential Leucine aminopeptidase activity measured in floc and litter samples collected in October 2015 from 3 STA-2 Cell 1 sampling locations. Each bar represents the mean ± SE of three cores.

It is too early to determine whether the observed differences in the patterns of carbon and nitrogen enzyme activity reflect an effect of season including differences in temperature, inflow water composition, or plant growth and senescence. Overall, the results of phosphatase activity are highly consistent with similar observations in the Everglades systems, and are consistent with the theory that phosphorus availability acts to suppress this enzyme activity. As a result, the approximately tenfold range in phosphatase activity in STA-2 shows significant promise for using this enzyme as a sensitive indicator of phosphorus availability.

It is important to note that these findings are preliminary. As the work progresses, a more fully developed analyses of enzyme activity in various ecosystem components will likely emerge. Understanding the patterns both spatially and temporally as they respond to changes in water column nutrients and seasonal temperature and biological changes will then enable interpretation of enzyme activities as they relate to phosphorus availability and microbial decomposition.

4.3 Work in Progress

Currently the floc and litter samples are being analyzed for organic matter content to assist in the interpretation of enzyme activities. Enzyme activity ratios and correlations of enzyme activities with other biogeochemical parameters measured in these ecosystem components is also being investigated. Additional sampling for enzyme activity is proceeding and other STA's, and samples of floc and litter will be analyzed during a flow experiment which is scheduled for Feb 2016 in STA-2 Cell 3.

5 Planned Activities

The following activities are planned for the next quarter (January 1 to March 31, 2016).

- Completion of spatial soil sampling in STA-2 Cell 1 and STA-3/4 Cell 3A starting January 2016. This will also include floc, soil and water sample collections from transect and benchmark sites.
- Water quality data analysis from the second flow event – impact of Lake Okeechobee water release to STAs.
- Initial review of soil data by all project personnel will commence upon receipt of results of chemical analysis.
- Complete the microbial biomass determination of floc and soil samples collected along the transect.
- Conduct mineralogical analysis of water samples collected from selected locations.
- Conduct enzyme analysis of floc and litter samples collected during flow experiments.

6 References

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