SEPTEMBER   **2016**   **QUARTERLY REPORT**

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 July – September 2016, 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 Year 2 of the project and included various activities that were initiated to meet the objectives laid out under multiple tasks.

Contents

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 surface water; (3) enhance the understanding of biotic and abiotic mechanisms and factors regulating P dynamics, especially in the lower reaches of the 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, studies will be conducted at select sites along soil P and vegetation gradients in WCA-2A for comparison. 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:
- Tasks 3 and 4. Year two soil sampling – Transect and benchmark sites
- Task 7a. Transect study: Surface water quality monitoring

2   Transect and Benchmark Soil Sampling [Task 3 and Task 4]

The objective of this task is to revisit established transect and benchmark locations and collect information pertaining to nutrient storages in floc and soils. A more comprehensive analysis of these samples will provide information that will help enhance our understanding of biogeochemical transformations occurring within the surface water, across the soil-surface water interface and within the soil column. Comparison of baseline soils conditions documented during the first two years of the study can offer insights into short term temporal changes in soil characteristics and associated P removal mechanisms. Soil sampling locations included three benchmark sites (inflow, midflow, and outflow) along the transect parallel to the flow direction, from the inflow to outflow points of the cell. The transect sites were co-located with several other ongoing studies such that all studies could mutually benefit from the information generated at these sites
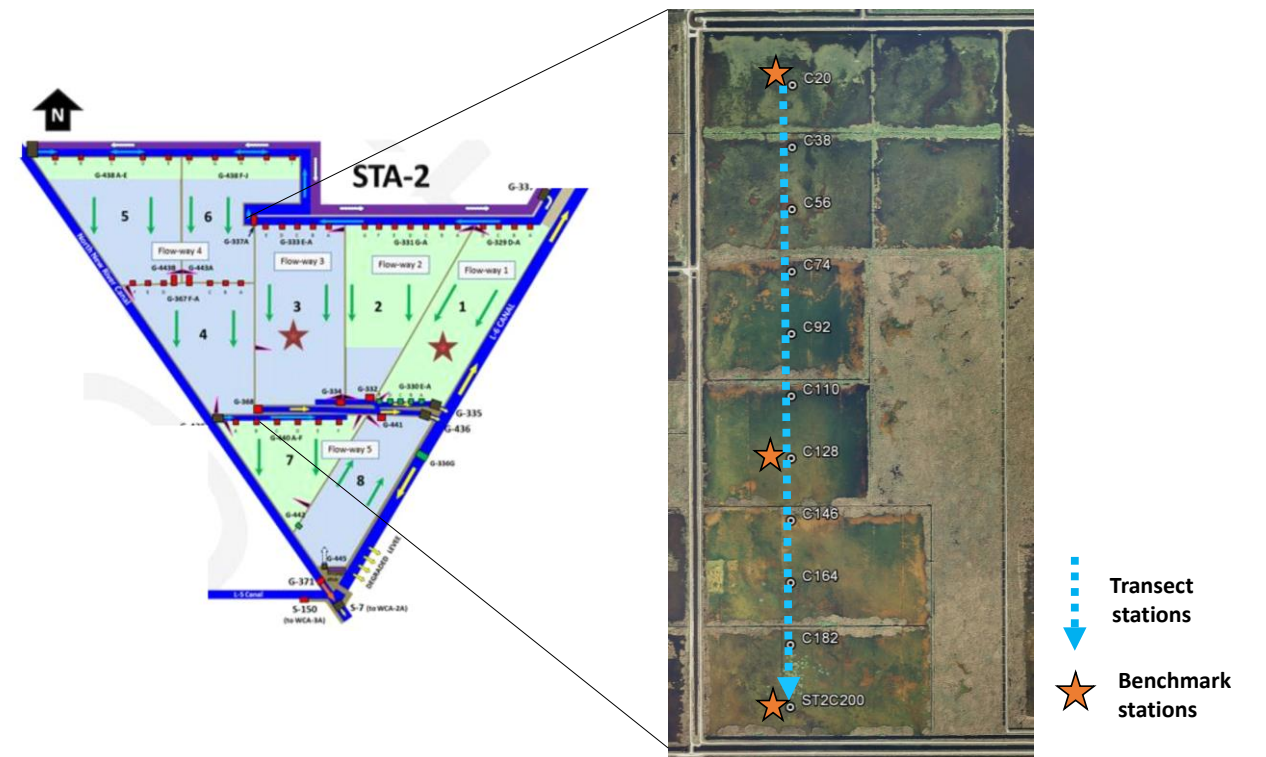
2.1   Work Completed During This Quarter

Year two transect and surface water samplings were conducted in STA-2 Cell 1 and Cell 3 in September 2016 with coordination between UF and District project personnel.

2.2   STA-2 – Cell 3

STA-2 Cell 3 has predominantly submerged aquatic vegetation (SAV) with a large patch of emergent aquatic vegetation (EAV; primarily *Typha domingensis*) in the eastern region of the cell. Floating aquatic vegetation (like *Pistia stratiotes* and *Nymphaea odorata*) were also observed in some open areas of the cell.

Transect soil and grab surface water samplings were conducted in STA-2 Cell 3 from 7th to 9th of September 2016 (Figure 2-1). Intact soil cores were collected along an established transect consisting of 11 stations (station ids – 20, 38, 56, 74, 92, 110, 128, 146, 164, 182 and 200). Three of these stations, C20 (inflow), C128 (midflow) and C200 (outflow) were sampled as benchmark
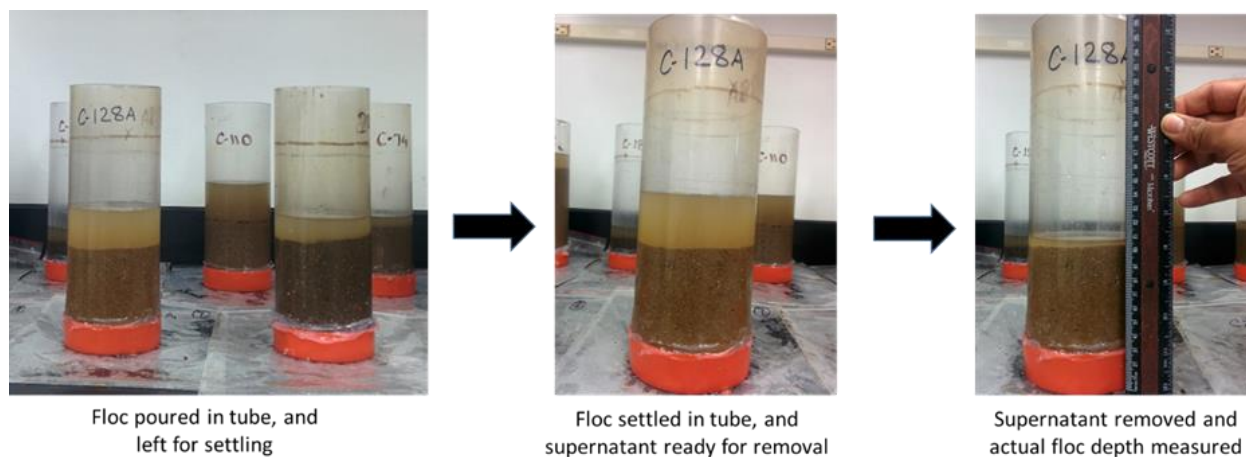
sites, where triplicate soil and water samples were taken [*See Appendix 1 for GPS coordinates of sampling locations*]. Four intact soil cores were taken from each location, to meet the amount of samples needed for the different studies. A total of 68 cores (17 x 4) were obtained. Soil cores were stored in a cold room (~4ºC) until they can be sectioned into plant litter (when present), floc, recently accreted soil (RAS) and pre-STA soil fractions.

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

Floc was characterized as the suspended unconsolidated material on top of consolidated RAS. It was poured into a plastic bag and underlying RAS layer was collected in a separate bag after recording its thickness. Floc depth was measured by allowing 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 discarded and thickness of settled floc was measured (Figure 2-2, steps 1 through 3).

Floc poured in tube, and
left for settling

Floc settled in tube, and
supernatant ready for removal

Supernatant removed and
actual floc depth measured

Figure 2-2: Three step process for determination of floc depth.

Pre-STA soils were divided into two sections: 0-5 cm and 5 - 15 cm, or up to compete depth of the soil core, whichever happen to be smaller (Table 2-1). Pre-STA soil was discernible from the overlying RAS and its thickness (depth) varied from one location to another depending on the total depth of the soil core.

Floc and soil sections from the four soil cores were thoroughly mixed and weighed before dividing into four portions. One portion was submitted to the District while three others were sent to the UF-Wetland Biogeochemistry Lab for analyses. Two portions were sent to WBL, Gainesville for P fractionation and microbial /enzyme analysis and one to Ft. Pierce for P-isotherm studies.

Grab surface water samples were processed and submitted to District laboratory for the analysis of total phosphorus (TP), total dissolved P (TDP), soluble reactive P (SRP), ammonium nitrogen (NH_4^+), nitrous oxides, NO_x (NO_3^-+NO_2), dissolved organic nitrogen (DON), total N (TN), dissolved organic carbon (DOC), alkalinity, calcium, magnesium, chloride, sodium, sulfate and chlorophyll-a.

Field parameters such as surface water pH, specific conductance, temperature and dissolved oxygen were measured using a hand held YSI. Soil and water depths were also recorded at each station at the time of sampling.

Table 2-1. STA-2 Cell 3: Depth of various sections after separating intact soil cores.

STA-2 Cell 3 Site #	Station id	Thickness of section (cm)			
		Floc	RAS	Pre-STA-1 (0-5 cm)	Pre-STA-2 (5-15 cm)
1*	C-20A	6.48	3.50	5.0	5.25
2*	C-20B	6.48	4.50	5.0	8.00
3*	C-20C	5.53	4.38	5.0	2.75
4	C-38	10.15	5.13	5.0	14.00
5	C-56	7.70	4.75	5.0	10.00
6	C-74	8.00	5.00	5.0	12.75
7	C-92	6.63	6.13	5.0	9.13
8	C-110	10.68	3.25	5.0	11.88
9*	C-128A	6.80	2.88	5.0	6.38
10*	C-128B	5.08	1.75	5.0	13.13
11*	C-128C	4.53	2.13	5.0	12.25
12	C-146	4.85	2.38	5.0	15.0
13	C-164	7.68	2.38	5.0	15.0
14	C-182	7.25	3.25	5.0	15.0
15*	C-200A	9.73	2.13	5.0	15.0
16*	C-200B	7.05	1.88	5.0	15.0
17*	C-200C	9.23	2.50	5.0	15.0

*Benchmark locations.

2.3   STA-2 – Cell 1

STA-2 Cell 1 is categorized as emergent aquatic vegetation (EAV) cell with primarily *Typha domingensis* with patches of *Cladium jamaicense* and *Nymphaea odorata*. Transect soil and grab surface water samplings were conducted in STA-2 Cell 1 from 20th to 22nd of September 2016 (Figure 2-3). Intact soil cores were collected along an established transect consisting of 11 stations (station ids – 34, 51, 69, 86, 104, 121, 138, 156, 173, 191 and 208). Three of these stations, 34 (inflow), 121 (midflow) and 208 (outflow) were sampled as benchmark sites, where triplicate soil and water samples were taken [*See Appendix 1 for GPS coordinates of sampling locations*]. Four intact soil cores were taken from each location, to meet the amount of samples needed for the different studies. A total of 68 cores (17 x 4) were obtained. Soil cores were stored in a cold room (~4ºC) until they can be sectioned into plant litter (when present), floc, recently accreted soil (RAS) and pre-STA soil fractions.
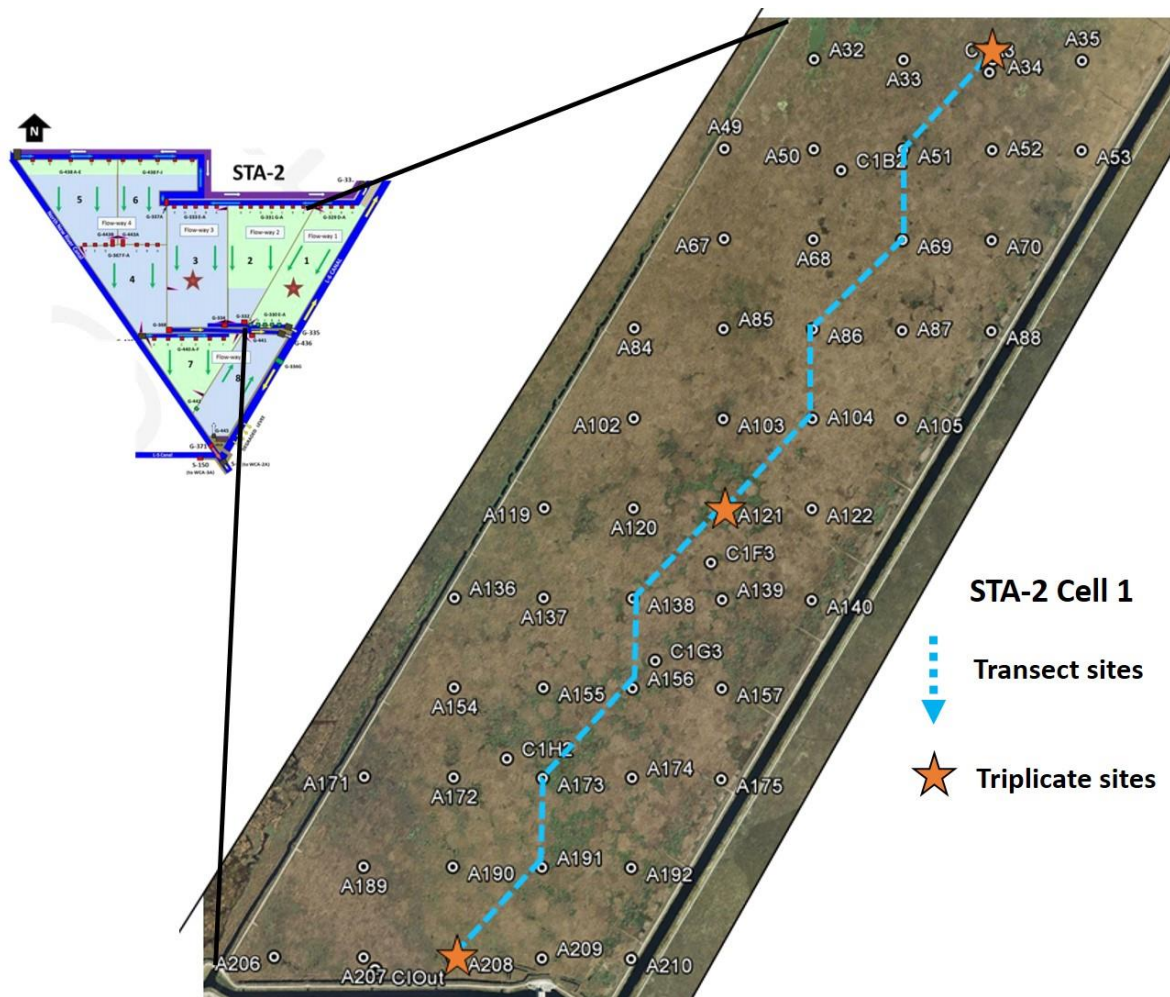
Figure 2-3. STA-2 Cell 1 – Soil and surface water sampling locations.

Floc was characterized as the suspended unconsolidated material on top of consolidated RAS. It was poured into a plastic bag and underlying RAS layer was collected in a separate bag after recording its thickness. Floc depth was measured by allowing 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 discarded and thickness of settled floc was measured (Figure 2-2, steps 1 through 3).

Pre-STA soils were divided into two sections: 0-5 cm and 5-15 cm, or up to compete depth of the soil core, whichever happen to be smaller (Table 2-2). Pre-STA soil was discernable from the overlying RAS and its thickness (depth) varied from one location to another depending on the total depth of the soil core.

Floc and soil sections from the four soil cores were thoroughly mixed and weighed before dividing into four portions. One portion was submitted to the District while three others were sent to the UF-Wetland Biogeochemistry Lab for analyses. Two portions were sent to WBL, Gainesville for P fractionation and microbial /enzyme analysis and one to Ft. Pierce for P-isotherm studies.

Grab surface water samples were processed and submitted to District laboratory for the analysis of total phosphorus (TP), total dissolved P (TDP), soluble reactive P (SRP), ammonium nitrogen (NH_4^+), nitrous oxides, NO_x (NO_3^-+NO_2), dissolved organic nitrogen (DON), total N (TN), dissolved organic carbon (DOC), alkalinity, calcium, magnesium, chloride, sodium, chloride sulfate and chlorophyll-a.

Field parameters such as surface water pH, specific conductance, temperature and dissolved oxygen were measured using a hand held YSI. Soil and water depths were also recorded at each station at the time of sampling (Figure 2-3).

Table 2-2. STA-2 Cell 1: Depth of various sections after separating intact soil cores.

STA-2 Cell 1 Site #	Station id	Thickness of section (cm)			
		Floc	RAS	Pre-STA-1 (0-5 cm)	Pre-STA-2 (5-15 cm)
1*	A-34A	5.35	6.50	5.0	13.5
2*	A-34B	9.70	5.50	5.0	12.25
3*	A-34C	8.15	4.38	5.0	12.5
4	A-51	5.98	4.13	5.0	12.25
5	A-69	5.98	1.38	5.0	12.75
6	A-86	3.98	1.88	5.0	15.00
7	A-104	7.28	2.88	5.0	12.25
8*	A-121A	5.78	2.00	5.0	15.0
9*	A-121B	6.18	2.63	5.0	15.0
10*	A-121C	4.75	2.25	5.0	14.63
11	A-138	3.13	3.25	5.0	15.0
12	A-156	4.20	1.38	5.0	15.0
13	A-173	3.85	3.88	5.0	12.75
14	A-191	2.35	3.25	5.0	15.0
15*	A-208A	1.10	2.00	5.0	15.0
16*	A-208B	1.55	2.50	5.0	15.0
17*	A-208C	1.05	2.13	5.0	12.75

*Benchmark locations.

3 Soil Phosphorus Fractionation [Task 5]

This task involves determination of the forms and distribution of P in the floc and RAS layers at the sampling locations using an operationally-defined P fractionation scheme (Figure 3-1). This information will be used to assess the relative proportion of reactive and stable P pools in the different soil layers and to explore correlative relationships between the various P pools, soil physical and chemical characteristics, flux rates, sorption and desorption characteristics, and surface water P species and concentration.

Chemical separation of the different P forms will be conducted on wet samples using the fractionation method developed by Ivanoff et al. (1998) and Richardson and Reddy (2013). All laboratory work related to P fractionation will be performed by the Wetland Biogeochemistry Lab, University of Florida.
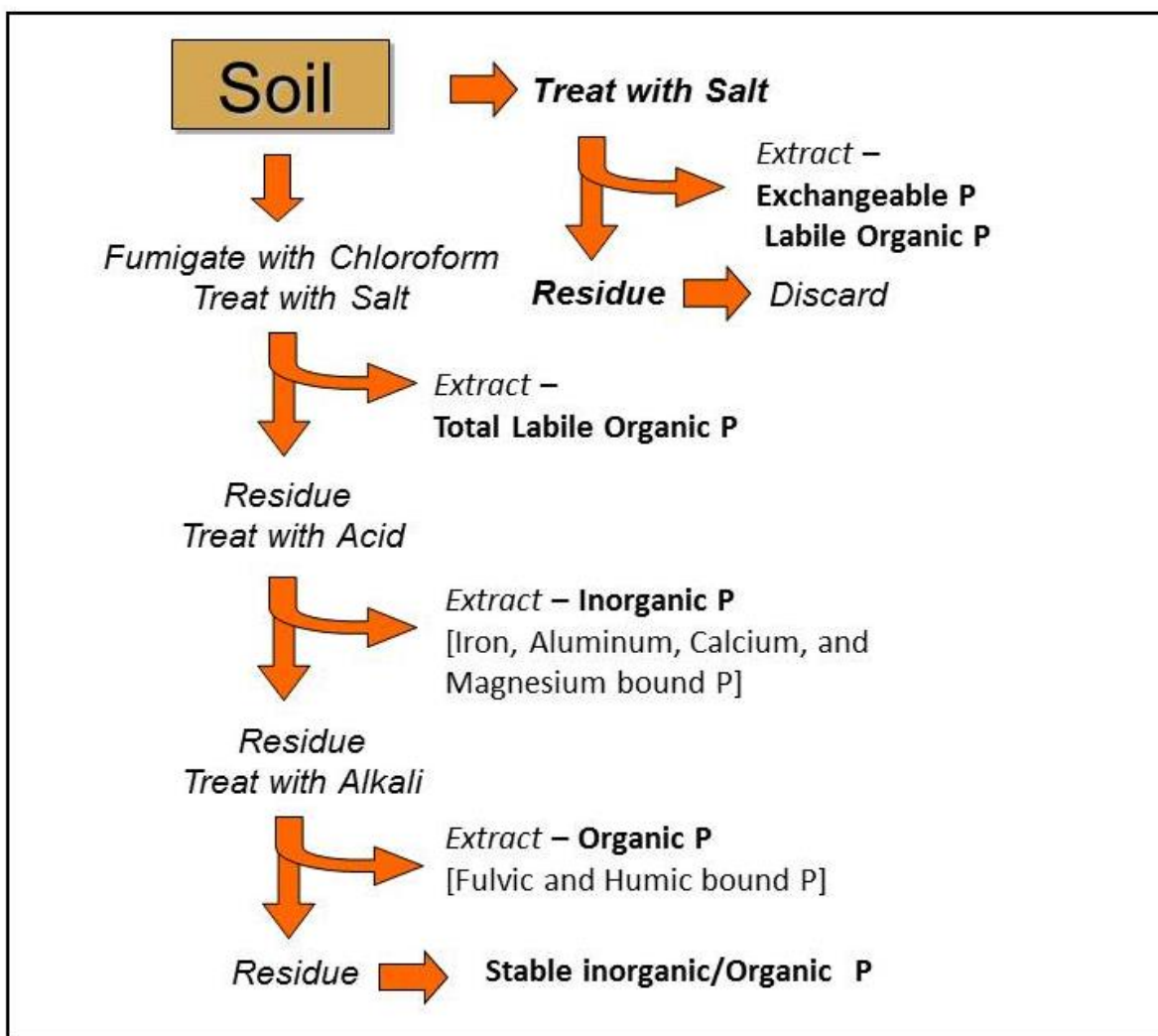

Figure 3-1: Schematic showing operationally defined P fractionation scheme used in wetland soils (Reddy and Delaune, 2008)

Soil samples were obtained from STA-2 Cell 1 and Cell 3 as described in Task 3 of this report. Soil and floc samples are subjected detailed fractionation scheme as shown in Figure 3-1. Preliminary results will be presented in the next quarterly report.

Table 3-1. Station names within the established transects from where soil cores are obtained for P fractionation during Year 2. Stations in 'bold" letter are sampled in triplicate.

Location	Stations
STA-2 Cell 1	**34,** 51, 69, 86, 104, **121**, 138, 156, 173, 191, and **208**
STA-2 Cell 3	**20,** 38, 56, 74, 92, 110, **128**, 146, 164, 182, and **200**
STA-3/4 Cell 3A	**A8,** A20**, A32,** A44**, A56**
STA-3/4 Cell 3B	**A7, A7c,** B7, B7c, **C7, C7c, D7** and **D7c**
WCA-2A	**F1, F2, F4, E5**, and **U3**

4   Phosphorus Sorption/Desorption Characteristics of STA soils [Task 6a]

Preliminary research was conducted to determine optimal P sorption/desorption parameters for the phosphorus sorption experiments. Soil, floc, and recently accreted soil were obtained from soil cores, mixed, and stored at 4°C. Preliminary investigations addressed optimal incubation conditions, either under an aerobic or anaerobic atmosphere (Table 4-1). Research indicated no significant difference in P sorption/desorption caused by incubation vessel atmosphere, so the aerobic atmosphere was utilized as it offers greater speed and efficiency of analysis.

Table 4-1. Preliminary data on the influence of aerobic and anaerobic conditions on P sorption on STA soils

			P sorbed	P sorbed
		P in 0.01 M KCl	ug/g	ug/g
		Conc (ug/ L)	aerobic	anaerobic
Soil 1	0.0 ug P/L in 0.01M KCl	0	-3.87	-3.67
Soil 1	10 ug P/L in 0.01M KCl	10	-2.01	-2.09
Soil 1	50 ug P/L in 0.01M KCl	50	-0.46	-0.67
Soil 1	100 ug P/L in 0.01M KCl	100	1.02	1.18
		Conc (ug/ L)	aerobic	anaerobic
Soil 2	0.0 ug P/L in 0.01M KCl	0	-1.42	-1.36
Soil 2	10 ug P/L in 0.01M KCl	10	-0.86	-0.97
Soil 2	50 ug P/L in 0.01M KCl	50	1.43	1.65
Soil 2	100 ug P/L in 0.01M KCl	100	3.73	3.32

Other experimental conditions being evaluated include comparison of the different equilibrating solutions i.e., 0.01 M KCl, straight or diluted outflow site water. Results of the preliminary runs will be summarized in the next quarterly report.

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

The objective of this task is to investigate changes in surface water nutrients under different flow scenarios and understand changes in P concentrations, and movement within a flow way. The approach is to collect high spatial-temporal resolution data using auto-samplers. Auto-samplers were deployed at six locations along a transect from inflow to outflow of Cell 3 (Figure 5-1 and Figure 5-2].
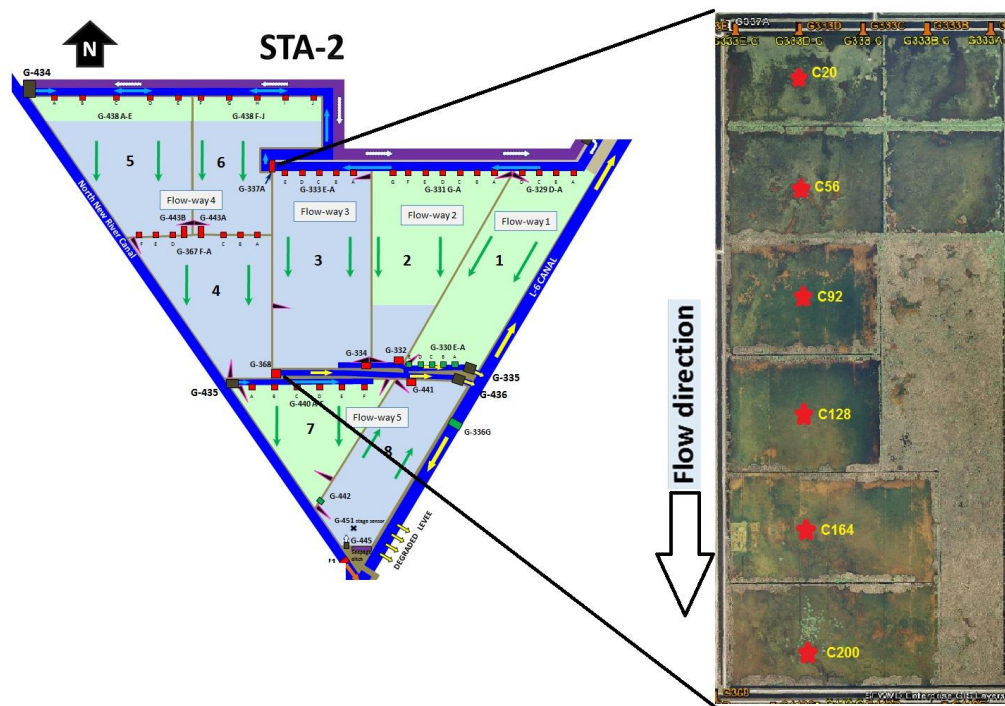

Figure 5-1: Water quality monitoring stations in STA-2 Cell 3 for continuous monitoring using autosamplers and for weekly grab water sampling.

The fourth flow event consisted of a No Flow-Flow-No Flow-Flow pattern for a 63-day period with a total flow of 71.12 x 10^5 and 61.55 x 10^5 m^3d^{-1} during each respective flow period (Table 5-1). During the flow event, water samples were collected every four hours (2 am, 6 am, 10 am, 2 pm, 6 pm and 10 pm) and analyzed for TP on discrete samples and for total nitrogen (TN) and total organic carbon (TOC) on daily composited samples. Weekly surface water grab samples were also collected from these sites for the analysis of TP, TN, SRP, Ca, Mg, NH_4^+, NOx, DOC, iron (Fe), sulfate, chloride, alkalinity, color and chlorophyll by the District lab.

During the flow event a submerged probe (EXO Sonde) was also deployed to record physical and optical surface water parameters at a temporal resolution of 15-min intervals. These parameters included temperature, pH, dissolved oxygen, specific conductivity, turbidity and chlorophyll. Generally, water temperature, pH, dissolved oxygen, turbidity and chlorophyll

showed diurnal fluctuations with peaks corresponding to the day-night cycle while specific conductivity did not show any diurnal patterns.
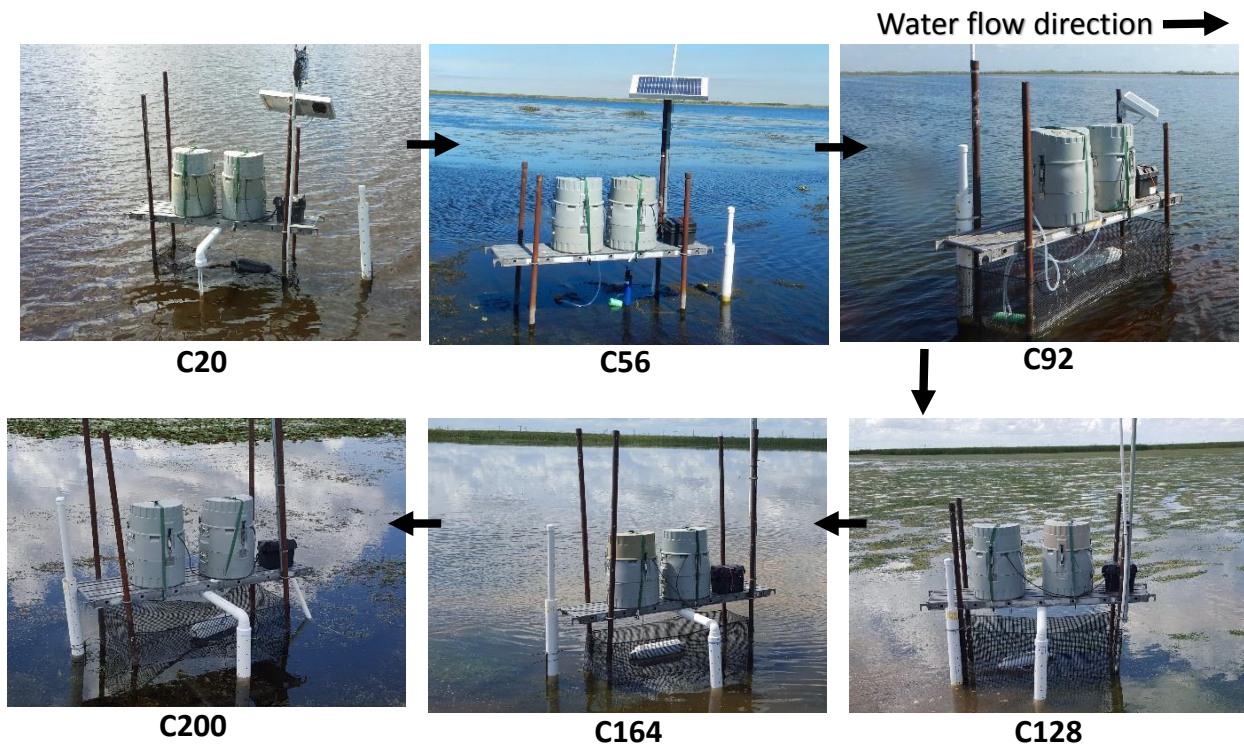
Water flow direction ➡

Figure 5-2: Auto-sampler platforms along the transect stations within STA-2 Cell 3.

Data collected during the fourth flow event (June 27 to August 19, 2016) were organized for statistical analysis after preliminary trend analysis. High temporal resolution data on field parameters from EXO-sondes were processed and are presented here.

5.1 Data Analysis – Methods

Water quality data from the auto-samplers and weekly grab samples were obtained from the District database. Data were organized into four groups to allow comparison of surface water nutrient concentrations among the different phases of the flow event in each of the six stations (Table 5-1).

Table 5-1. Mean (±SE), Total Flow, Hydraulic Loading Rate (HLR) and Phosphorus Loading Rate (PLR) for the fourth flow event in STA-2 Cell 3 (June 27-August 29, 2016)

Date	Phase	Flow	Flow Volume (x 10^5 m^3 d^{-1})		HLR (cm d^{-1})	PLR (mg m^{-2} d^{-1})
			Mean ± SE	Total		
Jun 27- Jul 2 2016	Phase 0	No Flow	0.00	0.00	0.00	0.00
Jul 3- Jul 24, 2016	Phase 1	Flow	3.23 ± 0.17	71.12	3.48 ± 0.19	1.60 ± 0.13
Jul 25-Aug 8 2016	Phase 2	No Flow	0.00	0.00	0.00	0.00
Aug 9-Aug 29 2016	Phase 3	Flow	2.93 ± 0.46	61.55	3.15 ± 0.49	2.89 ± 0.42

The data were screened for outliers using a non-parametric (distribution-free) approach (Julian and Hill, 2012). Values greater than the 99th percentile for a given station and period were identified as an outlier and removed from further statistical analysis. Unless otherwise noted all statistical operations were performed using the base stats R-package. Daily mean values for each parameter and station were compared between flow periods during each respective flow event using Dunn's test of multiple comparisons. The Dunn's test is a non-parametric multiple pairwise comparison after a Kruskal-Wallis test for stochastic dominance among groups and is analogous to one-way Analysis of Variance (ANOVA). All statistical operations were performed with R© (Ver 3.1.2, R Foundation for Statistical Computing, Vienna Austria). The critical level of significance was set to $\alpha = 0.05$. Spearman rank sum correlation analysis was performed on calculated total organic nitrogen (Table 5-2) and dissolved organic carbon. In addition to nutrient data, ion data were collected during this flow event. Surface water ion concentrations (i.e. sodium, potassium, calcium, magnesium, chloride, bicarbonate and sulfate) were converted from mass based (mg L^{-1}) to milli equivalents (meq L^{-1}) concentrations and an ion balance was performed for each sampling day and site. If the percent difference between the anions and cations were greater than ten percent (10%), the sample was excluded from further analysis due to uncertainty of the ion balance to reduce analytical uncertainties.

Table 5-2. Derived parameters estimated from analytically determined parameters.

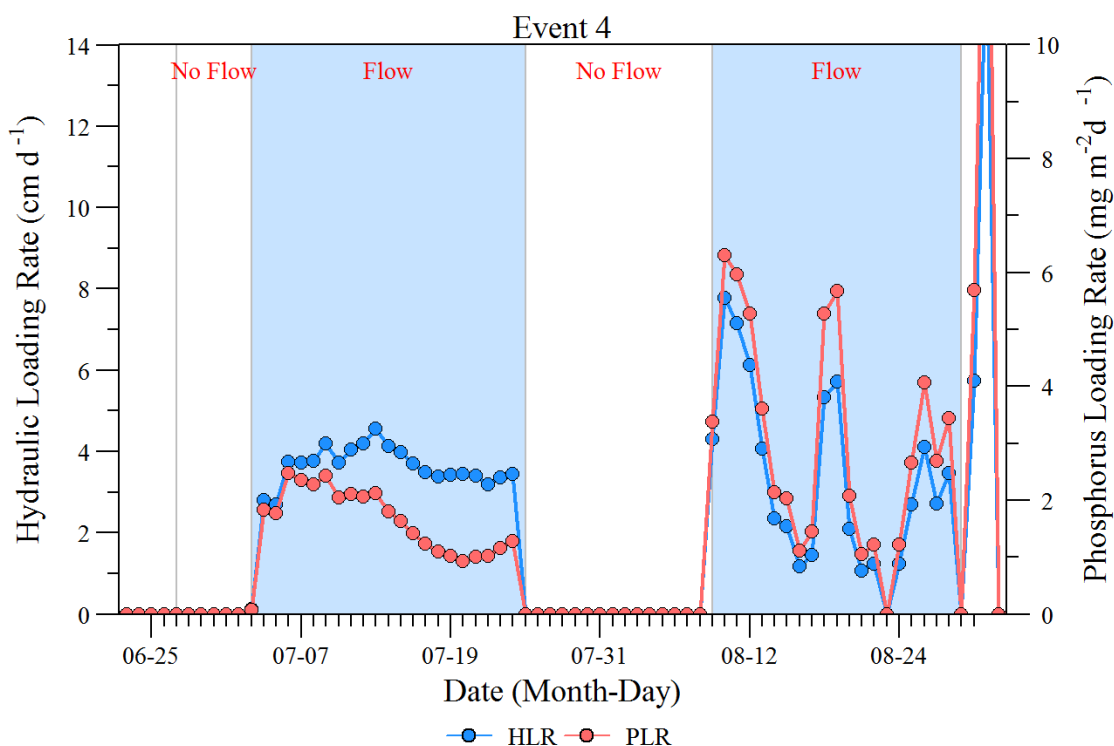| Parameter	Derivation
Particulate Phosphorus	Total Phosphorus – Total Dissolved Phosphorus
Dissolved Organic Phosphorus	Total Dissolved Phosphorus - Soluble Reactive Phosphate
Total Inorganic Nitrogen	Nitrate + Nitrite + Ammonia
Total Organic Nitrogen	Total Nitrogen - Total Inorganic Nitrogen
Bicarbonate	Alkalinity (as $CaCO_3$) x 1.22

Figure 5-3: Hydraulic loading rate (HLR) and phosphorus loading rate (PLR), STA-2, Cell 3, (June 27-August 29, 2016.)

The fourth flow event generated a total of 2,250 samples during a 63-day period. During the fourth flow event a relatively small number of suspected outliers were detected with approximately 1.5% of the samples identified as outliers using the station and flow period 99th percentile. Overall 2,250 samples were collected with 34 of these points identified as outliers (Table 5-3).

Table 5-3. Total number of autosampler total phosphorus samples that passed QA/QC screening collected during the fourth flow event, numbers in the parentheses are the total number of outlier samples per station and flow period.

Flow Period	C20	C56	C92	C128	C164	C200	Total
Phase 0	36 (1)	36(1)	33 (1)	34(1)	36 (1)	36 (1)	211 (6)
Phase 1	132 (2)	132 (2)	132 (2)	116 (1)	131 (2)	130 (2)	773 (11)
Phase 2	69 (0)	88 (1)	90 (1)	87 (1)	88 (1)	90 (1)	512 (5)
Phase 3	126 (2)	126 (2)	126 (2)	123 (2)	125 (2)	126 (2)	752 (12)

Similar to previous flow events, TP concentration declined along the flow path with a general decrease of daily mean TP observed at each station, however during this flow event stations (i.e. C92) at the transect mid-point experienced higher TP concentrations than the site near the inflow region of the cell (Figure 5-4 and Figure 5-5; χ^2=261.48, df=5, P<0.05). Generally, TP concentrations decreased during the flow event at each station with the exception of site C56 (Figure 5-5 and Figure 5-7). Total P concentrations observed at the C92 site were consistently higher relative to the other sites along the flow transect (Figure 5-7 and Figure 5-8). This elevated values may have resulted from some sampling error, i.e. the intake strainer for auto-sampler set too low, allowing intake of particulate debris into the sample bottle (personal communication with field staff). During this flow period, elevated TP concentration were observed at the first three stations during the flow event potentially attributed to by high velocity during periods of flow, wildlife disturbances, nutrient flux from the soil, decomposition of vegetation or algae observed near the inflow.
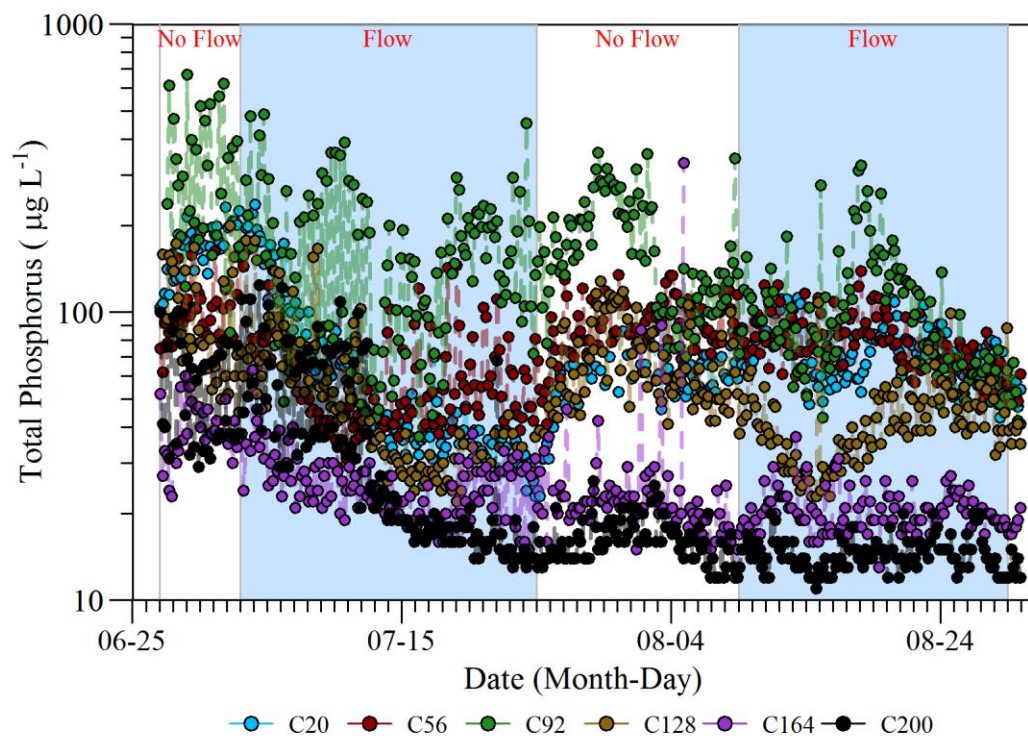
Figure 5-4: Autosampler total phosphorus concentration at locations along the STA-2 Cell 3 water quality transect during the fourth flow event. Note y-axis is on a log-scale and outliers were removed.
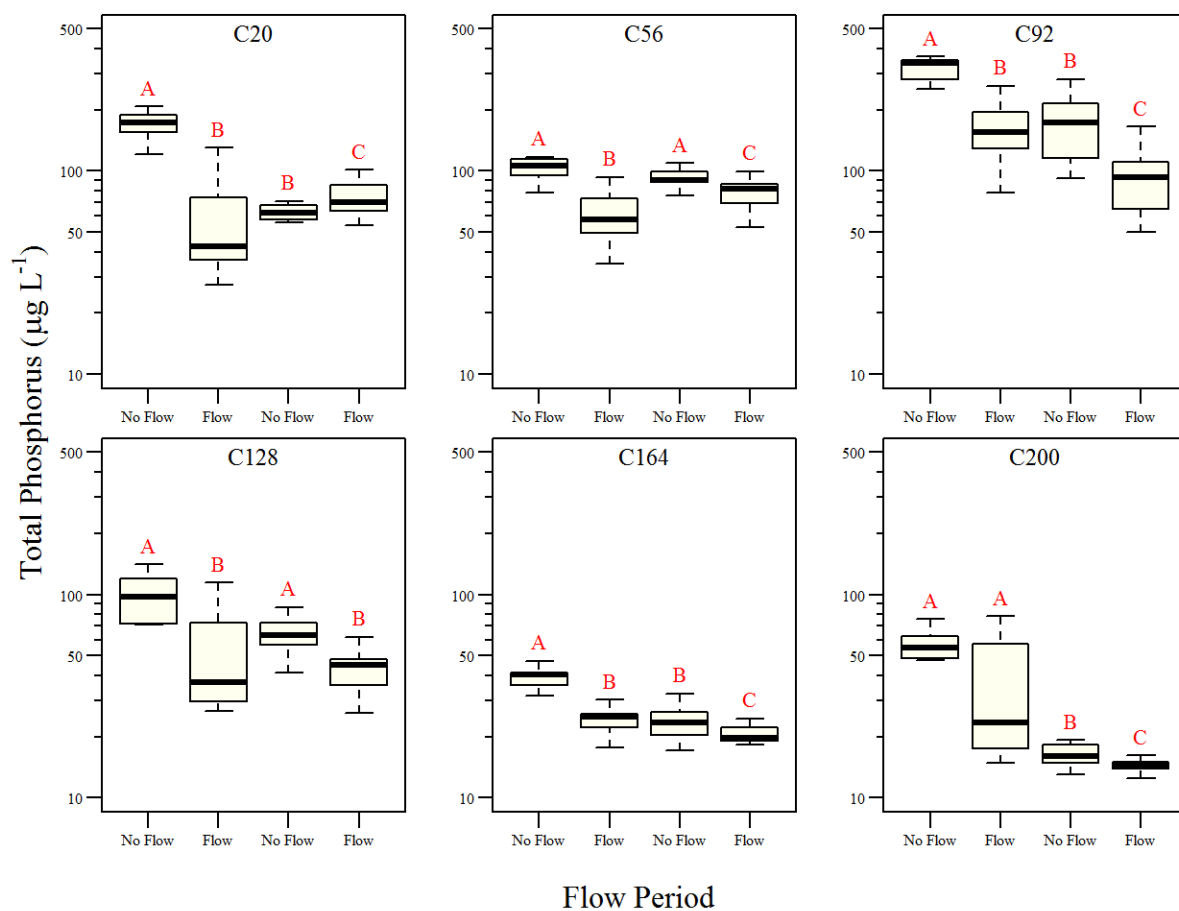
Figure 5-5: Boxplot of daily mean TP concentrations by flow period during the fourth flow event (June 27 to August 29, 2016). Letters indicate Dunn's Multiple Comparison results in daily TP concentrations between periods; different letters indicate statistically significant differences. Note y-axis is on a log-scale.
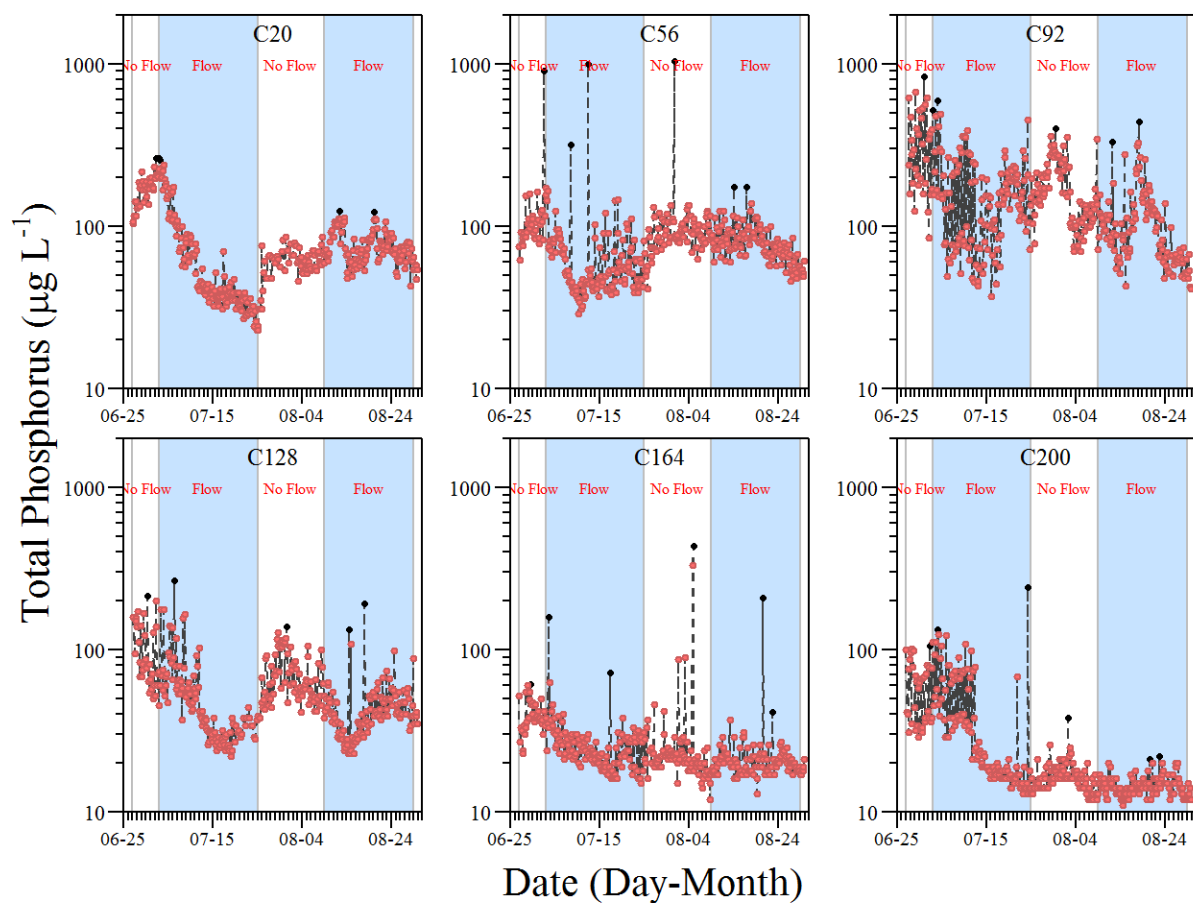
Figure 5-6: Autosampler total phosphorus concentrations sampled every four-hours at locations along the STA-2 Cell 3 water quality transect during the fourth flow event (June 27 to August 29, 2016). Note y-axis is on a log-scale, values that exceeded the station and flow period 99th percentile are identified as block and grey points.
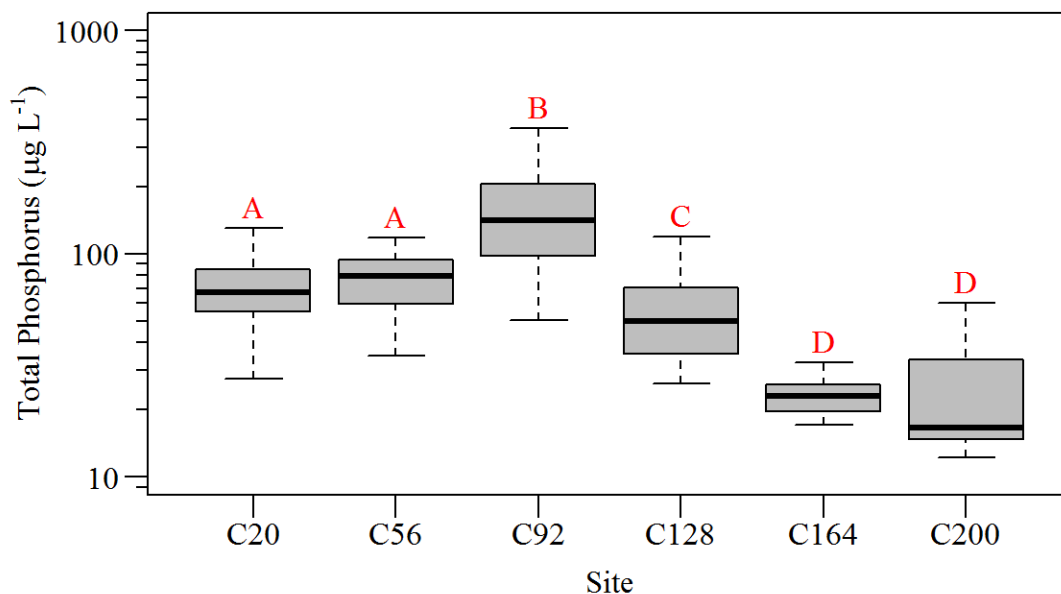
Figure 5-7: Boxplot of daily mean daily TP concentrations during the fourth flow event (June 27 to August 29, 2016). Letters indicate Dunn's Multiple Comparison results in daily mean TP concentration between sites; different letters indicate statistically significant differences. Note y-axis is on a log-scale.

5.1.1 Phosphorus and Nitrogen forms in grab samples

Total P, Total Dissolved P and Soluble Reactive P (SRP) in the surface water were determined on weekly grab samples, which helped to estimate Dissolved Organic P (DOP) and Particulate P (PP) in surface water. Soluble reactive P concentrations were greatest at the first two stations along the flow transect and highest during the first flow event at these stations. Except for a few occurrences, SRP was at the detection limit (2 μg L^{-1}). Particulate P constituted the largest proportion of TP in the surface water followed by DOP during all phases of this flow event (Figure 5-8). Highest TP concentrations were recorded during 'phase 0' (ranging from 61- 142 μg L^{-1}). Lowest TP concentrations were recorded during 'phase 3' (ranging from 12- 22 μg L^{-1}). During 'phase 0, and 1', PP was remarkably high at all stations along the transect, however it started to decline during 'phase 2' and was lowest during 'phase 3'.

Among all stations, proportion of TP as DOP was lower during 'phase 0' (13-30%) but increased as the flow event progressed such that DOP was 27 - 47 % of TP during 'phase 3'. Rapid growth and subsequent dying of floating filamentous algae (*Spirogyra spp.*) observed near the inflow of the cell during 'phase 3' may have contributed to the increased DOP and PP forms in the water. Furthermore, increased chlorophyll-a was observed during 'phase 3' at the first three sites (C20, C56 ad C92) (Figure 5-9 and Figure 5-10) and elevated dissolved organic carbon (DOC) concentration mid-cell during 'phase 3' (Figure 5-9). Particulate P concentrations were greatest during the initial No-Flow period presumably due to the prolonged period of no inflow to the cell due to limited water availability. This stagnant condition in combination with the growth and

die-off of algal and diatom communities could have contributed to the PP concentrations observed.
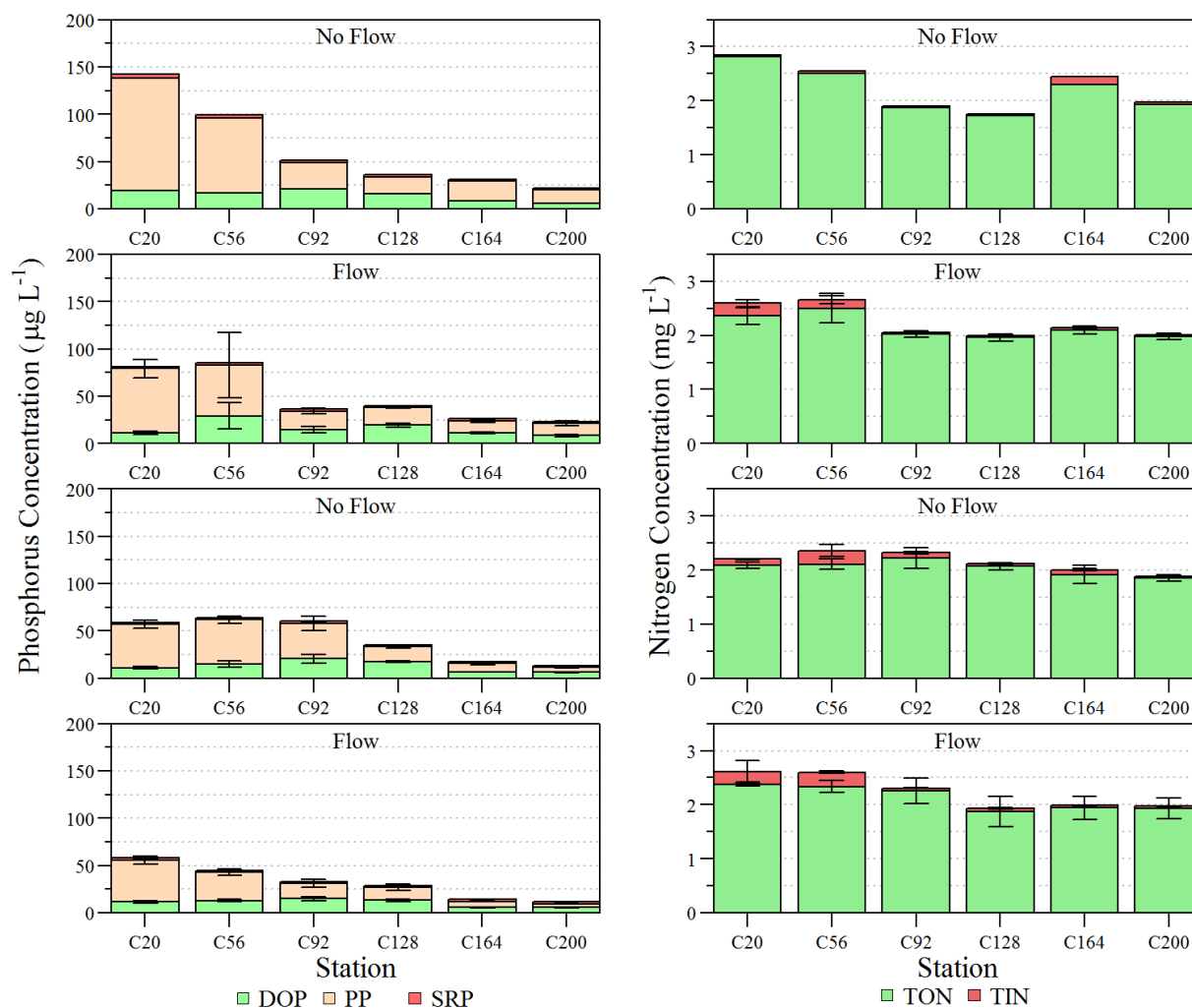

Figure 5-8: Phosphorus (Left) and Nitrogen (Right) fractions in surface water grab samples during fourth flow event.

In addition to the different P forms, total organic and total inorganic nitrogen (TON and TIN, respectively) were also investigate in the grab samples during the fourth flow event (Figure 5-8). Total nitrogen (TN) was variable between stations during different phases of the flow event, but the range of such differences were not noteworthy. Total N concentrations ranged between 1.5 to 4.8 mg L^{-1}. Total organic nitrogen comprises most of the TN derived from decomposition of organic matter and atmospheric nitrogen fixation by algae (Julian et al., 2016). Dissolved OC and TON were significantly positively correlated (Spearman's Correlation: $\rho=0.65$, $\rho<0.001$). Generally, TIN concentrations were highest at the front end of the cell (at C20 and C56).

5.1.2 Other nutrients and metals

Dissolved OC in the surface water did not change appreciably along the flow-way throughout the flow event (Figure 5-9) with concentrations ranging from 18 to38 mg L^{-1}. Generally, DOC concentrations were greater during flow periods than no-flow(stagnant) conditions presumably due to surface waters with high DOC concentrations attributed to run off (Figure 5-9).

Surface water total iron and aluminum concentrations were elevated at the site nearest the inflow location along the flow path (Figure 5-11). Total aluminum concentrations ranged from 8 to 135 µg L^{-1} with highest concentrations at the inflow and quickly reached MDL of 8 µg L^{-1}. Similarly, total iron concentrations ranged from 3 to 106 µg L^{1-} and remained relatively high up to the mid-point of the cell where concentrations reached MDL of 3 µg L^{-1}.

Generally, the surface waters of STA-2 Cell 3 are co-dominated calcium (Ca)-carbonate(HCO_3)-sodium(Na)-chloride(Cl) waters based on the stiff diagram of median ion concentrations (in milli equivalents) (Figure 5-12). Waters entering the cell are rich in Ca and HCO_3 presumably due to the underlying geology. Notice the relative change in shape of the stiff diagrams[1] between flow periods for site C20 (Figure 5-12). During no flow conditions, Ca and HCO_3 are relatively low compared to the other ions, as flow initiates, Ca and HCO_3 increase from basin water entering the cell. Calcium and HCO_3 concentrations quickly reduce along the flow path presumably driven by changes in pH and biotic activity. Ion data from site C56 during the first 'no flow' period were screened from the analysis due to an ion balance outside of the acceptable range therefore these data points were not included in the analysis. Therefore, Ca interactions with SRP in the surface water could dominate at the beginning of a treatment cell and be less apparent or relevant towards the back end of the cell.

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[1] Stiff diagrams are a graphical representation of the general chemistry of water. A polygonal shape is created from four parallel horizontal axes extending on either side of a vertical axis. Cations are plotted on the left of the vertical axis and anions are plotted on the right. The diagrams can be relatively distinctive for showing water composition differences or similarities. The width of the pattern is an approximation of total ionic strength. One feature is the tendency of a pattern to maintain its characteristic shape as the sample becomes diluted. It may be possible to trace the same types of ground water contamination from a source by studying the patterns.
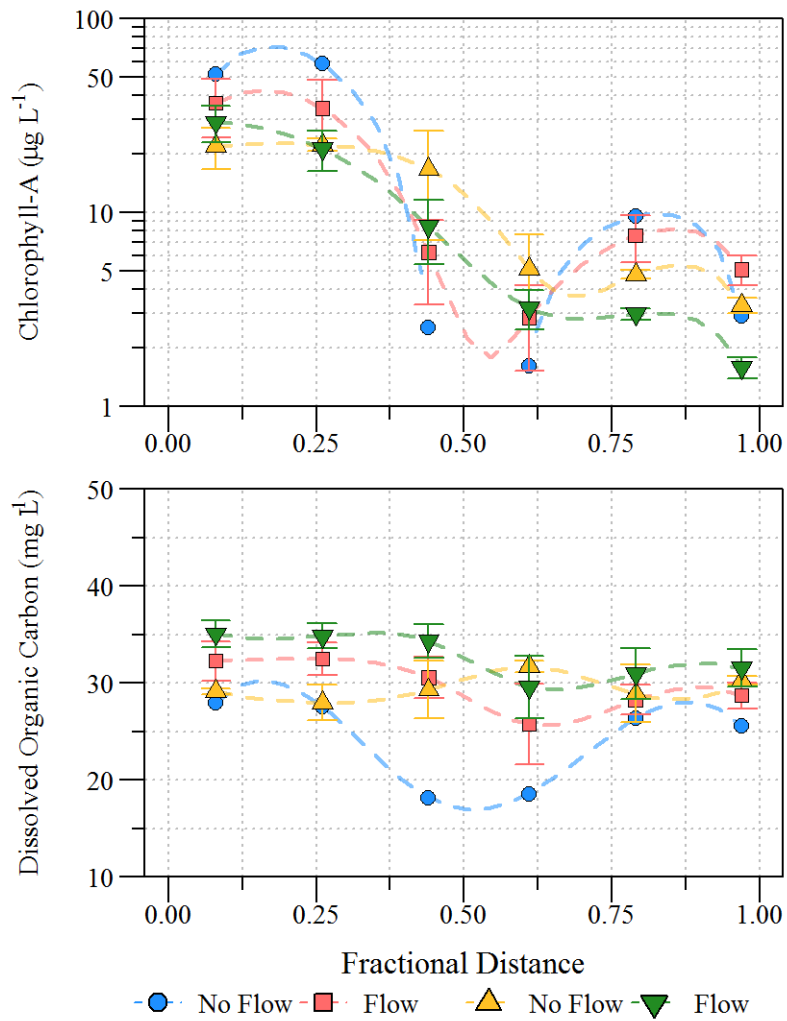
Figure 5-9: Flow period mean (±SE) chlorophyll-a and dissolved organic carbon concentrations during fourth flow event along the flow path.
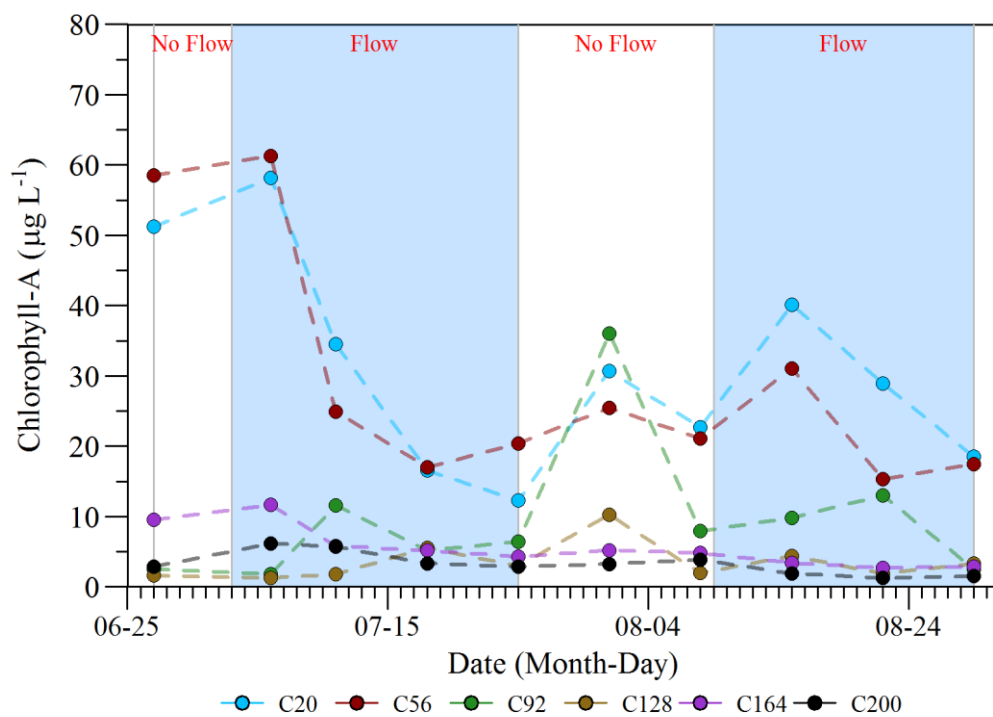
Figure 5-10: Change in chlorophyll-A concentrations at different stations during four phases of the flow event.

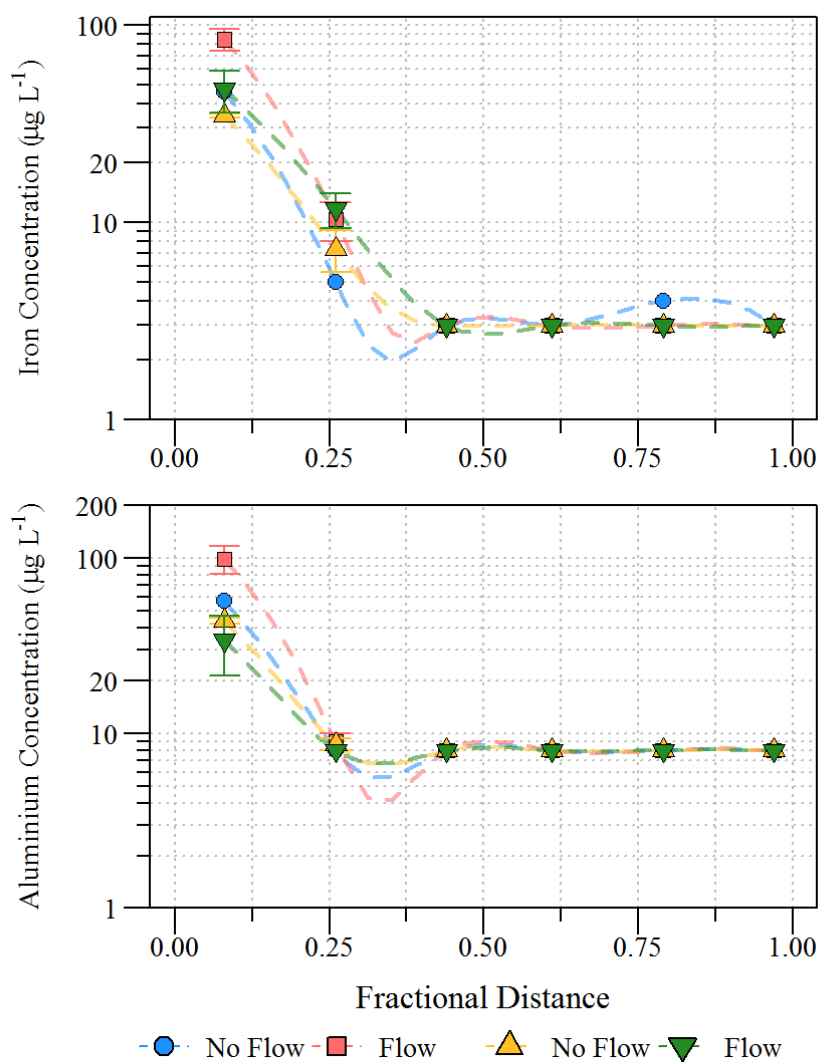Figure 5-11: Mean (± SE) surface water total iron and aluminum concentration for each flow period during the fourth flow event.
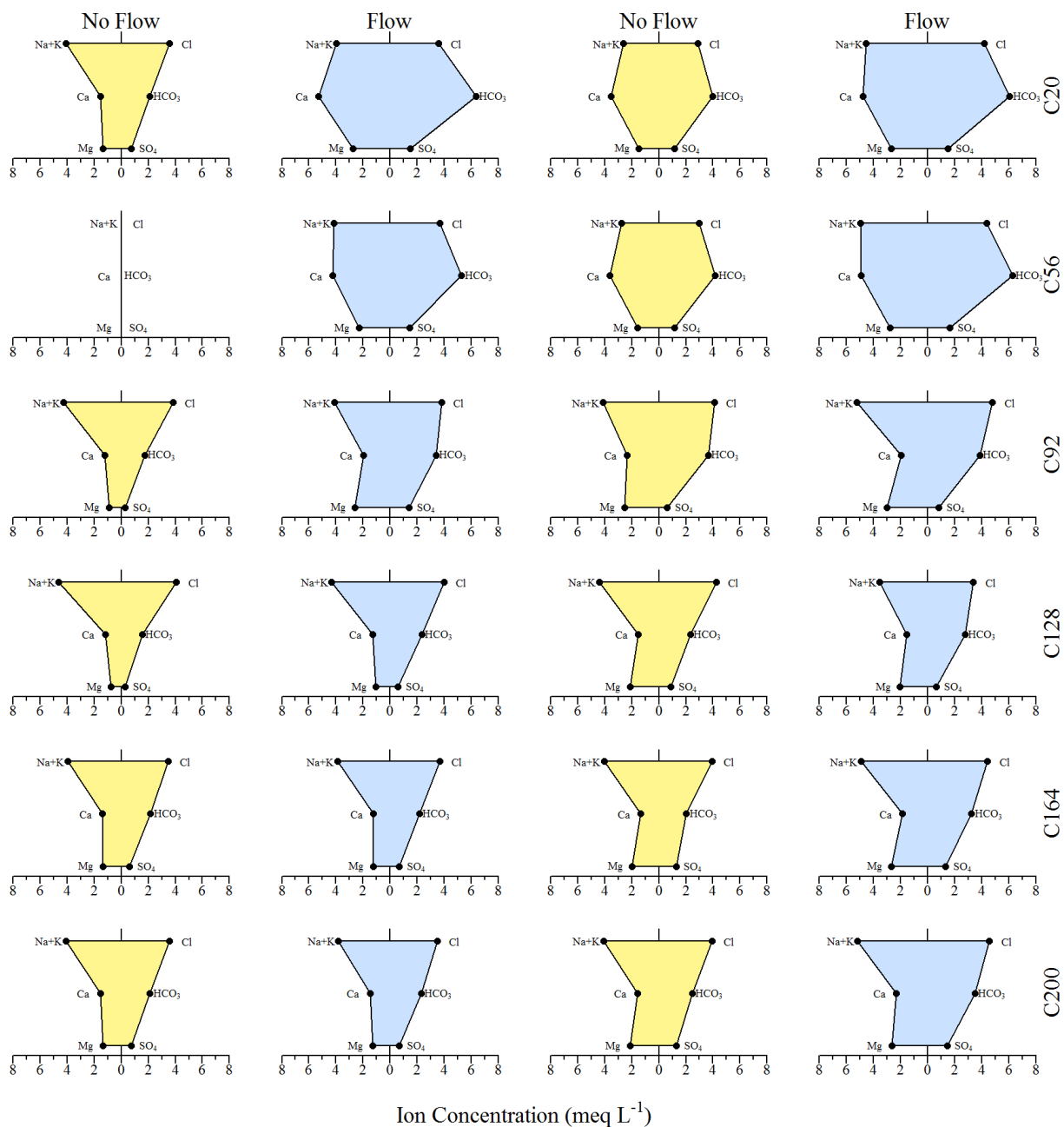
Figure 5-12: Median ion concentration for each station and flow period. Sites along the flow path are arranged from top to bottom and flow periods are arranged from left to right.

5.1.3 Physical attributes of water during flow event: EXO-Sonde data

Water quality data from sondes deployed at specific stations within each flow path (Table 5-4) were screened with methods similar to those used by the National Estuary Research Reserve (NERR) Program and the data management protocols developed by the Centralized Data Management Office (CDMO). These methods and protocols ensure that sampling, processing and data management techniques are comparable among stations (NOAA 2013). Two levels of quality assurance and quality control (QA/QC) were applied to the data. The first layer or primary QA/QC involves screening the data consistent with the limit of each individual sensor (Table 5-4), provides the upper and lower limits of each sensor. Data were flagged if values fell outside of the sensors optimal range of operation or if a particular parameter failed the post deployment calibration.

Table 5-4. Low and High Sensor values for the EXO YSI sonde. Values from YSI EXO operating manual.

Parameter	Lower Limit	Upper Limit	Units
Temperature	-5	45	Deg. C
Conductivity	0	200	ms/cm
Optical Dissolved Oxygen	0	500	% Air Saturation
	0	50	mg L^{-1}
fDOM	0	300	ppb QSU
pH	0	14	SU
Chlorophyll	0	400	µg L^{-1}
BGA-PC (phycocyanin)	0	100	µg L^{-1}
Turbidity	0	4000	NTU

The second layer of QA/QC or secondary QA/QC involves screening the data for suspect or missing data points. Data that would qualify under this level of QA/QC could be aberrant values not consistent with the current data stream such as a large spike in temperature for one data point in a 45-minute period with logged data at 15-minute intervals.

Primary QA/QC was completed using functions developed in R to fit this project's specific needs. After primary QA/QC was completed the data was then imported into an MS Access database where secondary QA/QC was completed. No data were deleted or removed from the original data files provided after data retrieval.

When the QA/QC is applied to the data collected during the three flow events, a high number of data points were retained for standard parameters including pH, Temperature, Specific Conductivity, Dissolved Oxygen, Chlorophyll and Turbidity (Table 5-5). Meanwhile a large volume of data from optical parameters such as fDOM and Blue-Green Algae (BGA-PC) were qualified as not useable due to data ranges, sensor responses, lack of calibration or improper calibration (Table 5-5). Based on this QA/QC process parameters with a high number of record retained will be used in further analysis including comparative statistical analysis and other analyses.

Table 5-5. Percent of useable (pass QA/QC) data collected during the fourth flow events.

Parameter	Units	Percent Useable
Temperature	Deg. C	100
Specific Conductivity	µs cm^{-1}	99.9
Optical Dissolved Oxygen	% Air Saturation	89.9
	mg L^{-1}	90.2
fDOM	ppb QSU	*42.0*
pH	SU	99.8
Chlorophyll	µg L^{-1}	99.9
BGA-PC (phycocyanin)	µg L^{-1}	*99.3*
Turbidity	NTU	70.6

Water quality sonde parameters (EXO Sonde) were collected almost throughout the entirety of the fourth flow event with site C200 during the second flow event (phase 3) and C200 midway through the first flow event (phase 1) and the entire second no-flow event (phase 3). Surface water temperature ranged between 24.7 and 39.5 °C (Figure 5-13).

Dissolved oxygen ranged between 0 and 40 mg/L, exhibited diel fluctuations and generally became depressed during no flow conditions. Sites C92 and C128 experienced the largest diel fluctuations in DO and site C164 generally didn't respond to the flow/no flow condition like other sites along the flow path (Figure 5-14).

Surface water pH ranged from 6.9 to 10.4, fluctuated along the diel cycle and exhibited similar patterns to DO with respect to response to flow and intensity of fluctuation (Figure 5-15). Specific conductance ranged from 483 to 1,377 µS cm^{-1} and responded to changes in flow regime. Sites C92 and C128 experienced significant changes in specific conductance values, more so than both upstream and downstream sites suggesting that either vegetation is contributing to specific conductance (pumping via reparation) or seepage could play a factor (Figure 5-16).

Chlorophyll-a values were greatest at the front end of the flow path (site C20) and relatively low at the back end with diel fluctuations of relative concentrations observed at each station (Figure 5-17). It appears that the EXO-sonde turbidity probe malfunctioned for sites C164 and C200 during deployment and failed post calibration, therefore these data is not presented. Similarly, fDOM data are not presented because majority of this data did not meet screening criteria due to data being recorded beyond the factor settings of the sensor.

Figure 5-13: Daily (red point and black lines) and 15-minute (light red line) surface water temperature during the fourth flow event (June 27 to August 29, 2016) at sites along the flow path in STA-2 Cell 3.

Figure 5-14: Daily (red point and black lines) and 15-minute (light red line) dissolved oxygen concentrations (mg/L) during the fourth flow event (June 27 to August 29, 2016) at sites along the flow path in STA-2 Cell 3.
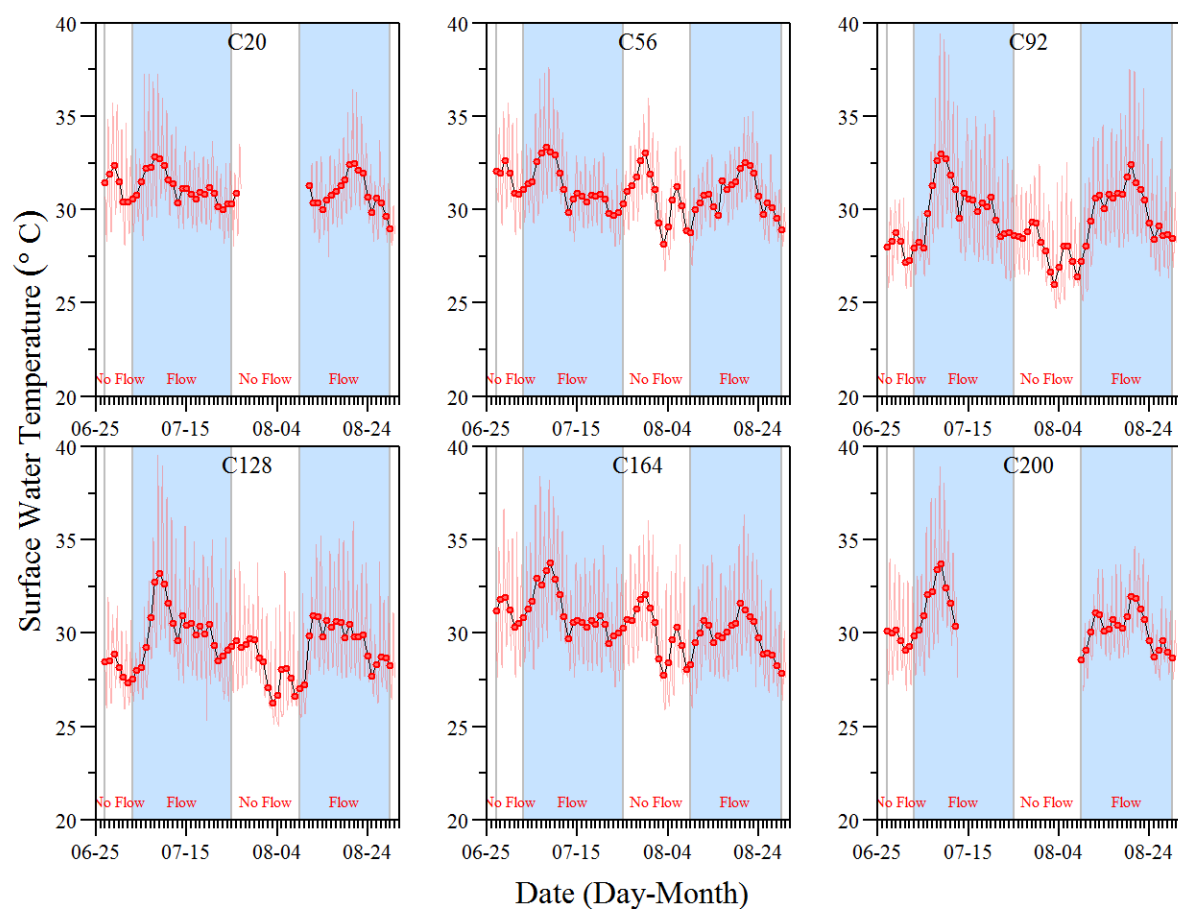
Figure 5-15: Daily (red point and black lines) and 15-minute (light red line) surface water pH during the fourth flow event (June 27 to August 29, 2016) at sites along the flow path in STA-2 Cell 3.
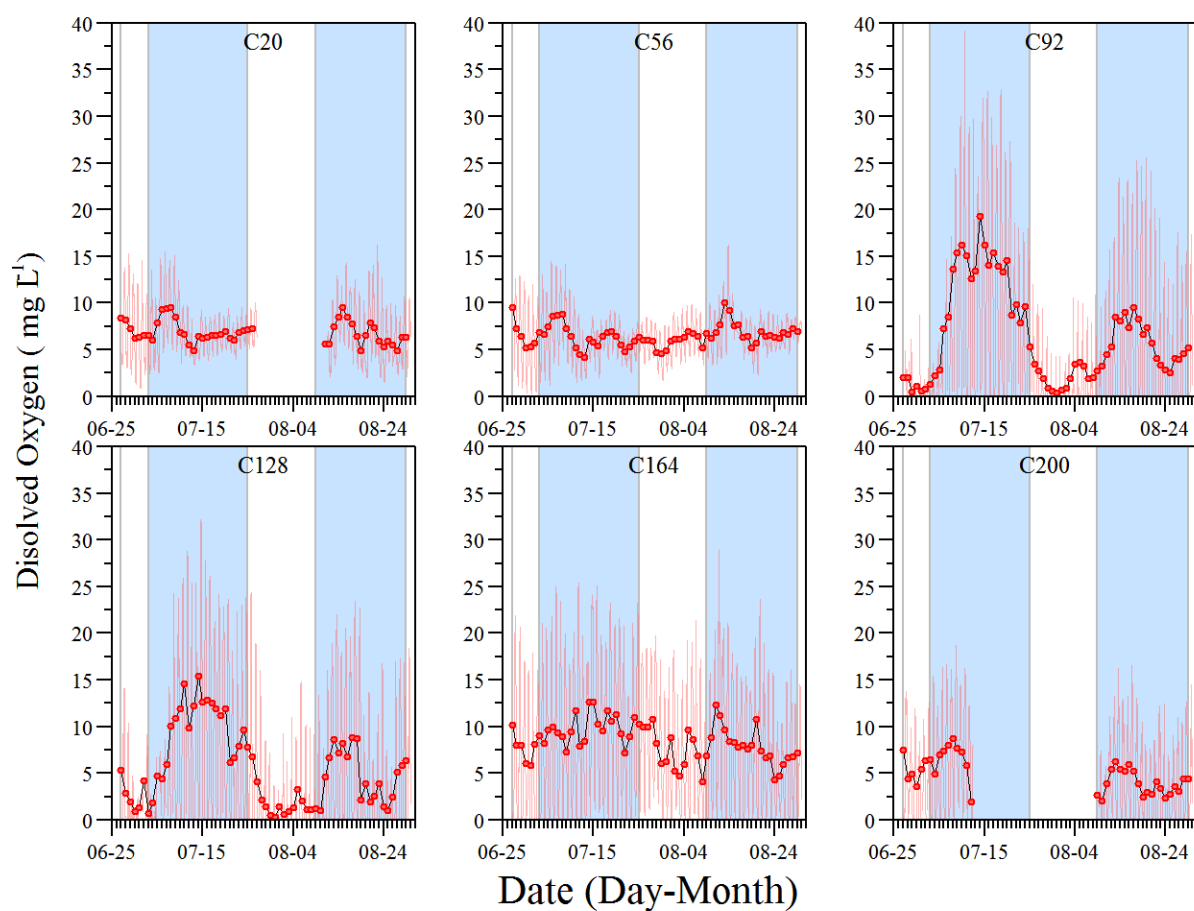
Figure 5-16: Daily (red point and black lines) and 15-minute (light red line) specific conductance during the fourth flow event (June 27 to August 29, 2016) at sites along the flow path in STA-2 Cell 3.
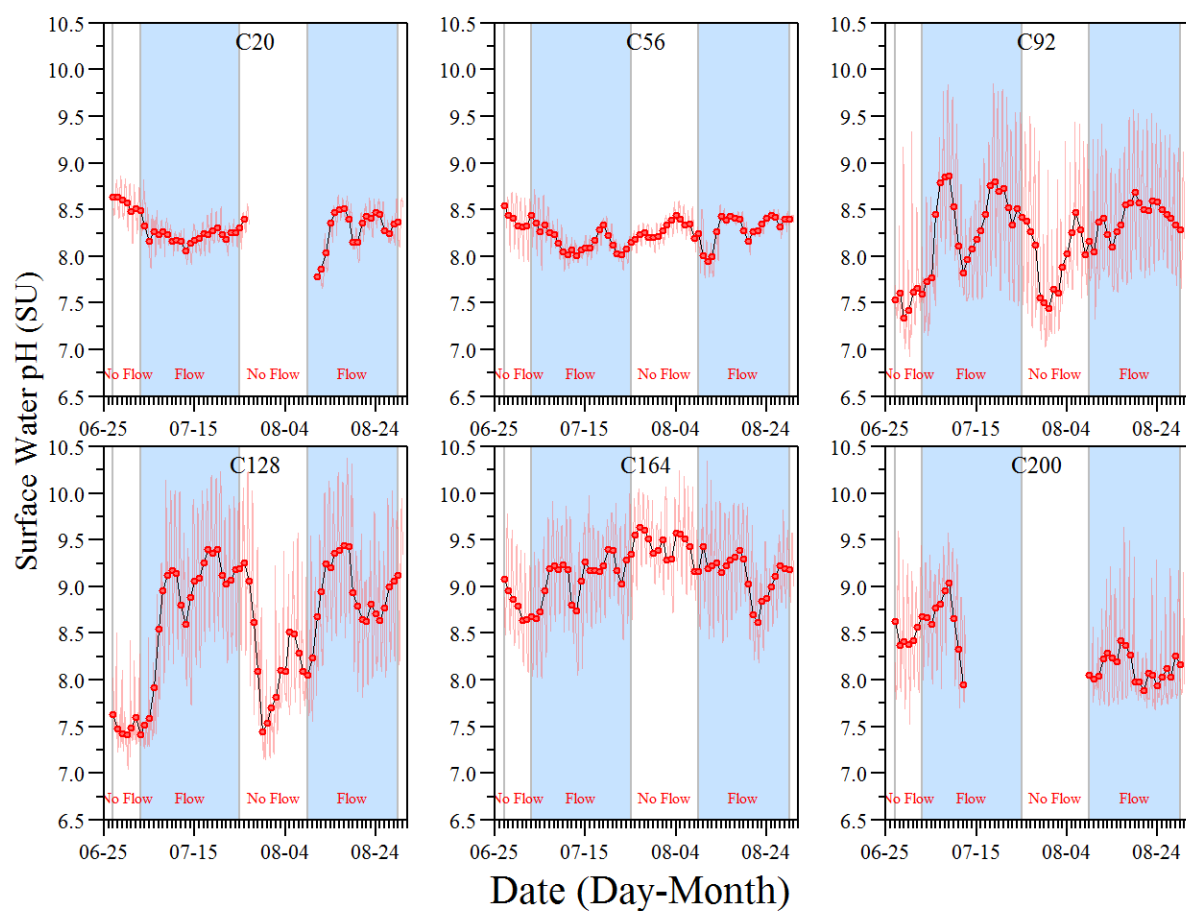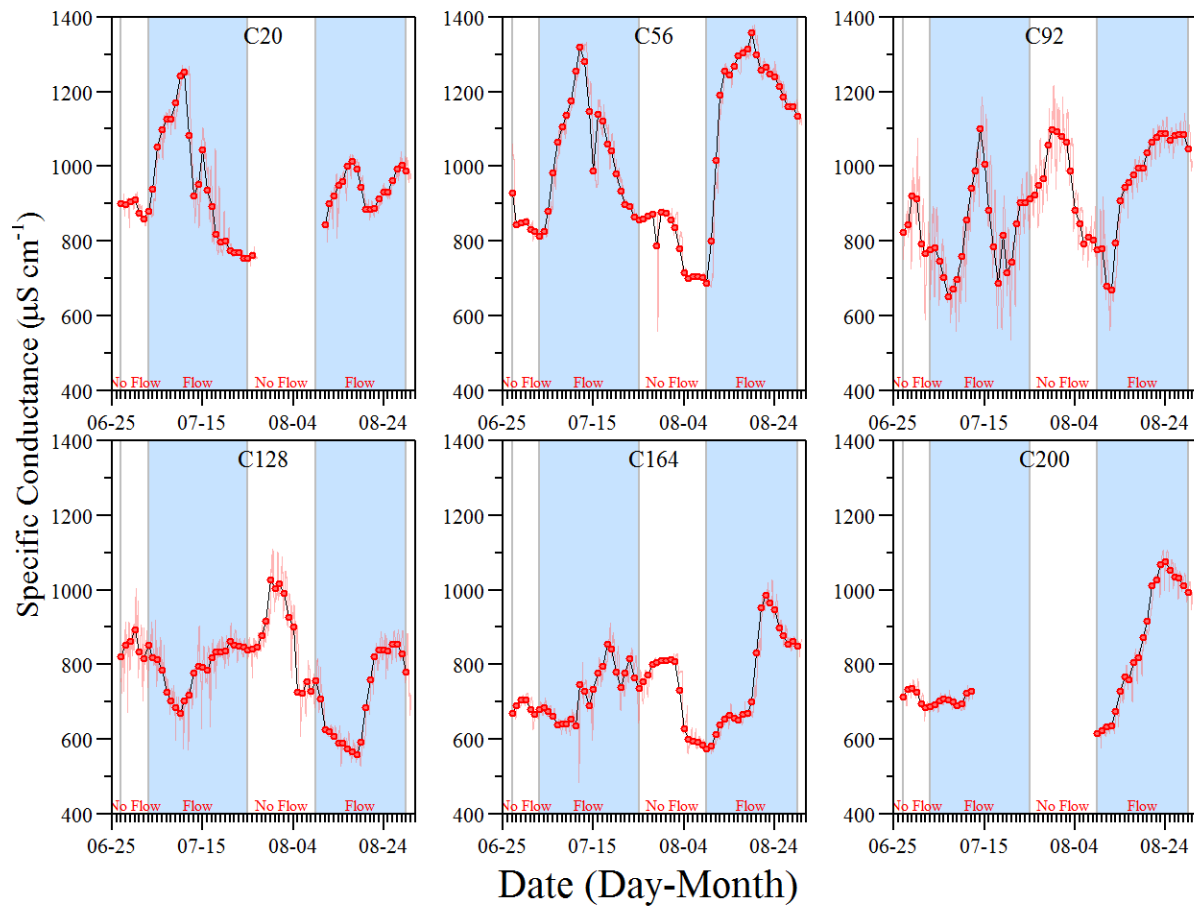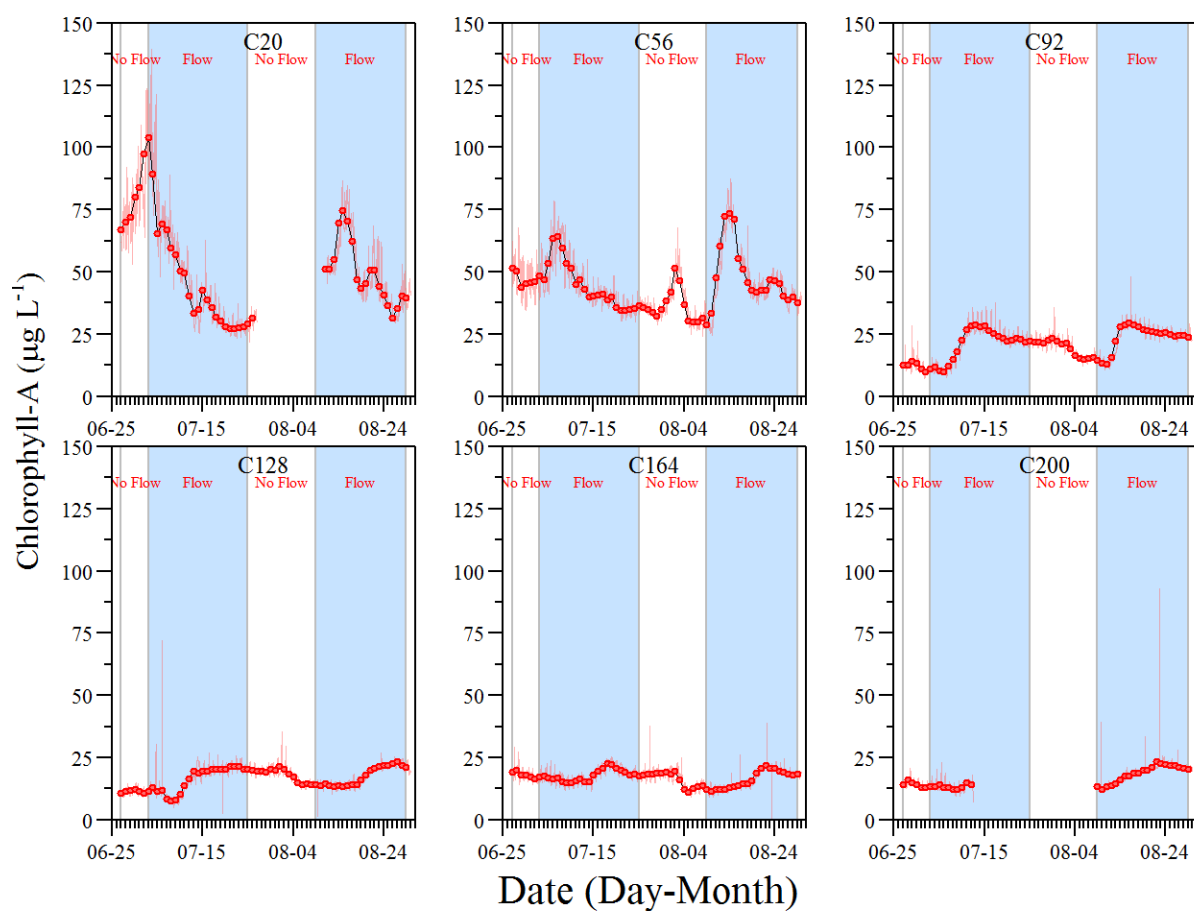
Figure 5-17: Daily (red point and black lines) and 15-minute (light red line) chlorophyll-a during the fourth flow event (June 27 to August 29, 2016) at sites along the flow path in STA-2 Cell 3.

6 Transect Study- Laboratory Enzyme Activity and Data Analysis [Task 7b]

Work Completed During This Quarter

The fourth flow event occurred from June 27 to August 29, 2016. During this period soil cores were collected on July 12, August 11, and August 25, representing periods of low flow, stagnant, and low flow conditions, respectively (Table 6-1). During each sampling event, three cores were collected from the three STA-2 Cell 3 benchmark sites (C20, C128, C200) and transported to the UF-WBL laboratory and stored at 4°C until processed (<24 hr).

Table 6-1. Flow conditions during sampling for floc enzyme activity change for the fourth flow event in STA-2 Cell 3 (June 27-August 29, 2016)

Flow period	Sampling date	Flow	Flow Volume ($\times 10^5$ m^3 d^{-1})		HLR (cm d^{-1})
			Mean ± SE	Total	
July 03-July 24, 2016	July 12	Low Flow	3.23 ± 0.17	71.12	3.48 ± 0.19
July 25-August 8, 2016	August 9	No Flow	0.00	0.00	0.00
August 09-August 29, 2016	August 25	Low Flow	2.93 ± 0.46	61.55	3.15 ± 0.49

Cores were sectioned to collect floc (upper 2cm) and processed for enzyme activities including phosphatase (mono- and di-esterase), β-glucosidase, and aminopeptidase. Enzyme activity analyses for these samples are complete, but data are not available for presentation until dry weight and microbial biomass determinations are completed.

7 Biogeochemical Processes: Laboratory and Field Studies (Task 8)

Transect soil sampling was conducted in STA-2 Cell 3 from September 7-9 at 11 sites including 3 benchmark sites (C20, C128, C200). At each benchmark site, 3 cores were collected and sectioned into- floc, recently accreted soil (RAS) and pre-STA soil fractions. Plant litter (when present) was collected. Samples were stored in plastic sample bags at ~4°C until they were shipped to UF-WBL. Floc depth was determined after allowing flocculent material to settle in a plastic core tube for 3-4 hours (Figure 2-2, See Chapter 3).

For this period, samples arrived in two shipments (total samples submitted were 68, there was no litter) on September 15 and September 21, 2016. To maintain a consistent treatment, analyses for all samples from STA-2 Cell 3 began on September 22, 2016. Samples received by UF on these dates were Floc, RAS, Pre STA-1 and Pre STA-2 (see Table 2-1) from STA-2 Cell 3. There were a total of 64 samples that were analyzed for microbial biomass-C, N, P, while select samples (n=43; See Table 7-1) were further analyzed for potentially mineralizable nitrogen (PMN), C-, N-, P- enzymes (Phosphatase, Bis-phosphatase, Aminopeptidase, Glucosidase).

Transect soil sampling was conducted in STA-2 Cell 3 from September 20-22 at 11 sites including 3 benchmark sites (C34, C121, C208). Litter samples were collected from all 11 sites and transported to UF-WBL on September 21, 2016. All litter samples (n= 17) were stored at 4°C until remaining floc and soil samples from this cell were received before beginning any analyses. Remaining samples were received in first week of October, 2016.

Table 7-1. Samples collected from transect and benchmark sites (*) in STA-2 Cell 3 were received by UF-WBL in September, 2016. All samples (n= 64) were analyzed for microbial biomass C/N/P, while red bold samples **x (n=43)** were analyzed for additional parameters (see text for details.

STA-2 Cell 3

Location	Rep	Litter	Floc	RAS	Pre STA1	Pre STA2
C20	3	--	**xxx**	**xxx**	**xxx**	xxx
C38	1	--	**x**	**x**	x	x
C56	1	--	**x**	**x**	x	x
C74	1	--	**x**	**x**	x	x
C92	1	--	**x**	**x**	x	x
C110	1	--	**x**	**x**	x	x
C128	3	--	**xxx**	**xxx**	**xxx**	xxx
C146	1	--	**x**	**x**	x	x
C164	1	--	**x**	**x**	x	x
C182	1	--	**x**	**x**	x	x
C200	3	--	**xxx**	**xxx**	**xxx**	xxx

8   Planned Activities

The following activities are planned for the next quarter (October to December, 2016).

- Completion of transect and benchmark soil sampling from STA-3/4 Cell 3A and 3B (Task 3).
- Lab processing and submission of samples to respective labs for analysis.
- Spatial sampling from WCA-2A (Dec, 2016). This will include floc, soil and water sample collections from selective sites from the established transect (Task 3).
- Water quality data analysis from the flow event in STA-2 Cell 3 that took place during 22 Feb and 11 April, 2016.
- Aquatic productivity estimates derived from EXO-Sonde data.
- Explorations of relationships between EXO-Sonde data and TP and TN trends using advanced analytical tools.
- For Task 5, soil and floc samples are currently being processed to determine P forms in samples collected from STA-2 and STA-3/4.
- For Task 6, experiments to determine P sorption capacities of soils are underway for samples collected from STA-2 and STA-3/4.
- For Task 7B, to complete the data analysis for samples (floc) collected for *P-Flow* study during Event 5 (October-November, 2016).
- For Task 7B, to complete the data analysis for samples (floc) collected for *P-Flow* study during Event 5 (November-December, 2016).
- For Task 8a, to complete the lab analysis for biogeochemical parameters in litter, floc and soil samples collected for *transect and benchmark* study from 4 sites (STA-2 Cell 1 and 3, STA-3/4 Cell 3A and 3B). Analysis completed till date will be made available in the next quarterly report (#6).
- Begin with data (calculations and statistics) analysis of the biogeochemical measurements determined (Task 10).

9 References

Ivanoff, D.B.; Reddy, K.R.; Robinson, S., 1998. Chemical fractionation of organic phosphorus in selected histosols. Soil Science. 163:36-45.

Julian, P.; Hill, S., 2012. A.R.M. Loxahatchee National Wildlife Refuge Total Phosphorus Outlier Analysis and Proposed Alternative Screening Criterion: Distribution Independent Outlier Analysis.: Everglades Technical Oversight Committee.

Julian, P.; Payne, G.; Xue, S., 2016. Chapter 3A: Water Quality in the Everglades Protection Areas. 2016 South Florida Environmental Report: South Florida Water Management District.

Richardson, C.J.; Reddy, K., 2013. Methods for soil phosphorus characterization and analysis of wetland soils. Methods in Biogeochemistry of Wetlands:603-638.

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. West Palm Beach, FL: South Florida Water Management Dist.

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, the discussion of results from the 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.

10 Appendices

10.1 Table of sampling locations in STA-2 Cell 1.

STA-2 Cell 1 Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
1	A34	26.41706	-80.4919	✔	✔✔✔
2	A51	26.41341	-80.496	✔	
3	A69	26.40973	-80.496	✔	
4	A86	26.40609	-80.5001	✔	
5	A104	26.40244	-80.5001	✔	
6	A121	26.39877	-80.5042	✔	✔✔✔
7	A138	26.39511	-80.5083	✔	
8	A156	26.39145	-80.5083	✔	
9	A173	26.38779	-80.5124	✔	
10	A191	26.38415	-80.5124	✔	
11	A208	26.38046	-80.5165	✔	✔✔✔

10.2 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	C20	26.41725	-80.5489	✔	✔✔✔
2	C38	26.41358	-80.5489	✔	
3	C56	26.40992	-80.5489	✔	
4	C74	26.40625	-80.549	✔	
5	C92	26.40258	-80.549	✔	
6	C110	26.39891	-80.549	✔	
7	C128	26.39525	-80.549	✔	✔✔✔
8	C146	26.39158	-80.549	✔	
9	C164	26.38791	-80.549	✔	
10	C182	26.38425	-80.5491	✔	
11	C200	26.38058	-80.5491	✔	✔✔✔

10.3 Table of sampling locations in STA-3/4 Cell 3A.

STA-3/4 Cell 3A Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
1	3_A20	26.39168	-80.6586	✔	
2	3_A32	26.38802	-80.6586	✔	
3	3_A44	26.38436	-80.6587	✔	
4	3_A56	26.3807	-80.6587	✔	✔✔✔
5	3_A8	26.39534	-80.6586	✔	✔✔✔

10.4 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	A7	26.37603	-80.6602	✔	✔✔✔
2	A7C	26.37412	-80.6602	✔	✔✔✔
3	B7	26.3723	-80.6602	✔	
4	B7C	26.3706	-80.6602	✔	
5	C7	26.36857	-80.6602	✔	✔✔✔
6	C7C	26.36857	-80.6602	✔	✔✔✔
7	D7	26.36484	-80.6602	✔	✔✔✔
8	D7C	26.3637	-80.6602	✔	✔✔✔