DECEMBER 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 October – December 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 second quarter of Year 2 of the project and included various activities that were initiated to meet the objectives laid out under multiple tasks.

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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 water quality data, and perhaps with a larger dataset including other STA cells selected for this project.*

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 in STA-3/4 and WCA-2A
- Task 6a. Initial work in connection to phosphorus sorption/desorption characteristics
- Task 7a. Transect study: Surface water quality monitoring
- Task 7b. Transect study: Enzyme and microbial biomass
- Task 8. Biogeochemical processes: Laboratory and field studies
- Task 10. Data synthesis and integration

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-3/4 Cell 3A and Cell 3B in October, 2016 and in WCA-2A in December, 2016, with coordination between UF and District project personnel.

2.2 STA-3/4 – Cell 3B

STA-3/4 Cell 3B is primarily characterized as a submerged aquatic vegetation (SAV) cell where SAV regions were surrounded by emergent vegetation (*Typha domingensis*) strips. In order to

capture differences in soil properties from these two different vegetated zones, additional sampling locations were identified in the vicinity of existing sampling grid points. These additional sites were depicted by a suffix ‘c’ indicating cattail dominant zone. Transect soil and grab surface water samplings were conducted in STA-3/4 Cell 3B on October 4, 2016 (Figure 2-1). Intact soil cores were collected along an established transect consisting of 8 stations (Station ids –A7, A7c, B7, B7c, C7, C7c, D7 and D7c). Three pairs of stations (A7 and A7c, C7 and C7c, D7 and D7c) each corresponding to inflow, midflow, and outflow of the cell 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 80 cores (20 x 4) were obtained. Soil cores were stored in a cold room (~4^{o}C) until they can be sectioned into plant litter (when present), floc, recently accreted soil (RAS) and pre-STA soil fractions.

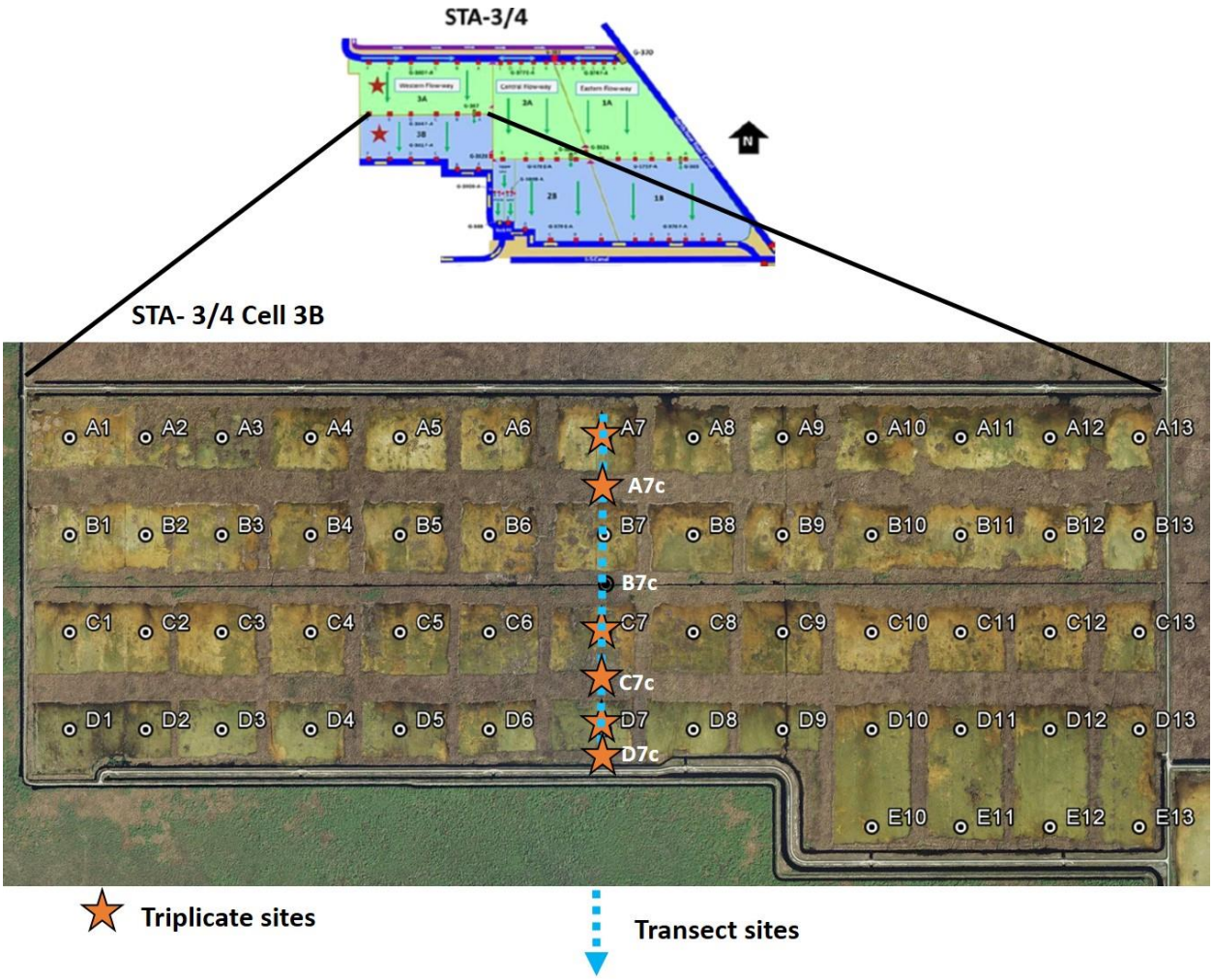

Figure 2-1: STA-3/4 Cell 3B – Soil and surface water sampling locations.

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).

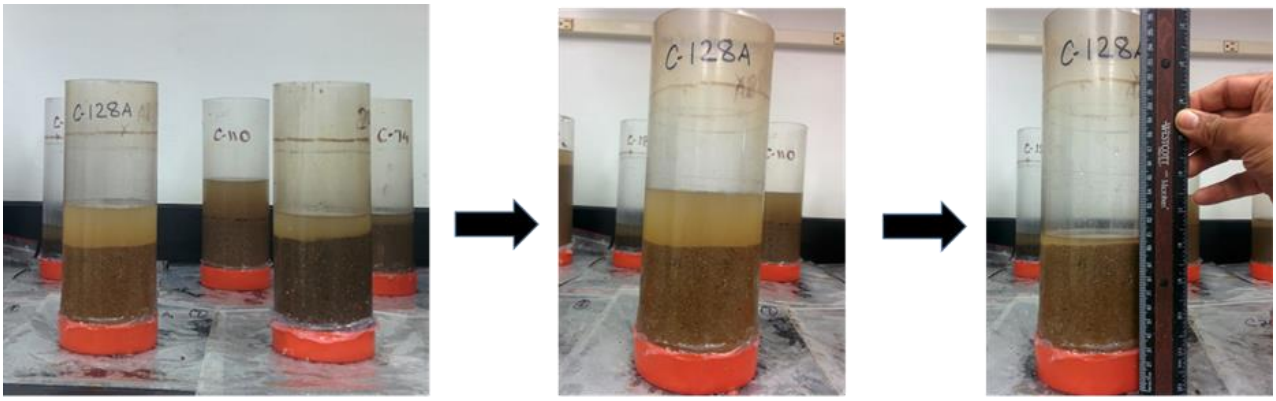

Figure 2-2: Three step process for determination of floc depth.

Pre-STA soils were divided into two sections: 0-5 cm and 5 - 15 cm, or up to complete depth of soil core, whichever happened 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 (Figure 2-1). Submerged aquatic vegetation (SAV), primarily *Chara* spp. was the predominant vegetation in open areas within the cell, however emergent vegetation (*Typha domingensis*) was present as vegetation strips forming quadrats surrounding SAV zones.

Table 2-1. STA-3/4 Cell 3B: Depth of various sections after separating intact soil cores. *Benchmark locations.

STA-3/4 Cell 3B Site #	Station id	Thickness of section (cm)			
		Floc	RAS	Pre-STA-1 (0-5 cm)	Pre-STA-2 (5-15 cm)
1*	A7A	12.28	1.50	5.0	13.75
2*	A7B	11.45	2.50	5.0	15.00
3*	A7C	11.60	2.00	5.0	15.00
4*	A7cA	3.43	2.13	5.0	15.00
5*	A7cB	4.78	1.75	5.0	15.00
6*	A7cC	2.85	2.63	5.0	15.00
7	B7	10.45	1.63	5.0	15.00
8	B7c	0.93	2.13	5.0	14.75
9*	C7A	7.88	1.75	5.0	11.50
10*	C7B	9.45	1.50	5.0	12.50
11*	C7C	9.10	2.13	5.0	13.13
12*	C7cA	1.60	2.63	5.0	12.13
13*	C7cB	3.95	1.50	5.0	14.63
14*	C7cC	2.43	1.75	5.0	14.75
15*	D7A	4.33	1.75	5.0	7.63
16*	D7B	7.03	2.00	5.0	8.25
17*	D7C	7.75	1.88	5.0	9.38
18*	D7cA	2.38	2.63	5.0	15.00
19*	D7cB	1.23	1.13	5.0	15.00
20*	D7cC	1.25	1.38	5.0	15.00

2.3 STA-3/4 – Cell 3A

STA-3/4 Cell 3A is categorized as emergent aquatic vegetation (EAV) cell with primarily *Typha domingensis* with patches of *Pistia* and *Salvinia.* Transect soil and grab surface water samplings were conducted in STA-3/4 Cell 3A on October 20, 2016 (Figure 2-3). Intact soil cores were collected along an established transect consisting of 5 stations (station ids – A8, A20, A32, A44 and A56). Three of these stations, A8 (inflow), A32 (midflow) and A56 (outflow) were sampled as benchmark sites, where triplicate soil and water samples were taken [*See Appendix 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 44 cores (11 x 4) were obtained. Soil cores were stored in a cold room (~4^{o}C) until they can be sectioned into plant litter (when present), floc, recently accreted soil (RAS) and pre-STA soil fractions.

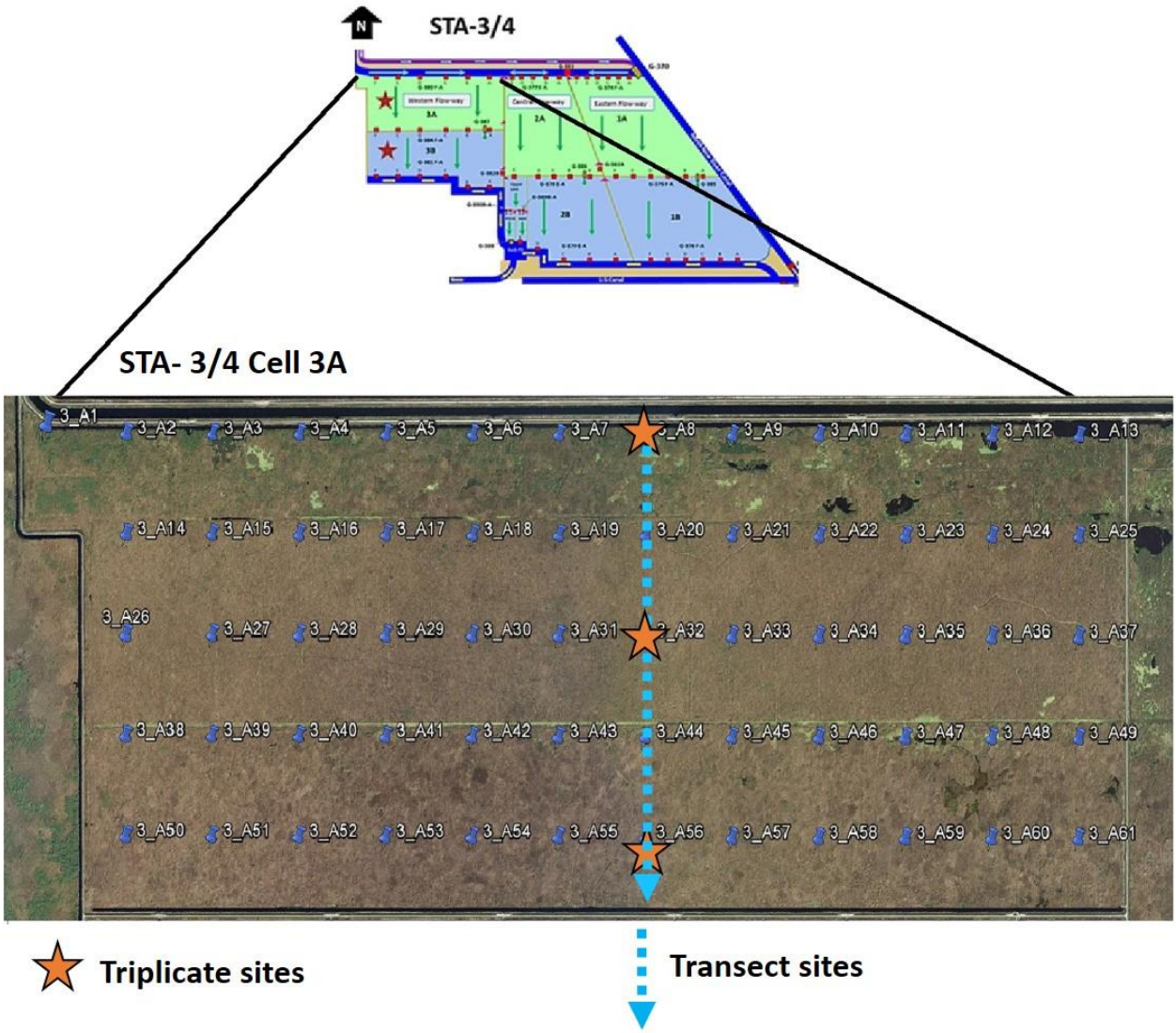

Figure 2-3. STA-2 Cell 1 – Soil and surface water sampling locations**.**

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 complete depth of soil core, whichever happened to be smaller (Table 2-2). 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 (Figure 2-3).

Table 2-2. STA-3/4 Cell 3A: Depth of various sections after separating intact soil cores.

STA-3/4 Cell 3A Site #	**Station id**	**Thickness of section (cm)**			
		Floc	**RAS**	**Pre-STA-1 (0-5 cm)**	**Pre-STA-2 (5-15 cm)**
1*****	A8A	13.80	10.00	5.00	6.00
2*****	A8B	23.98	14.50	4.75	5.50
3*****	A8C	21.18	15.00	5.00	5.50
4	A20	1.40	3.13	5.00	12.00
5*	A32A	1.85	5.00	5.00	12.00
6*	A32B	2.03	3.63	5.00	9.50
7*	A32C	1.63	5.63	5.00	10.25
8	A44	2.35	3.00	5.00	5.75
9*****	A56A	2.60	2.50	5.00	15.00
10*****	A56B	2.28	2.00	5.00	13.75
11*	A56C	3.10	3.50	5.00	15.00

*Benchmark locations.

2.4 Water Conservation Area (WCA) – 2A

Transect soil sampling took place in WCA-2A on December 6, 2016 (Figure 2-4) where soil cores and surface water samples were obtained along the transect consisting of 5 stations (Station ids – F1, F2, F4, E5, and U3). Selected sampling sites in WCA-2A were located along the soil P and vegetation gradient, where F1 and F2 were dominated by the presence of *Typha domingensis*, F4 site comprised a mix of *Typha domingensis* and *Cladium jamaicense*, and E5 and U3 primarily populated by *Cladium jamaicense* with some open water areas that had *Utricularia foliosa*. Three soil cores and three surface water samples were collected at all stations (*See Appendix for GPS coordinates of sampling locations*).

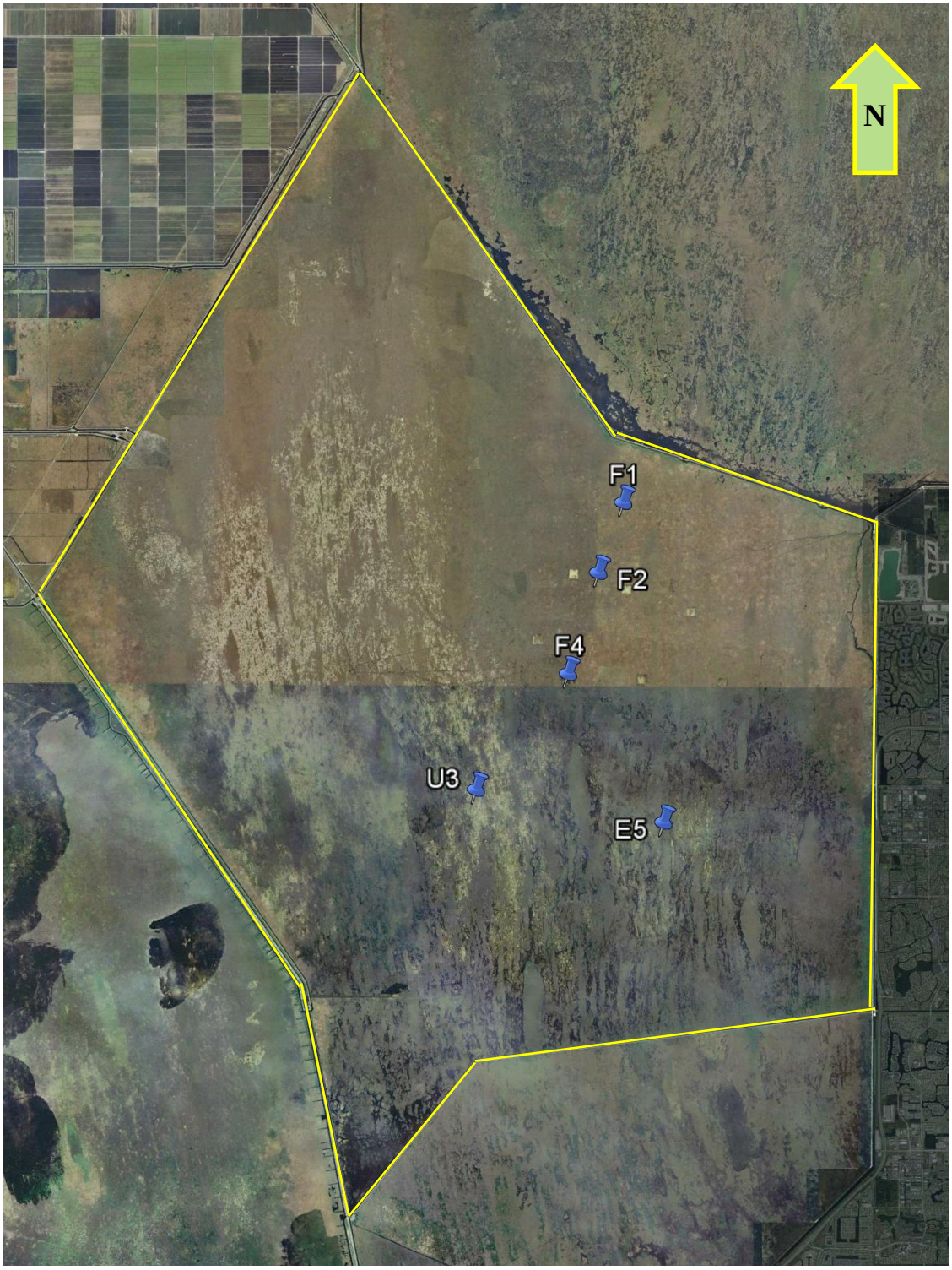

Figure 2-4. WCA-2A – Soil and surface water sampling locations.

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 (Table 2-3). 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). After separately collecting floc, underlying soil was divided into 0-5 cm, 5-10 cm and 10-20 cm layers.

Floc and soil sections from the two soil cores were thoroughly mixed and weighed before dividing into three portions. One portion was submitted to the District while two others were sent to the UF-Wetland Biogeochemistry Lab for analyses. One set of samples was sent to WBL, Gainesville for P fractionation and one set was sent to Ft. Pierce for P-sorption/desorption 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 2-4).

Table 2-3. WCA-2A: Depth of various sections after separating intact soil cores.

WCA-2A Site #	**Station id**	**Thickness of section (cm)**			
		Floc	**0-5 cm**	**5-10 cm**	**10-20 cm**
1	F1A	1.2	5	5	10
2	F1B	1.1	5	5	10
3	F1C	1.5	5	5	10
4	F2A	1.2	5	5	10
5	F2B	0.6	5	5	10
6	F2C	1.5	5	5	10
7	F4A	1.1	5	5	10
8	F4B	2.0	5	5	10
9	F4C	0.6	5	5	10
10	E5A	0.9	5	5	10
11	E5B	0.4	5	5	10
12	E5C	0.6	5	5	10
13	U3A	7.9	5	5	10
14	U3B	7.6	5	5	10
15	U3C	8.1	5	5	10

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 is being conducted at the Wetland Biogeochemistry Lab, University of Florida.

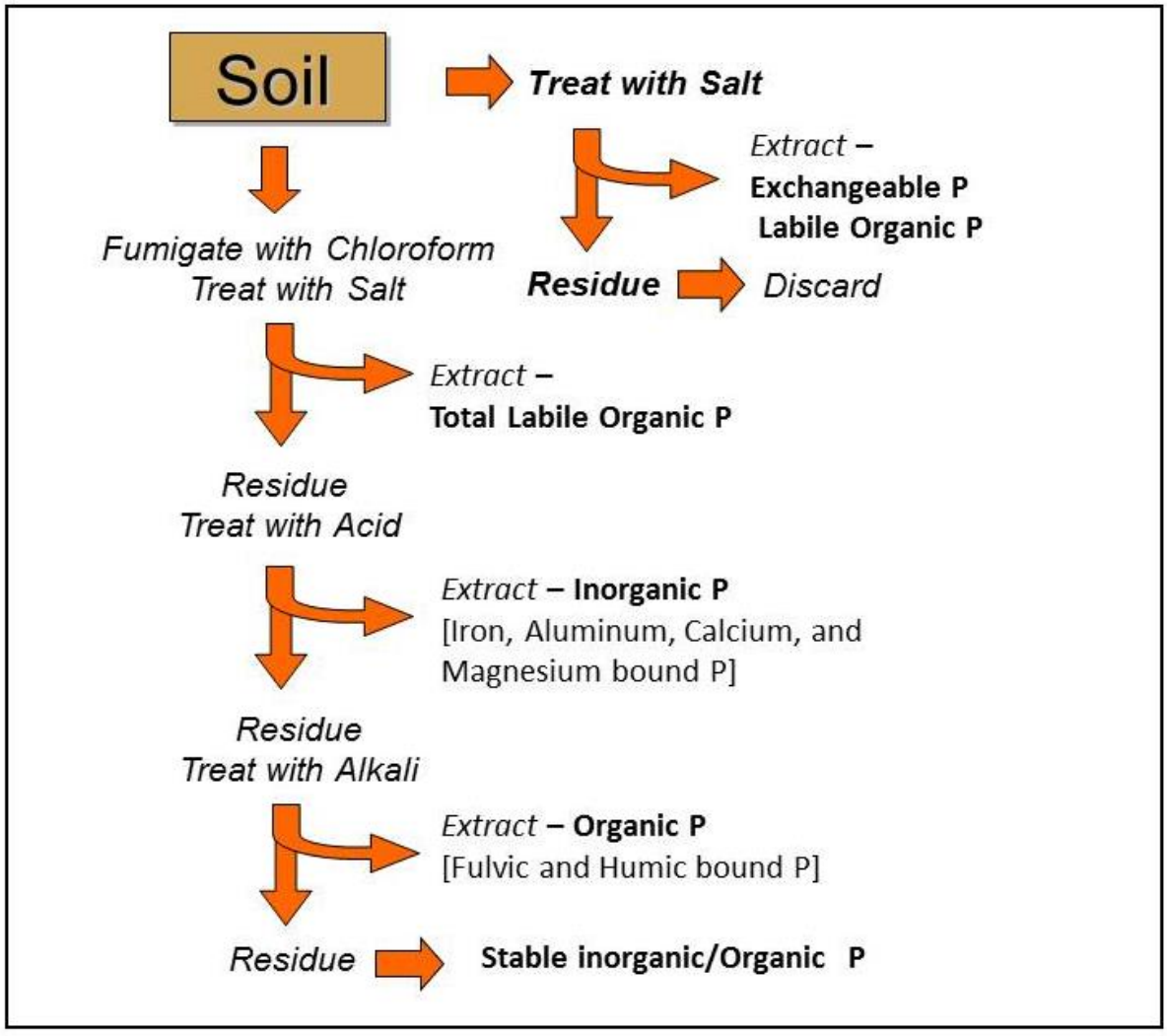

Figure 3-1: Schematic showing operationally defined P fractionation scheme used in wetland soils (Reddy and DeLaune, 2008).

Soil and floc samples were obtained from STA-2 Cell 1 and Cell 3 and STA-3/4 Cell 3A and 3B, and WCA-2A as described in Task 3 of this report. Soil and floc samples are subjected to detailed fractionation scheme as shown in Figure 3-1. Progress to-date and preliminary results are presented in this report.

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

Location	**Stations**	**Status**
STA-2 Cell 1	**34,** 51, 69, 86, 104, **121**, 138, 156, 173, 191, and **208**	Phosphorus fractionation is complete. Summary data tables are presented in this report
STA-2 Cell 3	**20,** 38, 56, 74, 92, 110, **128**, 146, 164, 182, and **200**	Phosphorus fractionation is complete. Summary data tables are presented in this report
STA-3/4 Cell 3A	**A8,** A20**, A32,** A44**, A56**	Phosphorus fractionation is on-going. Summary data tables will be presented in subsequent reports
STA-3/4 Cell 3B	**A7, A7c,** B7, B7c, **C7, C7c, D7** and **D7c**	Phosphorus fractionation is on-going. Summary data tables will be presented in subsequent reports
WCA-2A	**F1, F2, F4, E5**, and **U3**	Phosphorus fractionation is on-going. Summary data tables will be presented in subsequent reports

Table 3-2. Brief description of phosphorus forms extracted based on their solubility in acid or alkali solutions.

P- fraction	**Description**
$NaHCO_3$-Pi	0.5 *M* Bicarbonate extractable inorganic P. Also known as Olsen P. Considered as labile inorganic P. Readily available for use by macrophytes and periphyton.
$NaHCO_3$-TP	0.5 *M* Bicarbonate extractable total P (includes both organic P and inorganic P). Considered as labile inorganic and organic P. Readily available for use by macrophytes and periphyton.
$NaHCO_3$-Po	0.5 *M* Bicarbonate extractable organic P. Also known as labile organic P (LOP). LOP = [$NaHCO_3$-TP] – [$NaHCO_3$-Pi]. Rapidly mineralized to inorganic and made available for use by macrophytes and periphyton.
F- $NaHCO_3$-TP	Floc and soil samples are fumigated with chloroform to lyse microbial cells. Fumigated samples are extracted with 0.5 *M* bicarbonate and analyzed for total P (includes both organic P and inorganic P). Considered as labile inorganic and organic P. Readily available for use by macrophytes and periphyton.
F- $NaHCO_3$-Po	Fumigated + 0.5 *M* bicarbonate extractable organic P. Also known as total labile organic P (TLOP). TLOP = [F-$NaHCO_3$-TP] – [$NaHCO_3$-Pi]. Rapidly mineralized to inorganic and made available for use by macrophytes and periphyton.
MBP	Microbial biomass P. MBP = [F-$NaHCO_3$-TP] – [$NaHCO_3$-TP]. MBP values not adjusted to P extraction efficiency.
HCl-Pi	Acid (1 *M* HCl) extractable inorganic P. This represents calcium, magnesium, iron, and aluminum associated P.
HCl-TP	Acid (1 *M* HCl) extractable TP. This represents calcium, magnesium, iron, and aluminum associated P and acid hydrolysable organic P.
HCl – Po	Acid (1 *M* HCl) hydrolysable organic P is estimated as the difference between HCl-TP and HCl-Pi.
NaOH-TP	Alkali (0.5 *M* NaOH) extractable total P.
NaOH-FA-P	Alkali extracts treated with acid (conc. H_2SO_4) to partition organic P into fulvic acid (FA) and humic acid (HA) bound P. FA- presents acid soluble and alkali insoluble P.
NaOH-HA-P	Humic acid (HA) – P soluble in both acid and alkali. HA-P = [NaOH-TP] – [FA-P].
Residue- P	Acid (1 *M* HCl) and alkali (0.5 *M* NaOH) insoluble P. Considered as non-reactive P and stable pool.
Total Inorganic P	TPi = [$NaHCO_3$-Pi] + [HCl-Pi]
Total Organic P	TPo = [$NaHCO_3$-Po] + [MBP] + [HCl-Po] + [NaOH-FA-P] + [NaOH-HA-P] + [Residue- P]
Total P	Sum of Inorganic and Organic TP

3.1 Preliminary Results and Summary Data Tables

- Soil and floc samples from STA-2 Cell 3 are completely processed and analyzed for various P forms. Summary data tables are presented - Table 3-3 to Table 3-18.
- Soil samples from STA-2 Cell 1 are completely processed and analyzed for various P forms. Summary data tables are presented - Table 3-19 to Table 3-34.
- Some problems were encountered with floc samples from STA-2 Cell 1. Currently these floc samples are being re-analyzed and data will be reported in the next Quarterly report.
- Soil and floc samples from STA-3/4 and WCA-2A are in various stages of processing and analysis. Analysis of these samples will be completed in the next couple of months and results will be presented in the next quarterly report.
- Additional analysis for various routine chemical parameters is currently being conducted by the District laboratory. Data is not available at the time of this quarterly report preparation. As soon as these data become available, correlative relationships between P forms and select soil chemical properties will be explored.
- Interpretation of results will be presented in the second annual report due in July 2017.

Table 3-3. Concentration of Bicarbonate extractable Inorganic Phosphorus **($NaHCO_3$-Pi)**, also known as labile inorganic phosphorus (LIP) (mg/kg) of floc and soil samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). Soils were sampled by four components such as Floc, RAS, Pre-STA-1, and Pre-STA-2

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	276±41	194±40	14±2	11±4
38	750	174	124	15	17
56	1150	198	116	14	13
74	1550	141	137	15	21
92	2000	35	217	19	29
110	2380	64	39	26	19
128*	2800	36±14	34±9	15±3	24±2
146	3200	18	22	8	10
164	3600	20	18	8	9
182	4000	66	23	10	9
200*	4450	36±7	21±3	10±3	8±1

* Refers to benchmark sites with triplicate samples.

Table 3-4. Concentration of Bicarbonate extractable Total Phosphorus (**$NaHCO_3$-TP**) (mg/kg) of floc and soil samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV).

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA1	Pre-STA2
20*	350	331±47	227±40	18±4	17±4
38	750	189	131	17	21
56	1150	204	117	16.3	15
74	1550	152	144	22	24
92	2000	52	258	24	44
110	2380	77	42	36	25
128*	2800	46±10	45±7	21±5	36±2
146	3200	30	24	9	11
164	3600	31	22	10	12
182	4000	86.0	31	11	12
200*	4450	46±15	22±4	14±1	10±1

* Refers to benchmark sites with triplicate samples.

Table 3-5. Concentration of Bicarbonate extractable Organic Phosphorus **($NaHCO_3$-Po)**, also known as Labile Organic Phosphorus (LOP) (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). LOP was calculated from the difference of $NaHCO_3$-TP and NF-$NaHCO_3$-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	55.6±9.9	33.9±2.4	4.6±3.6	5.7±1.8
38	750	15.3	6.5	2.9	3.9
56	1150	6.5	0.5	2.1	2.1
74	1550	11.8	6.5	6.4	3.2
92	2000	16.6	41.6	5.4	14.7
110	2380	13.5	2.9	10.0	6.2
128*	2800	10.0±3.2	10.8±2.1	6.6±2.8	11.5±0.4
146	3200	12.0	2.3	1.6	0.4
164	3600	11.5	3.9	2.2	3.1
182	4000	19.8	7.7	0.9	2.8
200*	4450	10.5±8.7	1.3±1.2	3.4±1.3	2.2±0.3

* Refers to benchmark sites with triplicate samples.

Table 3-6. Concentration of Bicarbonate extractable Total Phosphorus **(F-$NaHCO_3$-TP)** (mg/kg) of floc and soils fumigated with chloroform to lyse microbial cells. Samples were obtained from STA-2 Cell-3 with submerged aquatic vegetation (SAV).

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	395±55	272±41	28±5	28±2
38	750	282	161	24	34
56	1150	370	168	20	21
74	1550	210	196	31	46
92	2000	92	304	44	73
110	2380	242	81	38	48
128*	2800	98±17	77±28	33±3	49±3
146	3200	130	57	16	15
164	3600	95	43	12	16
182	4000	201	32	16	18
200*	4450	118±22	31±3	15±1	17±3

* Refers to benchmark sites with triplicate samples.

Table 3-7. Concentration of Bicarbonate extractable Organic Phosphorus **(F-$NaHCO_3$-Po)**, also known as Total Labile Organic Phosphorus (TLOP) of fumigated samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). TLOP was calculated as a difference between F-$NaHCO_3$-TP and $NaHCO_3$-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	122±49	79±7	15±4	17±2
38	750	87	25	44	178
56	1150	42	12	29	55
74	1550	19	25	62	17
92	2000	27	14	17	22
110	2380	36	8	5	76
128*	2800	13±11	7±3	8±1	51±72
146	3200	5	7	7	11
164	3600	57	66	65	54
182	4000	112	71	76	99
200*	4450	117±52	49±11	11±5	17±8

* Refers to benchmark sites with triplicate samples.

Table 3-8. Concentration of Microbial Biomass Phosphorus **(MBP)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). MBP was calculated from the difference between F-$NaHCO_3$-TP and $NaHCO_3$-TP.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	66±58	45±10	10±2	11±2
38	750	93	30	7	14
56	1150	166	51	4	6
74	1550	57	52	9	22
92	2000	40	45	20	30
110	2380	165	40	2	23
128*	2800	52±9	33±27	11±4	13±2
146	3200	100	33	6	4
164	3600	64	21	2	4
182	4000	115	1	5	7
200*	4450	72±10	9±5	1±1	7±3

* Refers to benchmark sites with triplicate samples.

Table 3-9. Concentration of acid (1 *M* HCl) extractable Inorganic Phosphorus **(HCl-Pi)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This is the sum of calcium, magnesium, iron, and aluminum associated P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	410±25	351±62	105±18	111±18
38	750	458	386	205	286
56	1150	721	304	219	189
74	1550	346	328	62	38
92	2000	117	221	34	247
110	2380	170	150	97	164
128*	2800	104±14	118±14	47±2	57±5
146	3200	113	90	REDO	54
164	3600	91	123	84	75
182	4000	217	148	125	106
200*	4450	204±26	168±20	143±5	156±60

* Refers to benchmark sites with triplicate samples.

Table 3-10. Concentration of acid (1 *M* HCl) extractable Total Phosphorus **(HCl-TP)** (mg/kg) of samples from STA-2 Cell-3. This represents calcium, magnesium, iron, and aluminum associated P and acid hydrolysable organic P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	477±26	385±68	130±20	130±20
38	750	516	413	226	323
56	1150	768	313	244	201
74	1550	375	348	66	39
92	2000	121	242	51	257
110	2380	187	155	117	178
128*	2800	115±11	138±26	99±11	84±6
146	3200	118	111	REDO	95
164	3600	108	144	123	107
182	4000	239	165	171	143
200*	4450	207±22	192±23	196±3	198±66

* Refers to benchmark sites with triplicate samples.

Table 3-11. Concentration of acid (1 *M* HCl) extractable Organic Phosphorus **(HCl-Po)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). The hydrolysable organic P was estimated as the difference between HCl-TP and HCl-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	67±2	34±6	25±2	19±2
38	750	58	27	21	37
56	1150	47	9	25	11
74	1550	29	19	4	1
92	2000	5	21	17	10
110	2380	17	5	21	14
128*	2800	11±9	20±12	52±10	26±10
146	3200	5	21	REDO	40
164	3600	17	20	39	32
182	4000	21	16	46	37
200*	4450	4±4	24±4	53±2	42±12

* Refers to benchmark sites with triplicate samples.

Table 3-12. Concentration of alkali (0.5 *M* NaOH) extractable Total Phosphorus **(NaOH-TP)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV).

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	102±9	64±2	101±3	94±6
38	750	126	29	81	120
56	1150	92	61	102	85
74	1550	137	87	116	91
92	2000	51	58	138	89
110	2380	69	36	133	92
128*	2800	81±41	82±29	227±12	167±24
146	3200	65	70	117	123
164	3600	131	141	117	157
182	4000	150	101	146	181
200*	4450	128±21	104±8	165±18	173±36

* Refers to Benchmark sites with triplicate samples.

Table 3-13. Concentration of acid soluble and alkali insoluble phosphorus, also known as fulvic acid bound P **(FA-P)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This was done by treating alkali extracts with acid (conc. H_2SO_4) to partition organic P into fulvic acid (FA) and humic acid (HA) bound P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	75±15	44±14	45±3	38±7
38	750	84	28	38	115
56	1150	10	32	76	83
74	1550	79	57	96	45
92	2000	38	45	52	39
110	2380	67	30	60	48
128*	2800	49±13	68±22	139±16	93±7
146	3200	65	67	105	109
164	3600	89	90	76	86
182	4000	119	72	85	110
200*	4450	84±12	81±25	104±38	101±12

* Refers to benchmark sites with triplicate samples.

Table 3-14. Concentration of alkali soluble phosphorus, also known as humic acid bound phosphorus **(HA-P)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This represents phosphorus soluble in alkali, and calculated as a difference of NaOH-TP and FA-P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	28±20	20±12	56±4	56±1
38	750	42	1	43	5
56	1150	82	29	26	2
74	1550	58	30	20	46
92	2000	13	14	87	51
110	2380	2	7	74	44
128*	2800	32±29	14±10	88±27	74±25
146	3200	1	3	12	15
164	3600	41	51	42	72
182	4000	31	29	61	72
200*	4450	44±30	23±17	61±53	72±45

* Refers to benchmark sites with triplicate samples.

Table 3-15. Concentration of acid (1 *M* HCl) and alkali (0.5 *M* NaOH) insoluble phosphorus **(Residue-TP)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This is considered as non-reactive P and stable pool.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	141±23	104±21	25±4	24±4
38	750	169	126	41	44
56	1150	75	99	43	29
74	1550	117	117	27	28
92	2000	47	86	21	41
110	2380	90	100	28	30
128*	2800	38±8	71±6	22±4	18±2
146	3200	51	54	31	28
164	3600	62	61	42	37
182	4000	99	60	48	33
200*	4450	82±13	49±13	37±10	27±5

* Refers to benchmark sites with triplicate samples.

Table 3-16. Concentration of Total Inorganic Phosphorus **(TPi)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This is the sum of $NaHCO_3$-Pi and HCl-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	685±57	544±97	119±17	122±23
38	750	633	510	219	303
56	1150	919	420	233	202
74	1550	487	466	77	59
92	2000	153	438	53	276
110	2380	234	189	123	183
128*	2800	140±17	152±24	62±4	81±6
146	3200	130	112	NA	65
164	3600	111	141	92	84
182	4000	284	172	135	115
200*	4450	239±30	189±19	153±7	164±60

* Refers to Benchmark sites with triplicate samples.

Table 3-17. Concentration of Total Organic Phosphorus **(TPo)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This is the sum of $NaHCO_3$-Po, MBP, HCl-Po, NaOH-TP (FA-P + HA-P) and Residue- P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	432±66	280±32	165±5	152±7
38	750	462	218	153	218
56	1150	386	220	176	133
74	1550	352	281	162	146
92	2000	159	252	201	184
110	2380	354	184	193	165
128*	2800	192±45	216±53	318±17	236±26
146	3200	233	180	NA	196
164	3600	285	247	202	233
182	4000	404	187	246	260
200*	4450	296±42	187±20	259±14	251±43

* Refers to benchmark sites with triplicate samples.

Table 3-18. Concentration of Total Phosphorus **(TP)** (mg/kg) of samples from STA-2 Cell-3 with submerged aquatic vegetation (SAV). This is the sum of total organic and total inorganic phosphorus.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
20*	350	1117±96	825±123	284±21	274±27
38	750	1094	728	372	521
56	1150	1305	640	408	335
74	1550	838	747	239	204
92	2000	310	690	254	460
110	2380	588	373	317	348
128*	2800	332±62	368±72	381±16	317±23
146	3200	364	292	NA	260
164	3600	396	388	294	317
182	4000	687	358	381	375
200*	4450	535±64	375±39	412±20	415±83

* Refers to benchmark sites with triplicate samples.

Floc samples from STA-2 Cell 1 are undergoing analysis, so Table 3-19 to Table 3-34 do not have floc data.

Table 3-19. Concentration of Bicarbonate extractable Inorganic Phosphorus **($NaHCO_3$-Pi)**, also known as labile inorganic phosphorus (LIP) (mg/kg) of floc and soil samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). Soils were sampled by four components such as Floc, RAS, Pre-STA-I, and Pre-STA-II.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400	138±63	47±3	11±2	9±4
51	800	330	42	7	13
69	1200	354	27	10	11
86	1700	236	31	13	13
104	2200	188	12	13	13
121*	2700	88±21	27±8	13±3	17±1
138	3200	36	24	11	16
156	3700	46	18	12	13
173	4200	15	34	13	8
191	4600	12	18	14	11
208*	5200	7.7±4	18±19	13±2	16±2

* Refers to benchmark sites with triplicate samples.

Table 3-20. Concentration of Bicarbonate extractable Total Phosphorus (**$NaHCO_3$-TP**) (mg/kg) of floc and soil samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV).

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		71±14	14±2	13±1
51	800		69	15	13
69	1200		62	21	22
86	1700		41	17	15
104	2200		59	14	14
121*	2700		52±24	14±3	17±1
138	3200		70	30	22
156	3700		27	15	13
173	4200		46	14	10
191	4600		36	18	13
208*	5200		43±29	19±5	20±7

* Refers to benchmark sites with triplicate samples.

Table 3-21. Concentration of Bicarbonate extractable Organic Phosphorus **($NaHCO_3$-Po)**, also known as Labile Organic Phosphorus (LOP) (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). LOP was calculated from the difference of $NaHCO_3$-TP and NF- $NaHCO_3$-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		25±15	3±1	3±2
51	800		27	8	0
69	1200		35	11	11
86	1700		10	5	2
104	2200		47	2	1
121*	2700		26±18	2±1	1±1
138	3200		45	18	6
156	3700		9	3	0
173	4200		12	1	2
191	4600		18	3	2
208*	5200		24±11	6±3	5±5

* Refers to benchmark sites with triplicate samples.

Table 3-22. Concentration of Bicarbonate extractable Total Phosphorus **(F-$NaHCO_3$-TP)** (mg/kg) of floc and soils fumigated with chloroform to lyse microbial cells. Samples were obtained from STA-2 Cell-1 with emergent aquatic vegetation (EAV).

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		127±36	30±11	28±9
51	800		208	29	27
69	1200		123	26	26
86	1700		132	32	38
104	2200		173	30	24
121*	2700		190±132	28±8	26±2
138	3200		174	48	35
156	3700		126	35	30
173	4200		159	33	26
191	4600		114	38	40
208*	5200		197±26	49±16	40±10

* Refers to benchmark sites with triplicate samples.

Table 3-23. Concentration of Bicarbonate extractable Organic Phosphorus **(F-$NaHCO_3$-Po)**, also known as Total Labile Organic Phosphorus (TLOP) of fumigated samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). TLOP was calculated as a difference between F-$NaHCO_3$-TP and $NaHCO_3$-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		80±35	19±10	19±8
51	800		166	22	14
69	1200		96	16	15
86	1700		102	20	25
104	2200		161	17	11
121*	2700		162±126	15±5	9±3
138	3200		150	36	19
156	3700		108	23	16
173	4200		125	21	19
191	4600		96	23	29
208*	5200		179±9	37±16	24±8

* Refers to benchmark sites with triplicate samples.

Table 3-24. Concentration of Microbial Biomass Phosphorus **(MBP)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). MBP was calculated from the difference between F-$NaHCO_3$-TP and $NaHCO_3$-TP.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		56±28	16±11	16±8
51	800		139	14	14
69	1200		61	5	4
86	1700		91	15	13
104	2200		114	15	10
121*	2700		139±108	14±5	9±3
138	3200		105	18	13
156	3700		99	20	13
173	4200		113	20	16
191	4600		78	20	27
208*	5200		155±15	31±15	20±6

* Refers to benchmark sites with triplicate samples.

Table 3-25. Concentration of acid (1 *M* HCl) extractable Inorganic Phosphorus **(HCl-Pi)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This is the sum of calcium, magnesium, iron, and aluminum associated P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		266±13	44±2	15±2
51	800		270	47	17
69	1200		201	29	19
86	1700		178	35	17
104	2200		185	35	18
121*	2700		248±94	45±8	18±4
138	3200		212	50	15
156	3700		172	26	13
173	4200		96	54	13
191	4600		154	49	18
208*	5200		139±83	53±34	17±1

* Refers to benchmark sites with triplicate samples.

Table 3-26. Concentration of acid (1 *M* HCl) extractable Total Phosphorus **(HCl-TP)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This represents calcium, magnesium, iron, and aluminum associated P and acid hydrolysable organic P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		357±28	68±3	28±4
51	800		394	70	30
69	1200		281	46	28
86	1700		254	69	31
104	2200		257	60	31
121*	2700		321±89	71±5	33±10
138	3200		287	78	32
156	3700		206	41	23
173	4200		132	82	32
191	4600		185	71	30
208*	5200		178±74	79±34	33±5

* Refers to benchmark sites with triplicate samples.

Table 3-27. Concentration of acid (1 *M* HCl) extractable Organic Phosphorus **(HCl-Po)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). The hydrolysable organic P was estimated as the difference between HCl-TP and HCl-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		91±17	24±4	13±6
51	800		27	21	37
69	1200		80	17	9
86	1700		75	33	14
104	2200		72	25	12
121*	2700		73±6	27±5	14±7
138	3200		75	28	17
156	3700		34	15	9
173	4200		36	28	19
191	4600		32	22	12
208*	5200		39±15	26±6	17±4

* Refers to benchmark sites with triplicate samples.

Table 3-28. Concentration of alkali (0.5 *M* NaOH) extractable Total Phosphorus **(NaOH-TP)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV).

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		369±176	162±28	111±17
51	800		506	172	88
69	1200		480	161	91
86	1700		296	161	78
104	2200		347	166	97
121*	2700		363±142	144±29	79±3
138	3200		505	198	107
156	3700		222	147	98
173	4200		249	184	133
191	4600		253	207	173
208*	5200		321±12	261±39	172±18

* Refers to benchmark sites with triplicate samples.

Table 3-29. Concentration of acid soluble and alkali insoluble phosphorus, also known as fulvic acid bound P **(FA-P)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This was done by treating alkali extracts with acid (conc. H_2SO_4) to partition organic P into fulvic acid (FA) and humic acid (HA) bound P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		207±91	77±19	49±3
51	800		243	73	36
69	1200		221	67	50
86	1700		143	84	40
104	2200		245	88	46
121*	2700		170±65	61±21	33±4
138	3200		177	64	32
156	3700		78	51	40
173	4200		148	100	69
191	4600		108	54	53
208*	5200		94±10	89±9	66±20

* Refers to benchmark sites with triplicate samples.

Table 3-30. Concentration of alkali soluble phosphorus, also known as humic acid bound phosphorus **(HA-P)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This represents phosphorus soluble in alkali, and calculated as a difference of NaOH-TP and FA-P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		162±103	85±18	62±17
51	800		263	99	52
69	1200		259	94	42
86	1700		152	77	38
104	2200		102	79	50
121*	2700		193±106	83±31	45±6
138	3200		328	134	74
156	3700		143	96	57
173	4200		101	84	64
191	4600		144	152	120
208*	5200		227±22	172±31	106±19

* Refers to benchmark sites with triplicate samples.

Table 3-31. Concentration of acid (1 *M* HCl) and alkali (0.5 *M* NaOH) insoluble phosphorus **(Residue-TP)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This is considered as non-reactive P and stable pool.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		260±43	56±4	22±2
51	800		274	52	21
69	1200		266	45	22
86	1700		189	81	22
104	2200		264	80	38
121*	2700		240±49	69±3	30±5
138	3200		228	55	35
156	3700		126	44	21
173	4200		146	73	24
191	4600		122	64	26
208*	5200		118±9	62±10	30±2

* Refers to benchmark sites with triplicate samples.

Table 3-32. Concentration of Total Inorganic Phosphorus **(TPi)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This is the sum of $NaHCO_3$-Pi and HCl-Pi.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		313±12	55±4	24±6
51	800		312	53	30
69	1200		228	39	30
86	1700		209	48	30
104	2200		197	48	31
121*	2700		275±101	57±9	35±5
138	3200		237	62	31
156	3700		189	38	27
173	4200		131	67	21
191	4600		172	63	29
208*	5200		157±76	65±34	32±3

* Refers to benchmark sites with triplicate samples.

Table 3-33. Concentration of Total Organic Phosphorus **(TPo)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This is the sum of $NaHCO_3$-Po, MBP, HCl-Po, NaOH-TP (FA-P + HA-P) and Residue- P.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		800±252	261±34	166±16
51	800		1071	269	137
69	1200		922	239	137
86	1700		661	295	138
104	2200		844	288	158
121*	2700		838±288	255±27	132±6
138	3200		958	317	178
156	3700		490	229	145
173	4200		556	307	195
191	4600		502	316	241
208*	5200		657±2	386±68	243±31

* Refers to benchmark sites with triplicate samples.

Table 3-34. Concentration of Total Phosphorus **(TP)** (mg/kg) of samples from STA-2 Cell-1 with emergent aquatic vegetation (EAV). This is the sum of total organic and total inorganic phosphorus.

Transect Station	Distance from inflow (m)	Floc	RAS	Pre-STA-1	Pre-STA-2
34*	400		1113±264	315±37	190±11
51	800		1383	322	166
69	1200		1150	278	168
86	1700		871	343	168
104	2200		1041	336	189
121*	2700		1114±389	312±34	167±11
138	3200		1194	379	209
156	3700		679	267	171
173	4200		687	373	216
191	4600		674	379	270
208*	5200		814±76	452±83	275±33

* Refers to benchmark sites with triplicate samples.

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

Method development for P sorption/desorption isotherms progressed with evaluation of different extraction media including 0.01 M KCl, and various inflow and outflow site waters. Based upon scientific literature reviews, the use of outflow site water consistently for all STA cells would be deemed suitable as it contains a typical matrix that STA cells would experience, and the use of a single site water sample for incubations would provide consistency across all STAs to allow for comparison of values. However, actual testing of the media sources will continue and final results will be compared.

Meetings were held with SFWMD to discuss these issues, and another meeting will be held to make the final selection of methodology.

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 STA-2 Cell 3 (Figure 5-1 and Figure 5-2].

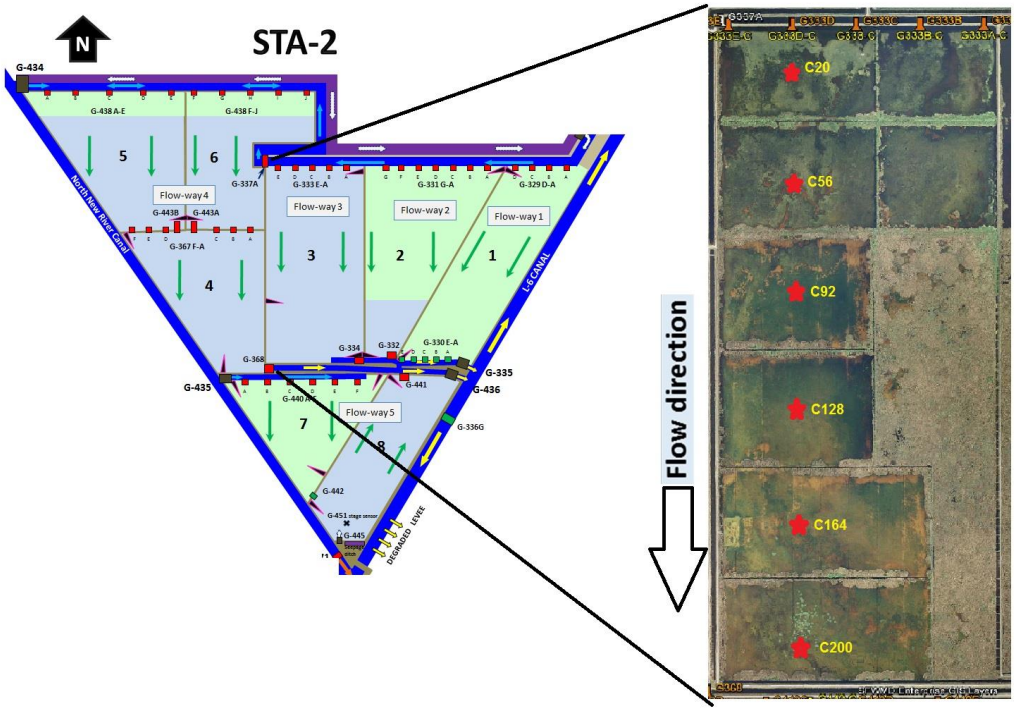

Figure 5-1: Surface water sampling locations – STA-2 Cell 3. Six stations where auto-samplers were deployed for continuous monitoring and weekly grab sampling.

5.1 Flow Event #5 (October 12 –November 22, 2016)

The fifth flow event was conducted in STA-2 Cell 3 to test the effect of high flow on an STA cell dominated by submerged aquatic vegetation (SAV). The flow event was divided into two distinct periods, the first being a high flow period spanning Oct 12 to Nov 3, 2016, followed by a stagnant flow period from Nov 4 to Nov 22, 2016 (Figure 5-3). During the high flow phase, average water inflows to the cell were 7.34 ± 2.5 $m^3 d^{-1}$, resulting in a mean hydraulic loading rate of 7.9 ± 0.3 cm d^{-1}. This resulted in an average P loading rate of 5.8 ± 0.4 mg P m^{-2} d^{-1}. There were no inflows into the cell during the stagnant phase of this flow event. Flows to the cell were determined by existing conditions, and availability of water (flows) in the upstream basin.

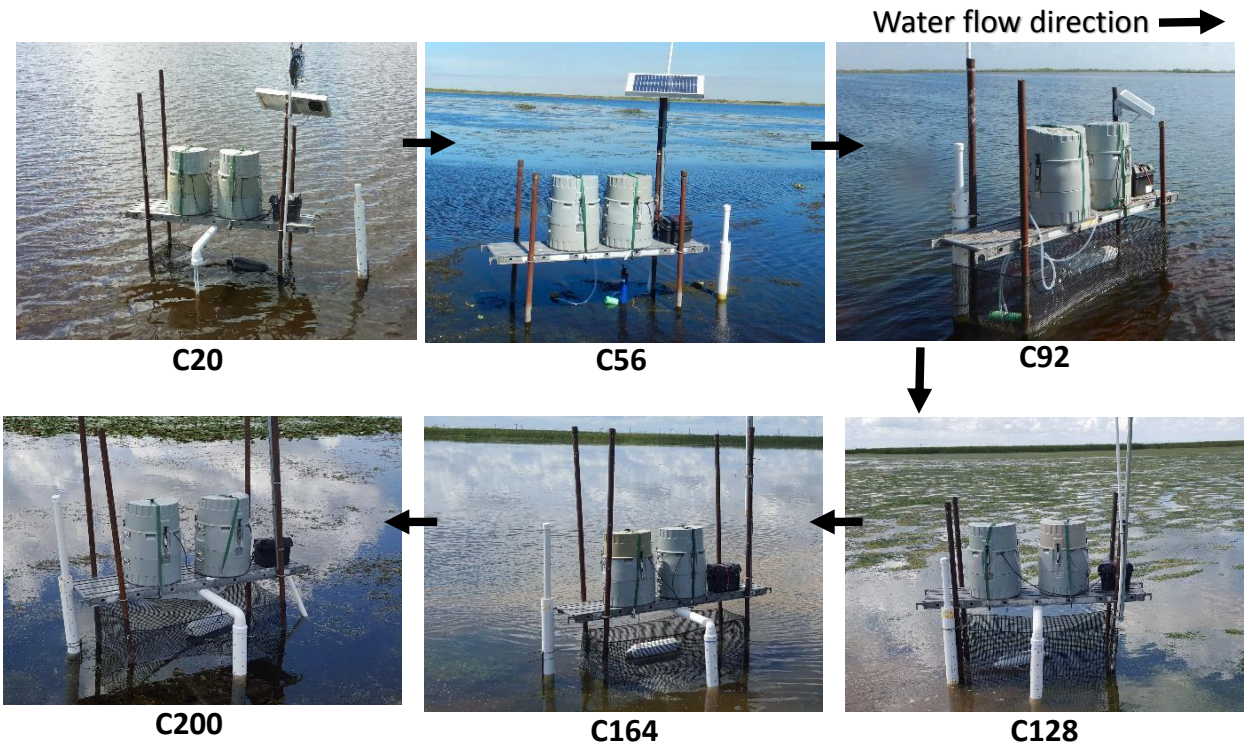

Figure 5-2: Auto-sampler stations along the transect stations within STA-2 Cell 3.

During the flow event, water samples were collected every 4 hours (2 am, 6 am, 10 am, 2 pm, 6 pm and 10 pm) and analyzed for TP. Weekly surface water grab samples were also collected from these stations 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 deployed to record physical and optical surface water parameters at a fine temporal resolution (15 min intervals). These parameters included temperature, pH, dissolved oxygen, specific conductance, 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.

Data collected during the fifth flow event (October 12 to November 22, 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.

5.2 Data Analysis – Methods

Water quality data from auto-sampler and grab samples were obtained from the District laboratory. Data was segregated into two groups corresponding to the two phases of flow experiment to allow differences in surface water nutrient concentrations between these flow phases (Table 5-1). Some parameters were analytically derived, while others were estimated from other parameters (Table 5-2).

Table 5-1. Mean (±SE), Total Flow, Hydraulic Loading Rate (HLR) and Phosphorus Loading Rate (PLR) for the fifth flow event in STA-2 Cell 3 (October 12 – November 22, 2016).

Date	Phase	Flow	Flow Volume ($\times 10^5$ m^3 d^{-1})		HLR (cm d^{-1})	PLR (mg m^{-2} d^{-1})
			Mean ± SE	Total		
Oct 12– Nov 3, 2016	Phase 1	High Flow	7.34 ± 2.5	168.9	7.90 ± 0.3	5.89 ± 0.4
Nov 4- Nov 22, 2016	Phase 2	No Flow	0.0	0.0	0.0	0.0

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). Spearman rank sum correlation analysis was performed on calculated surface water total organic nitrogen (estimated from total nitrogen – (nitrate + nitrite + ammonium)) and dissolved organic carbon (DOC). 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. 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 $\alpha = 0.05$.

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

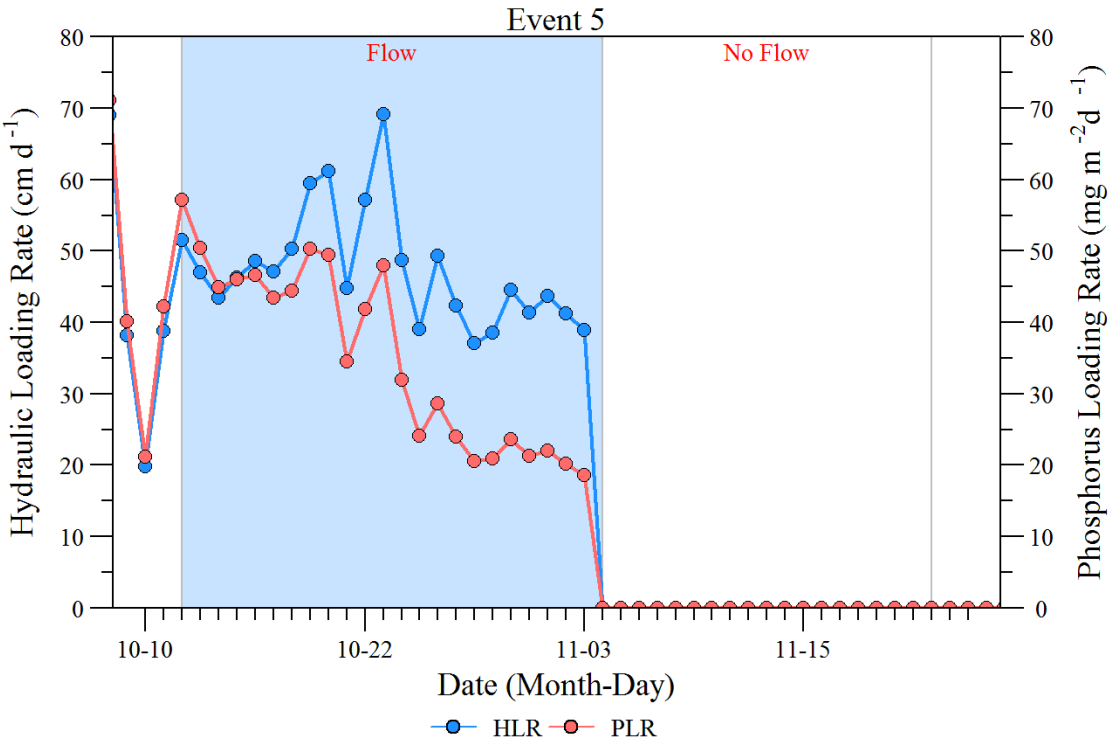

Figure 5-3: Hydraulic loading rate (HLR) and phosphorus loading rate (PLR), STA-2, Cell 3, (October 12 – November 22, 2016).

The fifth flow event generated a small number of autosampler TP data points due a shorter flow period. Overall 1,435 samples were collected with 22 of these points identified as outliers (Table 5-3).

Table 5-3. Total number of auto sampler total phosphorus samples that passed QA/QC screening collected during the fifth flow event. Number in parentheses is the total number of outliers per station and flow period.

Flow Period	C20	C56	C92	C128	C164	C200	Total
Phase 1	138 (2)	137 (2)	137 (2)	138 (2)	137 (2)	117 (2)	804 (12)
Phase 2	89 (1)	110 (2)	107 (2)	106 (2)	110 (2)	109 (1)	631 (10)

Much like the previous flow events, TP concentration significantly declined along the flow path with a general decrease of daily mean TP observed at each station (Figure 5-4 and Figure 5-5; χ^2= **159**, df=5, P<0.01). 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 were consistently higher at the C92 site relative to the other sites along the flow transect (Figure 5-7 and Figure 5-8) suggesting a higher "background" concentration attributable from soils, vegetation, etc During this flow period, elevated TP concentrations were observed at the first three stations during the flow event attributed to high velocity during flow period, wildlife disturbances, fluxing from the soil, decomposition of vegetation or algae observed near the inflow. As a whole, TP concentrations were gradually increasing under stagnant condition.

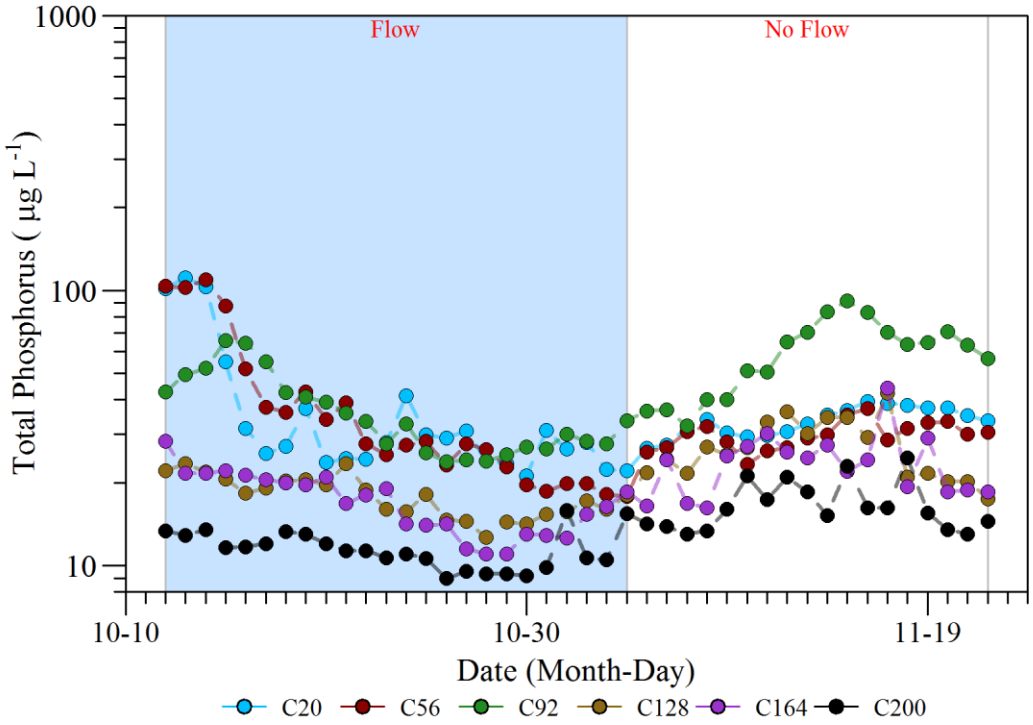

Figure 5-4: Daily mean autosampler total phosphorus concentration at locations along the STA-2 Cell 3 water quality transect during the fifth flow event. Note y-axis is on a log-scale and outliers were removed.

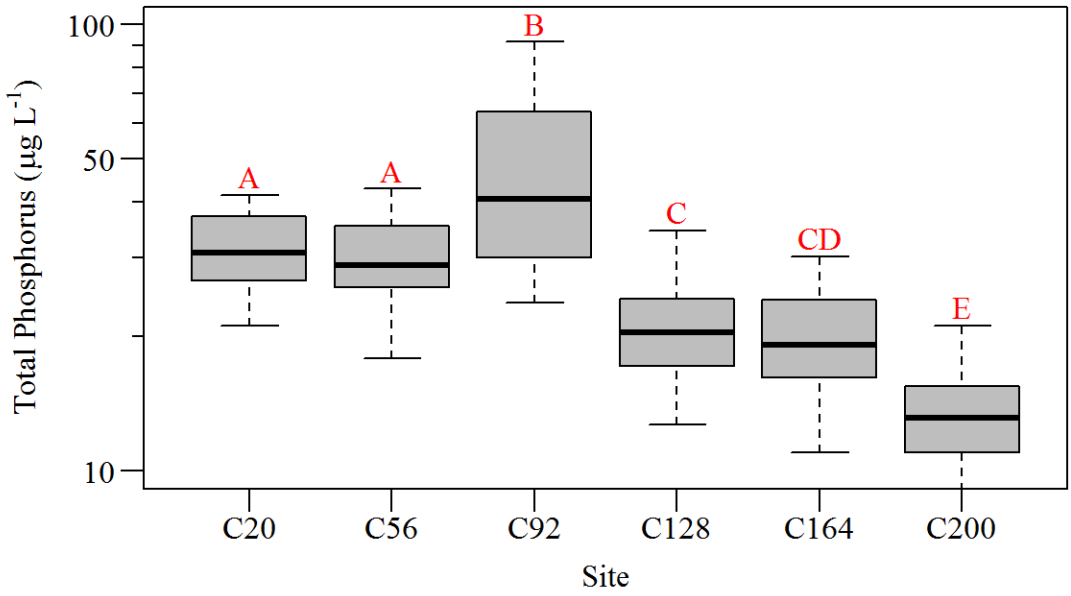

Figure 5-5: Boxplot of daily mean TP concentration by flow period during the 5th flow event (October 12 – November 22, 2016). Letters indicate Dunn's Multiple Comparison results in daily TP concentrations between periods; different letters indicate statistically significant differences. Note y-axis is on a log-scale.

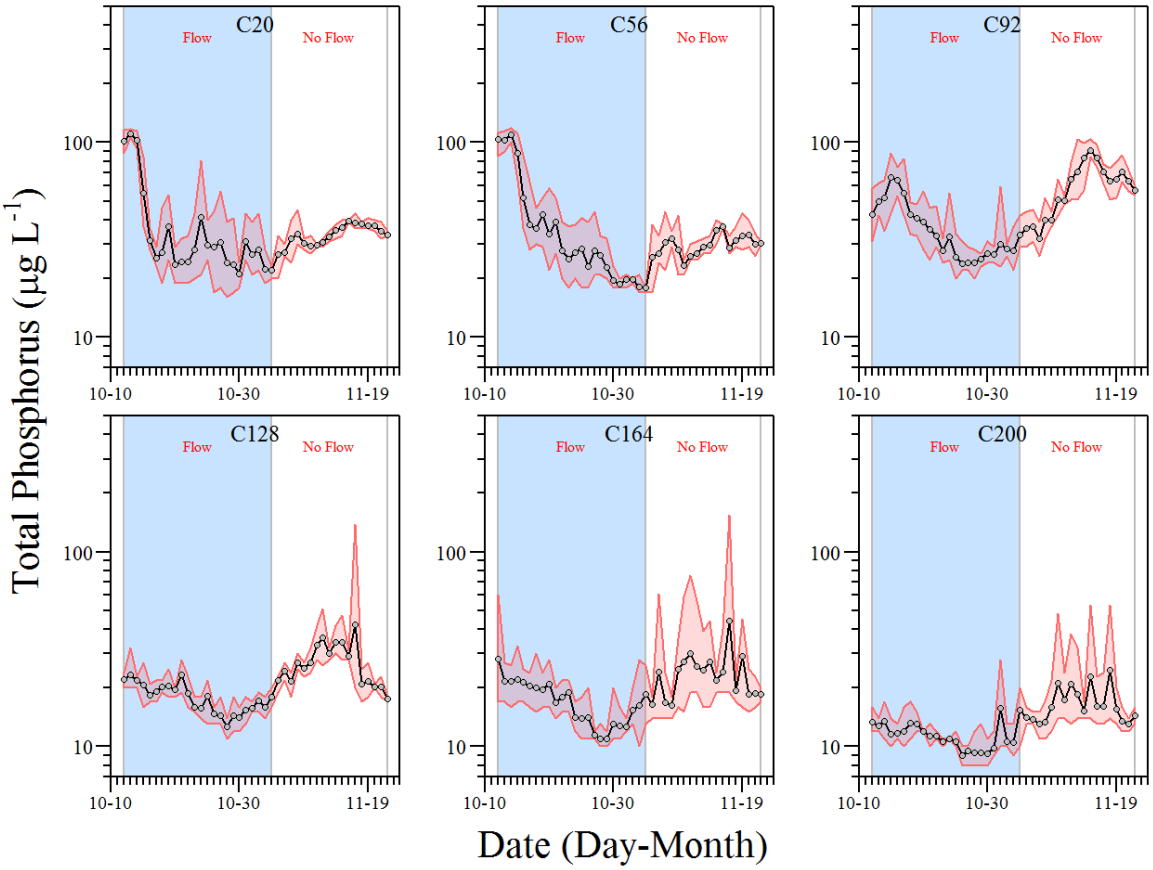

Figure 5-6: Daily mean (points) and range (red band) autosampler TP concentrations sampled every four-hours at locations along the STA-2 Cell 3 water quality transect during the fifth flow event (12 October – 22 November, 2016). Note y-axis is on a log-scale, values that exceeded the station and flow period 99th percentile were removed from daily mean and range calculations.

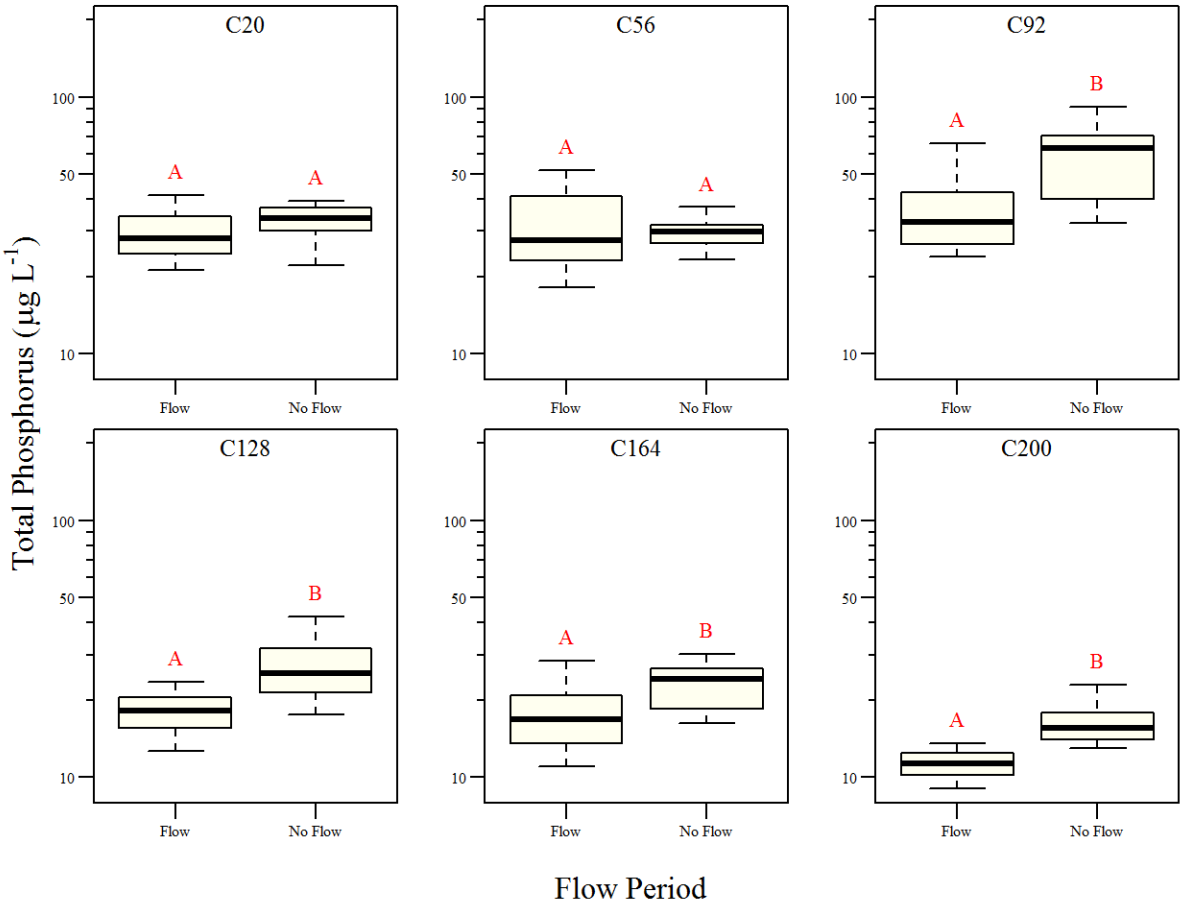

Figure 5-7. Boxplot of daily mean TP data during the fifth flow event (October 12 – November 22, 2016). Letters indicate Dunn's Multiple Comparison results in daily mean TP concentration between sites; different letters indicate statistically significant differences. Note y-axis is on a log-scale.

5.2.1 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. Particulate P (PP) constituted the largest proportion of TP in the surface water followed by dissolved organic P (DOP) during both phases of this flow event (Figure 5-8). Highest TP concentrations from grab samples were recorded during the no-flow period (68 µg L^{-1}; Site C92). Lowest TP concentration from grab samples were observed during the no-flow period (6 µg L^{-1}; C200). During both flow phases, PP comprised a large portion of TP. All P species were elevated during the "No Flow" period (Figure 5-7 and Figure 5-8).

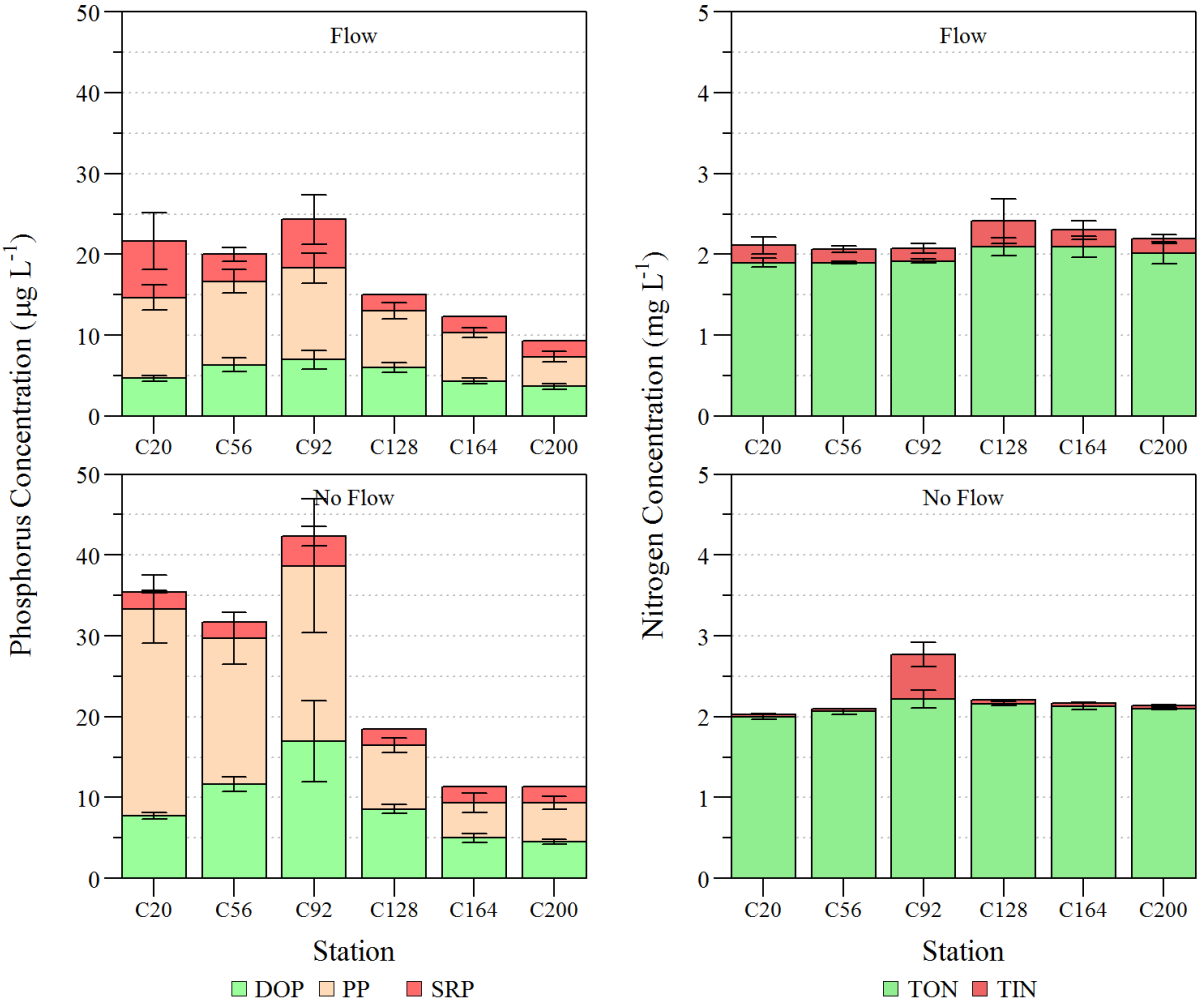

Figure 5-8: Phosphorus (Left) and Nitrogen (Right) fractions in surface water grab samples during fifth flow event.

In addition to the different P forms, trends in total organic and total inorganic nitrogen (TON and TIN, respectively) were also investigated on the grab samples during the fifth flow event (Figure 5-8). Total nitrogen (TN) concentrations varied slightly during and between flow conditions, with TON comprising the majority of the N pool. However, TIN concentrations were higher during the "Flow" period. During the "No Flow" period, TN concentrations were markedly elevated at C92 concurrent with high TP concentrations. Total N concentrations ranged from1.9 to 3.2 mg L^{-1}. Total organic nitrogen comprises most of the total nitrogen 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: $r = 0.75$, $\rho<0.001$). Generally, TIN concentrations were highest at the front end of the flow-way (i.e. C20 and C56).

5.2.2 Other nutrients and metals

Chlorophyll-A in surface water showed variable pattern along the flow way between the two phases of the flow event (Figure 5-9). During stagnant phase, Chl-A was relatively higher at the first three sampling stations in the treatment flow path, but lower at the remaining stations (Figure 5-10). Surface water dissolved organic C (DOC) (from grab samples) remained relatively constant across six sampling stations throughout the flow event (Figure 5-9) with concentrations ranging between 29 – 40 mg L^{-1}. Generally, DOC concentrations were greater during the "flow" period than during the "no-flow" period presumably due to the surface water with high DOC concentrations entering the cell during flow phase (Figure 5-9).

Surface water total iron and aluminum concentrations were elevated at the site nearest the inflow location (Figure 5-11). Total aluminum concentrations ranged from 8 to 126 µg L^{-1} with highest concentrations occurring at the inflow which quickly decreased to the MDL ~8 µg L^{-1}at the next station. Similarly, total iron concentrations ranged from 3 to 118 µg L^{1-} and remained relatively high until the mid-point of the cell where concentrations dropped to MDL ~3 µg L^{-1}.

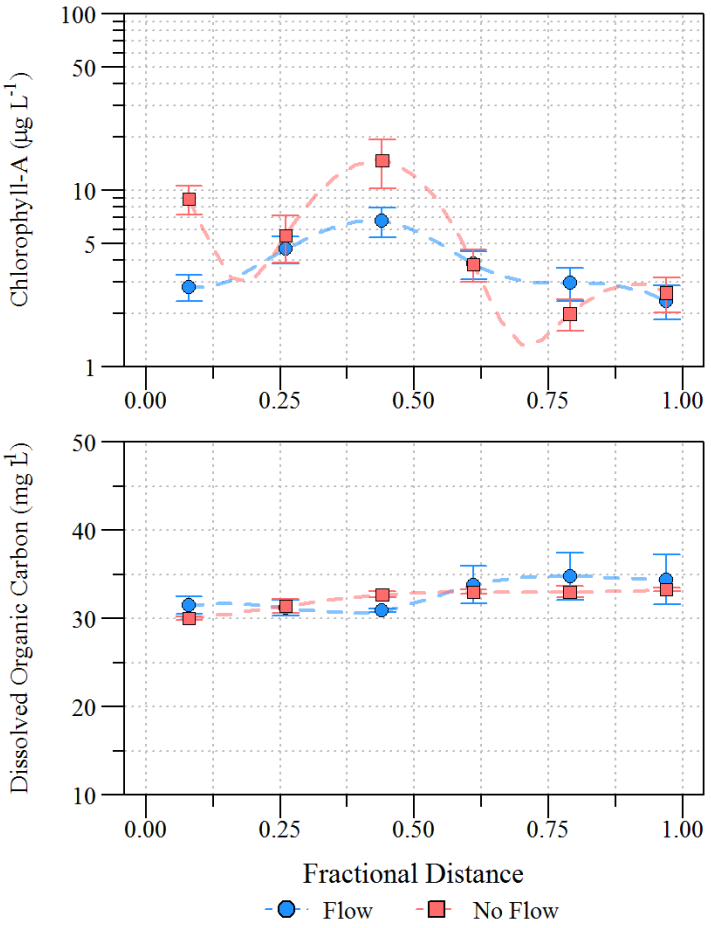

Figure 5-9: Flow period mean (±SE) chlorophyll-A and dissolved organic carbon concentrations during fifth flow event along the flow path.

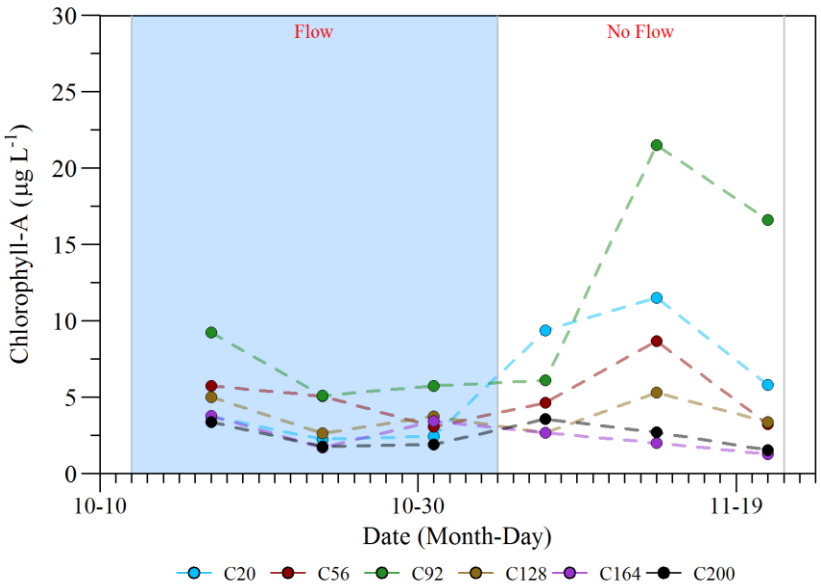

Figure 5-10: Change in chlorophyll-A concentrations at different stations during two phases of the flow event.

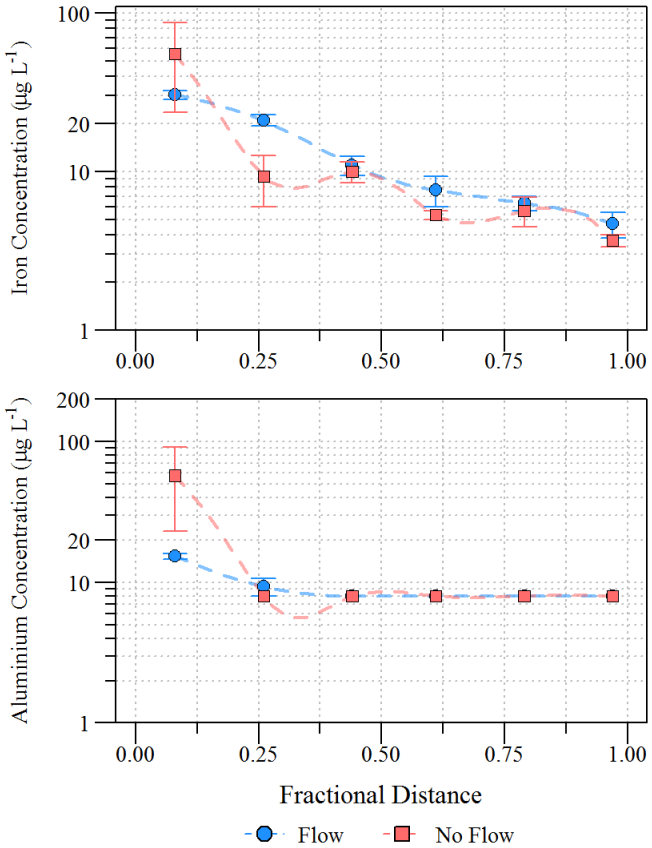

Figure 5-11: Mean (± SE) surface water total iron and aluminum concentrations for each flow period during the fifth flow event.

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 diagrams[1] of median ion concentrations (in milli equivalents) (Figure 5-12). Surface water entering the cell is rich in Ca and HCO_3 presumably due to the underlying geology - notice the relative change in shape of the stiff diagrams 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 increases from basin water entering the cell. Calcium and HCO_3 concentrations quickly reduce along the flow path, likely due to changes in pH and biotic activity. 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.

[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.

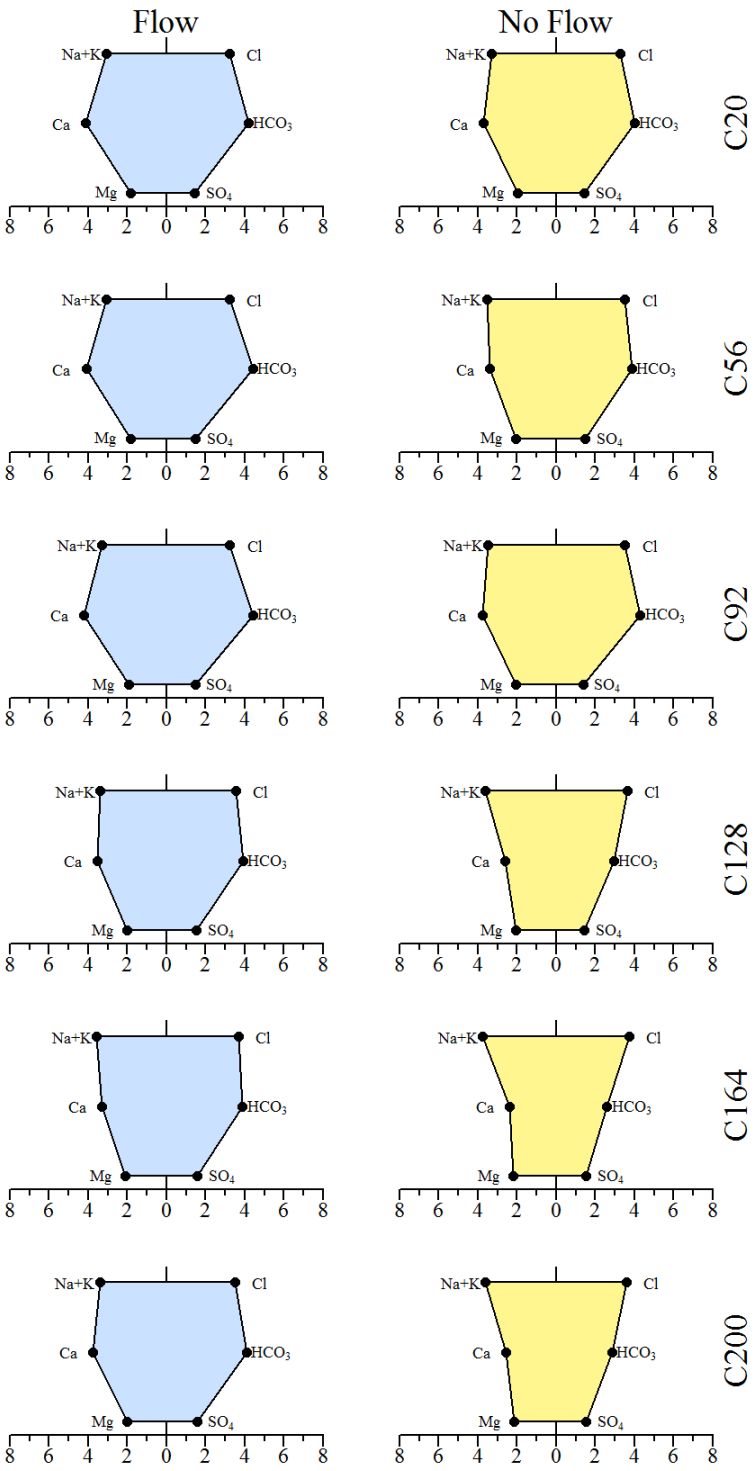

Figure 5-12: Median ion concentration for each station and flow period. Site along the flow path are arranged top to bottom and flow periods are arranged left to right.

5.2.3 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 EXO YSI 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 be qualified 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 fifth flow events.

Parameter	Units	Percent Useable
Temperature	Deg. C	99.4
Specific Conductivity	μs cm^{-1}	99.3
Optical Dissolved Oxygen	% Air Saturation	99.5
	mg L^{-1}	99.5
fDOM	ppb QSU	*0.0*
pH	SU	99.3
Chlorophyll	μg L^{-1}	98.3
BGA-PC (phycocyanin)	μg L^{-1}	*98.2*
Turbidity	NTU	80.7

Water quality sonde parameters (EXO YSI Sonde) were collected almost throughout the entirety of the fifth flow event except site C200 where a battery failure occurred early in the first phase leading into the second phase of the flow event. Surface water temperature ranged between 14.3 and 29.6 °C with clear diel changes (Figure 5-13).

Dissolved oxygen ranged between 0.1 and 379% saturation, and exhibited diel fluctuations. Unlike previous flow events, DO concentration either increased or remained the same during the "No Flow" period. Sites C92, C128, and C164 experienced the largest diel fluctuations in DO (Figure 5-14).

Surface water pH ranged between 7.2 to 9.7, 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 641 to 1446 μS cm^{-1} and responded to changes in flow regime (Figure 5-16).

Chlorophyll-a values were greatest at the cell mid-point (C92) 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 YSI sonde turbidity probe malfunctioned for site C20, therefore no data was collected for that site. Turbidity was greatest at site C92 during the "Flow" period (Figure 5-18). Unfortunately, fDOM data is not presented because all of this data did not meet screening criteria due to data being recorded beyond the factory settings of the sensor. Sensor adjustment or factory recalibration may be needed so that useable data could be collected in future flow events.

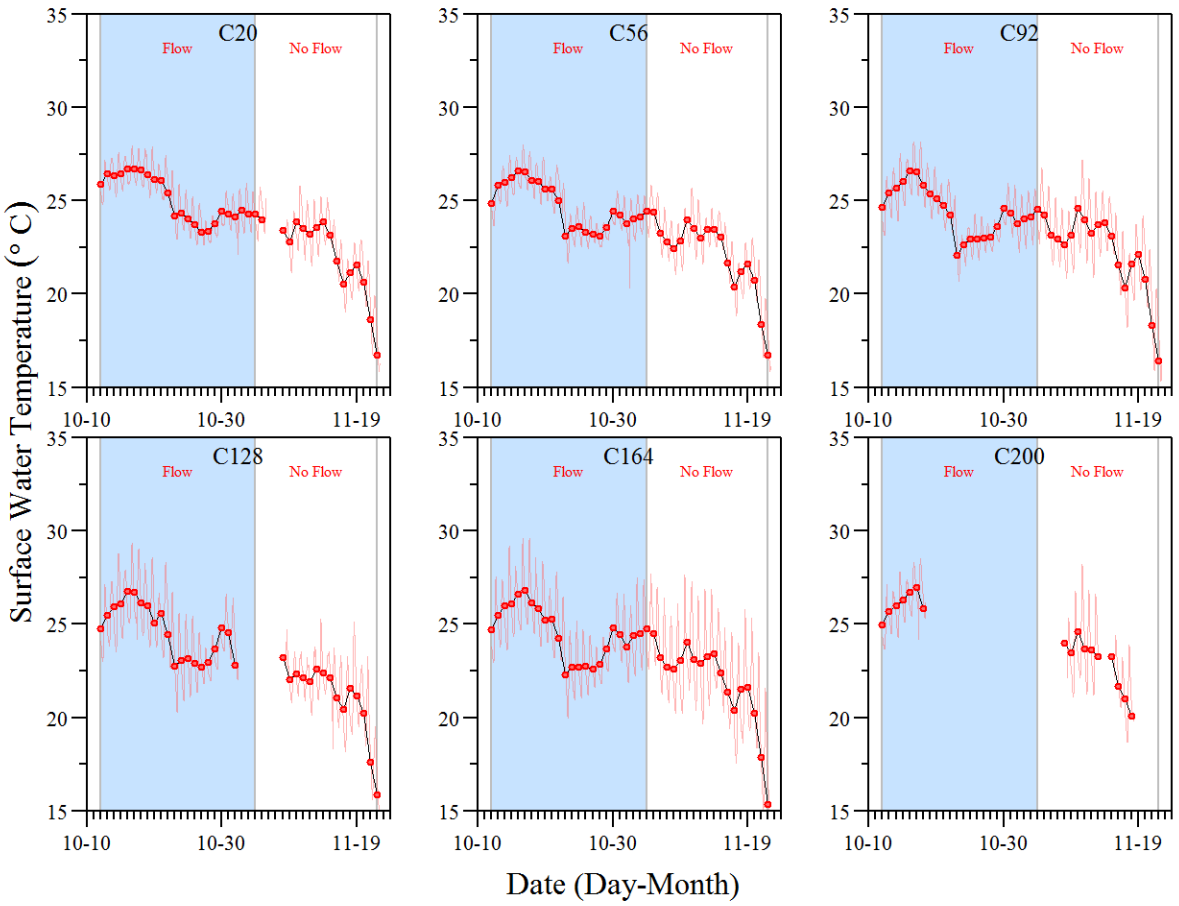

Figure 5-13: Daily (red point and black lines) and 15-minute (light red line) surface water temperature during the fifth flow event (12 October – 22 November, 2016) at sites along the flow path in STA-2 Cell 3.

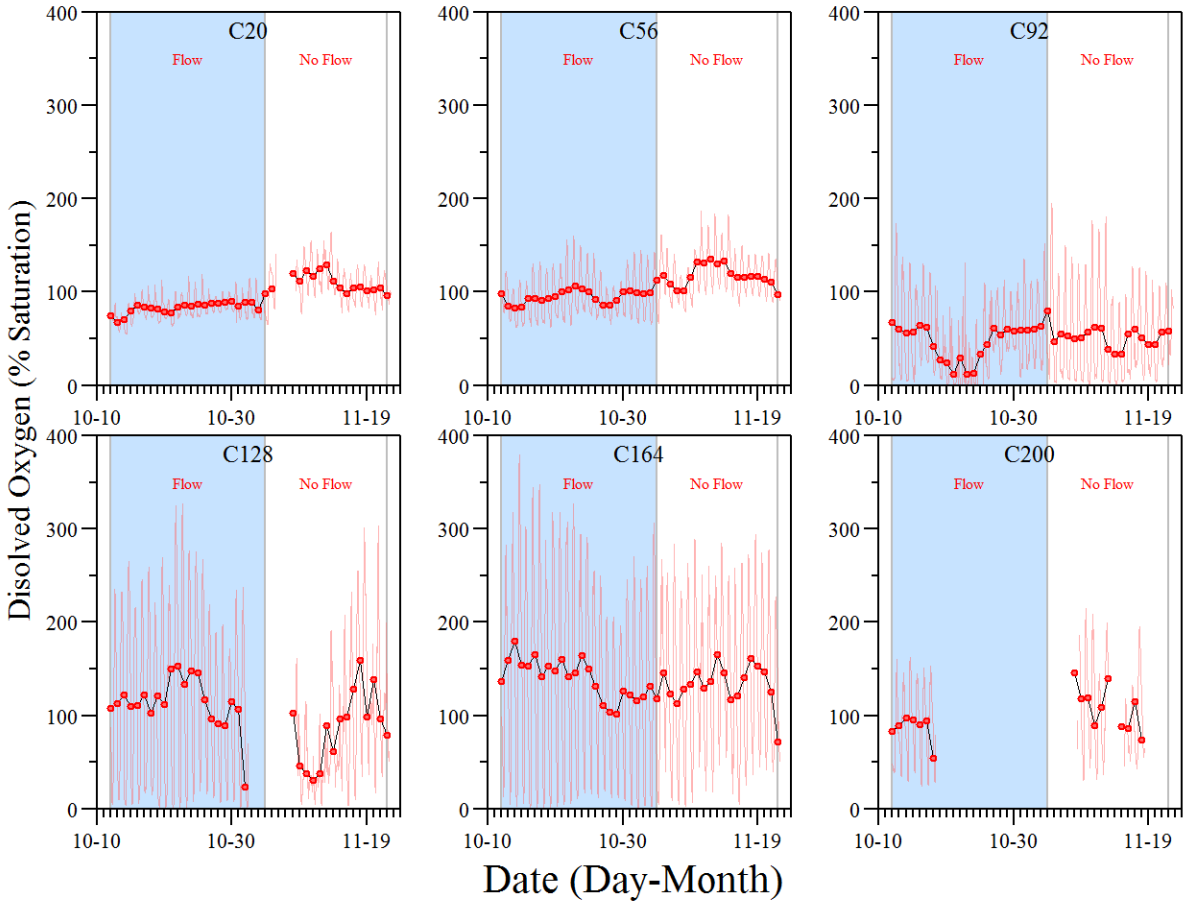

Figure 5-14: Daily (red point and black lines) and 15-minute (light red line) dissolved oxygen concentrations (% saturation) during the fifth flow event (12 October – 22 November, 2016) at sites along the flow path in STA-2 Cell 3.

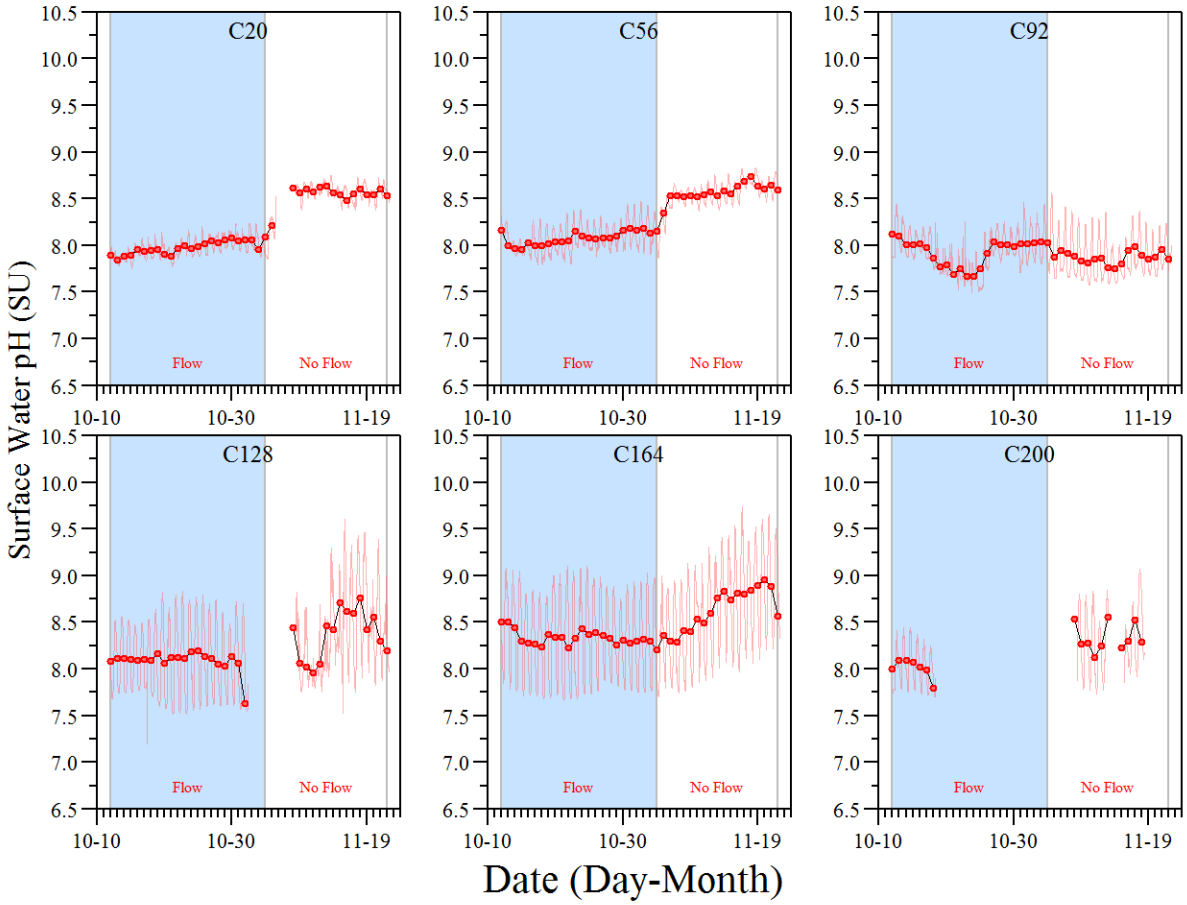

Figure 5-15: Daily (red point and black lines) and 15-minute (light red line) surface water pH during the fifth flow event (12 October – 22 November, 2016) at sites along the flow path in STA-2 Cell 3.

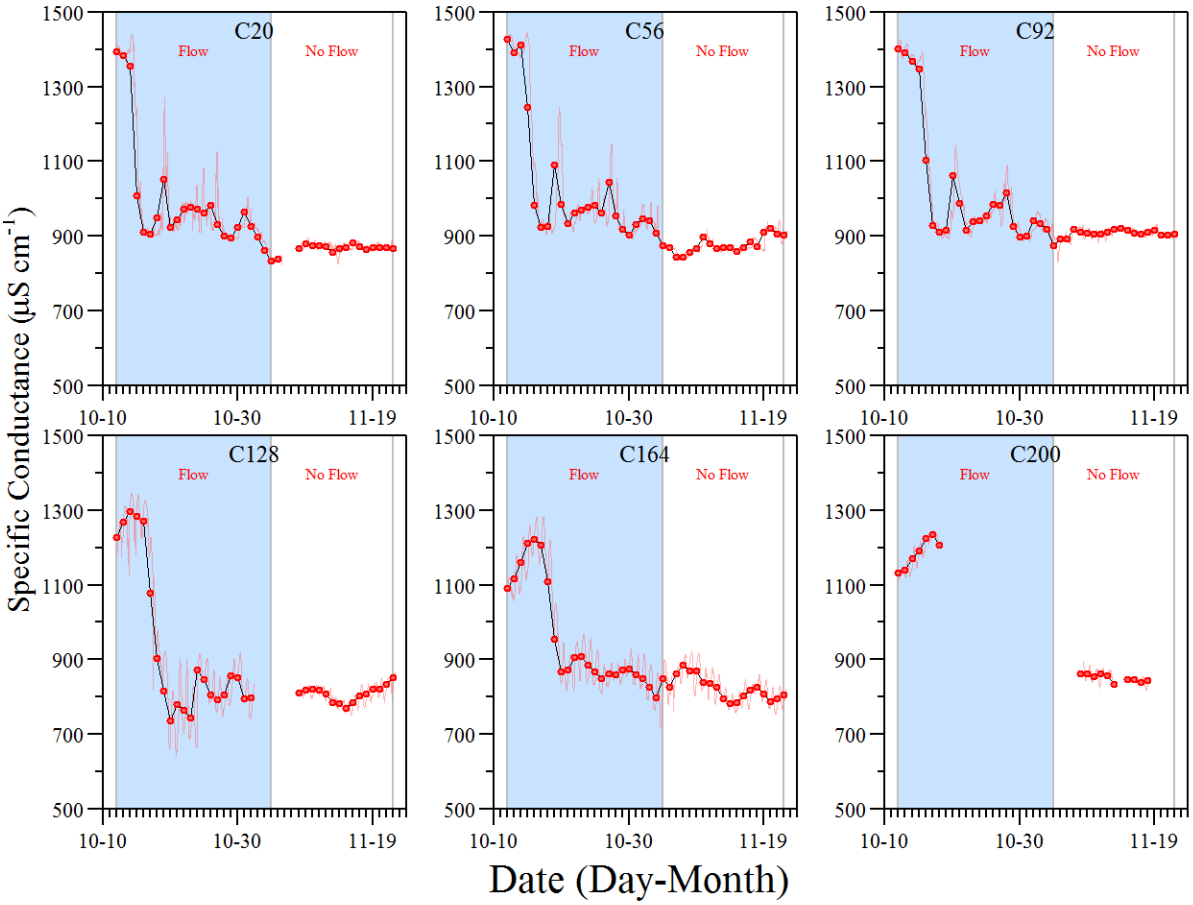

Figure 5-16: Daily (red point and black lines) and 15-minute (light red line) specific conductance during the fifth flow event (12 October – 22 November, 2016) at sites along the flow path in STA-2 Cell 3.

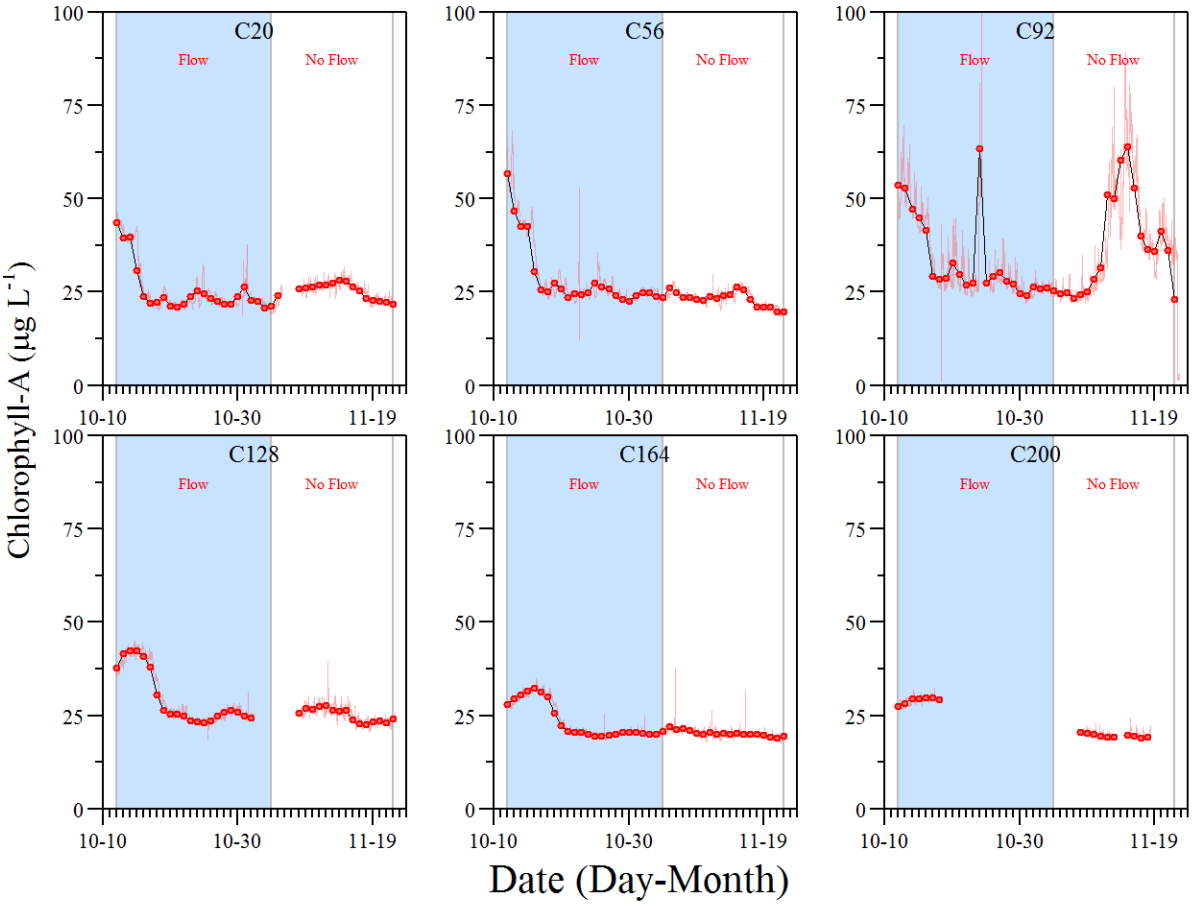

Figure 5-17: Daily (red point and black lines) and 15-minute (light red line) chlorophyll-a during the fifth flow event (12 October – 22 November, 2016) at sites along the flow path in STA-2 Cell 3.

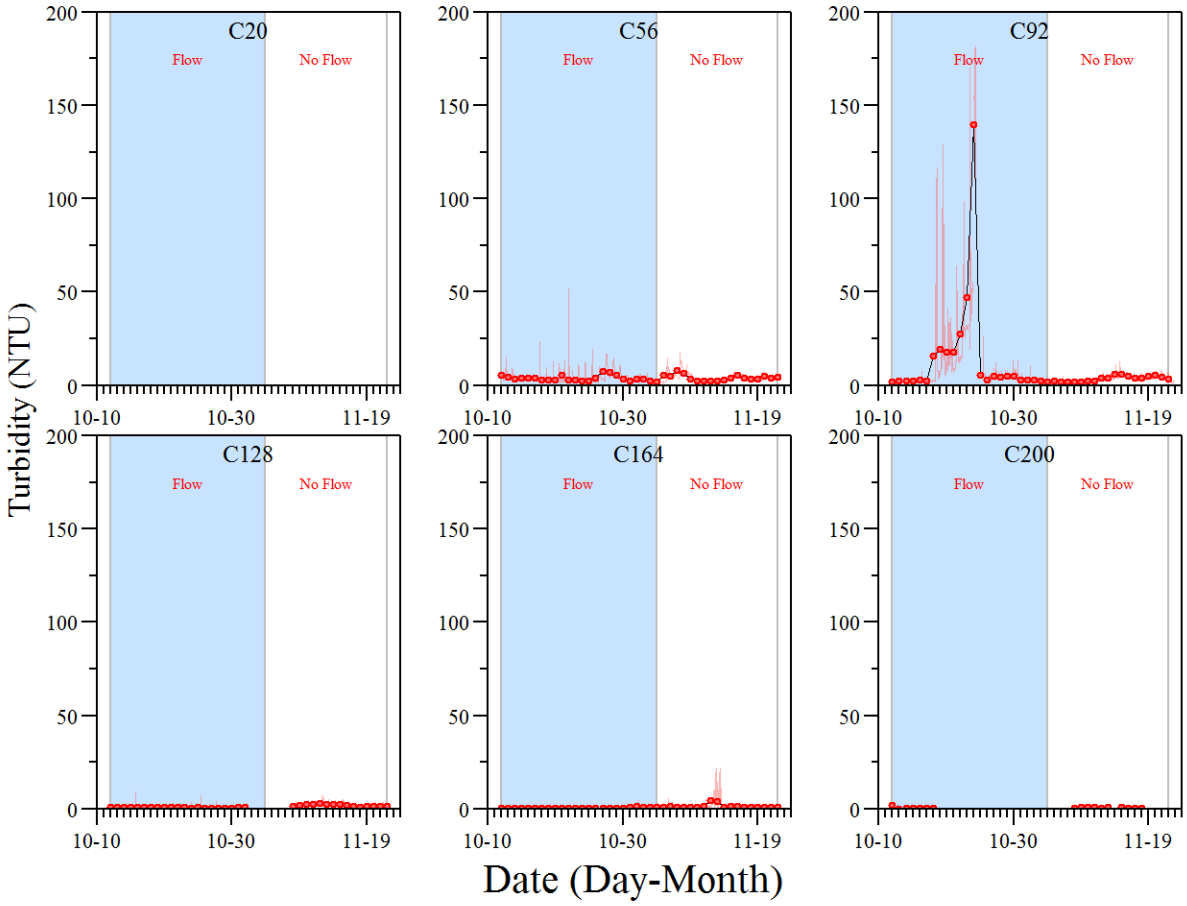

Figure 5-18. Daily (red point and black lines) and 15-minute (light red line) turbidity during the fifth flow event (12 October – 22 November, 2016) at sites along the flow path in STA-2 Cell 3.

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

The objective of this task is to determine the effect of water flow within the STAs on enzyme activities related to carbon (β Glucosidase), nitrogen (Aminopeptidase and N-acetyl glucosaminidase), and P (alkaline phosphatase (monoesterase), Bis-phosphatase (di-esterase) in floc and litter samples collected from the three benchmark sites (C-20, C128, and C200) in STA-2 Cell 3.

6.1 Work Completed During This Quarter

The fourth flow event extended 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, three cores were collected from the three STA-2 Cell 3 benchmark sites (C20, C120, C200) and transported to the UF-WBL laboratory. Intact cores were transported and stored at 4°C until processed (<24 hr).

Table 6-1. Flow conditions present during sampling for floc enzymes for the fourth flow event in STA-2 Cell 3 (27 June – 29 Aug, 2016)

Flow period	Sampling date	Flow	Flow Volume (x 10^5 m^3 d^{-1})		HLR (cm d^{-1})
			Mean ± SE	Total	
04 July - 24 July, 2016	July 12	Low Flow	3.23 ± 0.17	71.12	3.48 ± 0.19
25 July -14 Aug, 2016	August 9	No Flow	0.00	0.00	0.00
15 Aug - 29 Aug, 2016	August 22	Low (variable) 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), Bis-phosphatase (di-esterase), β-glucosidase, Leucine-aminopeptidase, and N-acetyl glucosaminidase. Enzyme analyses of these samples are complete, but some data values may not be available for presentation until dry weight and microbial biomass determinations are completed.

Table 6-2. Extracellular enzyme activities measured in floc samples based on dry weight for Flow Event 4. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase, NAG: N-acetyl-β-D-glucosaminidase. Values are averages of 3 replicates with standard error in parentheses. One replicate floc sample (*C20) was lost during processing therefore average is based on 2 values.

Flow	Site	AP	BisP	BG	LAP	NAG
		----------n mol $gdw^{-1}h^{-1}$----------				
Low	*C20	815(145)	325(45)	245(75)	2230(540)	195(35)
	C128	2497(446)	1113(127)	387(70)	3270(534)	517(78)
	C200	2980(112)	1957(313)	700(32)	4647(431)	507(68)
Stagnant	C20	387(62)	243(24)	147(18)	990(241)	107(7)
	C128	623(104)	417(54)	70(20)	960(211)	73(12)
	C200	1047(150)	580(142)	183(46)	1663(332)	147(38)
Low(variable)	C20	207(48)	247(37)	153(22)	1327(151)	113(15)
	C128	770(200)	650(214)	130(40)	1647(460)	140(40)
	C200	880(224)	850(231)	220(57)	2340(489)	190(139)

Table 6-3. Extracellular enzyme activities measured in litter samples based on dry weight for Flow Event 4. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase, NAG: N-acetyl-β-D-glucosaminidase. Values are averages of 3 replicates with standard error in parentheses.

Flow	Site	AP	BisP	BG	LAP	NAG
		----------n mol $gdw^{-1}h^{-1}$----------				
Low	C20	5763(1214)	2010(1055)	773(94)	36363(7145)	847(103)
	C128	13603(1530)	11447(1111)	1347(116)	29457(3288)	920(91)
	C200	12270(2182)	9413(1931)	847(133)	15063(1654)	517(67)
Stagnant	C20	12893(4001)	3090(941)	927(113)	56267(12850)	863(116)
	C128	15830(4923)	10537(3560)	973(224)	21590(3052)	767(358)
	C200	23107(3217)	18310(1946)	450(241)	16563(3592)	600(133)
Low(variable)	C20	6620(2294)	1877(631)	690(217)	61220(17709)	1170(407)
	C128	23553(3448)	20553(3959)	2360(439)	49650(11616)	1413(240)
	C200	19053(2018)	15643(3182)	1770(553)	25980(6465)	1117(165)

Table 6-4. Extracellular enzyme activities measured in floc samples based on microbial biomass carbon for Flow Event 4. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase, NAG: N-acetyl-β-D-glucosaminidase. Values are averages of 3 replicates with standard error in parentheses. One replicate floc sample (*C20) was lost during processing therefore average is based on 2 values.

Flow	**Site**	**AP**	**BisP**	**BG**	**LAP**	**NAG**
		----------nmol $gMBC^{-1}h^{-1}$----------				
Low	*C20	0.28 (0.03)	0.11(0.01)	0.08(0.00)	0.74(0.02)	0.07(0.01)
	C128	0.52(0.04)	0.24(0.02)	0.08(0.01)	0.69(0.05)	0.12(0.02)
	C200	0.37(0.03)	0.23(0.01)	0.08(0.00)	0.56(0.02)	0.06(0.00)
Stagnant	C20	0.11(0.02)	0.07(0.01)	0.04(0.01)	0.27(0.07)	0.03(0.00)
	C128	0.24(0.05)	0.16(0.03)	0.02(0.00)	0.35(0.06)	0.03(0.01)
	C200	0.22(0.01)	0.12(0.02)	0.03(0.01)	0.35(0.03)	0.03(0.00)
Low(variable)	C20	0.08(0.02)	0.10(0.02)	0.06(0.01)	0.51(0.1)	0.04(0.01)
	C128	0.17(0.05)	0.14(0.04)	0.03(0.01)	0.36(0.1)	0.03(0.01)
	C200	0.14(0.02)	0.14(0.03)	0.04(0.01)	0.36(0.05)	0.03(0.02)

The fifth experimental flow event occurred from October 12 to November 22, 2016. During this period intact soil cores were collected on November 1, 2016 and November 15, 2016 representing periods of high flow and stagnant respectively (Table 6-5). There was no litter collected during this flow event. 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, stored at 4°C overnight before being processed. Floc samples were collected from the top of the cores and analyzed for enzyme activities including phosphatase as mono-esterase (AP) and di-esterase (BisP), β-glucosidase (BG), and Leucine aminopeptidase (LAP) and N-acetyl glucosaminidase (NAG). Enzyme activity analyses for these samples are complete, and data are presented based on dry weight and microbial biomass carbon (Tables 6-6 and 6-7).

Table 6-5. Flow conditions present during sampling for floc enzyme activity for the fifth flow event in STA-2 Cell 3 (October 27 to Nov 20, 2016).

Flow period	**Sampling date**	**Flow**	**Flow Volume ($\times 10^5$ m^3 d^{-1})**		**HLR (cm d^{-1})**
			Mean ± SE	**Total**	
Oct 12 to Nov 3, 2016	Nov 1, 2016	High Flow	44.0 ± 1.6	1013.5	35.3 ± 2.7
Nov 4 to Nov 22, 2016	Nov 15, 2016	Stagnant	0.00	0.00	0.00

Table 6-6. Extracellular enzyme activities measured in floc samples based on dry weight for Flow Event 5. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase, NAG: N-acetyl-β-D-glucosaminidase. Values are averages of 3 replicates with standard error in parentheses.

Flow	**SITE**	**AP**	**BisP**	**BG**	**LAP**	**NAG**
		----------nmol $gdw^{-1}h^{-1}$----------				
High	C20	327(69)	477(107)	93(18)	1283(169)	277(30)
	C128	3147(421)	2233(315)	187(57)	6697(1947)	417(92)
	C200	1603(189)	1600(240)	193(36)	4697(715)	367(85)
Stagnant	C20	773(235)	310(52)	90(21)	1263(179)	33(9)
	C128	957(92)	1260(159)	63(9)	3403(433)	30(0)
	C200	837(314)	963(419)	140(33)	4750(404)	47(85)

Table 6-7. Extracellular enzyme activities measured in floc samples based on microbial biomass carbon for Flow Event 5. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase, NAG: N-acetyl-β-D-glucosaminidase. Values are averages of 3 replicates with standard error in parentheses.

Flow	**Site**	**AP**	**BisP**	**BG**	**LAP**	**NAG**
		----------nmol $gMBC^{-1}h^{-1}$----------				
High	C20	0.09(0.02)	0.14(0.03)	0.03(0.00)	0.37(0.03)	0.08(0.00)
	C128	0.54(0.06)	0.38(0.04)	0.03(0.01)	1.14(0.30)	0.07(0.01)
	C200	0.28(0.01)	0.28(0.02)	0.03(0.00)	0.82(0.03)	0.06(0.01)
Stagnant	C20	0.23(0.04)	0.10(0.02)	0.03(0.01)	0.40(0.05)	0.01(0.00)
	C128	0.20(0.01)	0.26(0.03)	0.01(0.00)	0.72(0.07)	0.01(0.00)
	C200	0.14(0.01)	0.16(0.02)	0.02(0.00)	0.78(0.18)	0.01(0.01)

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

The objectives of this study are to: (1) determine patterns of biogeochemical parameters, microbial activity and organic P mineralization as they relate to spatial gradients of nutrients and vegetation type in STA soils; and (2) document the patterns of decomposition processes and nutrient release in vertical profiles (aerobic/anaerobic transitions) of key STA regions.

7.1 Work Completed During This Quarter

Transect soil sampling was conducted in STA-2 Cell 3 over a period of 2 days (7-9 September). 11 sites including 3 benchmark sites (C20, C128, C200) (Table 7-1). Samples were submitted to UF-WBL on September 15, 2016. Samples were stored at 4C till they were transported to UFL-WBL. Transect soil sampling was conducted in STA 2 Cell 1 from September 19-22 at 17 sites including 3 benchmark sites (34, 121, 208) (Table 7-4). Samples were submitted to the UF-WBL on October 6, 2016. Transect soil sampling was conducted in STA 3/4 Cell 3B from October 4-6 at 20 sites including 6 benchmark sites (A7c, A7, C7c, C7, D7c, D7) (Table 7-9). Samples were submitted to UF-WBL on October 20, 2016. Transect soil sampling was conducted in STA3/4 Cell 3A from October 20-21, 2016 at 11 sites including 3 benchmark sites (A8, A32, A56) (Table 7-14). Samples were submitted to UF-WBL on October 27, 2016.

At each transect site, one intact soil core was collected, and at the benchmark sites, 3 replicate cores were collected. All cores were sectioned into- floc, recently accreted soil (RAS) and pre-STA soil fractions for STA 2 Cell 1 (n=68), STA 2 Cell 3 (n=68), and STA 3/4 Cell 3A (n=44). In STA 3/4 Cell 3B, all cores were sectioned into- floc, recently accreted soil (RAS) and 2 pre-STA soil fractions (n=80). Floc depth was determined after allowing flocculent material to settle in a plastic core tube for 3-4 hours (See Chapter 3). Plant litter samples were collected from STA 2 Cell 1, 3/4 Cell 3B (n=17) and STA 3/4 Cell 3A (n=11), but due to the difficulty in identifying SAV litter, they were not collected from STA 2 Cell 3. Samples were stored in plastic sample bags at ~4^{o}C until they were shipped to the UF-WBL.

Microbial biomass carbon, nitrogen and phosphorus values were determined for all samples. Other biogeochemical parameters including rates of enzyme activities (phosphatase, bis-phosphatase, aminopeptidase, glucosidase) respiration (aerobic and anaerobic), and potential mineralizable Phosphorus were determined in select samples. See tables below (Table 7-1 to Table 7-18). All lab analyses have been completed; however, the data analyses for some parameters (respiration, and potential mineralizable P) are still underway (to be analyzed=TBA). Parameter values for analyses completed to date are presented below.

Table 7-1. Samples collected from transect and benchmark sites (bold) in STA-2 Cell 3 were received by (UF-WBL) in September, 2016. All samples (n= 68) 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	Replicates	Litter	Floc	RAS	Pre-STA-1	Pre-STA-2
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

Table 7-2. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) based on dry weight for floc, soil and samples collected from transect and benchmark sites (bold) in STA-2 Cell 3. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such. Values with asterisks (*) indicate that some replicate samples are currently being rerun.

STA-2 Cell 3		**MBC**	**MBN**	**MBP**
Site	**Sample**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**
C-20	Floc	2458(287)	333(32)	66.2(33.6)
C-20	RAS	1315(67)	135(17)	44.7(5.4)
C-20	Pre-STA-1	230(89)	*23	10.0(1.4)
C-20	Pre-STA-2	452(53)	*35	11.1(1.2)
C-38	Floc	2335	174.8	93.1
C-38	RAS	919	73.4	30.0
C-38	Pre-STA-1	443	15.3	6.9
C-38	Pre-STA-2	1058	27.3	13.7
C-56	Floc	2132	227.1	166.2
C-56	RAS	771	24.3	51.3
C-56	Pre-STA-1	946	34.6	4.1
C-56	Pre-STA-2	613	29.0	6.3
C-74	Floc	2953	359.1	57.4
C-74	RAS	939	66.2	52.2
C-74	Pre-STA-1	416	24.8	9.2
C-74	Pre-STA-2	1236	39.9	22.1
C-92	Floc	2174	247.1	40.1
C-92	RAS	715	13.1	45.3
C-92	Pre-STA-1	651	20.7	19.6
C-92	Pre-STA-2	654	49.2	29.6
C-110	Floc	3477	439.8	164.5
C-110	RAS	1158	71.4	39.6
C-110	Pre-STA-1	780	50.4	2.0
C-110	Pre-STA-2	588	17.8	23.0
C-128	Floc	4611(864)	598(183)	51.8(5)
C-128	RAS	2258(424)	179(57)	32.6(16)
C-128	Pre-STA-1	915(267)	44.4(21)	11.2(2)
C-128	Pre-STA-2	1443(89)	76(22)	13.2(1)
C-146	Floc	6070	768.9	99.7
C-146	RAS	2423	192.2	33.2
C-146	Pre-STA-1	916	31.5	6.4
C-146	Pre-STA-2	931	131.0	4.2
C-164	Floc	5589	640.6	64.3
C-164	RAS	1822	179.2	20.9
C-164	Pre-STA-1	608	22.0	1.8
C-164	Pre-STA-2	393	54.6	3.9

C-182	Floc	6156	696.1	114.8
C-182	RAS	1237	61.9	1.3
C-182	Pre-STA-1	892	19.1	5.1
C-182	Pre-STA-2	418	8.1	6.7
C-200	Floc	3328(444)	360.0(54)	71.7(6)
C-200	RAS	1220(62)	42.7(22)	8.9(3)
C-200	Pre-STA-1	590(23)	*12	1.3(0.7)
C-200	Pre-STA-2	459(98)	*15	6.5(2)

Table 7-3. Extracellular enzyme activities measured based on dry weight for floc, and soil samples collected from transect and benchmark sites (bold) in STA-2 Cell 3. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-2 Cell 3		**AP**	**BisP**	**BG**	**LAP**
Site	**Sample**	**---------- (nmols/g dw/h) ----------**			
C-20	Floc	569.3(60)	323.7(129)	19.3(2)	693.7(119)
C-20	RAS	123.3(21)	54.3(2)	8.7(3)	152.7(44)
C-20	Pre-STA-1	83.0(14)	25.3(4)	11.7(5)	80.5(29)
C-38	Floc	145.0	60.0	57.0	335.0
C-38	RAS	98.0	79.0	47.0	171.0
C-56	Floc	93.0	76.0	72.0	324.0
C-56	RAS	190.0	89.0	26.0	83.0
C-74	Floc	239.0	131.0	107.0	786.0
C-74	RAS	147.0	96.0	97.0	112.0
C-92	Floc	479.0	568.0	84.0	899.0
C-92	RAS	227.0	112.0	5.0	228.0
C-110	Floc	1700.0	354.0	79.0	1431.0
C-110	RAS	148.0	100.0	2.0	132.0
C-128	Floc	1125(179)	1781(620)	87.7(20)	1577(334)
C-128	RAS	395(70)	204.7(18)	18.0(6)	234(54)
C-128	Pre-STA-1	191(8)	53.3(12)	32.0(5)	127(76)
C-146	Floc	1223.0	1858.0	125.0	1725.0
C-146	RAS	422.0	199.0	7.0	337.0
C-164	Floc	3851.0	746.0	37.0	1058.0
C-164	RAS	662.0	239.0	10.0	263.0
C-182	Floc	3835.0	2754.0	25.0	2282.0
C-182	RAS	241.0	101.0	7.0	131.0
C-200	Floc	665.0(106)	331.7(45)	201.3(34)	901.0(208)
C-200	RAS	169.0(24)	45.3(7)	17.0(11)	67.5(12)
C-200	Pre-STA-1	198.0(68)	40.3(10)	31.3(10)	27

Table 7-4. Samples collected from transect and benchmark sites (*) in STA-2 Cell 1 were received by (UF-WBL) in October, 2016. All samples (n= 85) were analyzed for microbial biomass C/N/P, while red bold samples **x (n=60)** were analyzed for additional parameters (see text for details).

STA-2 Cell 1						
Location	Replicates	Litter	Floc	RAS	Pre-STA-1	Pre-STA-2
C34	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
C51	1	**x**	**x**	**x**	x	x
C69	1	**x**	**x**	**x**	x	x
C86	1	**x**	**x**	**x**	x	x
C104	1	**x**	**x**	**x**	x	x
C121	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
C138	1	**x**	**x**	**x**	x	x
C156	1	**x**	**x**	**x**	x	x
C173	1	**x**	**x**	**x**	x	x
C191	1	**x**	**x**	**x**	x	x
C208	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx

Table 7-5. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) based on dry weight for litter samples collected from transect and benchmark sites (bold) in STA-2 Cell 1. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-2 Cell 1		MBC	MBN	MBP
Site	Sample	(mg/kg)	(mg/kg)	(mg/kg)
C34	Litter	14929(1629)	1709150)	203(24)
C51	Litter	12774	1492	187
C69	Litter	9752	1089	372
C86	Litter	16154	2027	317
C104	Litter	12910	1417	208
C121	Litter	10953(4220)	1278(800)	107 (79)
C138	Litter	15381	1618	413
C156	Litter	14581	1672	208
C173	Litter	9539	933	132
C191	Litter	12370	702	165
C208	Litter	11734(1539)	1159(278)	113(51)

Table 7-6. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) based on dry weight for floc and soil samples collected from transect and benchmark sites (bold) in STA-2 Cell 3. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-2 Cell 1		MBC	MBN	MBP
Site	Sample	(mg/kg)	(mg/kg)	(mg/kg)
C34	Floc	13173(171)	1380(101)	383(11)
C34	RAS	3300(374)	371(42)	56(16)
C34	Pre-STA-1	1680(338)	116(37)	16(6)
C34	Pre-STA-2	1817(442)	96(11)	15(4)
C51	Floc	23508	2078	928
C51	RAS	7006	808	139
C51	Pre-STA-1	1534	142	14
C51	Pre-STA-2	3562	189	14
C69	Floc	19983	1804	519
C69	RAS	5126	540	61
C69	Pre-STA-1	1852	131	5
C69	Pre-STA-2	2203	136	4
C86	Floc	23626	2526	929
C86	RAS	6183	699	91
C86	Pre-STA-1	1384	33	15
C86	Pre-STA-2	1238	82	23
C104	Floc	25507	3198	865
C104	RAS	5337	643	114
C104	Pre-STA-1	1322	57	15
C104	Pre-STA-2	1240	78	10
C121	Floc	29223(308)	3841(139)	815(173)
C121	RAS	6289(777)	648(104)	137(62)
C121	Pre-STA-1	1484(134)	75(27)	14(3)
C121	Pre-STA-2	1137(232)	56(26)	9(2)
C138	Floc	12391	1436	274
C138	RAS	4380	386	105
C138	Pre-STA-1	1022	5	18
C138	Pre-STA-2	358	44	13
C156	Floc	9253	871	459
C156	RAS	4737	500	99
C156	Pre-STA-1	1080	TBA	20
C156	Pre-STA-2	894	18	16
C173	Floc	16170	1948	480
C173	RAS	8447	729	113
C173	Pre-STA-1	1334	21	20
C173	Pre-STA-2	1127	44	16
C191	Floc	9560	1018	196

C191	RAS	4007	538	78
C191	Pre-STA-1	1617	86	20
C191	Pre-STA-2	TBA	57	27
C208	Floc	13916(949)	1729(253)	362(27)
C208	RAS	6875(635)	638(43)	155(9)
C208	Pre-STA-1	874(268)	57(30)	31(8)
C208	Pre-STA-2	TBA	59(29)	20(3)

Table 7-7. Extracellular enzyme activities measured based on dry weight for litter samples collected from transect and benchmark sites (bold) in STA-2 Cell 1. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-2 Cell 1		**AP**	**BisP**	**BG**	**LAP**
Site	**Sample**	**---------- (nmols/g dw/h) ----------**			
C34	Litter	791(266)	117(29)	436(99)	1524(440)
C51	Litter	427	80	412	929
C69	Litter	482	126	353	1654
C86	Litter	417	107	393	1372
C104	Litter	486	109	428	1158
C121	Litter	736(250)	152(51)	507(96)	1304(261)
C138	Litter	617	2307	368	3872
C156	Litter	482	2199	381	3730
C173	Litter	406	1984	338	2953
C191	Litter	403	3002	449	2586
C208	Litter	619(158)	3533(1269)	539(116)	2211(826)

Table 7-8. Extracellular enzyme activities measured based on dry weight for floc and soil samples collected from transect and benchmark sites (bold) in STA-2 Cell 1. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-2 Cell 1		**AP**	**BisP**	**BG**	**LAP**
Site	**Sample**	**---------- (nmols/g dw/h) ----------**			
C34	Floc	3535(853)	764(144)	1310(353)	4440(545)
C34	RAS	902(177)	184(37)	367(89)	886(156)
C34	Pre-STA-1	421(33)	110(8)	99(18)	237(16)
C51	Floc	3085	902	1127	5134
C51	RAS	1144	283	749	1540
C69	Floc	2123	825	957	8631
C69	RAS	1019	257	702	1723
C86	Floc	2415	928	726	7790
C86	RAS	797	181	487	1579
C104	Floc	3744	1221	694	4571
C104	RAS	590	168	516	1031
C121	Floc	2149(219)	1036(17)	718(96)	6112(66)
C121	RAS	1065(75)	380(22)	574(11)	1558(223)
C121	Pre-STA-1	550(39)	144(12)	160(37)	296(3)
C138	Floc	1500	803	689	2723
C138	RAS	959	341	592	951
C156	Floc	1661	792	507	2335
C156	RAS	825	369	381	885
C173	Floc	3552	2256	378	3366
C173	RAS	3432	977	481	1659
C191	Floc	1492	1370	275	2188
C191	RAS	1017	508	238	916
C208	Floc	4003(1018)	2551(678)	609(107)	3680(575)
C208	RAS	1681(63)	816(92)	620(76)	1909(64)
C208	Pre-STA-1	315(78)	121(5)	128(18)	337(88)

Table 7-9. Samples collected from transect and benchmark sites (*) in STA-3/4 Cell 3B were received by (UF-WBL) in October 18, 2016. All samples (n= 97) were analyzed for microbial biomass C/N/P, while Red bold samples **x (n=75)** were analyzed for additional parameters (see text for details).

STA-3/4 Cell 3B						
Location	Replicates	Litter	Floc	RAS	Pre-STA-1	Pre-STA-2
A7*	3	**xxx***	**xxx**	**xxx**	**xxx**	xxx
A7c	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
B7	1	**x**	**x**	**x**	x	x
B7c	1	**x**	**x**	**x**	x	x
C7	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
C7c	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
D7	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
D7c	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx

*Due to low sample size for litter from A7, replicates were combined to form one composite sample.

Table 7-10. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) based on dry weight for litter samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3B. For all benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-3/4 Cell 3B		MBC	MBN	MBP
Site	Sample	(mg/kg)	(mg/kg)	(mg/kg)
A7c	Litter	10999(716)	1103(32)	151(59)
B7	Litter	9069	351	126
B7c	Litter	9561	1217	96
C7	Litter	11305(3137)	645(302)	83(41)
C7c	Litter	16850(4918)	1181(149)	149(8)
D7	Litter	19957(868)	2104(325)	148(2)
D7c	Litter	13440(1958)	1706(308)	94(36)

*A7 no sample for analyses

Table 7-11. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) based on dry weight for floc and soil samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3B. For all benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such. (TBA= to be analyzed).

STA-3/4 Cell 3B		MBC	MBN	MBP*
Site	Sample	(mg/kg)	(mg/kg)	(mg/kg)
A7	Floc	3076(38)	332(36)	TBA
A7	RAS	1149(237)	68(18)	TBA
A7	Pre-STA-1	1325(459)	57(22)	TBA
A7	Pre-STA-2	1443(451)	78(24)	TBA
A7c	Floc	7795(793)	586(52)	TBA
A7c	RAS	4751(887)	327(102)	TBA
A7c	Pre-STA-1	2312(77)	62(21)	TBA
A7c	Pre-STA-2	1279(432)	23(14)	TBA
B7	Floc	2753	211	TBA
B7	RAS	2128	111	TBA
B7	Pre-STA-1	2519	127	TBA
B7	Pre-STA-2	2113	TBA	TBA
B7c	Floc	5822	362	TBA
B7c	RAS	4594	262	TBA
B7c	Pre-STA-1	1383	TBA	TBA
B7c	Pre-STA-2	1617	23	TBA
C7	Floc	3241(216)	248(17)	TBA
C7	RAS	1993(352)	81(43)	TBA
C7	Pre-STA-1	1356(276)	79(12)	TBA
C7	Pre-STA-2	767(72)	33(6)	TBA
C7c	Floc	6210(868)	670(115)	TBA
C7c	RAS	4396(848)	399(132)	TBA
C7c	Pre-STA-1	1883(148)	111(3)	TBA
C7c	Pre-STA-2	942(206)	54(5)	TBA
D7	Floc	4928(677)	526(71)	TBA
D7	RAS	3560(784)	188(23)	TBA
D7	Pre-STA-1	1541(124)	98(15)	TBA
D7	Pre-STA-2	883(60)	51(7)	TBA
D7c	Floc	8788(1102)	874(153)	TBA
D7c	RAS	3039(918)	179(44)	TBA
D7c	Pre-STA-1	1365(851)	60(38)	TBA
D7c	Pre-STA-2	1050(285)	66(7)	TBA

Table 7-12. Extracellular enzyme activities measured based on dry weight for litter samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3B. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such. (TBA= to be analyzed).

STA-3/4 Cell 3B Location	**Type**	**AP**	**BisP**	**BG**	**LAP**
			(nmols/g dw/h)		
A7	Litter	16405*	10090*	7059*	4684*
A7c	Litter	4916(1423)	TBA	5018(975)	7084(1563)
B7	Litter	8674	8674	8566	2147
B7c	Litter	3523	3523	4461	3771
C7	Litter	7915(2180)	TBA	6499(307)	2584(834)
C7c	Litter	3971(898)	4054(831)	5684(1325)	3989(733)
D7	Litter	13042(2742)	TBA	11819(3439)	6957(1028)
D7c	Litter	5473(1876)	TBA	7065(2123)	4520(865)

*Low sample size, therefore 3 replicates were combined.

Table 7-13 Extracellular enzyme activities measured based on dry weight for floc, and soil samples collected from transect and benchmark sites (bold) in STA-2 Cell 3. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such. (TBA= to be analyzed).

STA-3/4 Cell 3B	**Sample**	**AP**	**BisP**	**BG**	**LAP**
Site		(nmols/g dw/h)			
A7	Floc	757(5)	550(61)	222(66)	431(86)
A7	RAS	388(67)	209(24)	173(50)	119(6)
A7	Pre-STA-1	434(10)	TBA	186(28)	362(22)
A7c	Floc	820(87)	449(56)	1562(163)	1339(150)
A7c	RAS	702(4)	313(4)	1495(8)	844(39)
A7c	Pre-STA-1	366(14)	TBA	465(121)	369(18)
B7	Floc	407	375	213	289
B7	RAS	468	353	195	264
C7	Floc	732(92)	655(42)	339(72)	654(96)
C7	RAS	327(21)	345(59)	157(7)	247(39)
C7	Pre-STA-1	356(16)	TBA	138(14)	210(18)
D7	Floc	1263(181)	1141(190)	144(33)	661(120)
D7	RAS	932(108)	479(59)	231(103)	500(96)
D7	Pre-STA-1	423(64)	TBA	105(19)	301(41)
D7c	Floc	1978(649)	1184(417)	1599(424)	2796(1216)
D7c	RAS	620(140)	247(79)	307(111)	304(62)
D7c	Pre-STA-1	259(64)	TBA	26(16)	132(41)

Table 7-14. Samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3A were received by (UF-WBL) in November, 2016. All samples (n= 55) were analyzed for microbial biomass C/N/P, while Red bold samples **x (n=42)** were analyzed for additional parameters (see text for details).

STA-3/4 Cell 3A						
Location	Replicates	Litter	Floc	RAS	Pre-STA-1	Pre-STA-2
A8	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
A20	1	**x**	**x**	**x**	x	x
A32	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx
A44	1	**x**	**x**	**x**	x	x
A56	3	**xxx**	**xxx**	**xxx**	**xxx**	xxx

Table 7-15. Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) based on dry weight for litter samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3A. For all three benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-3/4 Cell 3A		MBC	MBN	MBP
Site	Sample	(mg/kg)	(mg/kg)	(mg/kg)
A8	Litter	23733(797)	2415(584)	237(62)
A20	Litter	19061	2590	333
A32	Litter	13922(3865)	1691(531)	194(50)
A44	Litter	20559	2661	144
A56	Litter	20462(2105)	2319(333)	362(17)

Table 7-16. Microbial biomass - carbon (MBC), -nitrogen (MBN) and – phosphorus (MBP) based on dry weight for floc and soil samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3A. For all benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such. (TBA= to be analyzed).

STA-3/4 Cell 3A		MBC	MBN	MBP
Site	Sample	(mg/kg)	(mg/kg)	(mg/kg)
A8	Floc	2441(139)	222(58)	TBA
A8	RAS	1122(290)	78(33)	TBA
A8	Pre-STA-1	966(211)	89(4)	TBA
A20	Floc	8935	808	TBA
A20	RAS	2000	86	TBA
A32	Floc	8146(1218)	605(35)	TBA
A32	RAS	2719(1115)	227(109)	TBA
A32	Pre-STA-1	1347(737)	58(16)	TBA
A44	Floc	12124	384	TBA
A44	RAS	7972	298	TBA
A56	Floc	10901(807)	943(44)	TBA
A56	RAS	6873(466)	580(79)	TBA
A56	Pre-STA-1	3035(240)	126(16)	TBA

Table 7-17. Extracellular enzyme activities measured based on dry weight for litter samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3A. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-3/4 Cell 3A					
Location	Replicates	**AP**	**BisP**	**BG**	**LAP**
			nmols/g dw/h		
A8	Litter	7251(3394)	2126(667)	21874(4661)	9166(1275)
A20	Litter	2340	839	4770	3956
A32	Litter	1530(801)	826(457)	3279(209)	1723(1045)
A44	Litter	304	308	3087	1551
A56	Litter	3501(976)	1874(558)	4865(1071)	7210(1454)

Table 7-18. Extracellular enzyme activities measured based on dry weight for floc and soil samples collected from transect and benchmark sites (bold) in STA-3/4 Cell 3A. AP: Alkaline phosphatase, BisP: phosphodiesterase, BG: beta-Glucosidase, LAP: Leucine aminopeptidase. For all benchmark sites (bold) values are averages of 3 replicate samples with standard error in parentheses. For all other transect points only one core was collected and the values are reported as such.

STA-3/4 Cell 3A		**AP**	**BisP**	**BG**	**LAP**
Location	Replicates	nmols/g dw/h			
A8	Floc	472(53)	127(12)	174(60)	473(18)
A8	RAS	312(43)	62(26)	76(29)	265(69)
A8	Pre-STA-1	230(24)	63(13)	17(3)	193(19)
A20	Floc	374	304	2164	2405
A20	RAS	278	132	2719	981
A32	Floc	574(6)	365(36)	2323(449)	2320(202)
A32	RAS	332(57)	210(44)	1852(164)	890(93)
A32	Pre-STA-1	92(35)	147(25)	378(78)	113(28)
A44	Floc	1201	619	3299	3168
A44	RAS	790	430	2323	2161
A56	Floc	801(60)	717(78)	2235(196)	2713(363)
A56	RAS	565(105)	533(113)	2332(208)	1555(173)
A56	Pre-STA-1	192(18)	150(76)	580(207)	295(35)

8 Data Integration and Synthesis (Task 10)

The objective of this task is to integrate data obtained from this project and legacy data into a cohesive framework in order to identify crucial soil and floc processes for P retention. Further key indicator variables are to be identified, which can be relatively easily measured but help monitor these key processes. The specific task is to link measurements via a conceptual/numerical framework here referred to as "analysis tool".

8.1 Work Completed During This Quarter.

8.1.1 Draft of a first numerical version of a conceptual model

A mathematical framework based on Paudel and Jawitz (2012) has been expanded such that it can represent a few major biogeochemical processes in a 1-d spatial dimension, such that it is congruent with spiraling theory. This approach allows upstream biogeochemical processes to feed downstream biogeochemical cycling, and it takes into consideration that the conditions change along the flow path. This is different of the traditional tank-in-series approach by not assuming any size of the tank over which it is assumed that tracers are mixed. On the other hand higher order mixing (e.g. dispersion, backflow) are not considered in the spiraling approach (although they could potentially feed added. However, spiraling theory can take into account changes in residence time along the flow, whereas with a tank-in-series the number of tanks would need to be set up as a dynamic property. A disadvantage is that there is a need to know the velocity (and ideally also the distribution) of the flow velocity along the flow path. The work here tests to what degree flow velocities need to be constrained. According to this 1-dimensional setup, the major determining equation for each possible P species that is transported along the flowpath (*P*, dissolved P, macrophytes, periphyton, floc and soil organic P) is as follows

$$\frac{\partial P}{dt} = S - \varepsilon * u * \frac{\partial P}{dx} - U \qquad \text{Eq. 1}$$

Where *Y* is P species, *S* is a (internal or external) source of P, *u* is the flow velocity, ε is a binary number (0 or 1) that indicates whether the particular species can be transported with flow, *x* is position along the flow path and *U* the potential sink of P. The full equations are given in the appendix (section 11.4).

In a first approach, we consider *P* as the total P in the water column. We couple Eq. 1 to Model 5 of Paudel and Jawitz (2012), with the following modifications:

- Macrophytes and periphyton uptake have a maximum uptake capacity, which indicates limitation by factors other than phosphorus.
- An internal source originating from pre-STA soils is incorporated
- There is an intermediate litter pool that catches floating particulates, and macrophyte and periphyton turnover.

At this point, the model's hydrology is extremely simplified with constant flow and water column height. This is meant to be improved, where residence times and height will be data-driven and hopefully available for sections within STAs (personal communication with Jin

Hajimirzaie, Zaki and Godin) and will be used for different sections creating a relationship between the biologically relevant velocity (u) residence time and section length (*length*).

$$u * HRT = length \quad \text{Eq. 2}$$

Where HRT is the hydrology residence time.

The model was setup such that the length of a STA is divided into 10 boxes where the biogeochemical reactions (S and U) are assumed to occur in the center of the box, while the advective transport ($-u\frac{\partial P}{\partial x}$) was calculated on the edges of the boxes using linear interpolation (staggered grid). The discretization was as follows:

$$-u\frac{\partial P}{\partial x} = \frac{u}{\Delta x}\left[\frac{P_{i-1}+P_i}{2} - \frac{P_i+P_{i+1}}{2}\right] \quad \text{Eq. 3}$$

Where Δx is the distance between the boxes' midpoints, P the P concentration in the water column and the subscripts represent the current box (i) the box upstream to the current one (i-1) and the box immediately downstream ($i+1$). At the upper boundary of an STA P_{i-1} reflects the inflow concentration, and at the lower boundary (ouflow) P_{i+1} is assumed to be Pi.

8.1.2 Sensitivity analysis

The model is a simplified numerical version of the conceptual model. Dependencies of on many dynamic variables are not incorporated yet. This includes temperature relationships, dynamic flows as well as partitioning between different P species (reactive phosphorus, dissolved organic phosphorus and particulate phosphorus). Similarly, parameters are fairly unknown (which can be constrained during data-model fusion). Here, the model was tested how a critical variable (P concentration at the outlet, after 13 year of STA operation) is determined by parameter choices using a global sensitivity analysis. This analysis asks, how much each individual parameter contributes to the predicted variability of the P concentration at the outflow. To that end we varied parameters by a factor of 3 around an initial (rough) estimate.

Most of the parameters are based on Paudel and Jawitz (2012). Specific parameter not in Paudel and Jawitz (2012)were estimated with the assumption that the 'true' value are within the factor of 6 parameter variability. These additional estimated parameters include water depth, hydrologic residence time, maximum macrophyte uptake rate, maximum periphyton uptake rate, rate of litter decay, fraction of macrophyte/periphyton returned to litter vs. water column, fraction of settling added to soil as opposed to litter. The annual P load (an important boundary condition) is synthetic yet realistic with respect to mean load and with a decline of load over time (Figure 8-1).

Table 8-1. Parameter values feeding into the analysis tool

Parameter	Description	Value	Units
H	Water depth	0.5	meter
HRT	Hydraulic residence time: note u is then calculated based on L/HRT, where length is the length of the basin	30	day
length	Length of the basin	5340	m
$k_{M,W}$	Macrophyte uptake rate	1	day^{-1}
$k_{M,,L}$	Macrophyte uptake from Litter	$2.2*10^{-4}$	day^{-1}
$k_{P,}$	Periphyton uptake rate	0.03	day^{-1}
$U_{M,max}$	Macrophyte maximum uptake	$1.9*10^{-3}$	$g\ m^{-2}\ day^{-1}$
$U_{P,max}$	Periphyton maximum uptake	$3.3*10^{-4}$	$g\ m^{-2}\ day^{-1}$
k_s	settling rate	0.18	day^{-1}
$f_{s,L}$	Fraction of settling going to litter pool	0.5	unitless
λ_m	Macrophyte turnover rate	0.0027	day^{-1}
λ_p	Periphyton turnover rate	0.01	day^{-1}
$f_{M,L}$	Fraction of macrophyte turnover returned to water (as opposed to litter)	0.44	unitless
$f_{A,,L}$	Fraction of periphyton turnover returned to water (as opposed to litter)	0.5	unitless
λ_{litter}	Turnover of litter P pool	0.002	day^{-1}
λ_{soil}	Turnover of soil P pool	$2.1*10^{-4}$	day^{-1}
S_0	Base release of P from pre-STA soil	$3.3*10^{-4}$	$g\ m^{-2}\ day^{-1}$

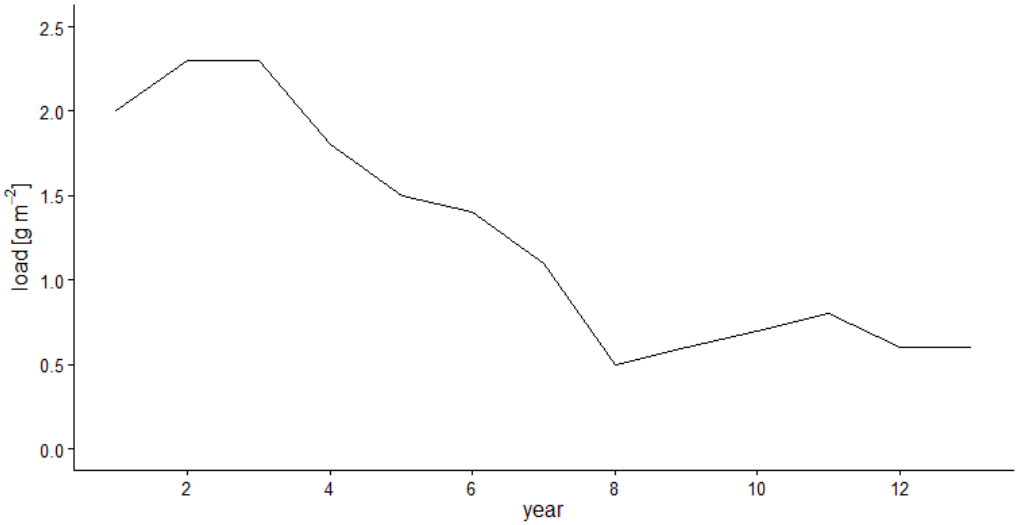

Figure 8-1. Load prescribed to the analysis tool for initial sensitivity test

Each of the parameters in Table 8-1 (except length of the cell, L), was allowed to vary by a factor of 3 (range [x*3^{-1}, x*3], where x is the parameter). Following Saltelli and Annoni (2010), however, instead of using the Sobol series, parameters were sampled using a latin hypercube sampling, using the R library 'lhs'. The number of runs were fixed to 1000. Total variability of concentration in year 13 was 98 mg P L^{-1}, when the parameter range was from $1/3\ x < x < 3x$, where x is the default parameter value (Table 8-1). The distribution was skewed with higher probabilities for higher values (Figure 8-2, upper panel).

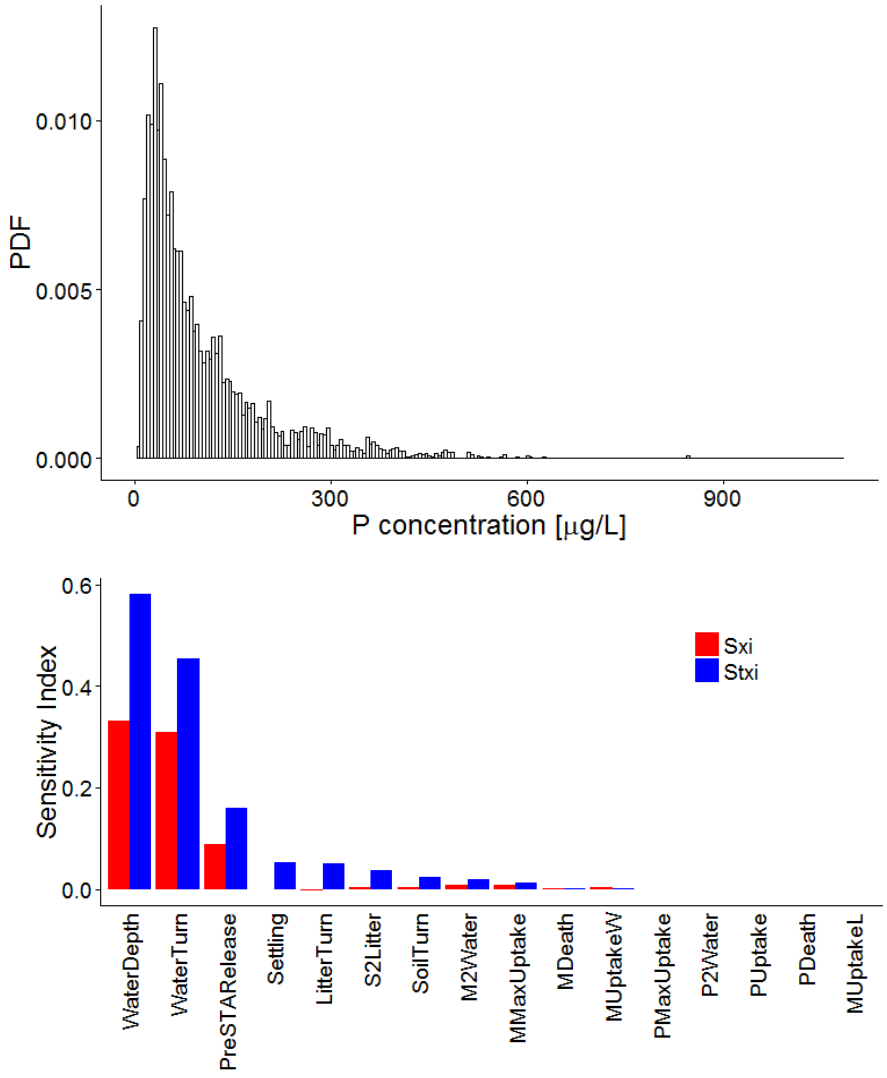

Figure 8-2. Result of global sensitivity analysis assessing parameter from Table 8-1 by varying each parameter within the range of factor of 3. Top: overall distribution of P concentration in outflow after 13 year of STA operation. Bottom: contribution of each parameter to the total variability with total sensitivity index (Stxi, and one at a time perturbation (Sxi). Key: Litter turn: λ_L, S2Litter: $f_{s,L}$; SoilTurn: λ_s; M2Water: 1-$f_{M,L}$; MMaxUptake: $U_{M,max}$; MDeath: λ_M; MuptakeW: kM,W; PMaxUptake: $U_{P,max}$; P2Water: 1-$f_{P,Lr}$; PUptake: k_A; PDeath: λ_A; MUptakeL: $k_{M,L}$

Figure 8-2 (lower panel) shows the contribution of each of the parameters to the uncertainty of outlet P concentration in year 13. The variable Sxi refers to the reduction of variance, if this particular parameter is fixed (i.e. it has no uncertainty). In contrast, the variable Stxi refers to the variance that remains, if all other parameters are fixed. Overall, the sensitivity analysis shows a strong sensitivity to water dynamics, followed by a parameter that sets how much P is released from pre-STA soil. Of all P removal rates, the rate of settling shows the strongest sensitivity, in particular this parameter is sensitive to the choice of other parameters (Stxi, blue bar). Turnover of litter and soil organic matter also play a minor role as does the partitioning of macrophyte death between surface water and litter and the partitioning of the settled P between litter and soil organic matter pool.

Expanding the sensitivity analysis to lower loads (based on a 10 fold decrease of inflow) and a tenfold decrease in input from pre-STA soils – which turns the system in a distinct oligotrophic system, still produces Water depth, Water residence time (WaterTurn in Figure 8-2 and Figure 8-3), as well as the level of release from pre-STA soils as the important variables that set the annual concentration after 13 years of STA operations (Figure 8-3).

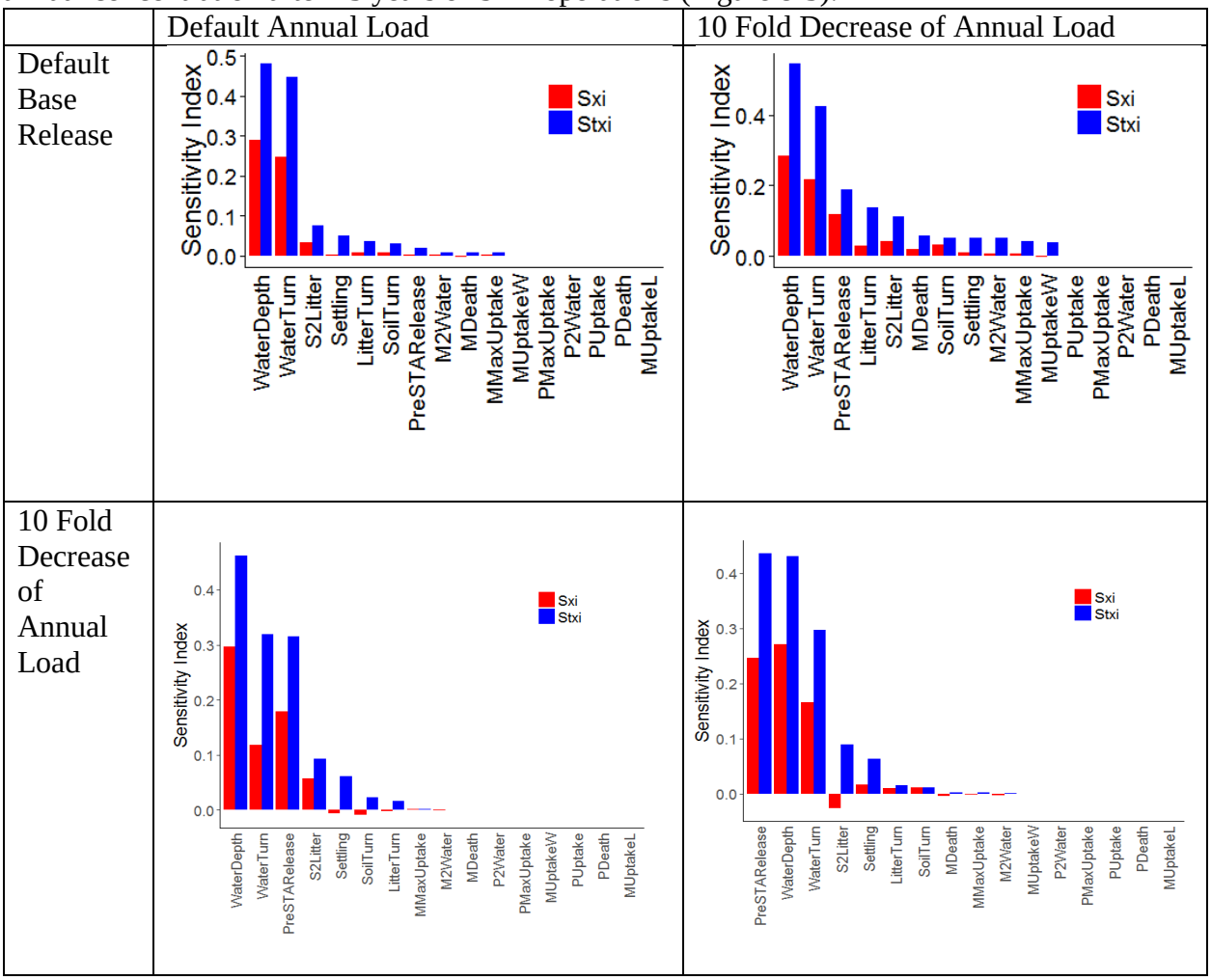

Figure 8-3. Result of global sensitivity analysis assessing parameters from Table 8-1 at 10 fold reduced load and release conditions.

8.2 Future Directions

The next steps include evaluation of formulations to estimate flow velocities from inflow and outflow rates, and combine with measurements of stage height.

Improve the biogeochemical model to account for more P species, so that they reflect measured data (different dissolved P species, floc).

Possibly improve P removal rates by differentiating processes (biotic uptake vs. abiotic co-precipitation).

Create software interface that directly reads DB Hydro Data to be incorporate into the mathematical framework.

9 Planned Activities

The following activities are planned for the next quarter (01 January to 31 March, 2017).

- **Task 3 and 4**, Year two benchmark sites soil sampling (Feb – March, 2017). Analysis of soils data that were collected in last quarter.
- **For Task 5**, soil and floc samples are currently being processed to determine P forms samples collected from 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 continue the data analysis for samples (floc) collected for *P-Flow* study during previous events. Explorations of relationships between EXO-Sonde data and TP and TN trends using advanced analytical tools.
- **For Task 8a**, to continue with data 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 to date (reruns of some samples, PMN/PMP and respiration, NAG and mineralizable P and N) will be made available in the subsequent reports as data becomes available.
- Begin with data (calculations and statistics) analysis of the biogeochemical measurements determined.
- **For Task 10**, implement better (prescribed) water dynamics into the analysis tool, using stage heights.
- Model improvement: partition dissolved P in water and soil column.

10 References

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11 Appendices

11.1 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	✓	✓✓✓

11.2 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	✓	✓✓✓

11.3 Table of Sampling Locations in WCA-2A.

WCA-2A Site #	Station ID	Latitude	Longitude	Transect station	Bench mark station (Triplicate cores)
1	F1	26.36066	-80.36935	✓	✓✓✓
2	F2	26.34266	-80.37650	✓	✓✓✓
3	F4	26.31670	-80.38518	✓	✓✓✓
4	E5	26.27849	-80.35783	✓	✓✓✓
5	U3	26.28693	-80.41156	✓	✓✓✓

11.4 Equations for Initial Simple Conceptual Model.

a) P in the water column

$$\frac{\partial P}{\partial t} = -u\frac{\partial P}{\partial x} + \frac{S_p}{H} - U_P + S$$

Where P is phosphorus concentration in the water column (g m^{-3}), t is time, u the average flow velocity, x the flow direction, S is the total local source (g m^{-2} day^{-1}), H the height of the water column (m) and U_p the uptake of P from the water column into macrophytes, periphyton and soils.

b) P in macrophytes

Macrophytes take up P from the water column, but are also able to access P stored in the litter through possibly through symbiosis with heterotrophs (fungi and bacteria). Macrophyte decay (death) is assumed to be proportional to the stored P.

$$\frac{dM}{dt} = U_{M,P} + U_{M,L} - \lambda_M M$$

Where M is the amount of P stored in macrophytes (g m^{-2}), U the uptake from the water column and litter, respectively, and λ_M a first order decay term.

The original Paudel and Jawitz (2012)model had macrophyte uptake as an exclusively P limited function, which under ample supply can lead to unrestricted growth. Thus uptake is constrained here to an upper limit that would indicate limitation by other sources than P, such as nitrogen or light. Thus

$$U_{M,P} = min\left(\frac{k_{M,W} * P}{H}, U_{M,max}\right)$$

Where *min* is the minimum function taking the lesser value of the 2 arguments, $k_{M,W}$ is the P limited uptake rate (day^{-1}), and $U_{M,max}$ is the maximum uptake rate occurring when P supply is sufficient. The division by water column height H is necessary, because of the conversion from concentration into a mass per area.

Similarly, uptake from litter is parameterized as

$$U_{M,L} = min\left(k_{M,L}, U_{M,max} - U_{M,P}\right)$$

Where $k_{M,L}$ is the uptake rate from litter (day^{-1}). It is assumed that macrophytes fulfill their P needs by the easier accessible P in the water column.

c) P in periphyton

Periphyton uptake and death is following the formulation of macrophyte uptake and decay, with the exception that Periphyton does not mine P in litter.

$$\frac{dA}{dt} = min\left(\frac{k_{A,W}P}{H}, U_{A,max}\right) - \lambda_A A$$

Where A is P in periphyton (g m^{-2}), $k_{A,W}$ is the uptake rate coefficient, $U_{A,max}$ a maximum periphyton growth, and λ_A the inverse of the turnover time of periphyton. Again, we assume that there is an upper limit to periphyton uptake, keeping periphyton growth in check if P is abundant.

d) P in litter

The litter pool is considered as dead plant material, receiving input from macrophyte death, periphyton death, and some material from settling. Litter is turning over at a constant rate.

$$\frac{dL}{dt} = \lambda_M f_{M,L} M + \lambda_A f_{A,L} A + \frac{k_S f_{S,L} P}{H} - \lambda_L L$$

Where L is the amount of P in litter, $f_{M,L}$ the fraction of plant decay added to the litter pool (as opposed to the water column), $f_{A,L}$ the fraction of periphyton turnover added to the litter, k_s the settling rate, $f_{S,L}$ the fraction of settling added to the litter, and λ_l the decay rate of litter.

e) P in recently accreted soil

Currently, soil only gets P directly from the water column, and turns over at a constant rate.

$$\frac{dS}{dt} = \frac{k_S(1 - f_{s,L})P}{H} - \lambda_s S$$

Where λ_s is the inverse turnover of soil organic matter.

f) Closing the phosphorus budget

The losses from macrophytes, periphyton, litter and soil feed into the source term S for the dynamics of P in the water column. In addition, there can be external sources S_0 (release from pre-STA soils, atmospheric deposition, birds).

$$S_p = \frac{1}{H}\left[\lambda_M M\left(1 - f_{M,L}\right) + \lambda_A A\left(1 - f_{A,L}\right) + \lambda_s S + \lambda_s S + S_0\right]$$

Sinks of P in the water column are as follows:

$$U = U_{M,P} + U_{A,P} + k_S P$$