Table

JUNE 2018 QUARTERLY REPORT

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

Work Order #: 4600003031-WO01

PREPARED FOR: South Florida Water Management District
3301 Gun Club Road
West Palm Beach, FL 33416

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

This quarterly progress report summarizes the activities performed during the period of April – June 2018, 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 third quarter of Year 3 of the project and included various activities that were initiated to meet the objectives laid out under multiple tasks.

Contents

Preliminary Data Disclaimer*: The data reported here are provisional and as such, are subject to change.*

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. Benchmark sites- soil chemical analysis - STA-2 and STA-3/4
- Task 6a – Alan Wright
- Task 6b – Alan Wright
- Task 7a. Transect study: water quality – K. R. Reddy
- Task 7b. Transect study: Enzyme and microbial biomass – Kanika Inglett
- Task 8. Biogeochemical processes: Laboratory and field studies – Patrick Inglett
- Task 9: Abiotic Degradation of Dissolved Organic Phosphorus and Carbon in STA-2 – Todd Osborne
- Task 10. Data synthesis and integration – Stefan Gerber

2 Benchmark Soil Sampling (Task 3 and Task 4)

The objective of this task is to revisit established 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. 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. Sampling of benchmark locations semi-annually provides information and offers insights on short term temporal changes in floc and soil characteristics and associated P removal mechanisms.

2.1 Work Completed During this Quarter

Year three benchmark soil, plant litter and surface water samples collected in STA-3/4 (Cells 3A and 3B) and STA-2 (FWs 1 and 3) were divided into two sets; one set was submitted to the District laboratory and the other to the UF Wetland Biogeochemistry laboratory for various analyses. Once we get all benchmark data into a standard format, we will conduct statistical analysis to determine interrelationships between soil layers, floc, and overlying water column. Results will be presented in the September quarterly report.

2.2 STA-3/4 – Cell 3A and Cell 3B

Benchmark soil, plant litter and surface water samplings were conducted in STA-3/4 Cell 3A and Cell 3B on March 6-7, 2018. Three benchmark stations, A8 (inflow), A32 (midflow), and A56 (outflow) in Cell 3A and three pairs of benchmark stations (A7 and A7c, C7 and C7c, D7 and D7c), corresponding to inflow, midflow, and outflow regions of Cell 3B, respectively, were sampled (**Figure 2-1**; *See Appendix for GPS coordinates of sampling locations*).

STA-3/4 Cell 3A is categorized as an emergent aquatic vegetation (EAV) cell with primarily *Typha domingensis* and patches of *Pistia* and *Salvinia*. STA-3/4 Cell 3B is a submerged aquatic vegetation (SAV)- based cell with *Chara* spp as the predominant vegetation. Strips of emergent vegetation primarily *Typha domingensis* surround the individual SAV areas within the cell. To capture differences in soil properties from these two different vegetated zones, additional sampling locations were identified near existing sampling grid points. These additional sites are depicted by a suffix 'c' indicating cattail dominant zone (for example- A7c, C7c, and D7c).

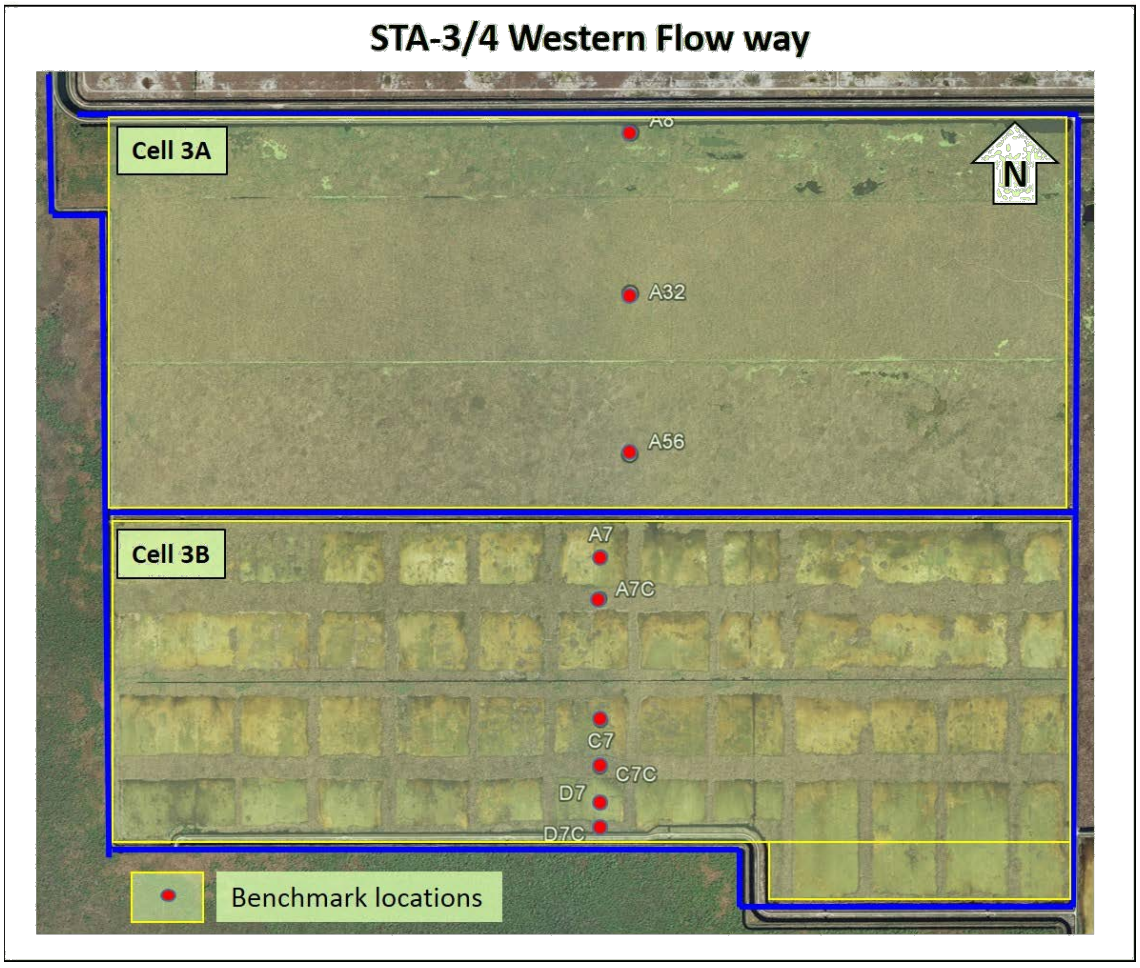

Figure 2-1. Benchmark locations in STA-3/4 Cells 3A and 3B where soil, plant litter, and surface water were collected.

Two intact soil cores were taken from each of the triplicate benchmark locations, to meet the amount of samples needed for the different studies. A total of 54 cores (27 x 2) were obtained from the two cells. Soil cores were stored in a cold room (~4^{o}C) until separated into plant litter (when present), floc, recently accreted soil (RAS), and pre-STA soil sections. Pre-STA soils were divided into two sections: 0-5 cm and >5 cm, or up to entire depth of soil core (**Table 2-1**). Pre-STA soil was separated from the overlying RAS and its thickness (depth) varied from one location to another depending on the total depth of the soil core.

Floc was characterized as the suspended unconsolidated material on top of consolidated RAS Floc depth was measured by allowing the suspended flocculent material to settle before determination of floc depth. Floc was poured into empty plastic tubes (same dimension as the soil core tubes) and allowed to settle for 4 hours. The supernatant water was discarded and thickness of settled floc was measured (**Figure 2-2**; Steps 1 through 3).

Floc and soil sections from two soil cores were thoroughly mixed and weighed before dividing into two sub-samples - one to be submitted to the District while the other sent to the UF-Wetland Biogeochemistry Laboratory (WBL) for analyses. Bulk plant litter samples were also sent to UF-WBL.

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*.

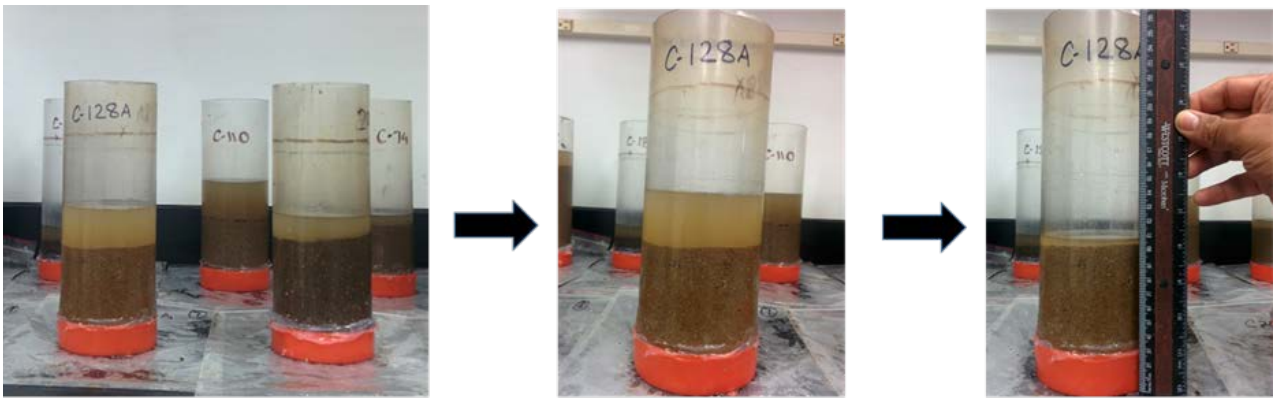

Figure 2-2: Three-step process for determination of floc depth.

Table 2-1. STA-3/4 Cells 3A and 3B: Depths of various sections after sectioning intact soil cores.

STA-3/4				
Station id	**Thickness of section (cm)**			
Cell 3A	**Floc**	**RAS**	**Pre-STA 1 (0-5 cm)**	**Pre-STA 2 (>5 cm)**
A8A	14.0	15.0	5.5*	No Data
A8B	17.8	16.0	5.0	10.5
A8C	17.7	14.0	5.0	10.3
A32A	4.7	3.3	5.0	11.5
A32B	7.0	5.0	5.0	15.0
A32C	3.8	3.8	5.0	15.0
A56A	13.1	6.0	5.0	15.0
A56B	13.2	6.0	5.0	15.0
A56C	9.3	2.5	5.0	13.5
Cell 3B				
A7A	10.9	3.0	5.0	13.8
A7B	10.8	3.8	5.0	12.0
A7C	13.7	5.0	5.0	12.0
A7cA	2.8	1.5	5.0	15.0
A7cB	5.5	4.0	5.0	13.0
A7cC	4.6	3.5	5.0	15.0
C7A	12.0	4.0	5.0	11.0
C7B	11.3	4.3	5.0	12.0
C7C	16.2	3.3	5.0	12.0
C7cA	4.2	3.0	5.0	12.0
C7cB	3.4	4.0	5.0	7.5
C7cC	5.6	4.3	5.0	7.3
D7A	11.6	4.8	5.0	8.3
D7B	9.1	4.3	5.0	11.5
D7C	10.8	6.3	5.0	9.3
D7cA	5.3	2.0	5.0	12.0
D7cB	1.8	3.0	5.0	9.0
D7cC	4.3	3.8	5.0	12.0

*For pre-STA-2 soil depth of 0.5 cm. This sample was added to pre-STA-1 soil, since the amount of soil collected was small.

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

Table 2-2. Field parameters recorded at the time of sampling.

STA-3/4	pH	Water temp.	Specific conductance	Dissolved oxygen	Water depth	Substrate thickness	Turbidity
Cell 3A	(SU)	(^{o}C)	($\mu s\ cm^{-1}$)	($mg\ L^{-1}$)	(cm)	(cm)	(NTU)
A8	7.4	23.2	652	4.5	90	64	12.70
A32	7.2	19.3	696	2.8	39	61	5.12
A56	7.4	20.5	762	5.9	38	69	0.56
Cell 3B							
A7	7.4	19.8	783	4.1	53	68	0.82
A7c	7.5	18.5	816	4.9	50	54	2.42
C7	7.5	19.0	844	3.6	38	48	1.94
C7c	7.4	19.8	847	3.5	44	43	2.25
D7	7.5	19.4	841	3.7	30	39	2.78
D7c	7.4	19.3	847	2.3	40	53	2.04

2.3 STA-2 – Flow-ways 1 and 3

Benchmark soil, plant litter, and surface water samplings were conducted in STA-2 Flow-ways 1 and 3 (FW 1 and FW 3) on March 20, 2018. Three benchmark stations, A34 (inflow), A121 (midflow), and A208 (outflow) were sampled in FW 1 and C20 (inflow), C128 (midflow) and C200 (outflow) were sampled in FW 3 (**Figure 2-3**; *See Appendix for GPS coordinates of sampling locations*).

STA-2 FW 1 is categorized as an emergent aquatic vegetation (EAV) cell composed primarily of cattail (*Typha domingensis)* with patches of *Cladium jamaicense* and *Nymphaea odorata,* whereas FW 3 is a submerged aquatic vegetation (SAV) cell, dominated by *Chara spp., Potamogeton spp.* and *Hydrilla verticillata*. Approximately 20% of the treatment area of FW 3 (southeastern region) has EAV consisting of *Typha domingensis* and *Cladium jamaicense*.

At every benchmark location, three replicate soil cores were obtained. However, to meet the quantity of samples needed for the different analyses, two soil cores from each of the triplicate benchmark locations were obtained. A total of 36 cores (18 x 2) were obtained from the two FWs. Soil cores were stored in a cold room (~4^{o}C) until sectioned into plant litter (when present), floc, recently accreted soil (RAS), and pre-STA soil. Pre-STA soils were divided into two sections: 0-5 cm and >5 cm, for up to entire depth of soil core (**Table 2-3**). The thickness of the pre-STA soil varied from one location to another depending on total depth of the soil core. Floc depth was measured as previously described (**Figure 2-2**; Steps 1 through 3). Collected samples were divided

into two sets; one set was submitted to the District laboratory and the other to the UF Wetland Biogeochemistry laboratory for various analyses.

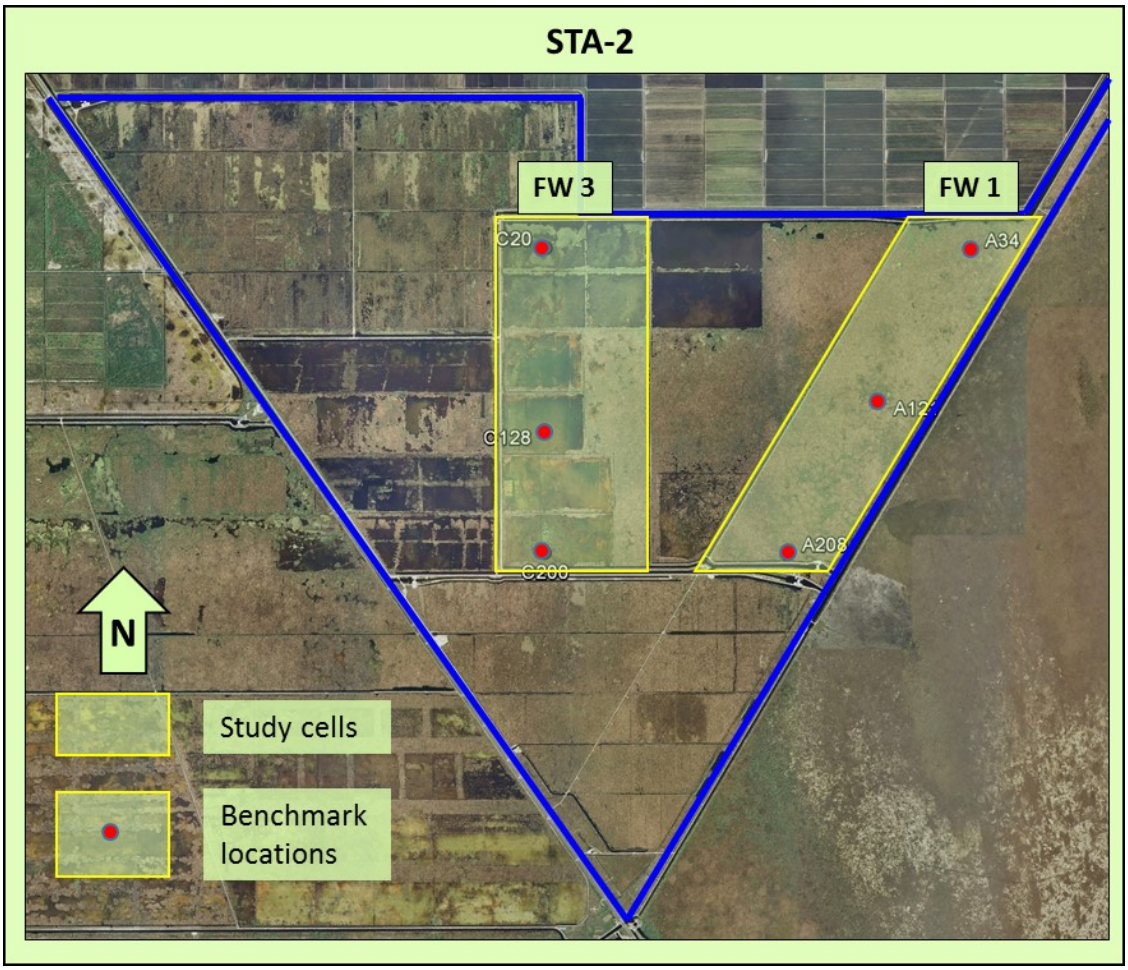

Figure 2-3. Benchmark locations in STA-2 FWs 1 and 3 where soil, plant litter, and surface water were collected.

Table 2-3. STA-2 FW 1 and FW 3: Depth of various sections after sectioning intact soil cores.

		Thickness of section (cm)			
STA-2	**Station id**	**Floc**	**RAS**	**Pre-STA 1 (0-5 cm)**	**Pre-STA 2 (>5 cm)**
FW 1	A-34A	4.5	3.0	5.0	10.5
	A-34B	2.1	4.0	5.0	10.0
	A-34C	3.0	5.3	5.0	10.0
	A-121A	10.0	3.5	5.0	10.0
	A-121B	5.8	4.5	5.0	10.0
	A-121C	7.5	6.3	5.0	10.0
	A-208A	6.7	3.5	5.0	10.0
	A-208B	10.3	5.0	5.0	10.0
	A-208C	5.1	6.3	5.0	10.0
FW 3	C-20A	2.1	6.5	5.0	9.0
	C-20B	2.6	8.3	5.0	9.5
	C-20C	2.6	7.5	5.0	13.0
	C-128A	15.1	5.0	5.0	12.0
	C-128B	13.0	9.0	5.0	12.0
	C-128C	14.0	4.5	5.0	12.0
	C-200A	12.5	4.0	5.0	12.0
	C-200B	7.5	4.0	5.0	12.0
	C-200C	12.2	3.8	5.0	12.0

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

Field parameters such as surface water pH, specific conductance, temperature and dissolved oxygen were measured using a hand held YSI. Soil and water depths were also recorded at each station at the time of sampling (**Table 2-4**).

Table 2-4. Field parameters recorded at the time of sampling.

STA-2	**pH**	**Water Temp.**	**Specific Conductance**	**Dissolved Oxygen**	**Water depth**	**Substrate thickness**	**Turbidity**
FW 1	(SU)	(^{o}C)	($\mu s\ cm^{-1}$)	($mg\ L^{-1}$)	(cm)	(cm)	(NTU)
A-34	7.3	24.5	673	2.7	44	143	8.6

A-121	7.4	23.9	726	2.7	67	104	4.8
A-208	7.3	22.3	631	1.8	70	161	0.65
FW 3							
C-20	8.12	23.6	1055	6.5	49	45	57.3
C-128	7.88	23.5	1505	3.6	42	70	36.6
C-200	7.35	24.2	1580	7.4	48	70	19.5

Table 2.1. Sampling dates of benchmark sites in study locations of STA-2, STA-3/4, and WCA-2A.

Sites	Sampling Dates	Data analysis
STA-2		
FW 3	October 13 to 15, 2015	Spatial sampling including transect and benchmark sites. Data submitted as part of Task 3
FW 1	February 9 to 11, 2016	Spatial sampling including transect and benchmark sites. Data submitted as part of Task 3
FW 3	September 7 to 9, 2016	Transect sampling including benchmark sites. Data submitted as part of Task 5.
FW 1	September 20 to 22, 2016	Transect sampling including benchmark sites. Data submitted as part of Task 5.
FWs 1 and 3	March 13 and 14, 2017	Sampling benchmark sites
FWs 1 and 3	October 10, 2017	Sampling benchmark sites
FWs 1 and 3	March 20, 2018	Sampling benchmark sites
STA-3/4		
Cell 3B	November 17 to 20, 2015	Spatial sampling including transect and benchmark sites. Data submitted as part of Task 3
Cell 3A	January 12 to 14, 2016	Spatial sampling including transect and benchmark sites. Data submitted as part of Task 3
Cell 3B	October 4, 2016	Transect sampling including benchmark sites. Data submitted as part of Task 5
Cell 3A	October 20, 2016	Transect sampling including benchmark sites. Data submitted as part of Task 5
Cells 3A and 3B	February 28, 2017	Sampling benchmark sites
Cells 3A and 3B	September 5 and 6, 2017	Sampling benchmark sites
Cells 3A and 3B	March 6 and 7, 2018	Sampling benchmark sites
WCA-2A	December 6, 2016	Sampling benchmark sites. Data submitted as part of Task 5

3 Phosphorus Sorption/Desorption Characteristics of STA Soils (Task 6a)

The lab experiment is complete and data analysis is ongoing. Complete report with results and interpretations will be included in the next quarterly report.

4 Phosphorus Exchange Between Soil and Water Column (Task 6a)

Laboratory work associated with the P dosing study is now complete. All water and soil porewater samples were submitted to the District laboratory for analyses. Complete report with results and interpretations will be included in the final project report due end of December 2018.

5 Transect Study - Water Quality Monitoring (Task 7a)

The flow event reported here took place in STA-2 FW 1 and was conducted to evaluate the biogeochemical response of an emergent aquatic vegetation dominated flow-way to low flow conditions (**Figure 5-1**). The flow event was divided into two distinct periods, the first being a low flow period spanning November 12 to November 27, 2017 followed by a no-flow period between November 28 to December 26, 2017 (**Figure 5-2**).

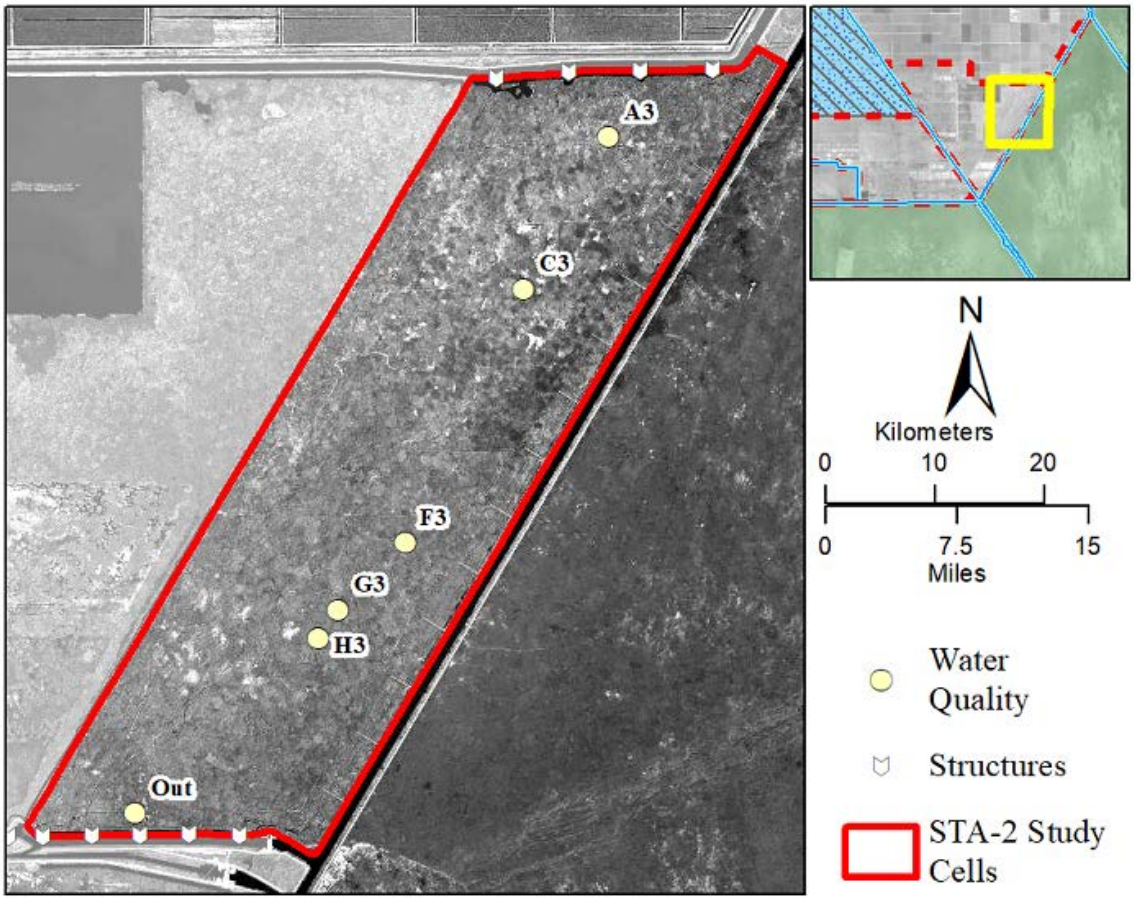

Figure 5-1. Surface water sampling locations in STA-2 FW 1 where autosamplers and sondes were deployed for water quality monitoring and weekly grab sampling.

While these flow periods are classified into "high", "low" or "no flow" conditions in the text, these classifications do not reflect the actual hydraulic or hydrologic conditions in-situ or the design capacity of inflow structure but are more indicative of relative water conditions within the context of the study.

5.1 Methods and Analyses

Flow data were downloaded from DBHYDRO (www.sfwmd.gov/dbhydro) for the period of the flow event. The water quality data were obtained from ERDP. Daily TP values were estimated from linear interpolation of bi-weekly TP grab samples. These daily TP estimates were multiplied by daily and multiplied by flow (with appropriate conversion factors applied) to estimate a daily load consistent with the Districts Java-based nutrient load program. Daily load and flow values were then divided by the area of the FW (1,840 acres) to estimate hydraulic (HLR) and phosphorus loading rates (PLR).

5.2 Flow-way Water Quality Assessments

The November to December 2017 event lasted 44 days, with a 15-day period of low flow followed by a no flow period of 28 days. During the "low flow" period, a total volume of 39.9 $x10^5$ m^3 for the 15-day period of flow entered FW 1 resulting in a daily average PLR of 0.96 ± 0.10 mg m^{-2} d^{-1} and HLR rate of 3.3 ± 0.4 cm d^{-1} (**Table 5-1** and **Figure 5-2**). Low flow is operationally defined as flow conditions in the range of >0 to 150 cfs. There are no inflows into system under no flow condition (0 cfs).

Autosamplers collected water samples at 4-hr intervals from November 14, 2017 to December 19, 2017. A total of 400 and 670 discrete samples were collected during low flow and no flow periods, respectively (**Table 5-2**). All collected samples were analyzed for TP.

Table 5-1. Total flow, hydraulic loading rate (HLR), and phosphorus loading rate (PLR) for the seventh flow event in STA-2 FW 1 (November 12 to December 26, 2018). Values are arithmetic mean ± standard error.

Date	Flow Period	Flow Volume (x 10^5 m^3 d^{-1})		HLR (cm d^{-1})	PLR (mg m^{-2} d^{-1})
		Mean ± SE	Total		
11/12/2017– 11/27/2017	Low Flow	2.5 ± 0.3	39.9	3.3 ± 0.4	0.96 ± 0.10
11/28/2017 – 12/26/2017	No Flow	0.0	0.0	0.0	0.0

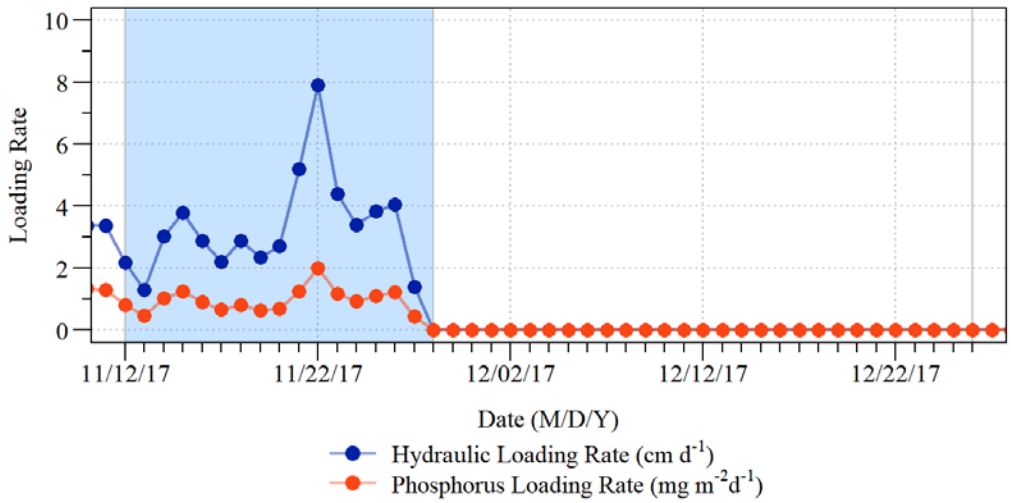

Figure 5-2. Daily hydraulic and phosphorus loading rates during the seventh flow event (November 12 to December 26, 2017) in STA-2 FW 1. Flow event was divided into two flow periods based on water flow conditions – low flow (Nov 12 - Nov 27, 2017) and no-flow (Nov 28-Dec 26, 2017).

Table 5-2. Number of water samples collected using autosamplers located along the flow path in STA-2 FW 1 (sampling period = November 14 – December 19, 2018).

Flow Period [Fractional Distance] (Distance form inflow, m)		A3 [0.08] (466)	C3 [0.23] (1,196)	F3 [0.55] (2,860)	G3 [0.68] (3,536)	H3 [0.73] (3,796)	OUT [0.94] (4,888)	Total
Low Flow	Sampling date	11/14-21	11/14-27	11/14-27	11/14-27	11/14-27	11/14-27	
	Collected	46	61	83	83	83	44	400
	Missing	2	23	1	1	1	40	68
	Total	48	84	84	84	84	84	468
No Flow	Sampling date	11/28-12/19	11/28-12/19	11/28-12/19	11/28-12/19	11/28-12/19	11/28-12/19	
	Collected	125	103	125	94	106	117	670
	Missing	7	29	7	8	20	15	86
	Total	132	132	132	102	126	132	756

Results are summarized in **Figures 5-3, 5-4, 5-5, and 5-6.** Daily mean TP concentrations of samples collected at each station for 'low flow' and 'no flow' periods show spatial gradients with high concentrations at stations near inflow (A3) and significantly decreased with distance from the inflow. No significant patterns were noted at stations F3, G3, and H3. However, outflow TP concentrations in the water column were significantly lower compared to upstream stations (p = 0.001).

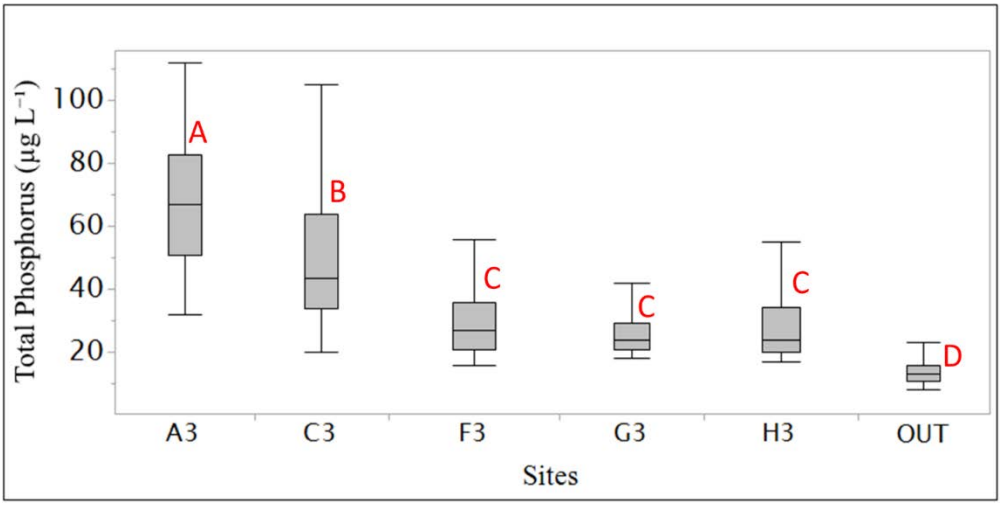

Figure 5-3: Boxplot of average TP concentration of all water samples (low flow and no flow periods) collected using autosamplers located along the flow path during the November 14 – December 26, 2017 flow event. Letters indicate Dunn's Multiple Comparison results in daily TP concentrations between periods; different letters indicate statistically significant differences.

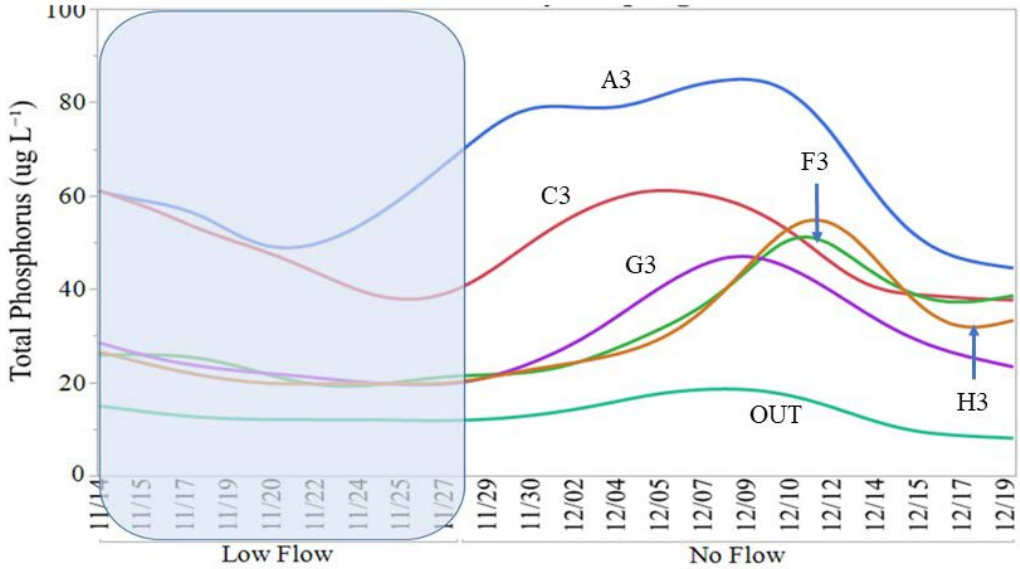

Figure 5-4: Daily mean total phosphorus concentration of water samples collected using autosamplers located along the flow path of STA-2 FW 1 (sampling period = November 14 – December 26, 2018). Data includes both 'low flow' and 'no flow' periods.

Total P concentration of surface water near inflow (Station A3) was high and increased with time during the 'low flow' period (**Figures 5-4 and 5-5)**. General trends showed that TP concentrations were lower during the 'low flow' period and increased steadily during 'no flow' period. This increase in TP levels was probably due to internal regeneration of P through mineralization of organic P associated with detrital matter and floc in the water column. In addition, there may be potential P flux from floc and RAS layers into the overlying water column.

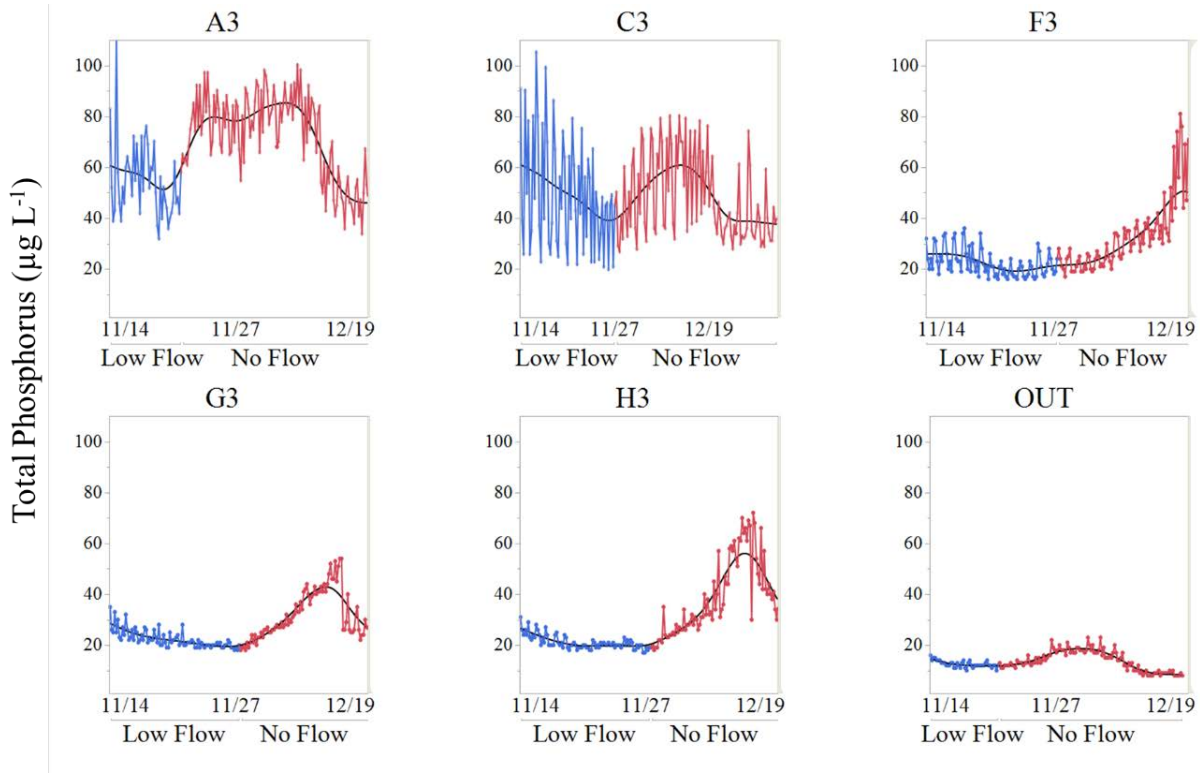

Figure 5-5: Daily mean TP concentrations of water samples collected using autosamplers located along the flow path during 'low flow' (November 14-27, 2017) and 'no flow' (November 28 – December 26, 2017) periods.

Mean TP concentrations during 'low flow' period were significantly lower than 'no flow' period at A3, F3, G3, and H3 stations and no significant differences were observed between flow conditions at stations C3 and the outflow (**Figure 5-6**). High degree of variability was noted in water samples collected at C3 station as compared to water samples collected at other locations. Inflow TP contains high levels of SRP, which is rapidly retained in the flow and vegetation during 'low flow' period and released back into water column during 'no flow' condition, as indicated by an increase in TP concentrations of the water column.

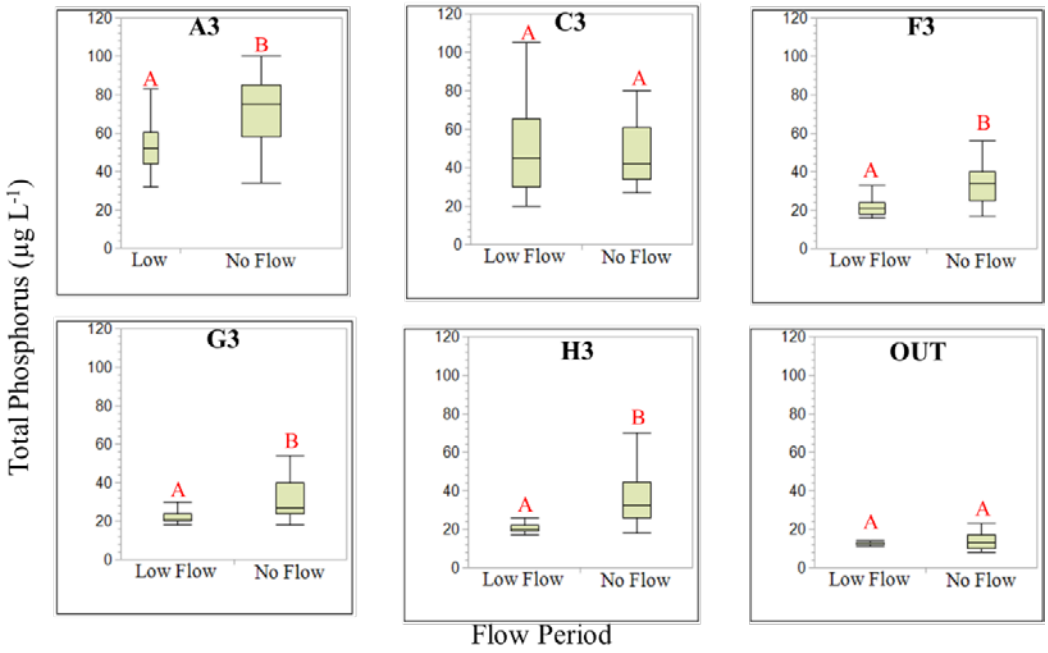

Figure 5-6. Boxplot of mean TP concentrations of all water samples collected using autosamplers located along the flow path during ow flow (November 14-27, 2017) and no flow (November 28 – December 26, 2017) periods. Letters indicate Dunn's Multiple Comparison results in daily mean TP concentrations between flow period; different letters indicate statistically significant differences.

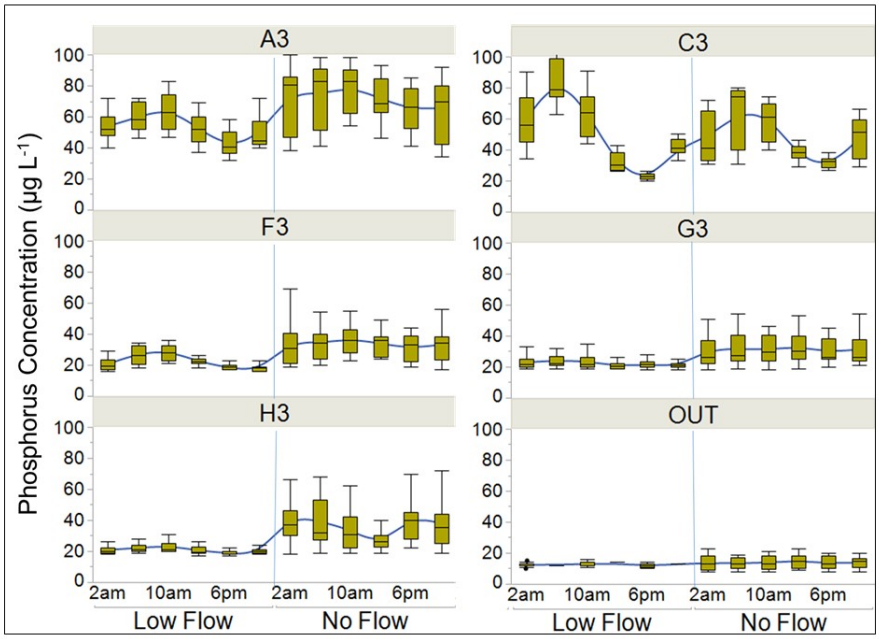

Figure 5-7. Boxplot of mean TP concentration of water samples collected at 4-hour intervals using autosamplers located along the flow path during low flow (November 14-27, 2017) and no flow (November 28 – December 26, 2017 periods. Values are mean of each 4-hour time interval within each day for both flow periods.

Data shown in **Figure 5-7** indicates diel patterns in TP concentrations of the water column at stations A3 and C3, with high values noted at 10 am in the morning and low concentrations at 6 pm in the evening. Similar diel patterns TP concentrations were shown at stations F3, G3, and H3, and no patterns were noted at the outflow station.

During the seventh flow event, grab samples were collected weekly and analyzed for a suite of water quality parameters including P forms, N forms, sulfate, TOC, DOC, Chlorophyll-a, ions, metals, alkalinity, alkaline phosphatase activity, color, and hardness. Grab sample TP concentrations ranged from 20 to 79 µg L^{-1} during the low flow period compared to 10 to 114 µg L^{-1} during the no flow period (**Figure 5-8**). Soluble reactive P concentrations ranged from 3 to 58 µg L^{-1} (17 to 76% of TP) during the low flow period compared to 2 to 76 µg L^{-1} (10 to 67% of TP) during the no flow period. Dissolved organic P concentrations ranged from 6 to 11 µg L^{-1} during the low flow period compared to 3 to 14 µg L^{-1} during the no flow period. Particulate P concentrations ranged from 4 to 19 µg L^{-1} during the low flow period compared to 8 to 24 µg L^{-1} during the no flow period. During the low flow event near A3 station, approximately 63 to 76% of TP was present as SRP, compared to 12 to 18% as DOP, and 9 to 24% as PP, respectively. During the no flow period near A3 station, approximately 46 to 67% of the TP was present as SRP compared to 12 to 19% as DOP, and 21 to 35% as PP, respectively. Under low flow condition near outflow station, approximately 17 to 22% of the TP was present as SRP compared to 44 to 50% as DOP, and 29 to 33% as PP, respectively. Under no flow condition near the outflow station, approximately 10 to 24% of the TP was present as SRP compared to 19 to 41% in DOP, and 35 to 7% in PP, respectively. In general, proportion of SRP pool decreased with distance from inflow under both low flow and no flow conditions, while the proportion of both DOP and PP as percent of total P increased. Proportions of DOP and PP at the outflow were higher under no flow compared to low flow condition.

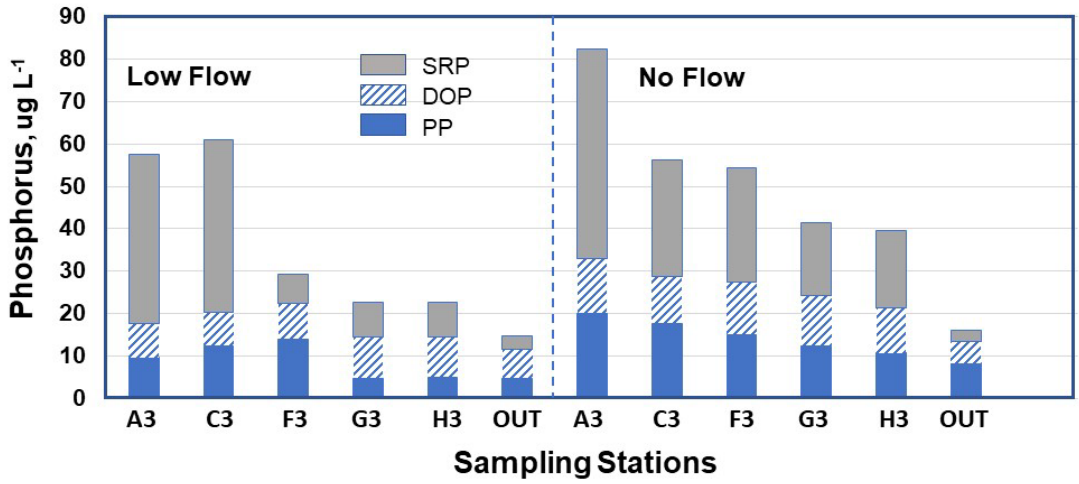

Figure 5-8. Phosphorus forms in the weekly grab samples collected along the flow path during low flow (November 14-27, 2017) and no flow (November 28 – December 26, 2017) periods. SRP = soluble reactive P; DOP = dissolved organic P; PP = particulate P; TP = sum of [SRP + DOP + PP]; and TDP = sum of [SRP +DOP].

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 (leucine aminopeptidase and N-acetyl-β-D-glucosaminidase), and phosphorus (phospho-monoesterase-alkaline phosphatase and diesterase/bis-phosphodiesterase) in floc and litter samples collected from STA cells along the three benchmark sites (inflow; midflow; and outflow).

6.1 Work Completed During this Quarter

During this quarter (April-June 2018), several video conferences were held to plan for the 8th sampling event to be conducted in STA-3/4 Cells 3A and 3B in June-July 2018. Sampling for the 3 flow periods proposed (Dates were modified after the reporting quarter).

Table 6-1. Proposed dates for sample collection (*litter* and *floc)* during the 8th flow event to be conducted in STA-3/4 Cells 3A and 3B (July 2018).

Flow period	Sampling date	Flow
Jun 18 –Jul 01, 2018	Jun 27, 2018	Flow (1-150 cfs)
Jul 02–15, 2018	Jul 11, 2018	Stagnant (0 cfs)
Jul 16–30, 2018	Jul 25, 2018	Post Stagnant (>300 cfs)

STA-3/4 Cell 3B Site Description

At sampling location B7, there was virtually no SAV and instead was open water (with a thick floc layer). At sampling location B7c cattail vegetation was present (**Figure 6-1**).

Figure 6-1. Sampling location B7 at STA-¾ Cell 3B with open water location (**left, B7**) and vegetated location B7c (**right**).

The outflow sites were D7 and D7c. At these sites the entire area is SAV (*Chara* spp.) intermixed with *Sagittaria* spp. Floc samples collected at site D7 represented the open water site and D7c represented the mixed vegetation sites. (**Figure 6-2**). There was no litter collected from the open water sites at either of the two sampling locations.

Figure 6-2. Sampling location D7 at STA-3/4 Cell 3B.

Table 6-2. Samples (*litter* and *floc)* collected for the first flow period on June 27, 2018 during the 8th flow event conducted in STA-3/4 Cell 3B (July 2018).

STA-3/4 Cell 3B	Vegetation Type	Litter	Floc
B7	Open water	No samples	xxx
B7c	Vegetated (Typha)	xxx	xxx
D7	Open water	No samples	xxx
D7c	Vegetated (mixed)	xxx	xxx

X represents each of the three replicate samples.

- Statistical analyses and manuscript preparation are in progress for the data collected from STA-2 FWs 1 and 3.
- A chapter describing the effect of flows on microbial enzyme activity is being prepared for inclusion in the 2019 SFER.

6.2 Future Activities

The following activities are planned for the next quarter (July1 to September 30, 2018).

- Continue sample collection for sampling event (# 8) 2018 in STA-3/4 Cells 3A and 3B. Complete the microbial biomass nutrient analyses and the enzyme assays for the June 27th samples.
- Completion of the chapter for the SFER report.

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

The objectives of this study are to 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 to 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 (April- June 2018).

Data analyses of respiration rates and enzyme activities in litter, floc, RAS and pre-STA soil samples collected in the last quarter (March-April 2018) is complete (**Tables 7-1 to 7-32).**

Sampling events in the four STA flow-ways/cells (STA-3/4 Cell 3A, STA-3/4 Cell 3B, STA-2 FW 1, and STA-2 FW 3) were completed in March 2018. Transect soil and litter sampling was conducted in STA-3/4 Cells 3A and 3B over a 2-day period (March 6-7 2018). Three (3) benchmark sites (A8, A32, A56) were sampled in STA-3/4 Cell 3A and six (6) benchmark sites (A7, A7c, C7, C7c, D7, D7c) were sampled in STA-3/4 Cell 3B. Soil cores were sectioned (for details see **Section 2**), and samples were stored at 4°C and transported to UF-WBL, Gainesville FL. Soil core sections were received by UF-WBL on March 15, 2018. On receipt, litter and soil samples were stored at 4°C until analysis the following day.

Sampling for STA-2 FWs 1 and 3 was conducted over a 2-day period (March 20-21, 2018). Soil cores were collected from three (3) benchmark sites (A34, A121, A208) in STA-2 FW 1 and from three (3) benchmark sites (C20, C128, C200) in STA-2 FW 3. Soil cores were sectioned (for details see **Section 2**), stored at 4^{o}C and shipped to UF-WBL, Gainesville, FL and they were received on March 26, 2018.

Description of the sampling methods are presented in Chapter 2. All analyses (for samples collected in March 2018) including microbial biomass C and N, enzyme activities, rates of respiration (aerobic and anaerobic), and potentially mineralizable phosphorus (PMP) and nitrogen (PMN) are presented in this report. Analysis of soil oxygen demand (SOD) also was initiated for all litter, floc, RAS, and pre-STA soils collected in March 2018 from STA-3/4 Cell 3B, STA-3/4 Cell 3A, STA-2 FW 1 and STA-2 FW 3.

Table 7-1. Samples collected from benchmark sites in *STA-3/4 Cell 3A* on March 6, 2018 were received by UF-WBL on March 15, 2018. Replicate samples collected for analysis at each sampling location are indicated by the symbol 'x'. (see **Figure 7-1** for sampling details; and appendix for GPS coordinates of sampling locations)

Location	Replicates	Litter	Floc	RAS	Pre-STA 1
A8	3	xxx	xxx	xxx	xxx
A32	3	xxx	xxx	xxx	xxx
A56	3	xxx	xxx	xxx	xxx

Table 7-2. Samples collected from benchmark sites in *STA-3/4 Cell 3B* on March 06, 2018 were received by UF-WBL on March 15, 2018. Replicate samples collected and analyzed at each sampling location are indicated by the symbol 'x'. (see **Figure 7-2** for sampling details; and appendix for GPS coordinates of sampling locations). Absence of litter samples at site A7 was noted.

Location	Replicates	Litter	Floc	RAS	Pre-STA 1
A7	3	---	xxx	xxx	xxx
A7c	3	xxx	xxx	xxx	xxx
C7	3	xxx	xxx	xxx	xxx
C7c	3	xxx	xxx	xxx	xxx
D7	3	xxx	xxx	xxx	xxx
D7c	3	xxx	xxx	xxx	xxx

Table 7-3. Samples collected from benchmark sites in *STA-2 FW 1* on March 20, 2018 were received by UF-WBL on March 26, 2018. Replicate samples collected and analyzed at each sampling location are indicated by the symbol 'x'. (see **Figure 7-3** for sampling details; and appendix for GPS coordinates of sampling locations).

Location	Replicates/site	Litter	Floc	RAS	Pre-STA 1
A34	3	xxx	xxx	xxx	xxx
A121	3	xxx	xxx	xxx	xxx
A208	3	xxx	xxx	xxx	xxx

Table 7-4. Samples collected from benchmark sites in *STA-2 FW3* on March 20, 2018 were received by UF-WBL on March 26, 2018. Replicate samples collected and analyzed at each sampling location are indicated by the symbol 'x'. (see **Figure 7-1** for sampling details; and appendix for GPS coordinates of sampling locations). No litter samples were collected.

Location	Replicates/ site	Litter	Floc	RAS	Pre-STA 1
C20	3	-	xxx	xxx	xxx
C128	3	-	xxx	xxx	xxx
C200	3	-	xxx	xxx	xxx

7.1.1 Results from March 2018 Sampling Event

STA-3/4 Cell 3A

Microbial Biomass

Table 7-5. Microbial biomass carbon (MBC), -nitrogen (MBN) and -phosphorus (MBP) in litter samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± standard error.

Location	Sample type	MBC (mg/kg)	MBN (mg/kg)	MBP (mg/kg)
A8	Litter	22201±10770	2113±931	136±29
A32	Litter	14476±2178	1085±200	112±21
A56	Litter	18873±2166	1195±166	182±45

Table 7-6. Microbial biomass-carbon (MBC), -nitrogen (MBN) and –phosphorus (MBP) in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample type	MBC (*mg/kg*)	MBN (*mg/kg*)	MBP (*mg/kg*)
A8	Floc	3842±150	391±26	63.7±5.1
	RAS	2677±302	100±16	35.7±6.2
	Pre-STA 1	667±183	40±14	7.5±3.5
A32	Floc	9141±509	689±37	108.7±13.3
	RAS	6070±411	270±20	55.8±4.2
	Pre-STA 1	626±179	43±16	9.1±3.5
A56	Floc	8152±1146	830±122	184.6±45.1
	RAS	5586±250	295±62	57.6±16
	Pre-STA 1	1801±97	96±7	15.1±3.2

Extracellular Enzyme activities

Table 7-7. Activities of 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: β-glucosidase, LAP: leucine aminopeptidase) in litter samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

		AP	BisP	BG	LAP
Location	Sample type	----------*μmols/g dw/h*----------			
A8	Litter	5.47±1.22	3.42±1.11	10.33±2.84	17.86±9.2
A32	Litter	1.41±0.2	0.72±0.22	3.83±0.89	4.51±2.6
A56	Litter	2.76±0.61	2.59±0.61	4.51±1.1	10.69±4.94

Table 7-8. Activities of 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: β-glucosidase, LAP: leucine aminopeptidase) in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

		AP	BisP	BG	LAP
Location	Sample type	----------*μmols/g dw/h*----------			
A8	Floc	1.5±0.27	1.2±0.29	0.46±0.13	1.7±0.21
	RAS	0.88±0.06	0.51±0.05	0.16±0.03	0.68±0.1
	Pre-STA 1	0.38±0.03	0.19±0.02	0.07±0.01	0.24±0.03
A32	Floc	2.04±0.48	0.82±0.15	1.45±0.12	4.2±0.85
	RAS	0.99±0.05	0.42±0.06	1.16±0.28	1.3±0.07
	Pre-STA 1	0.37±0.06	0.16±0.02	0.17±0.03	0.27±0.01
A56	Floc	1.43±0.36	0.93±0.31	1.02±0.04	4.14±1.02
	RAS	1.3±0.25	0.76±0.21	0.58±0.1	1.76±0.71
	Pre-STA 1	0.8±0.04	0.46±0.03	0.13±0.02	0.48±0.06

Mineralizable phosphorus and nitrogen

Table 7-9. Potentially mineralizable nitrogen (PMN) and –phosphorus (PMP) in litter samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample type	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
A8	Litter	22.57±21.62	14.26±12.3
A32	Litter	3.23±2.15	6.04±1.81
A56	Litter	0.93±1.18	1.76±0.48

Table 7-10. Potentially mineralizable nitrogen (PMN) and –phosphorus (PMP) in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample type	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
A8	Floc	-4.76±2.66	0.82±0.68
	RAS	-5.69±6.2	0.24±0.16
	Pre-STA 1	1.38±0.2	0.27±0.09
A32	Floc	0.44±3.5	1.76±0.57
	RAS	-1.77±1.19	0.27±0.07
	Pre-STA 1	0.59±0.21	0.05±0.02
A56	Floc	17.94±5.37	1.9±0.39
	RAS	-3.36±2.68	0.22±0.06
	Pre-STA1	1.31±0.2	0.05±0.03

Respiration

Table 7-11. Rates of aerobic and anaerobic respiration in litter samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample type	Aerobic respiration *umolesCO_2-C/gdw/day*	Anaerobic respiration *umolesCO_2-C/gdw/day*
A8	Litter	156±63.7	65.72±26.7
A32	Litter	119.2±6.6	42.16±11.35
A56	Litter	115.8±6.3	41.33±3.73

Table 7-12. Rates of aerobic and anaerobic respiration in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample type	Aerobic respiration *umolesCO_2-C/gdw/day*	Anaerobic respiration *umolesCO_2-C/gdw/day*
A8	Floc	61.4±6.4	5.02±0.44
	RAS	94.3±5.3	0.98±0.13
	Pre-STA 1	138.4±11	1.7±0.36
A32	Floc	105.9±43	2.34±0.19
	RAS	145±45.2	3.07±0.85
	Pre-STA 1	116.8±7.6	1.34±0.36
A56	Floc	118.1±21.8	4.3±0.51
	RAS	89.5±10.8	3.84±2.18
	Pre-STA 1	160.9±29.6	1.05±0.24

STA-3/4 Cell 3B

Microbial Biomass

Table 7-13. Microbial biomass carbon (MBC), -nitrogen (MBN) and -phosphorus (MBP) in litter samples collected from three benchmark sites in STA-3/4 Cell 3B in March 2018. Values are averages of 3 replicate samples ±the standard error.

Location	Sample	MBC (*mg/kg*)	MBN (*mg/kg*)	MBP (*mg/kg*)
A7c	Litter	7581±3238	612±287	70±35.1
C7	Litter	11995±2253	950±38	97±22.4
C7c	Litter	17964±3826	1492±409	148.8±21.5
D7	Litter	21968±1803	2708±303	232.2±8.6
D7c	Litter	16701±3501	1376±196	146±31.9

Table 7-14. Microbial biomass -carbon (MBC), -nitrogen (MBN) and –phosphorus (MBP) in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Site	Sample	MBC (*mg/kg*)	MBN (*mg/kg*)	MBP (*mg/kg*)
A7	Floc	3101±111	372±49	83.5±5.4
	RAS	1775±65	78±11	26.5±3.5
	Pre-STA 1	1633±374	106±8	19.7±2.9
A7c	Floc	11266±234	910±127	200±15.8
	RAS	8322±992	505±48	110.3±15.4
	Pre-STA 1	2563±635	144±61	25.2±4.6
C7	Floc	7708±1742	1188±292	191.2±25.9
	RAS	2344±254	121±10	35.6±6.7
	Pre-STA 1	1347±90	41±5	11.9±3.3
D7	Floc	10433±740	801±114	181.5±41
	RAS	7039±1154	423±105	86.3±17.7
	Pre-STA 1	2526±436	122±38	26.2±6.4
D7c	Floc	8654±780	936±52	204.3±24.3
	RAS	4059±1145	308±145	59.5±17.2
	Pre-STA 1	1539±70	103±5	12.5±3.1

Extracellular enzyme activities

Table 7-15. Activities for 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: β-glucosidase, LAP: leucine aminopeptidase) in litter samples collected from three benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample	AP	BisP	BG	LAP
		----------umols/gdw/h----------			
A7c	Litter	1.73±0.52	1.13±0.35	2.63±0.59	10.97±10.12
C7	Litter	2.14±0.21	1.92±0.02	2.21±0.48	4.21±1.68
C7c	Litter	6.26±1.75	5.34±1.18	4.53±0.36	8.38±2.67
D7	Litter	18.58±2.18	21.92±2.72	3.78±0.37	15.95±2.09
D7c	Litter	11.98±3.72	11.93±3.17	5±2.3	11.23±3.84

Table 7-16. Activities for 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: beta-glucosidase, LAP: leucine aminopeptidase) in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Site	Sample	AP	BisP	BG	LAP
		----------umols/gdw/h----------			
A7	Floc	0.52±0.09	0.57±0.03	0.15±0.04	1.3±0.11
	RAS	0.47±0.07	0.48±0.05	0.05±0.01	0.28±0.04
	pre-STA 1	0.4±0.02	0.39±0.02	0.19±0.07	0.47±0.08
A7c	Floc	1.99±0.13	1.04±0.06	1.16±0.1	4.85±0.94
	RAS	0.43±0.04	0.66±0.08	0.54±0.07	2.19±0.12
	pre-STA 1	0.46±0.04	0.21±0.02	0.31±0.06	0.62±0.11
C7	Floc	1.82±0.49	4.01±1.33	0.22±0.05	4.55±2.34
	RAS	0.32±0.05	0.51±0.04	0.04±0.01	0.23±0
	pre-STA 1	0.53±0.06	0.21±0.02	0.18±0.05	0.17±0.01
D7	Floc	1.37±0.09	1.13±0.07	0.86±0.06	4.62±1.14
	RAS	0.39±0.02	0.51±0.08	0.77±0.06	1.51±0.28
	pre-STA 1	0.46±0.04	0.3±0.06	0.27±0.05	0.66±0.11
D7c	Floc	4.07±0.2	5.26±0.88	0.37±0.05	2.96±0.64
	RAS	0.52±0.03	1.5±0.35	0.08±0.01	0.73±0.18
	pre-STA 1	0.64±0.03	0.25±0.03	0.19±0.02	0.31±0.04

Mineralizable phosphorus and nitrogen

Table 7-17. Potential mineralizable nitrogen (PMN) and –phosphorus (PMP) in litter samples collected from benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
A7c	Litter	1.94±2.41	2.25±0.25
C7	Litter	49.3±50.73	4.36±3.7
C7c	Litter	11.01±3.85	2.64±0.37
D7	Litter	48.46±7.46	3.25±0.14
D7c	Litter	0.37±3.65	2.88±0.36

Table 7-18. Potential mineralizable nitrogen (PMN) and –phosphorus (PMP) in floc and soil samples collected from three benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
A7	Floc	4.41±0.66	0.07±0.02
	RAS	0.98±0.11	0.02±0.01
	Pre-STA 1	2.06±0.27	0.04±0.01
A7c	Floc	26.44±6.28	3.02±0.44
	RAS	8.53±1.05	1.19±0.12
	Pre-STA 1	1.76±0.37	0.22±0.11
C7	Floc	9.06±1.56	0.02±0.01
	RAS	1.15±0.27	0.02±0
	Pre-STA 1	-2.4±4.28	0±0
D7	Floc	15.62±1.56	1.53±0.33
	RAS	-2.38±2.02	-0.26±0.4
	Pre-STA 1	-0.1±1.02	-0.03±0.03
D7c	Floc	23.09±4.45	0.02±0
	RAS	3.62±1.26	0±0
	Pre-STA 1	1.38±0.1	0.01±0

Respiration

Table 7-19. Rates of aerobic and anaerobic respiration in litter samples collected from all three benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Location	Sample	Aerobic respiration *umolesCO_2-C/gdw/day*	Anaerobic respiration *umolesCO_2-C/gdw/day*
A7c	Litter	131±49.8	26.71±11.72
C7	Litter	215.2±53.8	40.3±12
C7c	Litter	245.8±50.4	34.81±6.72
D7	Litter	317.1±17.3	56.38±12.21
D7c	Litter	231.5±109.3	67.4±45.94

Table 7-20. Rates of aerobic and anaerobic respiration in floc and soil samples collected from all benchmark sites in *STA-3/4 Cell 3B* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Site	Sample type	Aerobic respiration *umolesCO_2-C/gdw/day*	Anaerobic respiration *umolesCO_2-C/gdw/day*
A7	Floc	26.1±4.7	0.94±0.08
	RAS	79.4±14.8	0.66±0.16
	Pre-STA 1	74.1±7.4	1.32±0.16
A7c	Floc	235.7±54.5	6.29±3.41
	RAS	184.8±33.7	4.2±0.54
	Pre-STA 1	129.4±23.2	2.28±0.54
C7	Floc	37.1±6.7	1.84±0.32
	RAS	111.5±7.2	0.83±0.06
	Pre-STA 1	40.2±10.5	0.92±0.15
D7	Floc	195.9±28.1	6.26±0.65
	RAS	210.5±51.7	3.62±1.62
	Pre-STA 1	80.4±9	2.89±0.32
D7c	Floc	65±19.6	5.91±1.26
	RAS	90.6±7.6	2.21±0.13
	Pre-STA 1	43.9±9.6	1.16±0.14

STA-2 FW 1

Microbial Biomass

Table 7-21. Microbial biomass carbon (MBC), -nitrogen (MBN) and -phosphorus (MBP) in litter samples collected from three benchmark sites in *STA-2 FW 1* in March 2018. Values are averages of 3 replicate samples ±the standard error.

Site	Sample type	MBC (mg/kg)	MBN (mg/kg)	MBP (mg/kg)
A34	Litter	10439±1475	1214±206	156.2±39.5
A121	Litter	9692±4851	579±128	97.8±7.9
A208	Litter	12354±1107	915±136	139.6±7.7

Table 7-22. Microbial biomass carbon (MBC), -nitrogen (MBN) and -phosphorus (MBP) in floc and soil samples collected from three benchmark sites in *STA-2 FW 1* in March 2018. Values are averages of 3 replicate samples ±the standard error.

Site	Sample type	MBC (mg/kg)	MBN (mg/kg)	MBP (mg/kg)
A34	Floc	10217±160	979±68	191.1±4.3
	RAS	4760±295	508±25	94.2±3.5
	Pre-STA 1	1833±307	225±51	40.6±13.8
A121	Floc	25159±3332	3511±462	657.1±85.5
	RAS	5798±531	640±71	102.7±10.1
	Pre-STA 1	774±158	35±7	10±0.3
A208	Floc	13714±1517	1551±165	368.5±14.6
	RAS	5971±581	778±90	148.9±42.8
	Pre-STA 1	942±302	55±8	16.7±0.9

Extracellular enzyme activities

Table 7-23. Activities for 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: beta-glucosidase, LAP: leucine aminopeptidase) in litter samples collected from three benchmark sites in *STA-2 FW 1* in March 2018.

Site	Sample	AP	BisP	BG	LAP
		----------(umols/g dw/h)----------			
A34	Litter	1.16±0.36	0.72±0.25	1.85±0.66	6.35±0.27
A121	Litter	1.57±0.07	1.03±0.18	2.95±0.1	2±0.45
A208	Litter	6.05±1.34	6.16±1.48	4.05±0.55	6.57±0.75

Table 7-24. Activities for 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: beta-glucosidase, LAP: leucine aminopeptidase) in floc and soil samples collected from three benchmark sites in *STA-2 FW 1* in March 2018.

Site	Sample type	AP	BisP	BG	LAP
		----------(*μmols/g dw/h*)----------			
A34	Floc	2±0.24	0.84±0.09	1.21±0.05	5±0.43
	RAS	1.25±0.05	0.63±0.09	0.84±0.07	1.99±0.25
	Pre-STA 1	0.67±0.22	0.43±0.05	0.46±0.12	1.04±0.43
A121	Floc	3.11±0.24	1.56±0.3	1.23±0.31	11.2±1.3
	RAS	0.91±0.08	0.72±0.04	1.07±0.26	2.15±0.36
	Pre-STA 1	0.28±0.02	0.18±0.03	0.18±0.03	0.22±0.05
A208	Floc	2.81±0.49	3.11±0.29	1.2±0.07	5.23±0.72
	RAS	1.99±0.19	1.26±0.05	1.07±0.25	2.08±0.13
	Pre-STA 1	0.3±0.04	0.17±0.04	0.18±0.03	0.33±0.04

Mineralizable phosphorus and nitrogen

Table 7-25. Potential mineralizable nitrogen (PMN) and –phosphorus (PMP) in litter samples collected from all benchmark sites in *STA-2 FW 1* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Site	Sample type	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
A34	Litter	15.33±15.11	6.92±4.24
A121	Litter	1.83±1.51	5.48±0.25
A208	Litter	2.34±1.76	2.29±0.55

Table 7-26. Potential mineralizable nitrogen (PMN) and –phosphorus (PMP) in floc and soil samples collected from all benchmark sites in *STA-2 FW 1* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Site	Sample type	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
A34	Floc	10.69±7.64	3.11±1.09
	RAS	1.5±3.61	0.79±0.31
	Pre-STA 1	2.76±0.69	0.19±0.1
A121	Floc	58.04±2.71	8.19±1.18
	RAS	8.6±1.98	1.27±0.44
	Pre-STA 1	0.69±0.29	1.11±1.09
A208	Floc	12.67±8.36	2.21±0.51
	RAS	2.85±1.58	0.89±0.15
	Pre-STA 1	1.02±0.07	0.07±0.03

Respiration

Table 7-27. Rates of aerobic and anaerobic respiration in litter samples collected from all benchmark sites in *STA-2 FW 1* in March 2018. Values are averages of 3 replicate samples ± the standard error.

Site	Sample	Aerobic respiration *umolesCO_2-C/gdw/day*	Anaerobic respiration *umolesCO_2-C/gdw/day*
A34	Litter	170.5±24.7	40.41±9.5
A121	Litter	128.8±2.1	17.68±1.01
A208	Litter	154.7±11	28.84±5.65

Table 7-28. Rates of aerobic and anaerobic respiration in floc and soil samples collected from all benchmark sites in *STA-2 FW 1* in March 2018. Values are averages of 3 replicate samples ±the standard error.

Site	Sample type	Aerobic respiration *μmolesCO_2-C/gdw/day*	Anaerobic respiration *μmolesCO_2-C/gdw/day*
A34	Floc	98.4±13.4	11.14±4.74
	RAS	89.9±5.2	3.23±0.52
	Pre-STA 1	30.7±9.8	1.81±0.5
A121	Floc	71±8.7	7.78±1.44
	RAS	92.9±6.5	3.85±0.85
	Pre-STA 1	32.2±7.3	3.01±0.99
A208	Floc	92.5±15.9	8.89±4.29
	RAS	107.7±3.1	5.83±2.38
	Pre-STA 1	20.5±4.3	1.05±0.11

STA-2 FW 3

Microbial Biomass

Table 7-29. Microbial biomass carbon (MBC), -nitrogen (MBN) and -phosphorus (MBP) in litter samples collected from three benchmark sites in *STA-3/4 Cell 3A* in March 2018. Values are averages of 3 replicate samples ±the standard error.

Site	Sample type	MBC (*mg/kg*)	MBN (*mg/kg*)	MBP (*mg/kg*)
C-20	Floc	2651±284	270.5±53.1	51.62±7.97
	RAS	825±66	104.2±12	37.46±13.54
	Pre-STA 1	122±44	9.9±3.3	4.61±1.36
C-128	Floc	2949±403	349.3±37.9	83.85±5.41
	RAS	469±86	57.5±13.1	19.01±2.16
	Pre-STA 1	811±288	55.7±23.2	13.06±2.67
C-200	Floc	4936±630	571.1±59.3	137.73±13.72
	RAS	673±82	53.4±5.6	13.55±2.47
	Pre-STA 1	302±45	3.4±1.5	4.88±1.17

Extracellular enzyme activities

Table 7-30. Activities for 4 extracellular enzymes (AP: alkaline phosphatase, BisP: phosphodiesterase, BG: β-glucosidase, LAP: leucine aminopeptidase) in floc and soil samples collected from three benchmark sites in *STA-2 FW 3* in March 2018. Values are averages of 3 replicate samples ±the standard error.

Site	Sample	AP	BisP	BG	LAP
		----------(*μmols/g dw/h*)----------			
C-20	Floc	0.41±0.02	0.49±0.02	0.1±0.01	0.8±0.14
	RAS	0.16±0.01	0.42±0.02	0.06±0.01	0.35±0.01
	Pre-STA 1	0.11±0.02	0.07±0	0.02±0	0.17±0.02
C-128	Floc	0.53±0.05	0.66±0.06	0.1±0.01	1.07±0.27
	RAS	0.23±0.03	0.45±0.07	0.04±0.01	0.26±0.07
	Pre-STA 1	0.22±0.04	0.12±0.02	0.08±0.01	0.27±0.04
C-200	Floc	0.69±0.07	0.88±0.08	0.17±0.01	1.25±0.15
	RAS	0.23±0.05	0.35±0.06	0.06±0.01	0.24±0.05
	Pre-STA 1	0.15±0.06	0.09±0.03	0.1±0.02	0.13±0.04

Mineralizable phosphorus and nitrogen

Table 7-31. Potential mineralizable nitrogen (PMN) and –phosphorus (PMP) in floc and soil samples collected from all benchmark sites in *STA-2 FW 3* in March 2018. Values are averages of 3 replicate samples ±the standard error. There was no litter at the time of field sampling.

Site	Sample	PMN *μg NH_4-N/g dw/day*	PMP *μg PO_4-P/g dw/day*
C-20	Floc	1.28±2.75	0.28±0.18
	RAS	1.82±0.51	0.15±0.04
	Pre-STA 1	0.51±0.17	0.01±0
C-128	Floc	2.62±2.68	0.13±0.03
	RAS	1.31±0.55	0.02±0.01
	Pre-STA 1	1.42±0.38	0.01±0.02
C-200	Floc	4.1±1.15	0.16±0.01
	RAS	0.38±0.4	0±0
	Pre-STA 1	0.63±0.08	-0.01±0

Respiration

Table 7-32. Rates of aerobic and anaerobic respiration in floc and soil samples collected from all benchmark sites in *STA-2 FW 3*. Values are averages of 3 replicate samples ±the standard error. There was no litter at the time of field sampling.

Site	Sample	Aerobic respiration *umolesCO_2-C/gdw/day*	Anaerobic respiration *umolesCO_2-C/gdw/day*
C-20	Floc	17.8±3.7	1.15±0.49
	RAS	22.4±8.2	1.03±0.52
	Pre-STA 1	30.3±2.1	1.47±0.06
C-128	Floc	23.9±7.8	1.01±0.22
	RAS	17.6±0.9	0.64±0.13
	Pre-STA 1	42.4±1.6	1.51±0.16
C-200	Floc	41.6±0.9	1.37±0.36
	RAS	21.8±1.2	0.86±0.16
	Pre-STA 1	45.6±9.3	1.09±0.07

7.2 Future Work for Task 8.

For the next quarter, planned activities for Task 8 include completion of SOD analysis for all samples collected in March 2018. Once a completed dataset of all variables from 2016-2018 samplings is obtained, statistical analysis will be undertaken to assess site, seasonal, and depth variation among parameters. Multivariate approaches will also be explored with the combined dataset to derive patterns and correlations between microbial activities and the intrinsic nutrient

and stoichiometric properties of soils and microbial communities. Preparation of the final report will begin as results of these analyses become available.

8 Plant Litter Decomposition – Field Study (Task 8b)

As the initial phase of soil organic matter accretion, litter decomposition is a key factor regulating soil and organic P accretion in wetlands, including the Stormwater Treatment Areas of the Everglades. Litter material produced by different types of wetland vegetation can result in large differences in composition (i.e., tissue nutrients and macromolecular composition). For example, in STAs, major differences in litter quality between emergent aquatic vegetation (EAV) (more structural compounds) and submerged aquatic vegetation (SAV) (less structural compounds and more protein/lipid) can significantly affect the rate of decomposition and nutrient retention. In this manner, the mechanism of P retention and stability in STA soils can be directly attributed to litter decomposition processes.

Litter decomposition indicates turnover rates of vegetation community types, which can be useful in assessing P retention in wetlands. EAV has more structural molecular compounds and thus is generally more resistant to decomposition compared to SAV. The SAV has more protein and lipids, and less structural compounds, rendering its higher turnover rate. Since P retention and storage in vegetation, and burial and accretion of vegetation, is one of the primary storage mechanism for STAs, it is important to understand turnover rates of EAV and SAV vegetation and their relationship with biogeochemical properties and processes.

Litter bag methods are now routinely used to assess litter decomposition dynamics through a mass-loss process. Normally, litter bags are deployed over a period of months, or for enough time for material to become partially decomposed. Colonization of heterotrophic microorganisms on the remaining litter material occurs and is quantified as microbial biomass or through measurement of enzyme activity. The overall objective of this study is to determine the turnover rates of plant litter produced from EAV and SAV along a phosphorus gradient in the soil and water column of STAs and relate these rates to biogeochemical properties and site conditions. We hypothesized that the decomposition of EAV litter is slower than SAV and the overall decomposition rates of litter from both vegetation types are accelerated by nutrient loading. We selected STA-3/4 to conduct the litter decomposition study. STA-3/4 began operations in water year 2004 (WY2004)).

Workplan was submitted to the District for review and details on the progress of the study will be presented in the next quarterly report.

9 Abiotic Degradation of Dissolved Organic Phosphorus and Carbon in STAs (Task 9)

This purpose of this task is to determine the role of abiotic degradation of organic materials, specifically dissolved organic matter, in the cycling and ultimately retention of key nutrients. Phosphorus, and to a lesser extent nitrogen, are both of significant interest as the mineralization process from forms bound in organic matter to highly bioavailable forms (ortho-phosphate and nitrate/ammonia) may decrease the retention of these nutrients in the STAs resulting in an increase in the export to Everglades marshes.

To date, the following activities related to this task are ongoing:

1) Physical and chemical characterization of DOM from inflows, midflow and outflow sites within STA-2 Flow-ways 1 and 3.

2) Preliminary UV photolytic assays on standardized organic compounds and bulk inflow waters to determine N and P mineralization rates

3) Experimental determination of peroxides in bulk outflow waters and experimental reactions of hydrogen peroxide with raw inflow waters / analysis of nutrient mineralization

Results this Quarter

Preliminary characterization of SAV and EAV dominated flow-ways (STA-2 Flow-ways 1 and 3) suggests differences in processing of DOM between the dominant vegetative communities (**Table 9-1**). Directional changes in TP and TN both suggest mineralization processes are active but overall DOM levels (as DOC) remain mostly constant. The apparent change in nutrients without a concomitant DOC reduction suggests continual DOC inputs or potentially transformations.

Table 9-1. Results of DOM characterization from 2017 STA-2 Flow-ways 1 and 3.

Site (STA-2)	DOC mg l^{-1}	TP mg l^{-1}	TN mg l^{-1}	fDOM QSU	CDOM abs 318	SUVA abs 254	<10K Da (%)	10-100K Da (%)	>100K Da (%)
3-in	33.02	0.090	3.39	19.8	0.33	0.46	5.5	58.2	25.4
3-mid	27.19	0.052	2.87	25.4	0.28	0.34	8.4	51.0	23.2
3-out	26.44	0.018	2.06	25.9	0.20	0.27	7.4	57.1	20.0
1-in	36.37	0.110	3.84	20.1	0.32	0.46	4.5	52.9	28.9
1-mid	35.11	0.064	3.11	28.4	0.34	0.49	6.9	55.1	30.1
1-out	37.85	0.009	2.44	30.7	0.38	0.51	5.7	57.0	30.9

Measures of fluorescence DOM qualities, fDOM (expressed as quinone sulfate units), derived from EXO2 probes suggests an increase in fluorescent characteristics downstream in both flow ways. Chromophoric DOM (CDOM) and SUVA measures both suggest an increase in aromaticity of DOM downstream of inflow in EAV and a decrease in aromatic content downstream in SAV.

Photolytic Assay Trials

Experimental assays were performed using organic compounds known to have specific P and N to quantify potential rates of mineralization of organic N and P (**Figure 9-1**). Twenty-five mg/L solutions of RNA, phytic acid, and glucose 6 phosphate were made form deionized water and placed in 30 ml quartz glass tubes for UV exposure over 10 days in natural sunlight. Glass tubes

were placed in shallow basins with tap water flowing through to maintain constant temperature (**Figure 9-2**).

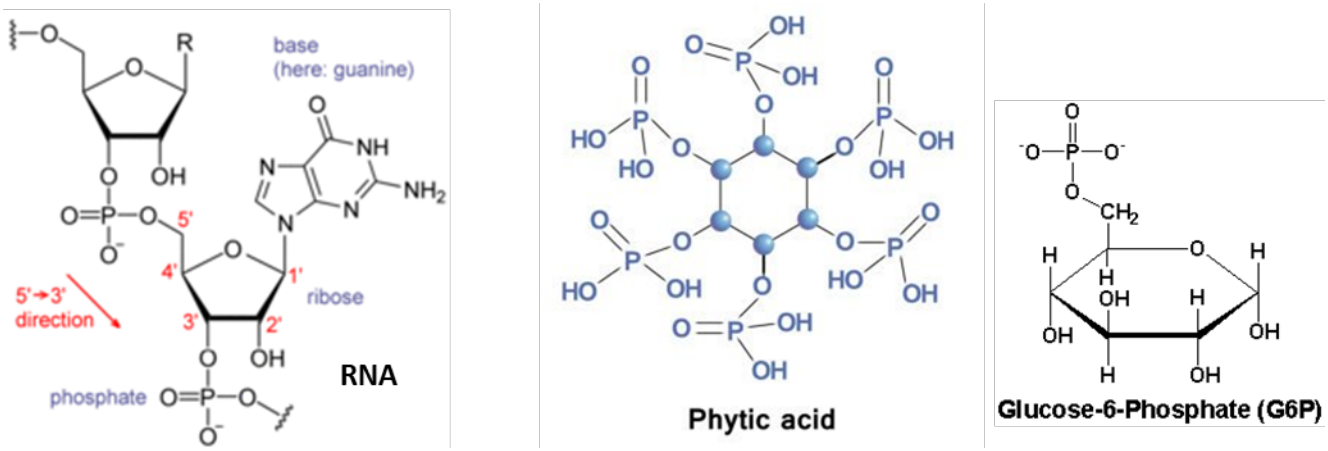

Figure 9-1. Standardized RNA, phytic acid, and glucose-6-phosphate compounds utilized in photolytic mineralization experiments to determine efficacy of ortho-phosphate generation from photolysis.

Figure 9-2. A. Flow through tanks to cool temperature of reaction vessels **B.** Photolytic reaction vessels made of quartz tubing exposed to sunlight. **C.** Photolytic reaction vessel control treatment tubes covered with aluminum foil.

Results of Standardized Organic Matter Assays

Results of trial exposures of standardized organic matter (RNA, Phytic acid, and glucose 6 phosphate) suggest that the compounds respond differently to UV light and microbial activity (**Figure 9-3**). Identical exposures using lab generated plant leachates resulted in photolytic losses of C from photolysis (**Table 9-2**). Nutrient changes (NOx and $-PO_4$) were analyzed in the laboratory (**Table 9-3**).

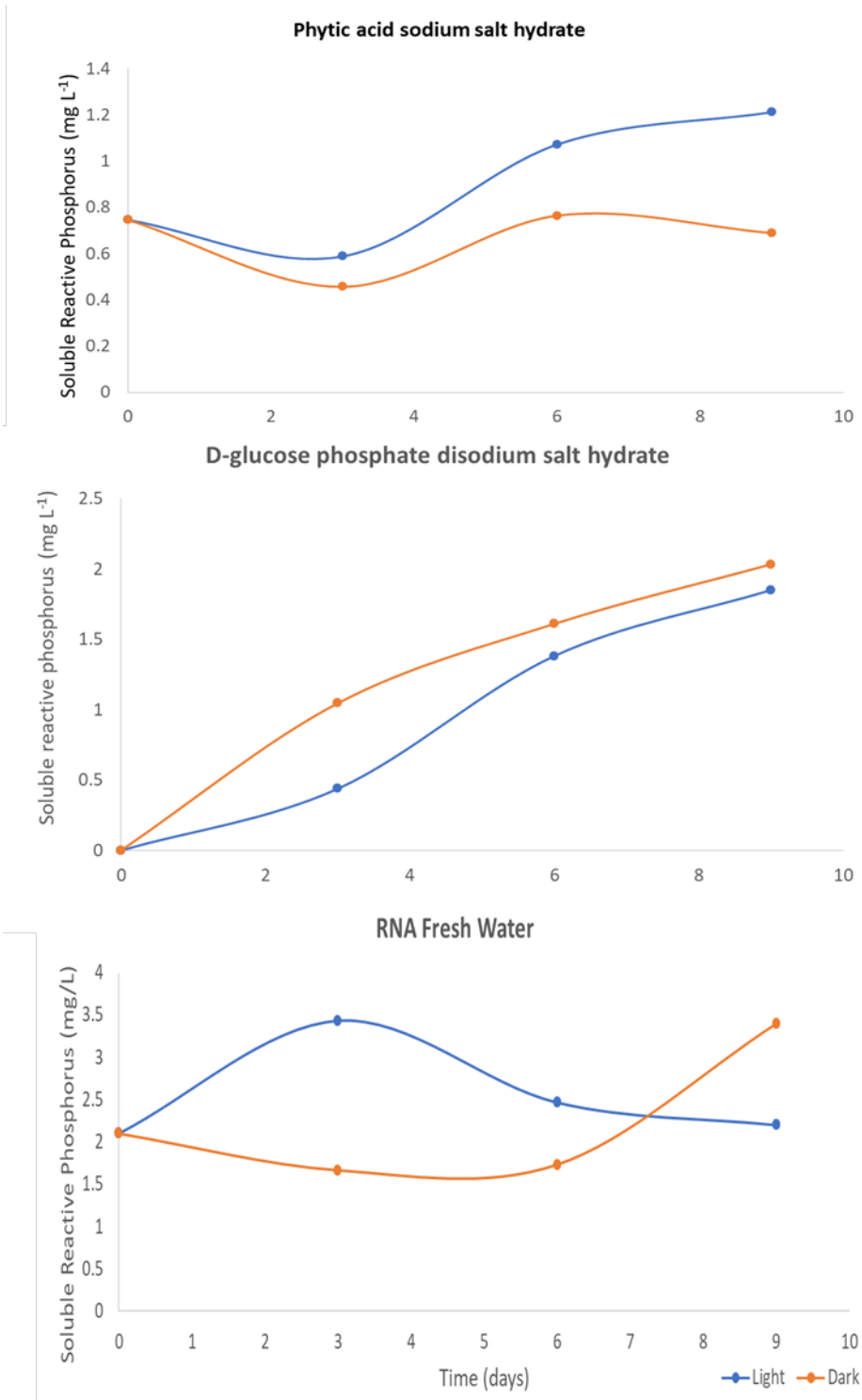

Figure 9-3. Soluble reactive phosphorus (SRP, mg/L) under light and dark conditions for RNA, phytic acid, and glucose 6 phosphate exposed to 10 days of UV light from natural sunlight.

Table 9-2. DOC concentrations (mg/L) in exposure tubes for plant leachates from *Typha latifolia*, *Cladium jamaiscence*, and *Naja guadalupensis* at time = 0, 7, and 14 days. Mean +/- std dev ( )

Species	DOC (t=0)	DOC (t=7) UV	DOC (t=7) Dark	DOC (t=14) UV	DOC (t=14) Dark
TYP	108	89 (5)	105 (4)	80 (6)	107 (4)
CLAD	94	81 (6)	92 (6)	78 (9)	90 (7)
NAJA	102	98 (4)	92 (9)	92 (9)	102 (4)

Table 9-3. Nutrient content for preliminary UV exposure experiments. Nitrate+nitrite (NOx) and ortho-phosphate concentrations (mg/L) in exposure tubes for plant leachates from *Typha latifolia*, *Cladium jamaiscence*, and *Naja guadalupensis* at time = 0, 7, and 14 days. Mean +/- std dev ( )

Species	PO_4 (t=0)	PO_4 (t=7) UV	PO_4 (t=7) Dark	PO_4 (t=14) UV	PO_4 (t=14) Dark
TYP	0.029	0.019 (0.012)	0.022 (0.015)	0.015 (0.011)	0.017 (0.010)
CLAD	0.011	0.017 (0.010)	0.010 (0.014)	0.008 (0.009)	0.012 (0.011)
NAJA	0.021	0.024 (0.010)	0.018 (0.009)	0.010 (0.009)	0.012 (0.008)
Species	NOx (t=0)	NOx (t=7) UV	NOx (t=7) Dark	NOx (t=14) UV	NOx (t=14) Dark
TYP	0.79	1.09 (0.19)	0.59 (0.11)	0.81 (0.19)	0.31 (0.18)
CLAD	0.45	0.56 (0.15)	0.39 (0.10)	0.35 (0.14)	0.26 (0.19)
NAJA	0.99	0.94 (0.25)	0.51 (0.15)	0.75 (0.22)	0.15 (0.16)

Determination of Hydrogen Peroxide in Outflow Waters

Water samples from the outflows of STA 2 Flow-ways 1 and 3 were tested for hydrogen peroxide content using the thiosulfate titration method. Initial results indicate no peroxide content in Flow-way 1 (EAV) outflow waters (0.0- 0.02 mg l^{-1} H_20_2), however, there appeared to be small amounts present in the outflow waters of STA 2 Flow-way 3 (SAV) that ranged from 2.3- 3.5 mg l^{-1} H_20_2). These positive results for the SAV flow-way gave sufficient evidence to initiate a short bioassay experiment with inflow waters from flow-way 3. This experiment used raw water plus 5 mg l^{-1} H_20_2 and raw water without H_20_2 to determine if peroxide presence will enhance DOM decomposition. After 5 days, water from each reactor was analyzed for DOC. Results indicate that 5 mg l^{-1} H_20_2 additions resulted in 41% greater DOC mineralization compared to no H_20_2 treatment. Currently, a full-scale experiment is planned to elucidate the role of peroxide in DOC mineralization in STA waters.

Planned Activities for Next Quarter

1. Amendment of methods for UV experiment and re-run to clarify results, with special effort to reduce standard error of results. In particular, we will try several anti-microbial

compounds to try and reduce microbial activity in control and treatment reactors which confounds interpretation of results from current data set.

2. Initiate full peroxide treatment experiment to determine role in SRP and NOx / NH4+ generation from natural and plant extract derived DOM.

10 Data Integration and Synthesis (Task 10)

Summary

The objective of this task is to integrate data obtained from this project and legacy data into a cohesive modeling framework to identify crucial soil and floc processes for phosphorus (P) retention. Literature shows that there is a need to account for short-circuits in STAs. We presented a framework in the last report and tested short circuiting on transient P retention in our 1d spiraling model. Without parameterization (not adjusting the parameters to obtain matches between model and data, but instead using published literature values), we found that the effects of short circuits on P outlet concentrations variabilities are smaller than effects of those the full spiraling model versus a simple first order (k-C*) model.

We further show statistical analyses to relate P and other biogeochemical relevant state variables with each other, including principal component analysis. We propose using structural equation modeling as a tool to further evaluate data and use the relationships to test the spiraling model.

Testing effects of short-circuits

a) Hydrology

We used the default parameter sets in our 1d spiraling model, where we obtained parameters from the literature (UF, 2017). We tested the effect of short-circuits in STA-2 FW 1on hydraulic loading (**Figure 10.1).** The ensemble members (colored lines) describe a probabilistic hydraulic loading, calculated based on the tank-in-series approach and using an auto-correlation process (UF, 2018).

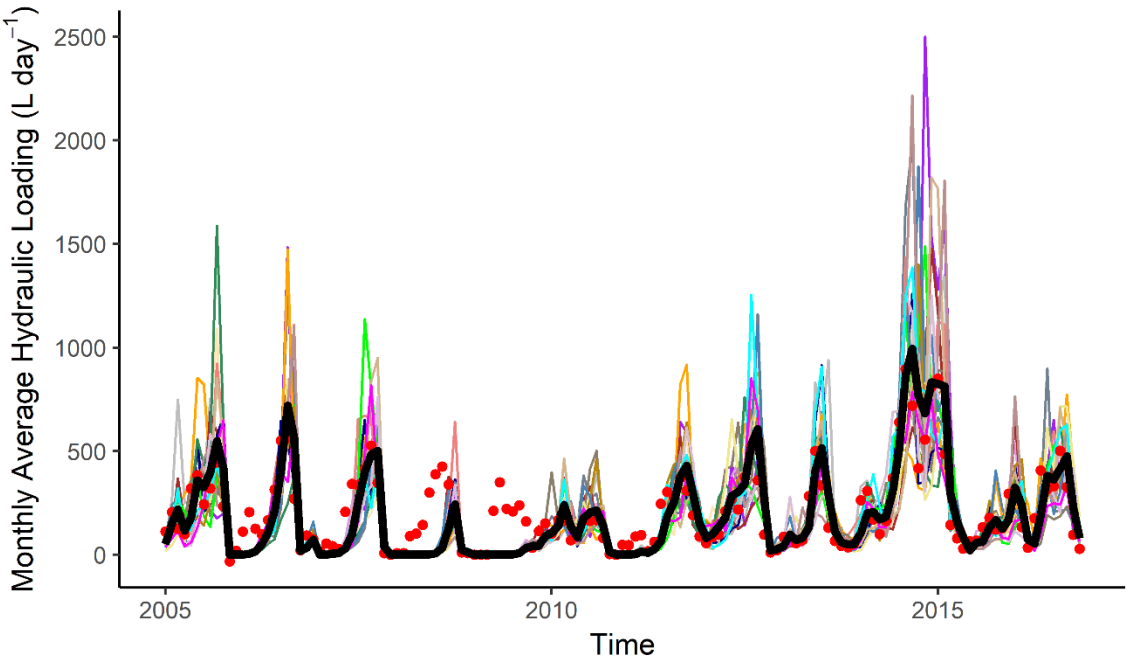

Figure 10.1. Time series of monthly averaged hydraulic loading generated for 20 ensembles (colored lines) with a relaxation of 20 days and 4 tanks in series (shape parameter is 4 in the gamma distribution). The black line is an average of all the colored lines. The red points are hydraulic loading data used to estimate interpolate hydraulic loading and estimate an average residence time for the gamma residence time distribution (RTD).

We find that the ensemble mean captures monthly loading values, except for periods where there was a prolonged minimal loading. The agreement depends on the strength of the temporal autocorrelation (parameter *relaxation*). Here relaxation means the rate with which the influence of a previous load affects the current load. A relaxation rate of 20 days means that current load is calculated by the previous day's load plus the difference between current and previous day's load times 0.05 day^{-1} (1/20 days) (see also Bykovski, 2016). Here we chose 20 days since this is about the average hydrologic residence time. Much of the tests with hydrology were shown in the last quarterly Report (UF, 2018).

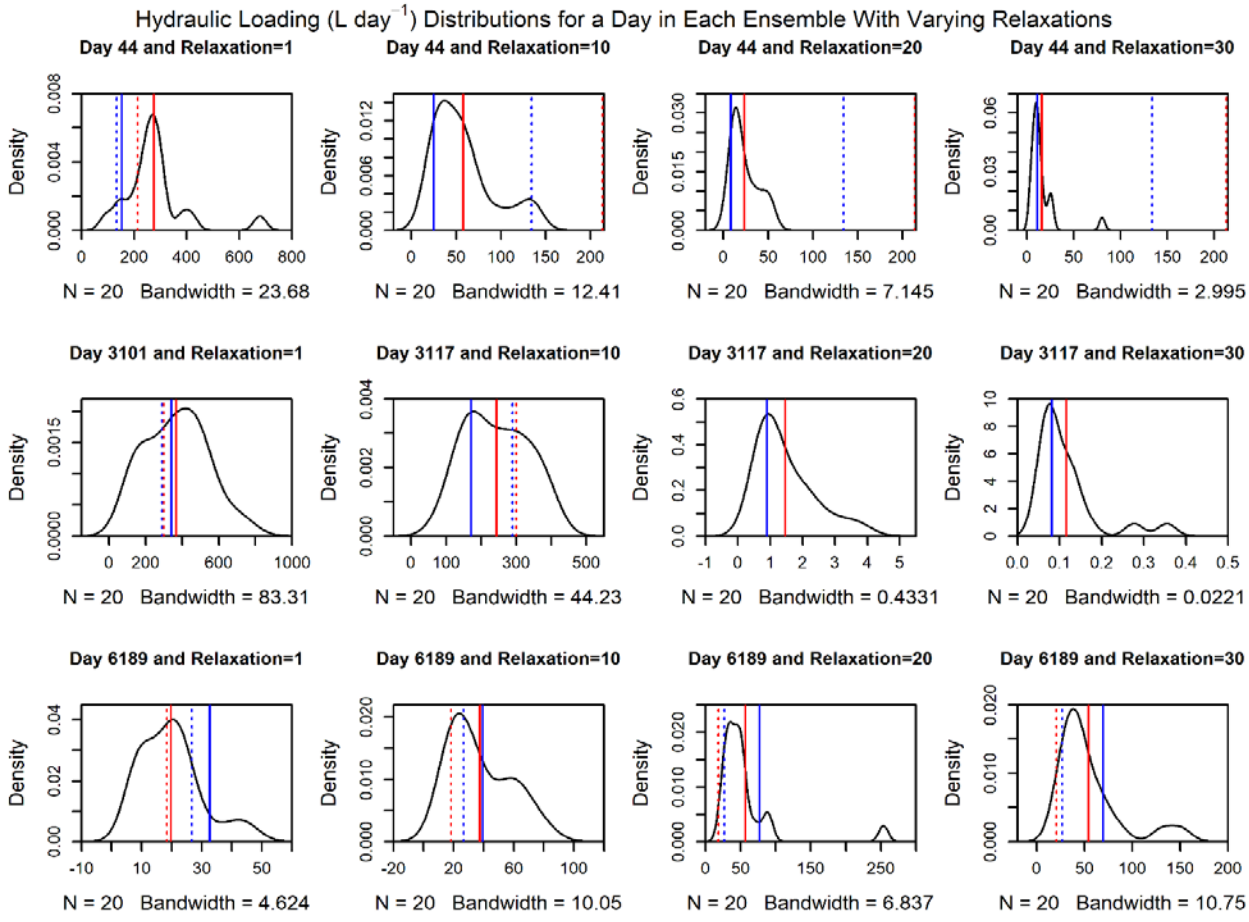

Figure 10.2. Probability density functions of ensemble hydraulic loading rate following the method of Bykovski, (2016) where loads where determined from random draws of residence time distributions that are relaxed on various time scales (1, 10, 20 and 30 days columns) as observed on various days along the time axis in Figure 10.1 (days 44, 3101, 8189). Results are compared

against observed loading rates. The red dashed line is the data's hydraulic loading rate. The red solid line is the model's ensemble mean. The blue dashed line is the data mean for hydraulic loading values in the past 20 days relative to a given day. The blue solid line is the model's ensemble mean for hydraulic loading values in the past 20 days relative to a given day. N represents the number of ensembles. Bandwidth indicates the resolution of the x axis in the figure. Distributions were plotted determined using R's hist function with bandwidth (resolution of x axis) and number of bins indicated in each plot. Note that some plots are missing dashed vertical lines because they are out of range.

We evaluated the ensemble outcome for predicted velocities (here expressed as loads), by looking at the probability density function (**Figure 10.2**) for a few specific days. We find that flow rates are not necessarily gamma distributed as this would have been expected from a single NTIS model. While part of the distribution curve may be attributed to the low ensemble numbers of 20, antecedent conditions also play a critical role. While it seems that shorter relaxation is a good choice, it would not represent short circuits effectively: We expect a series of water parcels arrive at the outlet to have experienced different mean velocities, however the shorter the relaxation time (compared to the mean residence time), the more the individual parcel's velocity moves towards the mean, essentially creating a plug and flow model. To represent short circuits, we need a certain range in velocities.

a) P retention

To test effects of short circuits on P retention, we applied both our 1-d spiraling model, and a simple model, where P is removed based on a single rate constant. We first compared the two based on plug-and flow estimation.

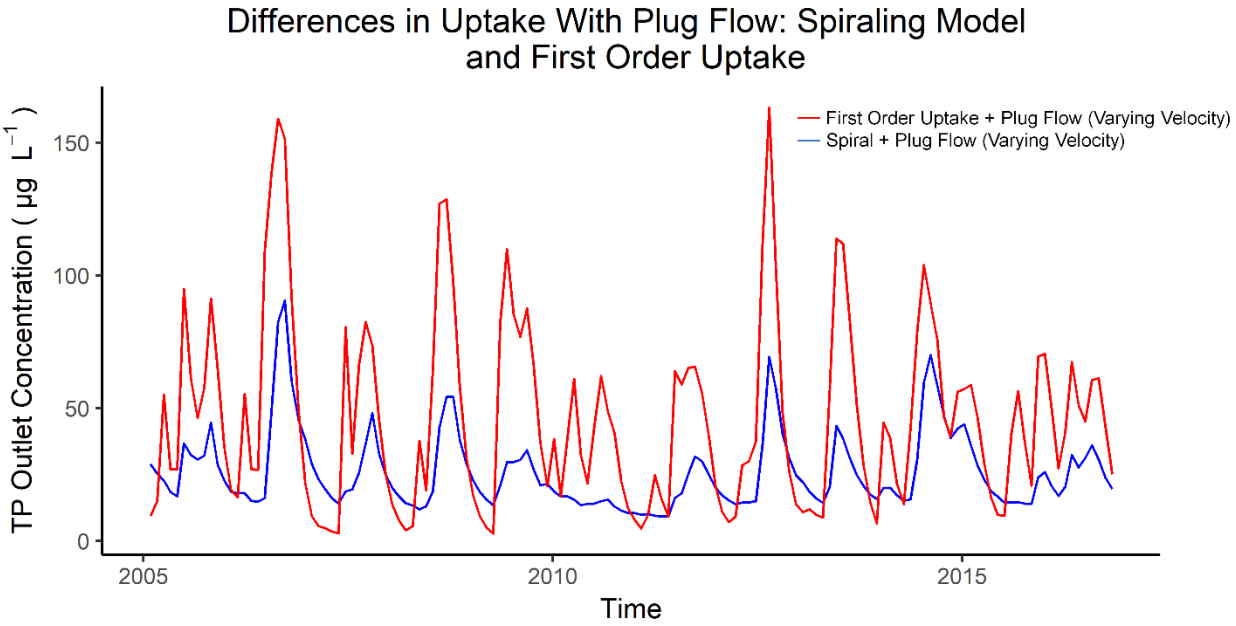

Figure 10.3. Time series from 2005-2016 of TP outlet concentration comparing between first order uptake (red line) and spiraling uptake (blue line). Both models have a plug flow with varying velocity set up.

Here, we adjusted the uptake parameter in the simple first order uptake model to approximate values of outlet TP concentration in the 1d spiraling model. Note that these values are off (the 1d spiral model is parameterized from literature value, and parameter estimation of the whole model is ongoing). However, we think that the tampering of the variability in outlet concentration (**Figure 10.3**) is a robust feature, because slower pools such as floc, vegetation and periphyton contribute to a more constant internal load, a feature that is missing in a simple first order model.

Yet, the 1d spiral model results depend on external load, and on hydrologic load (**Figure 10.4**.) It is the variable load causes the fluctuation in P outlet concentration when the velocity is held constant, while the difference between variable and constant flow can be attributed to flow velocity. Note, that these comparisons do not include short-circuits.

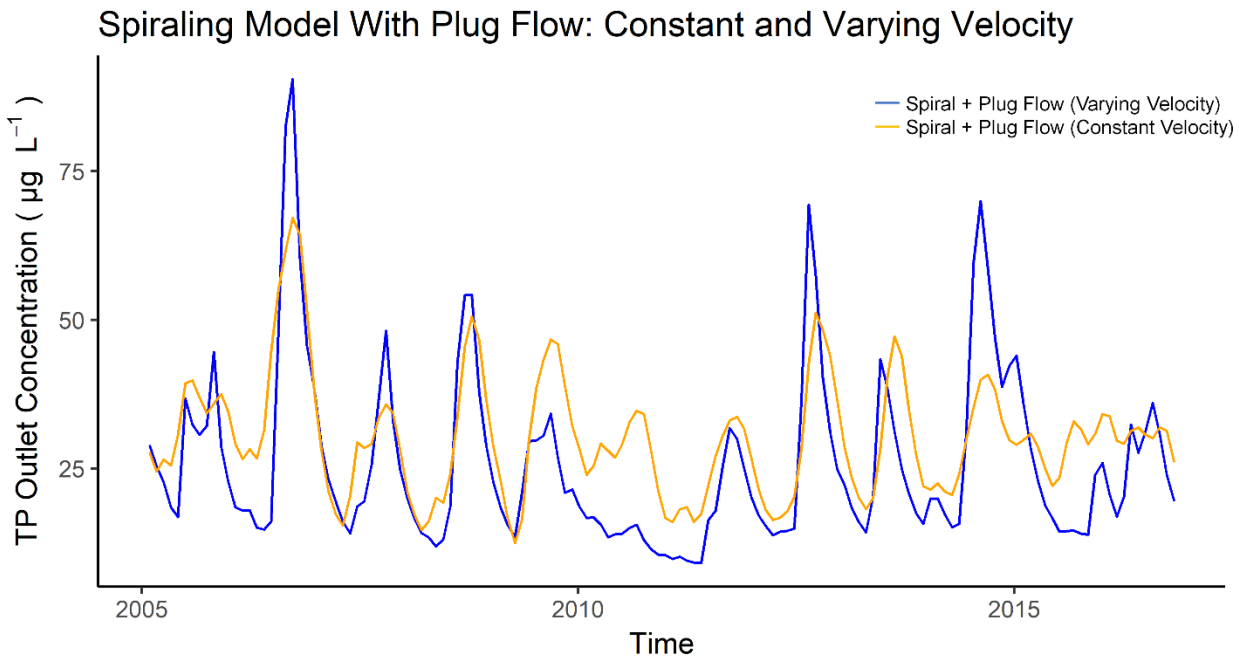

Figure 10.4 Time series from 2005-2016 of TP outlet concentration with spiraling uptake and comparing between plug flow with constant (orange line) and varying velocity (blue line).

To consider short circuits, we use a relaxation coefficient of 20 days first since this is the approximate travel time of the average parcel (**Figure 10.5**). The shape parameter for the residence time distribution is chosen to be 4 (number of tanks). We apply this to both the 1d spiraling model and the simple uptake model, and we compare against the plug and flow solution. Interestingly, we see very little difference between the plug and flow solution vs. the short-circuit solution (NTIS) with respect to the P outlet concentration. The difference between a simple first order model and the more complex 1d model appears much bigger than the differences induced by short-circuits, which holds across several relaxation times (**Figure 10.6**). Given the uncertainty in other parameters, the plug and flow representation applying mean flow velocities is a sufficient representation of the system.

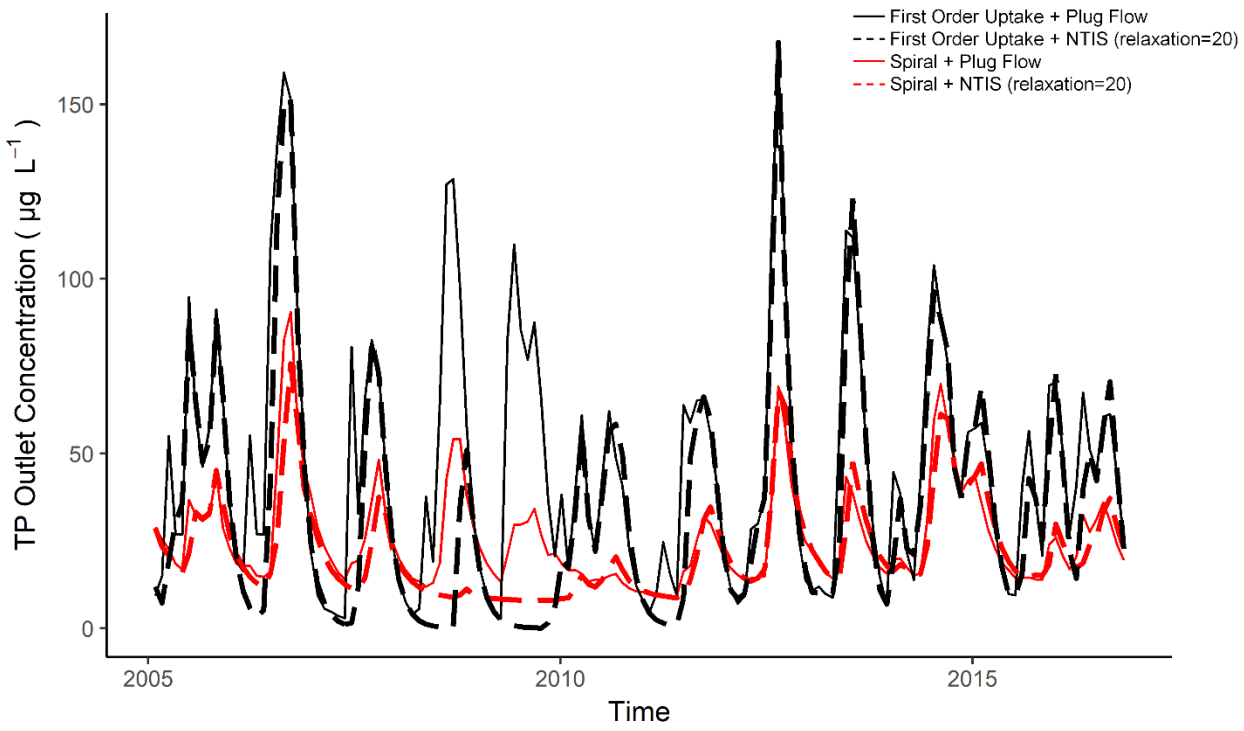

Figure 10.5. Time series from 2005-2016 of TP outlet concentration in four different models with combinations of uptake (first order uptake, spiraling uptake) and velocity (plug flow with varying velocity, NTIS with relaxation of 20).

The picture does not change if we introduce different relaxation times (**Figure 10.6**). This has critical consequence. If there is no significant model improvement by introducing short-circuits, large gains in efficiency in model inversion and data-model fusion can be obtained. In essence, increasing the simulation analyses by a factor of 20.

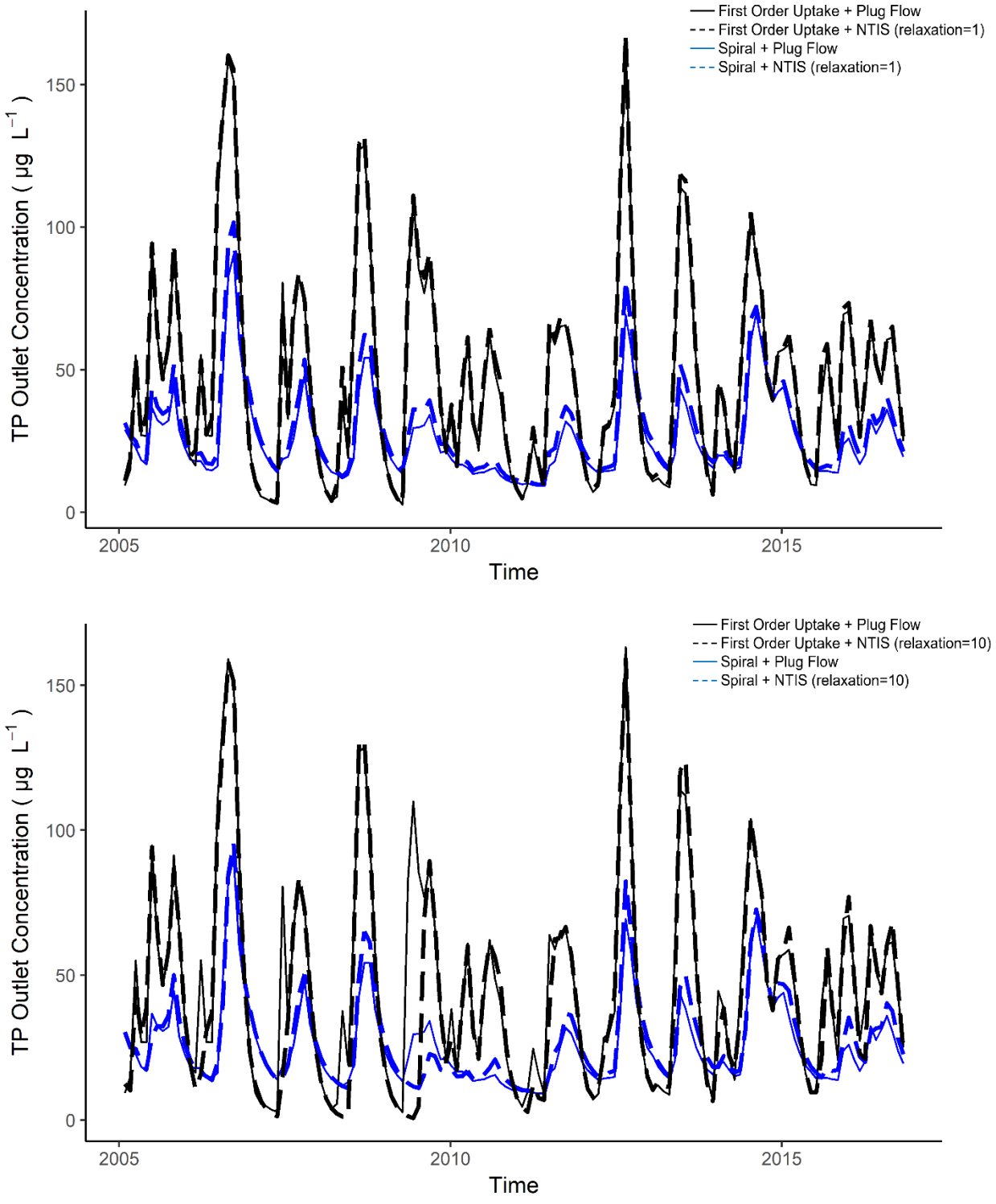

Figure 10.6. As **Figure 5**, but different relaxation times of 1 day (top) and 10 days (bottom).

Exploring Statistical Relationships

A complementary approach to the modeling "bottom up" approach is exploring statistical relationships. In the following we show three approaches that may be useful for hypothesis testing as well as for evaluation of the conceptual model. The relationships shown in this report are at this point for demonstration purposes, we do not draw any conclusions, but the may be helpful to construct hypotheses.

Correlation analyses of variables measured in the water column, in the floc and in the soil for STA-2 FWs 1 and 3 are quite different (**Figure 10.7**). What immediately becomes clear is that much of the correlations in the water column have opposite signs when comparing the two FWs. Relationships among variables are more homogenous across FW in the Floc and the soil layer (**Figures 10.8 and 10.9**).

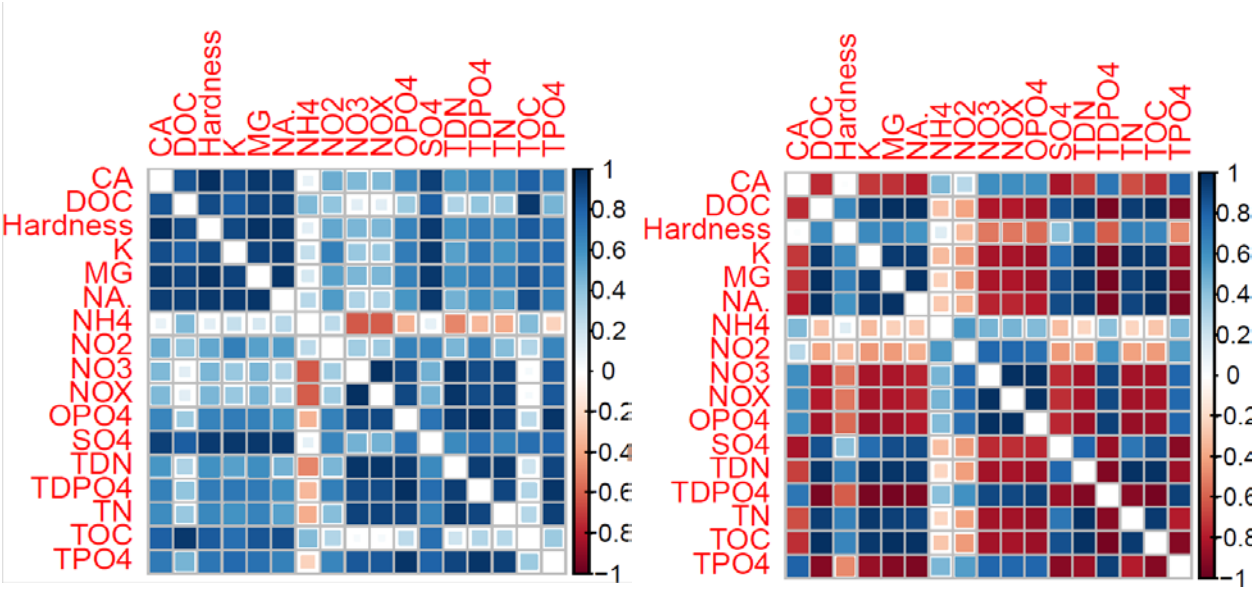

Figure 10.7. Correlation analysis of different variables across the transect for the water column for STA-2 FW 1(**left**) and FW3 (**right)**. The color and size of the square indicate sign and strength of the correlation coefficient. The following variables were tested (OPO4: Orthophosphate, TDN: Total dissolved nitrogen, TDPO4: Total dissolved phosphorus, TN: Total nitrogen, TOC: Total organic carbon, TPO4: total phosphorus.

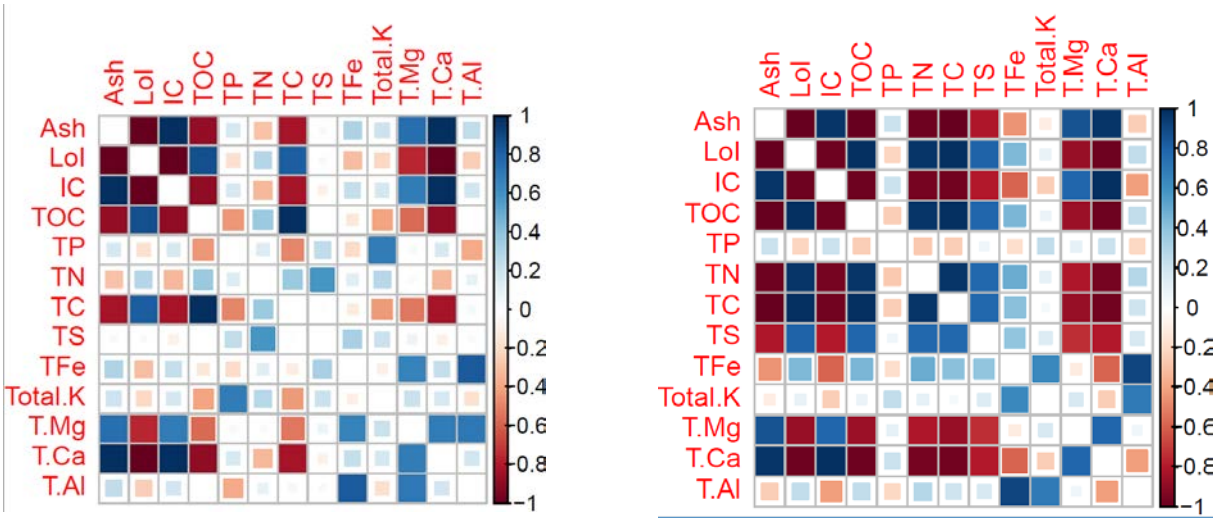

Figure 10.8. Correlation analysis for different variables across the stations for Floc in FW 1 (**left**) and FW 3 (**right**) of STA-2. The color and size of the square indicate sign and strength of the correlation coefficient. The following nomenclature is used: T in the beginning refers to total. Normal chemical element notation is used. Ash denotes ash fraction, IC inorganic carbon and OC organic carbon.

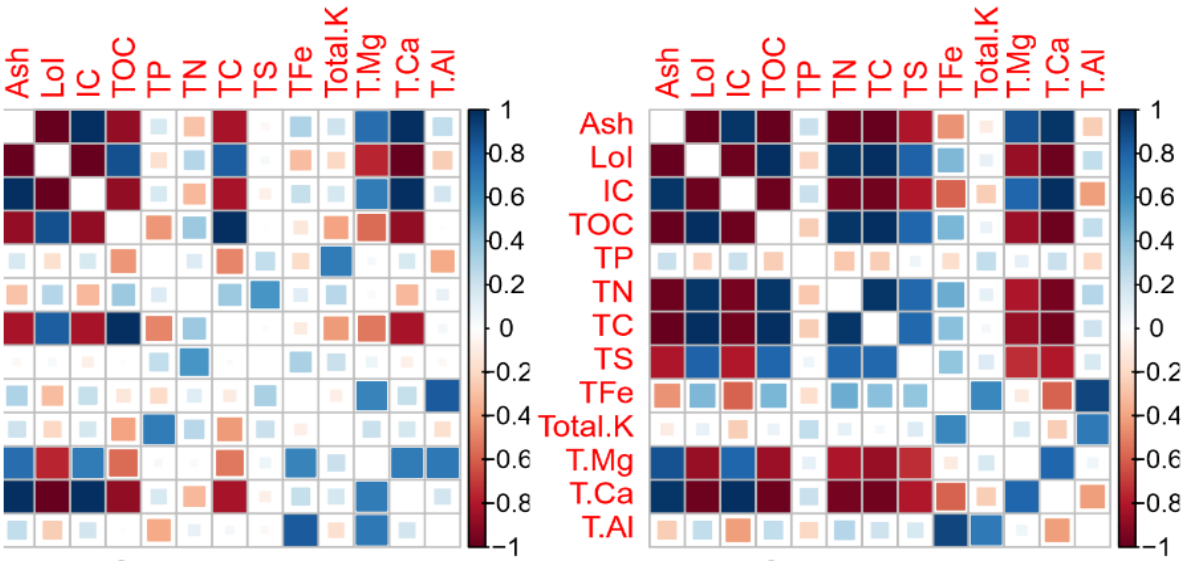

Figure 10.9. Same as **Figure 10.8**, but for the recently accreted soil (RAS)layer.

We further conducted a principal component analysis for the four layers, water column, floc, recently accreted soil and pre-STA soil for STA-2 FW 1, STA-2 FW 3, STA-3/4 Cells 3A and 3B (**Figure 10.10**). In each case the first two principal components (PCs) explain >90% of the variance. In the water column, the PCs align for the different flowways but also along the flowpath. In floc and soil, it is difficult the discern a clear pattern. There is some organization between FWs, but not clear separation. Distance along the flowpath appears to be a less important factor in floc and soil layer as there is a less visible alignment as in the water column.

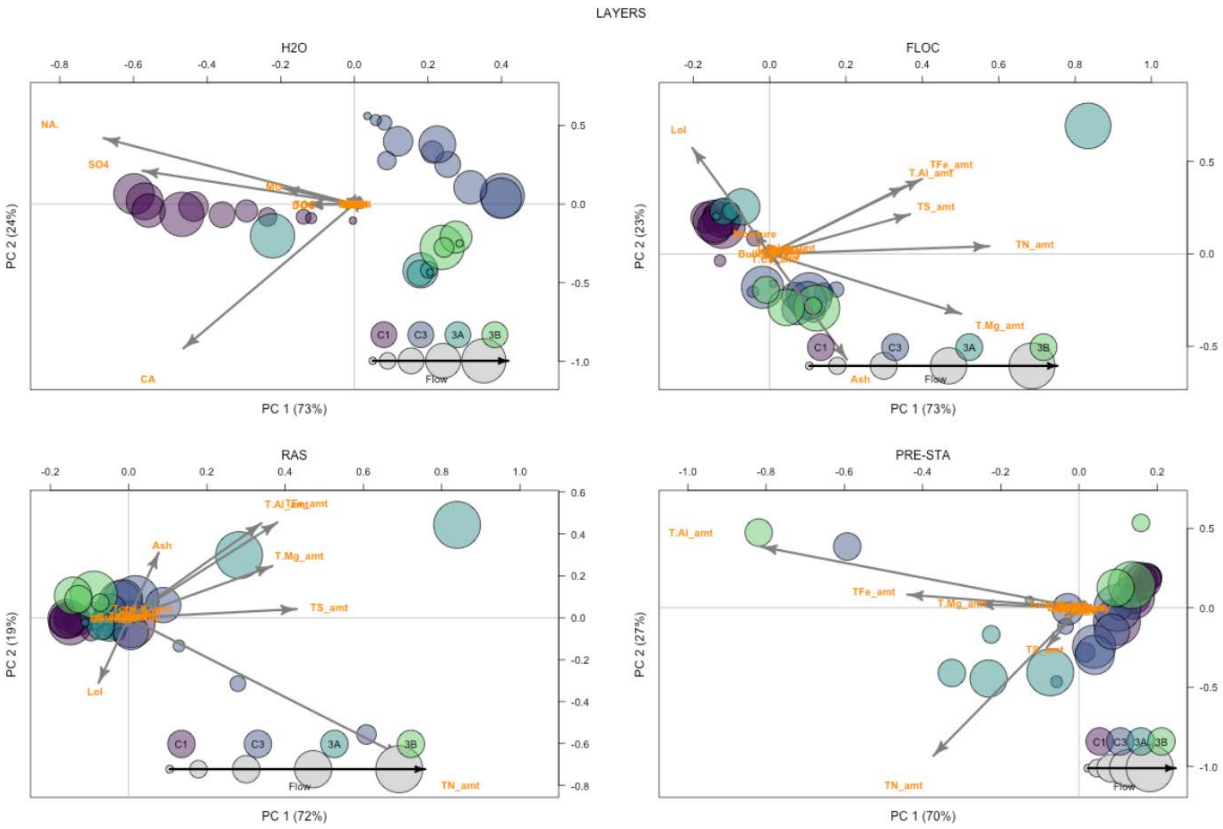

Figure 10.10. Principal component analysis of the spatial data for water column, floc, recently accreted soil and pre-STA soil. The x-axis shows the first principal component, the y axis the 2nd principal component. The dots are colored coded by FW, and size indicates distance along the flowpath (small close to inlet, large close to outlet). Arrows indicate loading onto the principal component axes by selected variables. The suffix "amnt" refers to the variable in units mass per area instead of concentration (mass element per mass material).

We note that both the correlation matrix and the principal component analysis are correlational. In order to test hypotheses, there is a need to consider predicted and predicting variables. In a complex system with lots of interdependencies it may happen that a variable may be both, a variable of interest depending on others and a predictor. To overcome this problem, we are testing structural equation modeling (Lefcheck, 2016), that allows for variables to be both predictor and being predicted. Two examples are 1) include almost all spatial variables to predict the macro-elements in water column floc and soil (**Figure 10.11**), and 2) restrict the analysis to predictions of macro-elements in the recently accreted soil (RAS, **Figure 10.11**), while predictors are still covariates in

the same layer, the layer above (Floc), and the layer below. Both figures are for illustration purposes and we currently evaluate the design. One issue that may come up with large number of variables but limited number of observation are type II errors. We are currently exploring ways to address this.

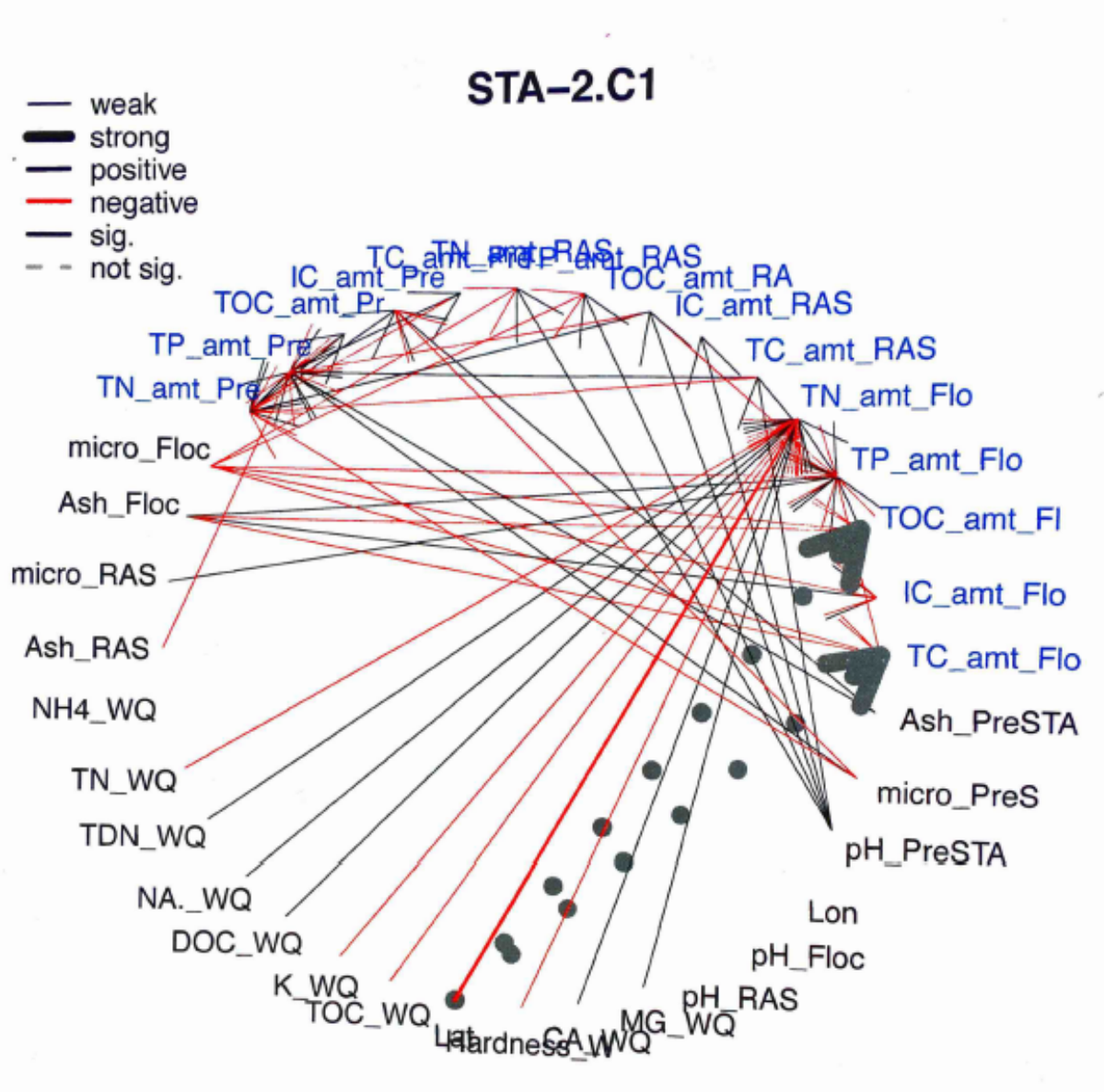

Figure 10.11. Example of structural equation modeling. The arrows denote prediction and relationships and are classified as weak influence (little effect), strong influence (more effect), and noting correlation (positive or negative). The blue variables are predicted variables (but these can also be predictors. Nomenclature is as follows: The first part of the string until ("_") denotes the variable as in Figure 10.8, the last part (after the last "_" denotes the location (WQ: Water

column, Fl: Floc, RAS: recently accreted soil, Pre-STA: pre-STA soil). Where there is “amt” in the name, the total element mass is being used (mass per area).

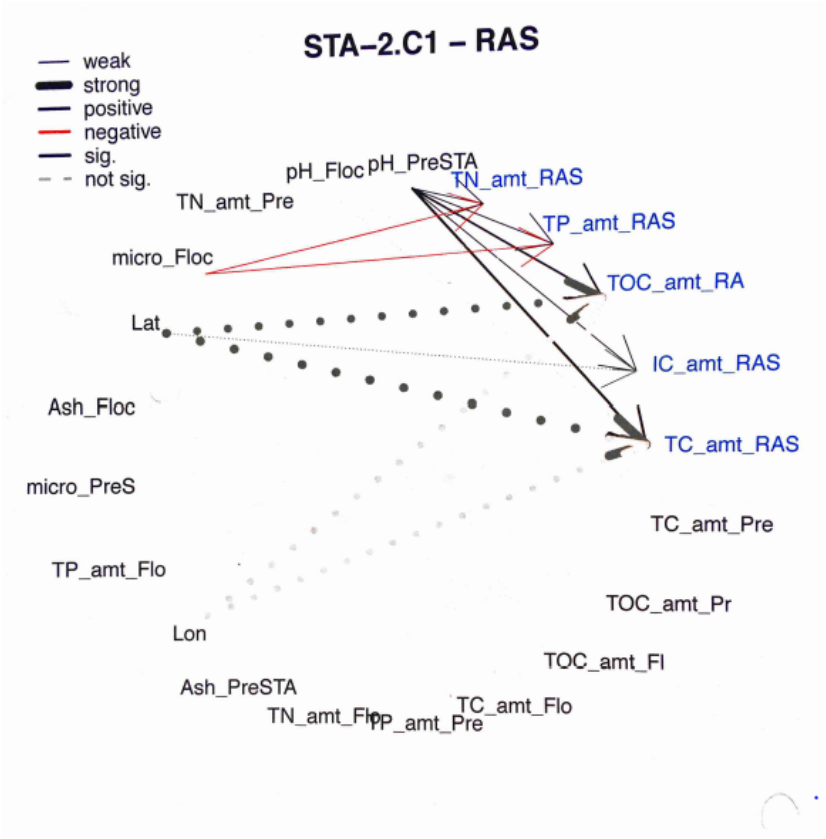

Figure 10.12. Same as **Figure 11**, but with reduced predicted variables and reduced predictors. Predicted variables include macro-elements in the recently accreted soil, while predictors are various variables in layers above and below.

11 Planned Activities

The following activities are planned for the next quarter (July 1, 2018 to September 30, 2018).

- **Tasks 3 and 4**. Analysis of soils data that were collected in all seasons.
- **For Task 5**. Submit a manuscript after it is reviewed by the district.
- **For Task 6a.** Complete calculations and analysis of P sorption data for STA-2 and STA-3/4 and finalize the report.
- **For Task 6b.** Complete column experiments on P exchange between soil and water column.
- **For Task 7a.** Continue data analysis for water quality data
- **For Task 7b**. Complete statistical analysis and interpretation of microbial enzyme assays data measured on various samples from all completed flow events. Begin preparations for next flow event (tentatively scheduled for May 2018) in STA-3/4.
- **For Task 8a**. Continue data analysis for biogeochemical parameters in litter, floc and soil samples collected at *benchmark* sites in March 2018.
- **For Task 8b.** Start the field experiments on plant litter decomposition in Cells 3A and 3B.
- **For Task 9.** Continue the experiments for chemical characterization of DOM and photolysis effects in STA-3/4 Cells 3A and 3B.
- **For Task 10.** Implement better (prescribed) water dynamics into the analysis tool, using stage heights and model improvement: partition dissolved P in water and soil column.

12 References

Bykhovsky, D., 2016. Simple generation of Gamma, Gamma–Gamma, and K distributions with exponential autocorrelation function. Journal of Lightwave Technology. 34:2106-2110.

Lefcheck, J. S. 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods in Ecology and Evolution 7:573–579.

UF-WBL, 2017. Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs). 2nd Annual Report. Work Order # 4600003031-WO01. West Palm Beach, FL: South Florida Water Management Dist.

UF-WBL, 2018. Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs). 11th Quarterly Report. Work Order # 4600003031-WO01. West Palm Beach, FL: South Florida Water Management Dist.