Quantifying the Recalcitrance and Lability of Phosphorus within Stormwater Treatment Areas (Biomarker Study)

Project Work Plan

Work order 4600004016-WO02

Submitted to:

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PROJECT DESCRIPTION AND OBJECTIVES

The primary goals of this work are to evaluate relationships between organic matter (OM) and phosphorus (P) and to evaluate sources and potential turnover of P within the Stormwater Treatment Areas (STAs).

To accomplish these goals, we will (1) characterize water quality of STA source waters; (2) measure biomarkers from faunal excretion and bioturbation samples; and (3) assess relationships between P and OM in an *in-situ* decomposition/leaching study.

The specific tasks and objectives of this study are:

Task 1: Project kick-off meeting

Task 2: Project work plan

Task 3: Characterize P and OM in STA source waters

Task 4: Evaluate the potential of faunal biomarkers as tracers of fish inputs of OM and P

Task 5: Evaluate biogeochemical signatures in a decomposition/leaching study to understand sources and turnover of P and OM of STA waters under controlled conditions.

(STOP/GO) Task 6: (optional 1-year extension) Characterize P and OM lability and recalcitrance in STA outflow water.

DESCRIPTION OF TASKS

Task 1. Project Kick-off Meeting [completed]

The UNIVERSITY Principal Investigator and other key scientists involved with the project shall virtually attend a project kickoff meeting with the District project team within three weeks after issuance of this Work Order. During the kickoff meeting, specific details regarding the study, timelines, project deliverables, and expectations will be discussed. Contact information for key personnel and their roles and responsibilities from both the UNIVERSITY and the District project teams shall be provided during the kick-off meeting.

Task 1 Deliverables [completed]

A draft memorandum summarizing minutes of the kick-off meeting and a list of action items for both the District and the UNIVERSITY.

Final memorandum summarizing minutes of the kick-off meeting and a list of action items for both the District and the UNIVERSITY.

Task 2: Project Work Plan [This document]

The UNIVERSITY shall develop a Draft Project Work Plan in accordance with the project objectives and discussions at the kickoff meeting. District staff shall review and provide comments within two weeks following receipt of this Draft Project Work Plan. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Project Work Plan within two weeks of receiving such comments.

Task 2 Deliverables

Draft Project Work Plan
Final Project Work Plan

Task 3: Characterize P and OM in STA source waters to understand differences in the quality of STA source waters and their potential effect on STA performance. **[in progress]**

Variability in the quality of STA source water may influence STA performance. P speciation and OM quality will be measured in eight STA source waters: Lake Okeechobee canal discharge (Station S-354), distribution canals downstream of structure discharge from STA-1W (Station G-302), STA-1E (Station S-319), STA-2 (Station S-6), STA 3/4 (Station G-370), STA 5/6 (Station G-508), A1 FEB (Station G-372) and L8 FEB (Station G-538). Stations are summarized in Figure 1 and Table 1. Samples were collected on November 4-5, 2020. Triplicate water samples were collected for each of the eight source waters (Figure 1). Whole water was filtered through glass fiber filters (GF/Fs) to isolate particulate organic matter (POM) and dissolved organic matter (DOM) fractions. Ultrafiltration was conducted using a Amicon stirred cell and Millipore ultrafiltration membranes (10 kDa), according to Ged and Boyer (2013) to separate DOM into two pools of different apparent molecular weight (AMW; < 10 kDa and >10 kDa). A < 10 kDa

and > 10 kDa AMW cutoff was selected based on the results of Ged and Boyer (2013) who found that the majority of dissolved organic phosphorus (DOP) was associated with the <1 kDa and >10 kDa AMW pool, and a lower proportion of DOP was associated with intermediate size fractions. Centrifugal ultrafiltration membranes can be biased for the low molecular weight pool (<1 kDa), and they suggest that the high proportion of P in the < 1kDa pool was attributed to soluble reactive phosphorus (SRP) or possibly ultrafiltration bias. However, they found that the larger AMW pool (>10 kDa) had an excitation emission matrix (EEMs) signal indicating that it could be associated with large biomolecules. Since this SOW is focused on understanding in situ production, the 10 kDa cutoff would be more appropriate to capture the production of large biomolecules associated with P, without the potential bias of lower kDa membrane cutoffs. This will result in four size fractions: (1) POM; (2) whole DOM (i.e. water filtered through a GF/F, nominal pore size 0.7 µm); (3) < 10 kDa DOM fraction (i.e. low apparent molecular weight, LMW); (4) > 10 kDa DOM fraction (i.e. high apparent molecular weight, HMW). An aliquot of each sample will also be filtered through a 0.45 µm filter to be processed for total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and dissolved organic carbon (DOC). Both the 0.7 µm and 0.45 µm filtrate will be analyzed for TDP.

After filtration there will be 96 samples (8 source waters, 3 replicates, 4 size fractions; Table 1). Samples will be analyzed for bulk measures as follows: POM (i.e. material captured on the GF/F) will be analyzed for total phosphorus (TP) and total inorganic phosphorus (TPi). Total organic phosphorus (TPo) will be calculated by difference, as described below; unfiltered water samples will be analyzed for TP (at the District's laboratory, see Table 2); filtered water samples will be analyzed for TDP and SRP at the District's laboratory (see Table 2), DOC, and TDN (at the University of Florida); the ultrafiltration samples (LMW and HMW) will be analyzed for TP, TDP, and SRP at UF's Wetland Biogeochemistry Laboratory. Particulate phosphorus (PP) will be calculated as the difference between TP and TDP while DOP will be calculated as the difference between TDP and SRP. A subset of 4 source waters will be analyzed for microbial bioassays and enzyme additions, to identify enzyme hydrolysable pools (4 source waters, 3 replicates, 12 samples total; more details below). One replicate of POM and DOM in all source waters (8 source waters, 1 replicate, two size fractions = 16 samples) will be analyzed for P speciation (P NMR and/or X-ray absorbance spectroscopy; XAS). The decision whether samples will be analyzed by one or both of these methods will be made based on whether each method has sufficient sensitivity for dissolved water samples, and depending on beam time availability for XAS, which is currently delayed due to COVID-19. DOM samples will be concentrated and freeze-dried to provide a solid media for X-ray absorbance spectroscopy. One replicate from each source water and DOM size fraction (i.e. whole DOM, LMW, and HMW; 8 source waters, 1 replicate, 3 size fractions = 24 samples) will be analyzed for EEMS via fluorescence spectroscopy. Signatures of OM turnover (amino acids, stable isotopes) will be analyzed for POM for all source waters (8 source waters, 3 replicates, 1 size fraction = 24 samples).

Microbial bioassays will consist of incubations of filtered (0.2 µm) source water in stirred flasks with a microbial inoculum (5-10% volume of unfiltered water sample) for periods of up to 2 weeks. Incubations will begin as soon as possible (within 1 week) upon sample receipt. During incubations, flasks will be monitored for bacterial growth, respiration and DOC and nutrient changes (dissolved inorganic nitrogen [DIN], SRP). Enzyme bioassays will use the same filtered source water incubated with addition of commercially available purified enzymes. In this work the emphasis will be placed on phosphorus enzyme additions (phosphatase,

phosphodiesterase); however, other hydrolytic enzymes also will be explored including, leucine aminopeptidase (N), N-Acetyl-β-Glucosaminidase (N), β-glucosidase (C) and peroxidase. The incubations for enzyme additions will begin as soon after sample receipt as possible (within 1 week) and occur along with the microbial bioassays for a period of up to 2 weeks. Enzyme additions will occur either directly to the source water in the flask or to the source water contained within a length of dialysis tubing held in a DI water matrix. Non enzyme amended samples will then be used to determine enzyme affected changes in the source water nutrient concentrations (DOC, DIN, SRP, TDP, TDN) in the flask or accumulation of hydrolysis products (DIN, SRP, DOC) in DI matrix. These changes in nutrient form and concentration will be used to quantify the portion of nutrients susceptible to enzymatic breakdown within the dissolved fraction of the various source waters.

During Task 3, each PI will conduct initial methods tests to confirm that their proposed methods are suitable for the decomposition/leaching study proposed in Task 5. We do not expect initial method validation to take longer than 2 months. A summary of samples and analyses are included in Table 1 and a summary of District laboratory services is included in Table 2.

Task 3 Deliverables [in progress]

Spreadsheets of raw data including QA/QC values. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses.

Task 4. Evaluate the potential of faunal biomarkers as tracers of fish inputs of OM and P and understand how fish bioturbation and excretion affects P concentrations. **[in progress]**

Faunal contributions to P concentrations may be significant (Evans et al. 2019). To assist with this effort, we will partner with the principal investigator of the faunal study, Joel Trexler, to assess markers of faunal OM and P inputs, as recent faunal surveys and experiments have demonstrated the abundance of aquatic fauna in the STAs and have suggested that just 6 common fish species can recycle 53% of the daily P entering STA-2 (Evans et al., 2019). The faunal study members will collect samples from bioturbation and excretion experiments for biomarker analyses. The faunal excretion experiments are planned in 2020 and bioturbation experiments are planned in 2021, with dates subject to change as the COVID-19 situation evolves (Summary schedule of tasks, below). Our team will provide guidance to the faunal contract for proper processing/storage of samples for biomarker analyses. The faunal study will measure appropriate bulk nutrients from bioturbation and excretion samples. This biomarker study will then analyze the same samples for stable isotopes and fecal sterols to determine if fish-specific markers can be found that have also been observed within STA samples. For the excretion experiment, triplicate samples will be collected from the initial and final time points of the large fish size class treatment (Blue Tilapia). Whole water samples will be filtered at the University of Florida, and DOM and POM will be collected. For the bioturbation experiment, triplicate samples will be taken from the high fish density treatment and the control. Whole water samples will be filtered at the University of Florida, and DOM and POM will be collected. In addition, floc, soil, and periphyton will be collected for the bioturbation experiment. All faunal study samples will be analyzed for stable isotopes, dissolved organic carbon, total dissolved nitrogen, particulate organic carbon and nitrogen, and dissolved and particulate fecal sterols,

when possible. Samples are summarized in Table 3. This task will occur in parallel with Task 3 (see the Summary Schedule of Tasks below) and will not influence the STOP/GO decisions of other tasks in this statement of work.

Task 4 Deliverables [in progress]

Spreadsheets of raw data including QA/QC values. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses.

Task 5. Evaluate biogeochemical signatures in a decomposition/leaching study to understand sources and turnover of P and OM of STA waters under controlled conditions. **[planned]**

This task will improve our understanding of internal processes that drive the production and transformations of DOP and PP in the STAs. To explore the mechanisms of internal processing of P and OM in the STAs, we will apply the suite of analyses described in Task 3 to a decomposition/leaching study, with subsequent tests of photolability (Task 5a) and bioavailability (Task 5b). Following is a detailed description of the decomposition/leaching study.

The study will be established within two STA flow-ways/cells: one dominated by emergent aquatic vegetation (EAV), and one dominated by submerged aquatic vegetation (SAV). "Litterbags" will be created for both litter and floc (litterbag design described below), placed *in situ*, and destructively harvested at three timepoints: an initial timepoint, 20 days, and 40 days. Three replicates will be collected at each timepoint, although additional litterbags will be deployed at the start of the experiment, to account for any potential losses in the field. If no litterbags are lost, then extra litterbags will serve as additional replicates when possible. Litterbags will either be anchored directly into the sediment using stainless steel staples or affixed to a floating ring that will be anchored into the sediment using PVC if possible, or coated rebar if PVC is not stable enough (Figure 2). The best method for deployment will be determined via a field test prior to the experiment initiation. For each timepoint, an aliquot of the litterbag contents (i.e. floc and litter) and site water will be collected for a "pre-leachate" sample. Then, litterbags will be placed in bottles filled with site water for a short period of time (no longer than 48 hours, depending on personnel time and feasibility of site access) and incubated *in situ*. Analyses will require liters of water samples, so smaller bottles (500 to 1000mL volume) will be used and composited to reach the required sample volume (approx. 5 L per sample). After the designated leaching time, "post-leaching" litterbags and water will be retrieved for further analysis. Leaching experiments will only be conducted for the samples collected at that timepoint, all other litterbags will remain *in situ* until the next timepoint. For each timepoint, there will be 36 samples collected, as there will be two vegetation types (EAV, SAV), three sample types (litter, floc, water), two leachate times (pre- and post-leachate), and three replicates. After collection, the lability and recalcitrance of P and OM of these samples will be analyzed for photolability (Task 5a) and signatures of biological production/turnover (Task 5b). The experimental design is summarized in Figure 3.

Litterbags will be created using a short (prototype will be 5 cm in length, but once the site has been selected, this length can be modified to reflect the average floc depth of the site) polycarbonate tube, with either ends of the tube sealed with a material of appropriate mesh size

(0.5 to 1mm mesh size) to allow for water exchange and macroinvertebrate access but to minimize the loss of floc and litter material. Analyses will require 10's of grams of wet weight material, so litterbags will be created in a sufficient diameter to provide enough material for analyses (at minimum 10 cm inner diameter, but possibly larger). If the dimensions of individual litterbags cannot be constructed to meet the required amount of sample material, then we will deploy extra litterbags that will be composited to meet the necessary amount of sample material. This determination will be made after an initial field deployment test. Litter and floc will be collected from each site and added to the litterbags on the same day of collection, sealed, and deployed in the field. EAV and SAV litter and floc will only be deployed in their respective sites (i.e. EAV litter and floc will be incubated only to EAV site) to maintain a feasible number of samples.

Task 5a. Photolability

At each decomposition/leaching study timepoint, whole water samples will be transported in the dark to the Whitney Marine Laboratory for solar exposure tests. One replicate from the field will be subject to the photolability tests. Ultrafiltration will be conducted to separate DOM into two AMW size fractions (see Task 1). Whole water, DOM, and 2 DOM AMW fractions (LMW and HMW) from one replicate of the decomposition/leaching water samples (12 field samples) will be subject to two treatments of UV exposure (high, low) to evaluate OM and associated PP and DOP transformations when subject to solar exposure (12 water samples, 4 size fractions, 2 UV treatments = 96 total samples). The solar simulator produces light from 300-800 nm, and the high exposure treatments will be conducted as uncovered reaction vessels, while the low exposure treatments will be covered with mylar to reduce solar exposure.

Samples subject to UV exposure will be directly measured for TP, SRP, and TDP. Values for PP and DOP will be calculated by difference as described for Task 3. One pre- and post-exposure (< 24 hours) sample per treatment will be analyzed using UV fluorescence and absorbance, and excitation-emission matrices (EEMs) will be analyzed to identify signatures of OM for both particulate and dissolved phases. A humification index will be calculated according to Ohno and Bro (2006) by dividing emission intensity in the 435-480nm region by emission intensity in the 300-345nm region. EEMS signatures that are indicative of large biopolymers also will be identified. If possible, aliquots of pre- and post-exposure samples also will be analyzed for P speciation, if sufficient sample material is available. The samples from the photolability test and DOM size fractionation will be analyzed at UF's Wetland Biogeochemistry Laboratory to minimize transit time and allow samples to be analyzed rapidly after collection.

Task 5b. Signatures of biological production/turnover

Over the course of the decomposition/leaching study, samples will be analyzed to identify signatures of biological production and turnover. Our earlier work found that amino acid biomarkers tracked OM degradation and that bacterial amino acids (muramic acid, murA, and diaminopimelic acid, DAPA) were positively correlated with organic P, suggesting potential relationships between fresh, biologically-derived OM pools and organic P, although this relationship appeared to vary between STAs and reference sites (Morrison et al., 2019). This work supports past work that highlighted the importance of bacterial biomass P contributions to particulate phosphorus (PP) concentrations in other areas of the Everglades (Noe and Childers, 2007) and more recent work that found 40% of STA DOP was in the HMW fraction, which was attributed to either colloids or large biopolymers like phospholipids, further suggesting a

potential biological contribution to DOP pools within the water column (Ged and Boyer, 2013). However, Reddy et al. (2019) found that microbial P only comprises 15-25% of soil P, suggesting that microbially-derived P may not be stored long-term.

Samples from the decomposition/leaching study (Task 5) will be analyzed for amino acid biomarkers, stable isotopes, and microbial bioassays as indicators of biological activity and microbial nutrient demands. To minimize cost, only the initial and final (40 days after litterbag deployment) time points will be analyzed for microbial bioassays. Triplicates of solid samples (litter bag, floc bag, POM) will be analyzed for TOC, TC, TN, amino acids, TP and TPi. One replicate of solid samples (litter bag, floc bag, POM) will be analyzed for XAS, P-NMR, and microbial biomass C,N,P. Triplicate water samples will be analyzed for TP, TDP, SRP, DOC, TDN, and dissolved amino acids. One replicate of each water sample will be analyzed for fluorescence spectroscopy, XAS, and P-NMR, and a subset of water samples will be analyzed for microbial bioassays and enzyme additions. Relationships between microbial analyses and biomarkers will be evaluated through correlations and multivariate analyses between biological signatures and SRP, DOP, PP, EEMs, and P speciation data (when available). Additional investigations also will be undertaken to evaluate relationships between dissolved and particulate OM signatures and dissolved and particulate P pools, as well as P speciation (when available) to investigate if fresh OM production is associated with increased P.

Using the methods described earlier (Task 3), microbial bioassays will be used to evaluate microbial growth and enzyme transformations in the initial and final timepoints. Incubations will be conducted in laboratory conditions for water samples collected from the decomposition/leaching study with differences between microbial production and potential enzymatic nutrient conversion reflecting the potential for organic nutrient turnover in an STA. In addition to microbial bioassays, enzyme additions (as described in Task 3) will also be conducted for phosphatase (P) and phosphodiesterase (P), with consideration of leucine aminopeptidase (N), *N*-Acetyl-β-Glucosaminidase (N), β-glucosidase (C), phenol oxidase (C), and peroxidase (C).

Additional investigations will be undertaken to investigate if fresh OM production is associated with increased P by evaluating relationships between dissolved and particulate OM signatures and dissolved and particulate P pools, as well as P speciation (when available).

SERVICES REQUIRED FROM THE DISTRICT LABORATORY

District laboratory services are only requested for Task 3 to provide context regarding P detection limits, particularly for P-speciation techniques. We request that the SFWMD water chemistry laboratory measure TP for unfiltered water samples and SRP, and TDP on filtered water samples, according to the definitions provided below. PP and DOP will be calculated by difference (PP = TP-TDP; DOP = TDP – SRP). Triplicate site water samples have already been collected and submitted to the District's laboratory during the week of 11/02/2020, approved under the Environmental Monitoring Review Process (EMRT number 202009-2).

Definitions (from SFWMD Science Plan for the Everglades STA, July 2018):

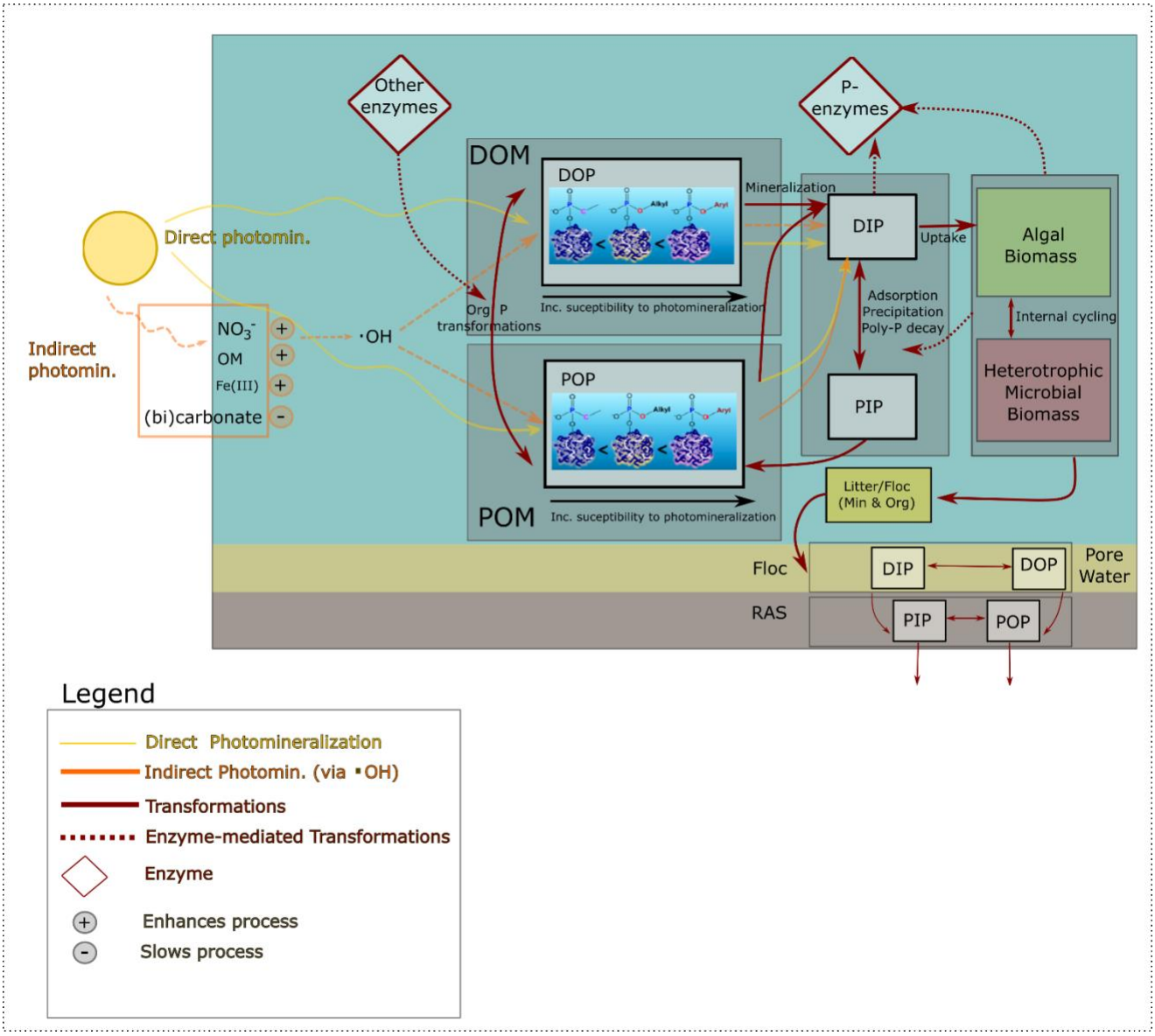
Particulate phosphorus (PP): Particulate-bound P, not passing through a 0.45-micrometer filter, that can include both organic and inorganic forms; usually a calculated value: PP = TP – total dissolved P.

Total dissolved phosphorus (TDP): Total P in water sample filtered through a 0.45-micrometer membrane filter and analyzed after sample acid digestion process; may include soluble reactive P and DOP.

Soluble reactive phosphorus (SRP): The dissolved form of P measured in a water sample after being filtered through a 0.45- micrometer membrane filter; generally represents the most readily available form of phosphorus.

Total phosphorus (TP): Total amount of P in a system or in an environmental sample, includes both organic and inorganic forms of P.

CONCEPTUAL MODEL OF P LABILITY IN STA NUTRIENT CYCLING



Schematic of P lability in the STAs, modified from C. Saunders and Zhang et al., (2019). Photochemical (direct and indirect) as well as enzymatic turnover is shown.

PROJECT PERSONNEL ROLES AND RESPONSIBILITIES

Dr. Elise Morrison will oversee project coordination and communication with the SFWMD. She will be responsible for hiring and supervising the postdoc on the project.

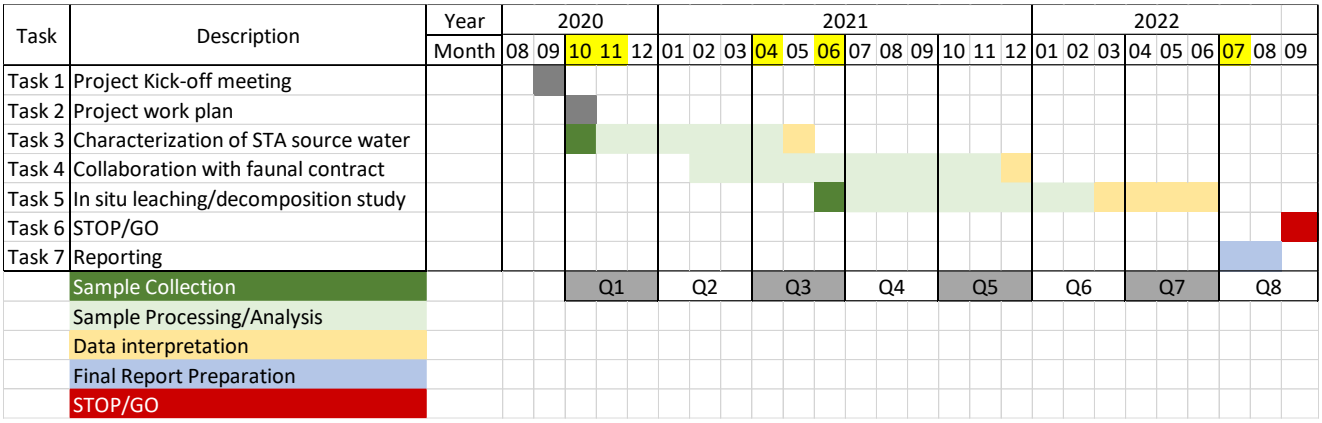
Dr. Thomas Bianchi will be responsible for ensuring that the biomarker and bulk carbon and nitrogen data are collected and analyzed. He will be responsible for supervising the graduate student on the project and will help co-advise the postdoc on the project with Drs. Morrison and Osborne.

Dr. Patrick Inglett will be responsible for TP, TDP, and SRP analysis, as well as microbial bioassays and enzyme additions.

Dr. Todd Osborne will be responsible for running samples on the solar simulator, running samples on the Aqualog, and co-advising the postdoc on the project with Drs. Morrison and Bianchi.

Dr. Jonathan Judy will be responsible for running samples for P-NMR and XAS.

SCHEDULE OF ACTIVITIES



Task-based deliverable deadlines (tentative)

10/2/2020	Project Kickoff deliverable due [complete]
11/20/2020	Project plan due
6/2/2021	Task 3 deliverables due
4/2/2021	Task 4 deliverables due
7/2/2022	Task 5 deliverables due

Quarterly reporting deadlines (tentative)

1/2/2021	First quarterly report
4/2/2021	Second quarterly report
7/2/2021	Third quarterly report
10/2/2021	Fourth quarterly report
1/2/2022	Fifth quarterly report
4/2/2022	Sixth quarterly report
7/2/2022	Seventh quarterly report

UNIVERSITY'S CONTINGENCY PLAN

In the event that there is staff turnover at the University of Florida, the University will notify the District as soon as possible to discuss how staff turnover may impact the progress of the project. If the position in question is not that of a PI or co-PI, then a hiring committee will be formed by project PIs, a hiring announcement will be drafted, and a suitable replacement will be sought as soon as possible through the University of Florida's Human Resources Department. If the position in question is that of a PI or co-PI, then PIs will discuss options with the District and the University of Florida's HR Department, which may include (1) continuing the PI or co-PI's role in the project while at their new institution; (2) finding a suitable replacement co-PI at the University of Florida who can take on the necessary responsibilities; (3) revising the scope of this project to revise or remove the responsibilities of the position in question; or (4) other options not listed here as determined by UF HR, the District, and other PIs.

UNIVERSITY'S QA/QC PROCEDURE

The University will strive to ensure that the quality of staff work is acceptable, and all project deliverables are complete and accurate. Each PI is responsible for ensuring that their staff adhere to the QA/QC guidelines outlined in their laboratory's standard operating procedures (SOPs).

STANDARD OPERATING PROCEDURES (SOPS)

Standard operating procedures have been provided as separate documents, and include:

- Bianchi amino acid hydrolysis
- Bianchi dissolved organic carbon and total dissolved nitrogen
- Bianchi fecal sterols
- Bianchi laboratory filtration
- Bianchi PPL solid phase extraction
- Bianchi Stable isotopes
- Bianchi Ultrafiltration
- Judy: NMR and XAS

Other previously published SOPs that will be used include:

- SFWMD-RSSP-FLD-SOP-0013-01-Water Quality Grab Sampling
- USGS Open File Report 2018-1096 Procedures for Using the Horiba Scientific Aqualog®Fluorometer to Measure Absorbance and Fluorescence from Dissolved Organic Matter
- Standard Method 4500-P PHOSPHORUS (2017)

APPENDIX: FIGURES AND TABLES

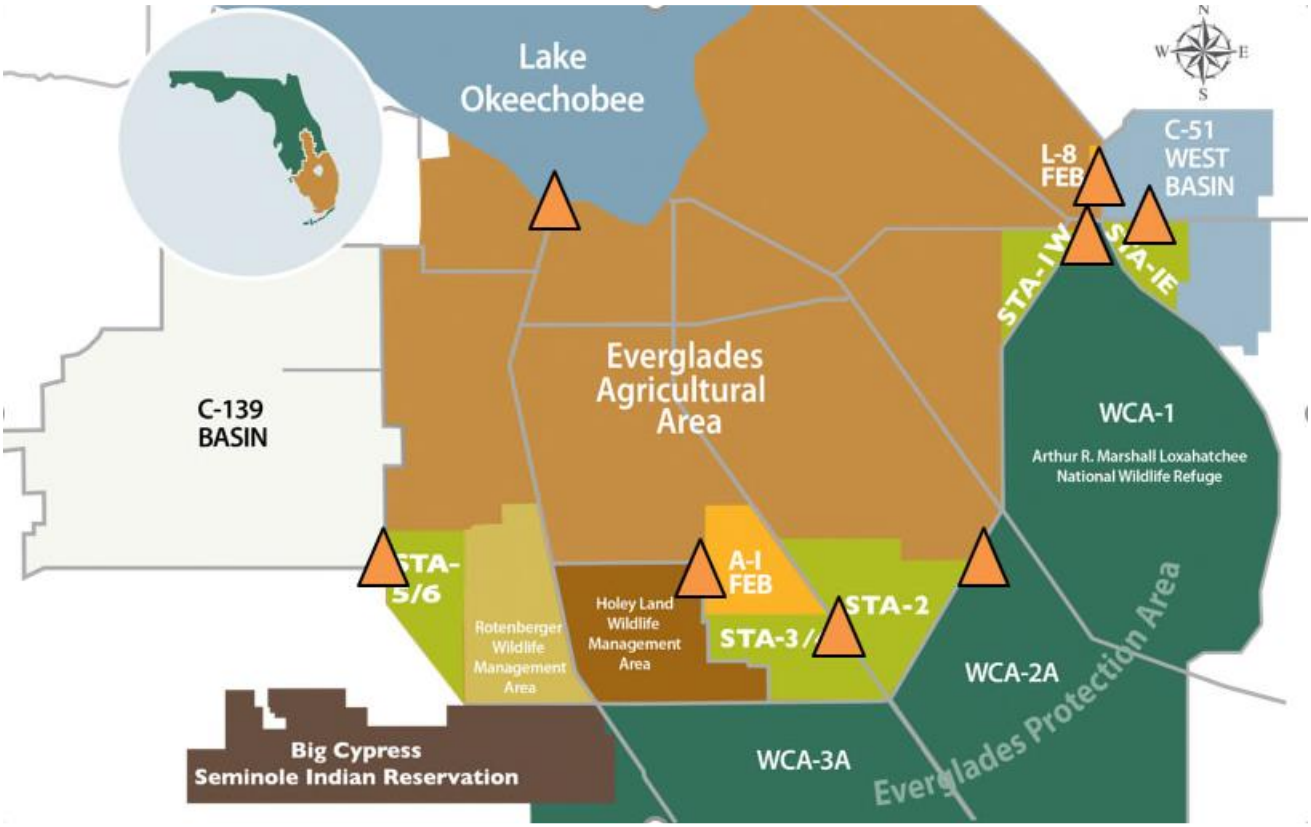


Figure 1. Sites analyzed for Task 3: source water characterization. Site names and samples are summarized in Table 1.

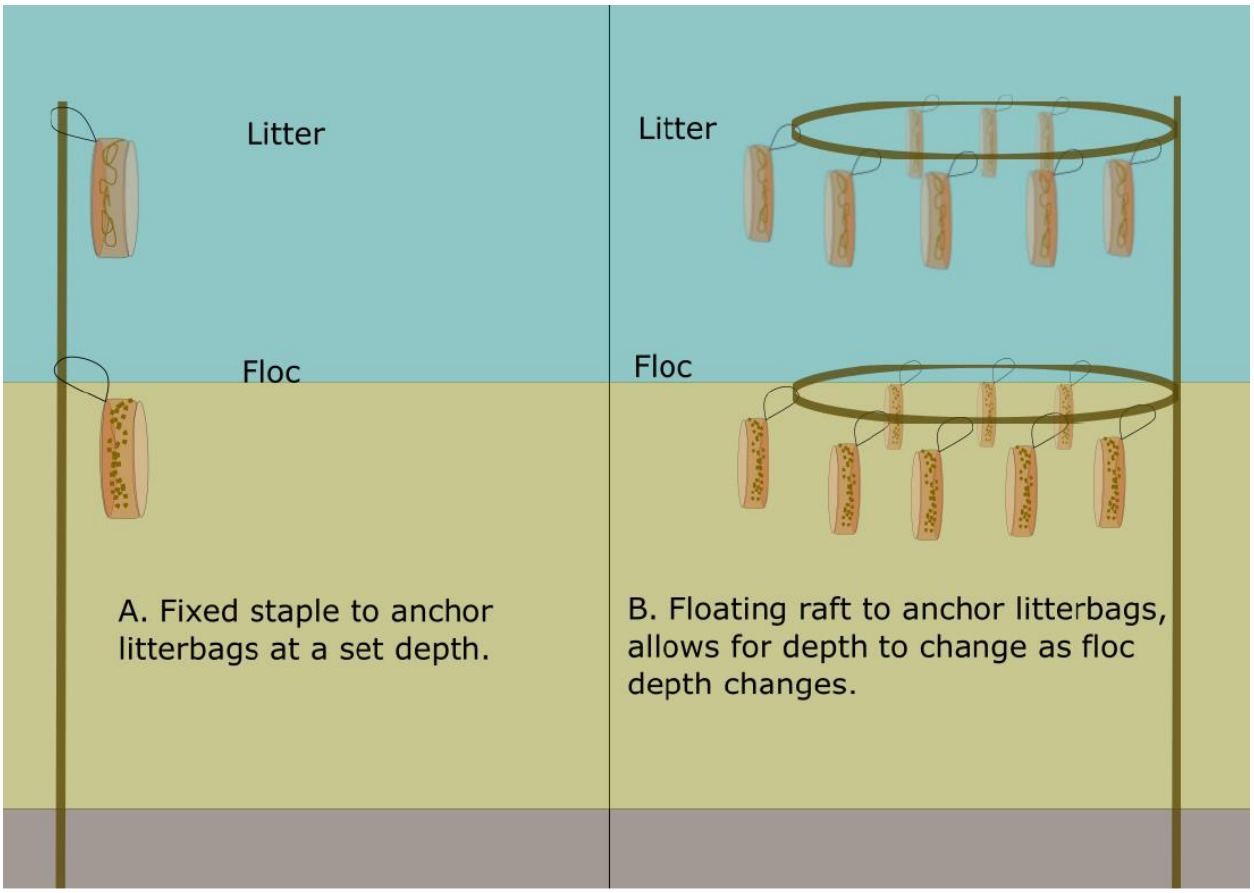


Figure 2. Two litterbag deployment designs. Option A has litterbags stapled into substrate to maintain a fixed depth. Option B has litterbags deployed in an array on a floating “raft” that allows for litterbag depth to change as water level and floc depth changes.

General notes:

Deploy all litter/floc bags on Day 0
Destructively sample/leach at day 0, 20, and 40

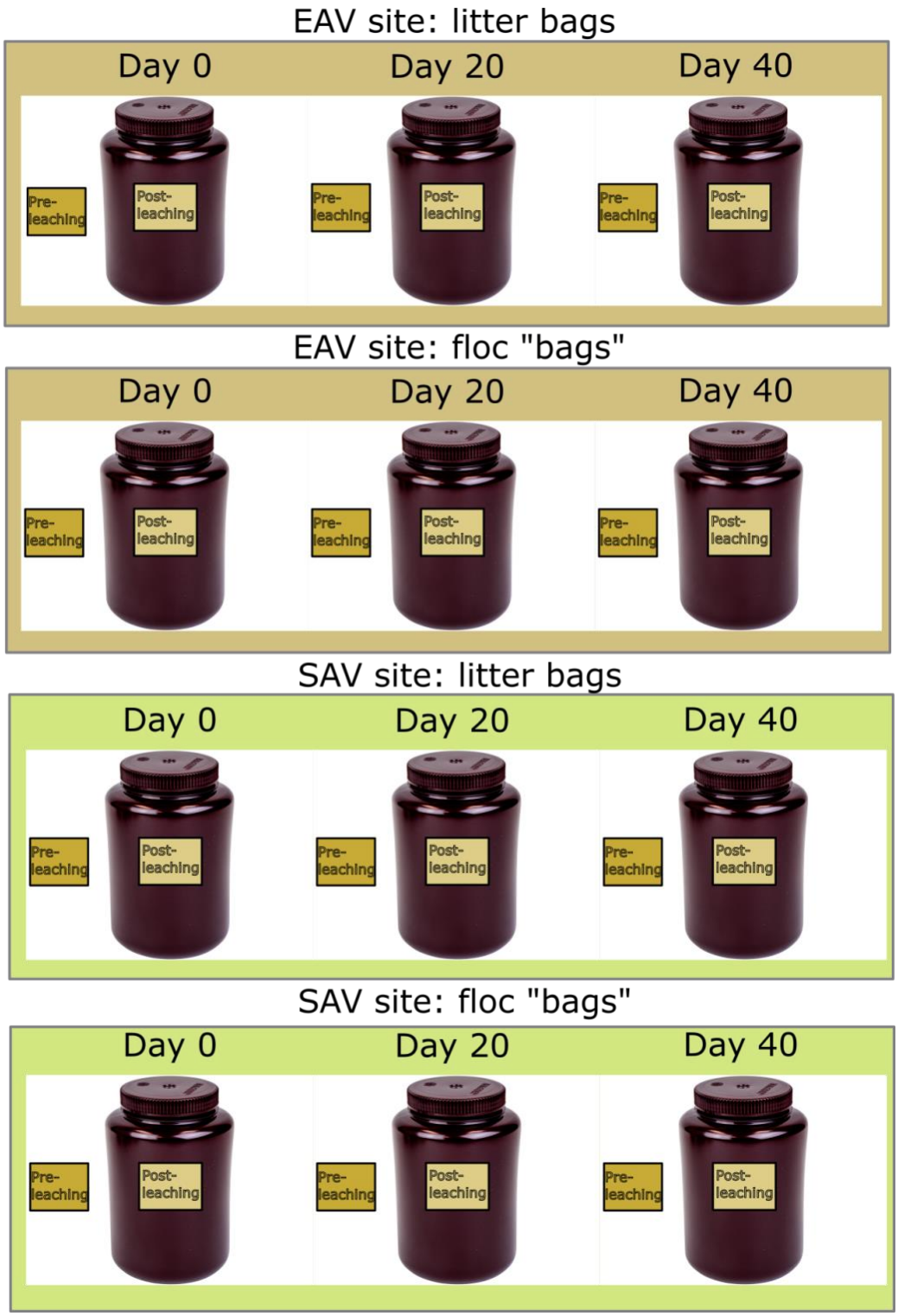


Figure 3. Schematic of Task 5 experimental design.

Table 1. Station codes and descriptions for Task 3.

Station Code	Description
S-354	Lake Okeechobee outflow
G-302	STA-1W
S-319	STA-1E
S-6	STA-2
G-370	STA-3/4
G-508	STA-5/6
G-372	A1 FEB
G-538	L8 FEB

Table 1. Analyses proposed for Task 3.

Hydrologic Unit	SampleType	UF-Bianchi		UF-Judy		UF-Osborne			UF-Inglett			
		Amino acids, stable isotopes	Ultrafiltration	XAS spectra	P-NMR	DOC	TDN	Fluorescence Spectroscopy	SRP	TDP (0.45 um filtrate)	TDP (0.7 um filtrate)	Microbial bioassay, enzyme additions
Lake Okeechobee canal discharge	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 1W (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 1E (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 2 (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 3/4 (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 5/6 (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
A1 FEB (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
L8 FEB (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	Number of Samples per Analysis	**48**	**24**	**16**	**16**	**72**	**72**	**24**	**48**	**48**	**48**	**12**
		Amino acids, stable isotopes	Ultrafiltration	XAS spectra	P-NMR	DOC	TDN	Fluorescence Spectroscopy	SRP	TDP (0.45 um filtrate)	TDP (0.7 um filtrate)	Microbial bioassay, enzyme additions
		UF-Bianchi		UF-Judy		UF-Osborne			UF-Inglett			

Table 2. District laboratory services requested for Task 3.

Hydrologic Unit	SampleType	TP (water)	TDP (water)	SRP (water)
Lake Okeechobee canal discharge	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 1W (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 1E (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 2 (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 3/4 (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 5/6 (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
A1 FEB (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
L8 FEB (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
	Number of Samples per Analysis	**24**	**24**	**24**

Table 3. Analyses to be run for Task 4. Samples in the “Trexler lab” column will be collected by Trexler et al. but analyzed by the District.

Experiment Type	Faunal size class or Fish density	Timepoint	SampleType	Analyses and who will run them										
				Trexler Lab					UF-Bianchi					
				TN	NH_4	TP	SRP	TDP	DOC	TDN	Stable isotopes (C, N)	TC, TOC, TN	Fecal sterols	High-res mass spec
Excretion	Large	Initial	Whole water	3		3								
			DOM		3		3	3	3	3			3	1
			POM								3	3	3	
	Large	Final	Whole water	3		3								
			DOM		3		3	3	3	3			3	1
			POM								3	3	3	
Bioturbation	High Density	Initial	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
		Final	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
	Control	Initial	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
		Final	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
		TASK 2	**Total samples**	42	18	54		18	18	18	54	54	60	6
				TN	NH_4	TP	SRP	TDP	DOC	TDN	Stable isotopes (C, N)	TC, TOC, TN	Fecal sterols	High-res mass spec
				Trexler Lab					UF-Bianchi					

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Quantifying the Recalcitrance and Lability of Phosphorus within Stormwater Treatment Areas (Biomarker Study)

Project Work Plan

Work order 4600004016-WO02

Submitted to:

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PROJECT DESCRIPTION AND OBJECTIVES

The primary goals of this work are to evaluate relationships between organic matter (OM) and phosphorus (P) and to evaluate sources and potential turnover of P within the Stormwater Treatment Areas (STAs).

To accomplish these goals, we will (1) characterize water quality of STA source waters; (2) measure biomarkers from faunal excretion and bioturbation samples; and (3) assess relationships between P and OM in an *in-situ* decomposition/leaching study.

The specific tasks and objectives of this study are:

Task 1: Project kick-off meeting

Task 2: Project work plan

Task 3: Characterize P and OM in STA source waters

Task 4: Evaluate the potential of faunal biomarkers as tracers of fish inputs of OM and P

Task 5: Evaluate biogeochemical signatures in a decomposition/leaching study to understand sources and turnover of P and OM of STA waters under controlled conditions.

(STOP/GO) Task 6: (optional 1-year extension) Characterize P and OM lability and recalcitrance in STA outflow water.

DESCRIPTION OF TASKS

Task 1. Project Kick-off Meeting [completed]

The UNIVERSITY Principal Investigator and other key scientists involved with the project shall virtually attend a project kickoff meeting with the District project team within three weeks after issuance of this Work Order. During the kickoff meeting, specific details regarding the study, timelines, project deliverables, and expectations will be discussed. Contact information for key personnel and their roles and responsibilities from both the UNIVERSITY and the District project teams shall be provided during the kick-off meeting.

Task 1 Deliverables [completed]

A draft memorandum summarizing minutes of the kick-off meeting and a list of action items for both the District and the UNIVERSITY.

Final memorandum summarizing minutes of the kick-off meeting and a list of action items for both the District and the UNIVERSITY.

Task 2: Project Work Plan [This document]

The UNIVERSITY shall develop a Draft Project Work Plan in accordance with the project objectives and discussions at the kickoff meeting. District staff shall review and provide comments within two weeks following receipt of this Draft Project Work Plan. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Project Work Plan within two weeks of receiving such comments.

Task 2 Deliverables

Draft Project Work Plan
Final Project Work Plan

Task 3: Characterize P and OM in STA source waters to understand differences in the quality of STA source waters and their potential effect on STA performance. **[in progress]**

Variability in the quality of STA source water may influence STA performance. P speciation and OM quality will be measured in eight STA source waters: Lake Okeechobee canal discharge (Station S-354), distribution canals downstream of structure discharge from STA-1W (Station G-302), STA-1E (Station S-319), STA-2 (Station S-6), STA 3/4 (Station G-370), STA 5/6 (Station G-508), A1 FEB (Station G-372) and L8 FEB (Station G-538). Stations are summarized in Figure 1 and Table 1. Samples were collected on November 4-5, 2020. Triplicate water samples were collected for each of the eight source waters (Figure 1). Whole water was filtered through glass fiber filters (GF/Fs) to isolate particulate organic matter (POM) and dissolved organic matter (DOM) fractions. Ultrafiltration was conducted using a Amicon stirred cell and Millipore ultrafiltration membranes (10 kDa), according to Ged and Boyer (2013) to separate DOM into two pools of different apparent molecular weight (AMW; < 10 kDa and >10 kDa). A < 10 kDa

and > 10 kDa AMW cutoff was selected based on the results of Ged and Boyer (2013) who found that the majority of dissolved organic phosphorus (DOP) was associated with the <1 kDa and >10 kDa AMW pool, and a lower proportion of DOP was associated with intermediate size fractions. Centrifugal ultrafiltration membranes can be biased for the low molecular weight pool (<1 kDa), and they suggest that the high proportion of P in the < 1kDa pool was attributed to soluble reactive phosphorus (SRP) or possibly ultrafiltration bias. However, they found that the larger AMW pool (>10 kDa) had an excitation emission matrix (EEMs) signal indicating that it could be associated with large biomolecules. Since this SOW is focused on understanding in situ production, the 10 kDa cutoff would be more appropriate to capture the production of large biomolecules associated with P, without the potential bias of lower kDa membrane cutoffs. This will result in four size fractions: (1) POM; (2) whole DOM (i.e. water filtered through a GF/F, nominal pore size 0.7 µm); (3) < 10 kDa DOM fraction (i.e. low apparent molecular weight, LMW); (4) > 10 kDa DOM fraction (i.e. high apparent molecular weight, HMW). An aliquot of each sample will also be filtered through a 0.45 µm filter to be processed for total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and dissolved organic carbon (DOC). Both the 0.7 µm and 0.45 µm filtrate will be analyzed for TDP.

After filtration there will be 96 samples (8 source waters, 3 replicates, 4 size fractions; Table 1). Samples will be analyzed for bulk measures as follows: POM (i.e. material captured on the GF/F) will be analyzed for total phosphorus (TP) and total inorganic phosphorus (TPi). Total organic phosphorus (TPo) will be calculated by difference, as described below; unfiltered water samples will be analyzed for TP (at the District's laboratory, see Table 2); filtered water samples will be analyzed for TDP and SRP at the District's laboratory (see Table 2), DOC, and TDN (at the University of Florida); the ultrafiltration samples (LMW and HMW) will be analyzed for TP, TDP, and SRP at UF's Wetland Biogeochemistry Laboratory. Particulate phosphorus (PP) will be calculated as the difference between TP and TDP while DOP will be calculated as the difference between TDP and SRP. A subset of 4 source waters will be analyzed for microbial bioassays and enzyme additions, to identify enzyme hydrolysable pools (4 source waters, 3 replicates, 12 samples total; more details below). One replicate of POM and DOM in all source waters (8 source waters, 1 replicate, two size fractions = 16 samples) will be analyzed for P speciation (P NMR and/or X-ray absorbance spectroscopy; XAS). The decision whether samples will be analyzed by one or both of these methods will be made based on whether each method has sufficient sensitivity for dissolved water samples, and depending on beam time availability for XAS, which is currently delayed due to COVID-19. DOM samples will be concentrated and freeze-dried to provide a solid media for X-ray absorbance spectroscopy. One replicate from each source water and DOM size fraction (i.e. whole DOM, LMW, and HMW; 8 source waters, 1 replicate, 3 size fractions = 24 samples) will be analyzed for EEMS via fluorescence spectroscopy. Signatures of OM turnover (amino acids, stable isotopes) will be analyzed for POM for all source waters (8 source waters, 3 replicates, 1 size fraction = 24 samples).

Microbial bioassays will consist of incubations of filtered (0.2 µm) source water in stirred flasks with a microbial inoculum (5-10% volume of unfiltered water sample) for periods of up to 2 weeks. Incubations will begin as soon as possible (within 1 week) upon sample receipt. During incubations, flasks will be monitored for bacterial growth, respiration and DOC and nutrient changes (dissolved inorganic nitrogen [DIN], SRP). Enzyme bioassays will use the same filtered source water incubated with addition of commercially available purified enzymes. In this work the emphasis will be placed on phosphorus enzyme additions (phosphatase,

phosphodiesterase); however, other hydrolytic enzymes also will be explored including, leucine aminopeptidase (N), N-Acetyl-β-Glucosaminidase (N), β-glucosidase (C) and peroxidase. The incubations for enzyme additions will begin as soon after sample receipt as possible (within 1 week) and occur along with the microbial bioassays for a period of up to 2 weeks. Enzyme additions will occur either directly to the source water in the flask or to the source water contained within a length of dialysis tubing held in a DI water matrix. Non enzyme amended samples will then be used to determine enzyme affected changes in the source water nutrient concentrations (DOC, DIN, SRP, TDP, TDN) in the flask or accumulation of hydrolysis products (DIN, SRP, DOC) in DI matrix. These changes in nutrient form and concentration will be used to quantify the portion of nutrients susceptible to enzymatic breakdown within the dissolved fraction of the various source waters.

During Task 3, each PI will conduct initial methods tests to confirm that their proposed methods are suitable for the decomposition/leaching study proposed in Task 5. We do not expect initial method validation to take longer than 2 months. A summary of samples and analyses are included in Table 1 and a summary of District laboratory services is included in Table 2.

Task 3 Deliverables [in progress]

Spreadsheets of raw data including QA/QC values. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses.

Task 4. Evaluate the potential of faunal biomarkers as tracers of fish inputs of OM and P and understand how fish bioturbation and excretion affects P concentrations. **[in progress]**

Faunal contributions to P concentrations may be significant (Evans et al. 2019). To assist with this effort, we will partner with the principal investigator of the faunal study, Joel Trexler, to assess markers of faunal OM and P inputs, as recent faunal surveys and experiments have demonstrated the abundance of aquatic fauna in the STAs and have suggested that just 6 common fish species can recycle 53% of the daily P entering STA-2 (Evans et al., 2019). The faunal study members will collect samples from bioturbation and excretion experiments for biomarker analyses. The faunal excretion experiments are planned in 2020 and bioturbation experiments are planned in 2021, with dates subject to change as the COVID-19 situation evolves (Summary schedule of tasks, below). Our team will provide guidance to the faunal contract for proper processing/storage of samples for biomarker analyses. The faunal study will measure appropriate bulk nutrients from bioturbation and excretion samples. This biomarker study will then analyze the same samples for stable isotopes and fecal sterols to determine if fish-specific markers can be found that have also been observed within STA samples. For the excretion experiment, triplicate samples will be collected from the initial and final time points of the large fish size class treatment (Blue Tilapia). Whole water samples will be filtered at the University of Florida, and DOM and POM will be collected. For the bioturbation experiment, triplicate samples will be taken from the high fish density treatment and the control. Whole water samples will be filtered at the University of Florida, and DOM and POM will be collected. In addition, floc, soil, and periphyton will be collected for the bioturbation experiment. All faunal study samples will be analyzed for stable isotopes, dissolved organic carbon, total dissolved nitrogen, particulate organic carbon and nitrogen, and dissolved and particulate fecal sterols,

when possible. Samples are summarized in Table 3. This task will occur in parallel with Task 3 (see the Summary Schedule of Tasks below) and will not influence the STOP/GO decisions of other tasks in this statement of work.

Task 4 Deliverables [in progress]

Spreadsheets of raw data including QA/QC values. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses.

Task 5. Evaluate biogeochemical signatures in a decomposition/leaching study to understand sources and turnover of P and OM of STA waters under controlled conditions. **[planned]**

This task will improve our understanding of internal processes that drive the production and transformations of DOP and PP in the STAs. To explore the mechanisms of internal processing of P and OM in the STAs, we will apply the suite of analyses described in Task 3 to a decomposition/leaching study, with subsequent tests of photolability (Task 5a) and bioavailability (Task 5b). Following is a detailed description of the decomposition/leaching study.

The study will be established within two STA flow-ways/cells: one dominated by emergent aquatic vegetation (EAV), and one dominated by submerged aquatic vegetation (SAV). "Litterbags" will be created for both litter and floc (litterbag design described below), placed *in situ*, and destructively harvested at three timepoints: an initial timepoint, 20 days, and 40 days. Three replicates will be collected at each timepoint, although additional litterbags will be deployed at the start of the experiment, to account for any potential losses in the field. If no litterbags are lost, then extra litterbags will serve as additional replicates when possible. Litterbags will either be anchored directly into the sediment using stainless steel staples or affixed to a floating ring that will be anchored into the sediment using PVC if possible, or coated rebar if PVC is not stable enough (Figure 2). The best method for deployment will be determined via a field test prior to the experiment initiation. For each timepoint, an aliquot of the litterbag contents (i.e. floc and litter) and site water will be collected for a "pre-leachate" sample. Then, litterbags will be placed in bottles filled with site water for a short period of time (no longer than 48 hours, depending on personnel time and feasibility of site access) and incubated *in situ*. Analyses will require liters of water samples, so smaller bottles (500 to 1000mL volume) will be used and composited to reach the required sample volume (approx. 5 L per sample). After the designated leaching time, "post-leaching" litterbags and water will be retrieved for further analysis. Leaching experiments will only be conducted for the samples collected at that timepoint, all other litterbags will remain *in situ* until the next timepoint. For each timepoint, there will be 36 samples collected, as there will be two vegetation types (EAV, SAV), three sample types (litter, floc, water), two leachate times (pre- and post-leachate), and three replicates. After collection, the lability and recalcitrance of P and OM of these samples will be analyzed for photolability (Task 5a) and signatures of biological production/turnover (Task 5b). The experimental design is summarized in Figure 3.

Litterbags will be created using a short (prototype will be 5 cm in length, but once the site has been selected, this length can be modified to reflect the average floc depth of the site) polycarbonate tube, with either ends of the tube sealed with a material of appropriate mesh size

(0.5 to 1mm mesh size) to allow for water exchange and macroinvertebrate access but to minimize the loss of floc and litter material. Analyses will require 10's of grams of wet weight material, so litterbags will be created in a sufficient diameter to provide enough material for analyses (at minimum 10 cm inner diameter, but possibly larger). If the dimensions of individual litterbags cannot be constructed to meet the required amount of sample material, then we will deploy extra litterbags that will be composited to meet the necessary amount of sample material. This determination will be made after an initial field deployment test. Litter and floc will be collected from each site and added to the litterbags on the same day of collection, sealed, and deployed in the field. EAV and SAV litter and floc will only be deployed in their respective sites (i.e. EAV litter and floc will be incubated only to EAV site) to maintain a feasible number of samples.

Task 5a. Photolability

At each decomposition/leaching study timepoint, whole water samples will be transported in the dark to the Whitney Marine Laboratory for solar exposure tests. One replicate from the field will be subject to the photolability tests. Ultrafiltration will be conducted to separate DOM into two AMW size fractions (see Task 1). Whole water, DOM, and 2 DOM AMW fractions (LMW and HMW) from one replicate of the decomposition/leaching water samples (12 field samples) will be subject to two treatments of UV exposure (high, low) to evaluate OM and associated PP and DOP transformations when subject to solar exposure (12 water samples, 4 size fractions, 2 UV treatments = 96 total samples). The solar simulator produces light from 300-800 nm, and the high exposure treatments will be conducted as uncovered reaction vessels, while the low exposure treatments will be covered with mylar to reduce solar exposure.

Samples subject to UV exposure will be directly measured for TP, SRP, and TDP. Values for PP and DOP will be calculated by difference as described for Task 3. One pre- and post-exposure (< 24 hours) sample per treatment will be analyzed using UV fluorescence and absorbance, and excitation-emission matrices (EEMs) will be analyzed to identify signatures of OM for both particulate and dissolved phases. A humification index will be calculated according to Ohno and Bro (2006) by dividing emission intensity in the 435-480nm region by emission intensity in the 300-345nm region. EEMS signatures that are indicative of large biopolymers also will be identified. If possible, aliquots of pre- and post-exposure samples also will be analyzed for P speciation, if sufficient sample material is available. The samples from the photolability test and DOM size fractionation will be analyzed at UF's Wetland Biogeochemistry Laboratory to minimize transit time and allow samples to be analyzed rapidly after collection.

Task 5b. Signatures of biological production/turnover

Over the course of the decomposition/leaching study, samples will be analyzed to identify signatures of biological production and turnover. Our earlier work found that amino acid biomarkers tracked OM degradation and that bacterial amino acids (muramic acid, murA, and diaminopimelic acid, DAPA) were positively correlated with organic P, suggesting potential relationships between fresh, biologically-derived OM pools and organic P, although this relationship appeared to vary between STAs and reference sites (Morrison et al., 2019). This work supports past work that highlighted the importance of bacterial biomass P contributions to particulate phosphorus (PP) concentrations in other areas of the Everglades (Noe and Childers, 2007) and more recent work that found 40% of STA DOP was in the HMW fraction, which was attributed to either colloids or large biopolymers like phospholipids, further suggesting a potential biological contribution to DOP pools within the water column (Ged and Boyer, 2013).

However, Reddy et al. (2019) found that microbial P only comprises 15-25% of soil P, suggesting that microbially-derived P may not be stored long-term.

Samples from the decomposition/leaching study (Task 5) will be analyzed for amino acid biomarkers, stable isotopes, and microbial bioassays as indicators of biological activity and microbial nutrient demands. To minimize cost, only the initial and final (40 days after litterbag deployment) time points will be analyzed for microbial bioassays. Triplicates of solid samples (litter bag, floc bag, POM) will be analyzed for TOC, TC, TN, amino acids, TP and TPi. One replicate of solid samples (litter bag, floc bag, POM) will be analyzed for XAS, P-NMR, and microbial biomass C,N,P. Triplicate water samples will be analyzed for TP, TDP, SRP, DOC, TDN, and dissolved amino acids. One replicate of each water sample will be analyzed for fluorescence spectroscopy, XAS, and P-NMR, and a subset of water samples will be analyzed for microbial bioassays and enzyme additions. Relationships between microbial analyses and biomarkers will be evaluated through correlations and multivariate analyses between biological signatures and SRP, DOP, PP, EEMs, and P speciation data (when available). Additional investigations also will be undertaken to evaluate relationships between dissolved and particulate OM signatures and dissolved and particulate P pools, as well as P speciation (when available) to investigate if fresh OM production is associated with increased P.

Using the methods described earlier (Task 3), microbial bioassays will be used to evaluate microbial growth and enzyme transformations in the initial and final timepoints. Incubations will be conducted in laboratory conditions for water samples collected from the decomposition/leaching study with differences between microbial production and potential enzymatic nutrient conversion reflecting the potential for organic nutrient turnover in an STA. In addition to microbial bioassays, enzyme additions (as described in Task 3) will also be conducted for phosphatase (P) and phosphodiesterase (P), with consideration of leucine aminopeptidase (N), *N*-Acetyl-β-Glucosaminidase (N), β-glucosidase (C), phenol oxidase (C), and peroxidase (C).

Additional investigations will be undertaken to investigate if fresh OM production is associated with increased P by evaluating relationships between dissolved and particulate OM signatures and dissolved and particulate P pools, as well as P speciation (when available).

SERVICES REQUIRED FROM THE DISTRICT LABORATORY

District laboratory services are only requested for Task 3 to provide context regarding P detection limits, particularly for P-speciation techniques. We request that the SFWMD water chemistry laboratory measure TP for unfiltered water samples and SRP, and TDP on filtered water samples, according to the definitions provided below. PP and DOP will be calculated by difference (PP = TP-TDP; DOP = TDP – SRP). Triplicate site water samples have already been collected and submitted to the District's laboratory during the week of 11/02/2020, approved under the Environmental Monitoring Review Process (EMRT number 202009-2).

Definitions (from SFWMD Science Plan for the Everglades STA, July 2018):

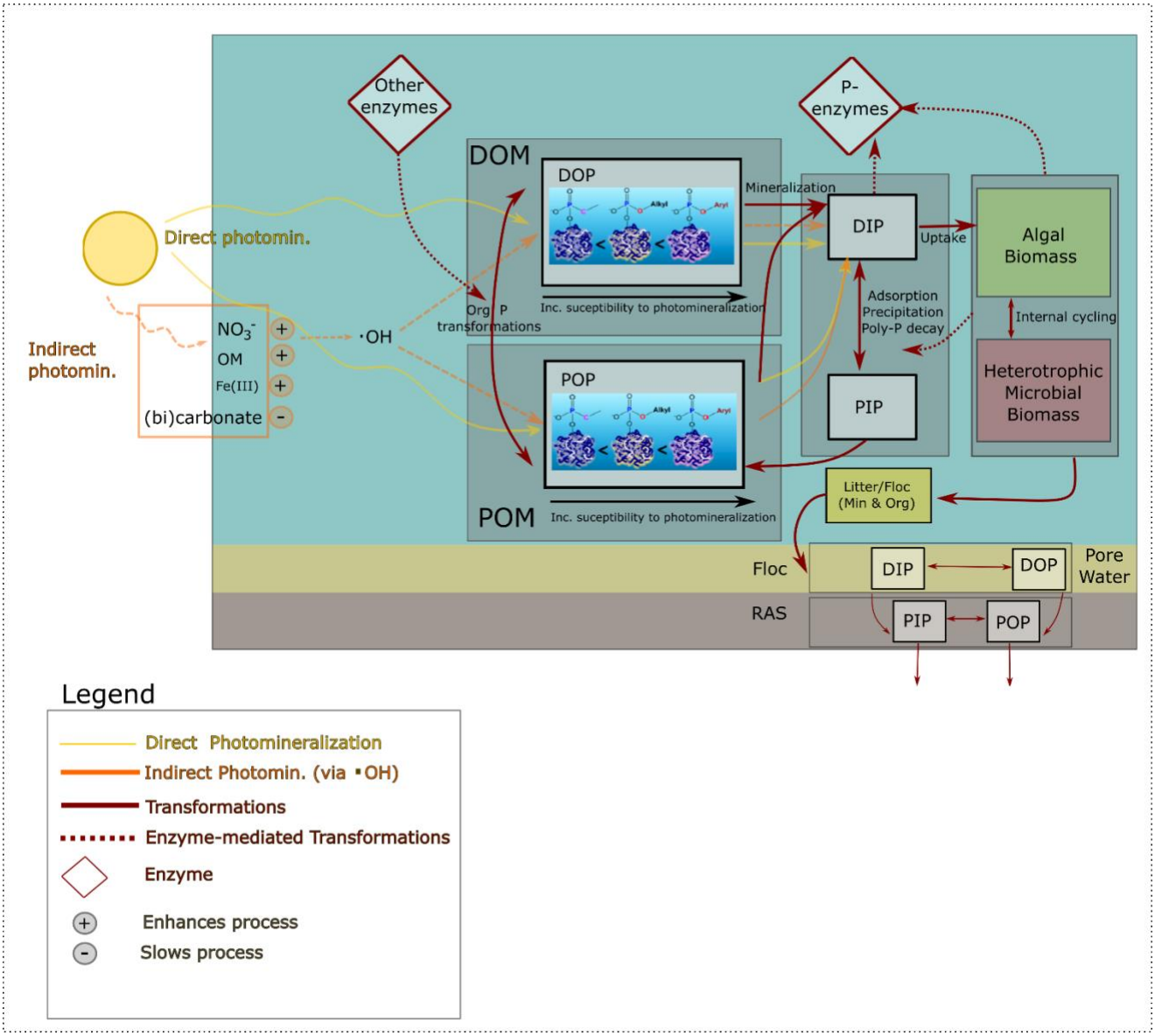
Particulate phosphorus (PP): Particulate-bound P, not passing through a 0.45-micrometer filter, that can include both organic and inorganic forms; usually a calculated value: PP = TP – total dissolved P.

Total dissolved phosphorus (TDP): Total P in water sample filtered through a 0.45-micrometer membrane filter and analyzed after sample acid digestion process; may include soluble reactive P and DOP.

Soluble reactive phosphorus (SRP): The dissolved form of P measured in a water sample after being filtered through a 0.45- micrometer membrane filter; generally represents the most readily available form of phosphorus.

Total phosphorus (TP): Total amount of P in a system or in an environmental sample, includes both organic and inorganic forms of P.

CONCEPTUAL MODEL OF P LABILITY IN STA NUTRIENT CYCLING



Schematic of P lability in the STAs, modified from C. Saunders and Zhang et al., (2019). Photochemical (direct and indirect) as well as enzymatic turnover is shown.

PROJECT PERSONNEL ROLES AND RESPONSIBILITIES

Dr. Elise Morrison will oversee project coordination and communication with the SFWMD. She will be responsible for hiring and supervising the postdoc on the project.

Dr. Thomas Bianchi will be responsible for ensuring that the biomarker and bulk carbon and nitrogen data are collected and analyzed. He will be responsible for supervising the graduate student on the project and will help co-advise the postdoc on the project with Drs. Morrison and Osborne.

Dr. Patrick Inglett will be responsible for TP, TDP, and SRP analysis, as well as microbial bioassays and enzyme additions.

Dr. Todd Osborne will be responsible for running samples on the solar simulator, running samples on the Aqualog, and co-advising the postdoc on the project with Drs. Morrison and Bianchi.

Dr. Jonathan Judy will be responsible for running samples for P-NMR and XAS.

SCHEDULE OF ACTIVITIES

Task	Description	Year	2020					2021											2022								
		Month	08	09	10	11	12	01	02	03	04	05	06	07	08	09	10	11	12	01	02	03	04	05	06	07	08	09
Task 1	Project Kick-off meeting																											
Task 2	Project work plan																											
Task 3	Characterization of STA source water																											
Task 4	Collaboration with faunal contract																											
Task 5	In situ leaching/decomposition study																											
Task 6	STOP/GO																											
Task 7	Reporting																											
	Sample Collection				Q1			Q2			Q3			Q4			Q5			Q6			Q7			Q8		
	Sample Processing/Analysis																											
	Data interpretation																											
	Final Report Preparation																											
	STOP/GO																											

Task-based deliverable deadlines (tentative)

10/2/2020	Project Kickoff deliverable due [complete]
11/20/2020	Project plan due
6/2/2021	Task 3 deliverables due
4/2/2021	Task 4 deliverables due
7/2/2022	Task 5 deliverables due

Quarterly reporting deadlines (tentative)

1/2/2021	First quarterly report
4/2/2021	Second quarterly report
7/2/2021	Third quarterly report
10/2/2021	Fourth quarterly report
1/2/2022	Fifth quarterly report
4/2/2022	Sixth quarterly report
7/2/2022	Seventh quarterly report

UNIVERSITY'S CONTINGENCY PLAN

In the event that there is staff turnover at the University of Florida, the University will notify the District as soon as possible to discuss how staff turnover may impact the progress of the project. If the position in question is not that of a PI or co-PI, then a hiring committee will be formed by project PIs, a hiring announcement will be drafted, and a suitable replacement will be sought as soon as possible through the University of Florida's Human Resources Department. If the position in question is that of a PI or co-PI, then PIs will discuss options with the District and the University of Florida's HR Department, which may include (1) continuing the PI or co-PI's role in the project while at their new institution; (2) finding a suitable replacement co-PI at the University of Florida who can take on the necessary responsibilities; (3) revising the scope of this project to revise or remove the responsibilities of the position in question; or (4) other options not listed here as determined by UF HR, the District, and other PIs.

UNIVERSITY'S QA/QC PROCEDURE

The University will strive to ensure that the quality of staff work is acceptable, and all project deliverables are complete and accurate. Each PI is responsible for ensuring that their staff adhere to the QA/QC guidelines outlined in their laboratory's standard operating procedures (SOPs).

STANDARD OPERATING PROCEDURES (SOPS)

Standard operating procedures have been provided as separate documents, and include:

- Bianchi amino acid hydrolysis
- Bianchi dissolved organic carbon and total dissolved nitrogen
- Bianchi fecal sterols
- Bianchi laboratory filtration
- Bianchi PPL solid phase extraction
- Bianchi Stable isotopes
- Bianchi Ultrafiltration
- Judy: NMR and XAS

Other previously published SOPs that will be used include:

- SFWMD-RSSP-FLD-SOP-0013-01-Water Quality Grab Sampling
- USGS Open File Report 2018-1096 Procedures for Using the Horiba Scientific Aqualog®Fluorometer to Measure Absorbance and Fluorescence from Dissolved Organic Matter
- Standard Method 4500-P PHOSPHORUS (2017)

APPENDIX: FIGURES AND TABLES

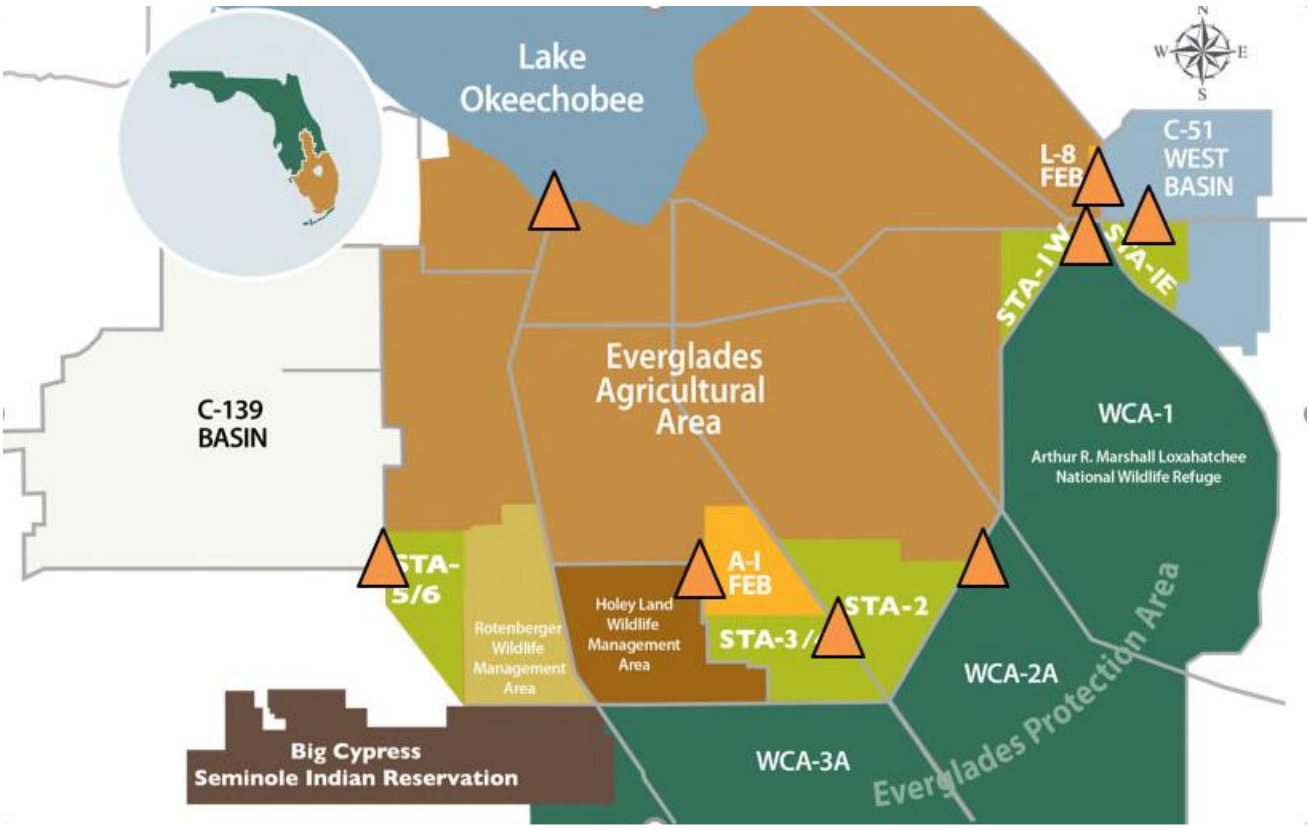


Figure 1. Sites analyzed for Task 3: source water characterization. Site names and samples are summarized in Table 1.

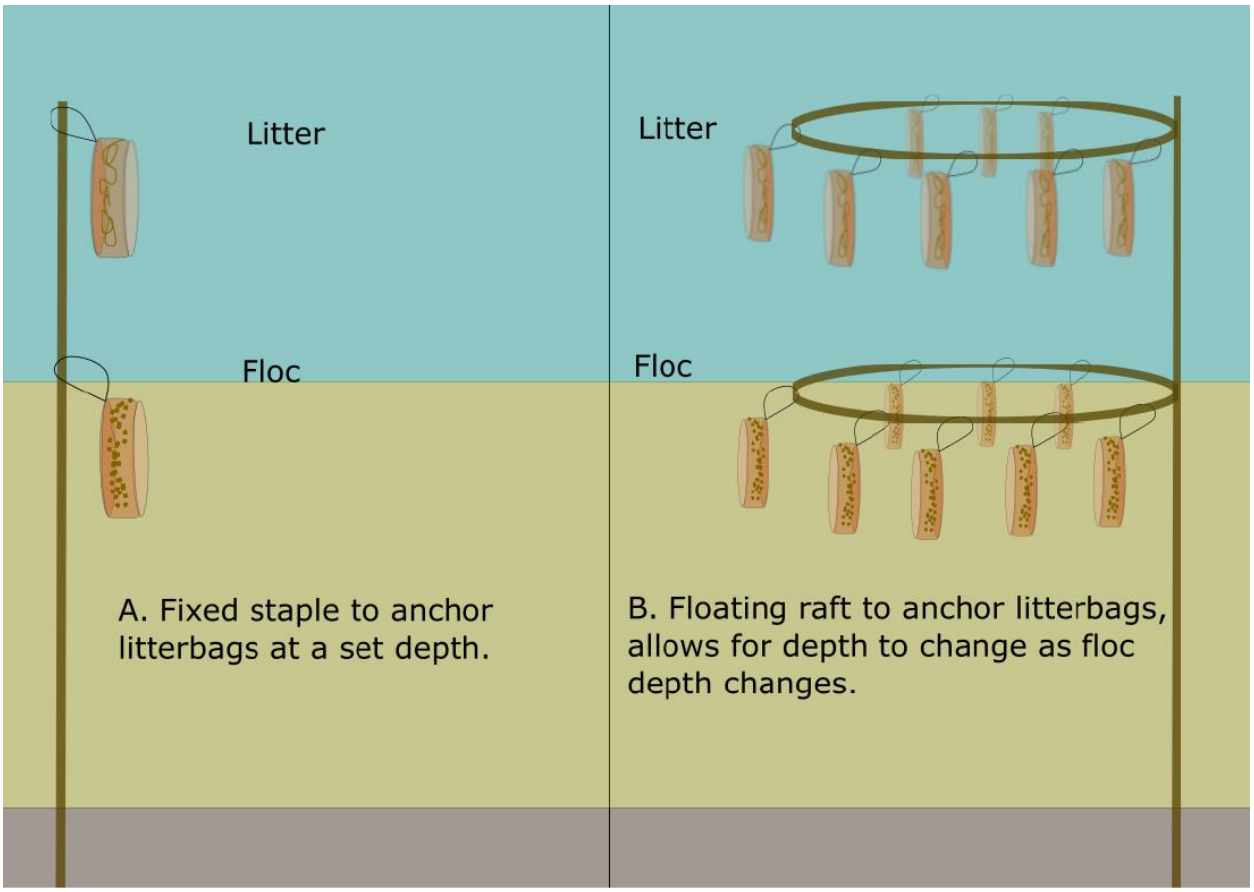


Figure 2. Two litterbag deployment designs. Option A has litterbags stapled into substrate to maintain a fixed depth. Option B has litterbags deployed in an array on a floating “raft” that allows for litterbag depth to change as water level and floc depth changes.

General notes:

Deploy all litter/floc bags on Day 0
Destructively sample/leach at day 0, 20, and 40

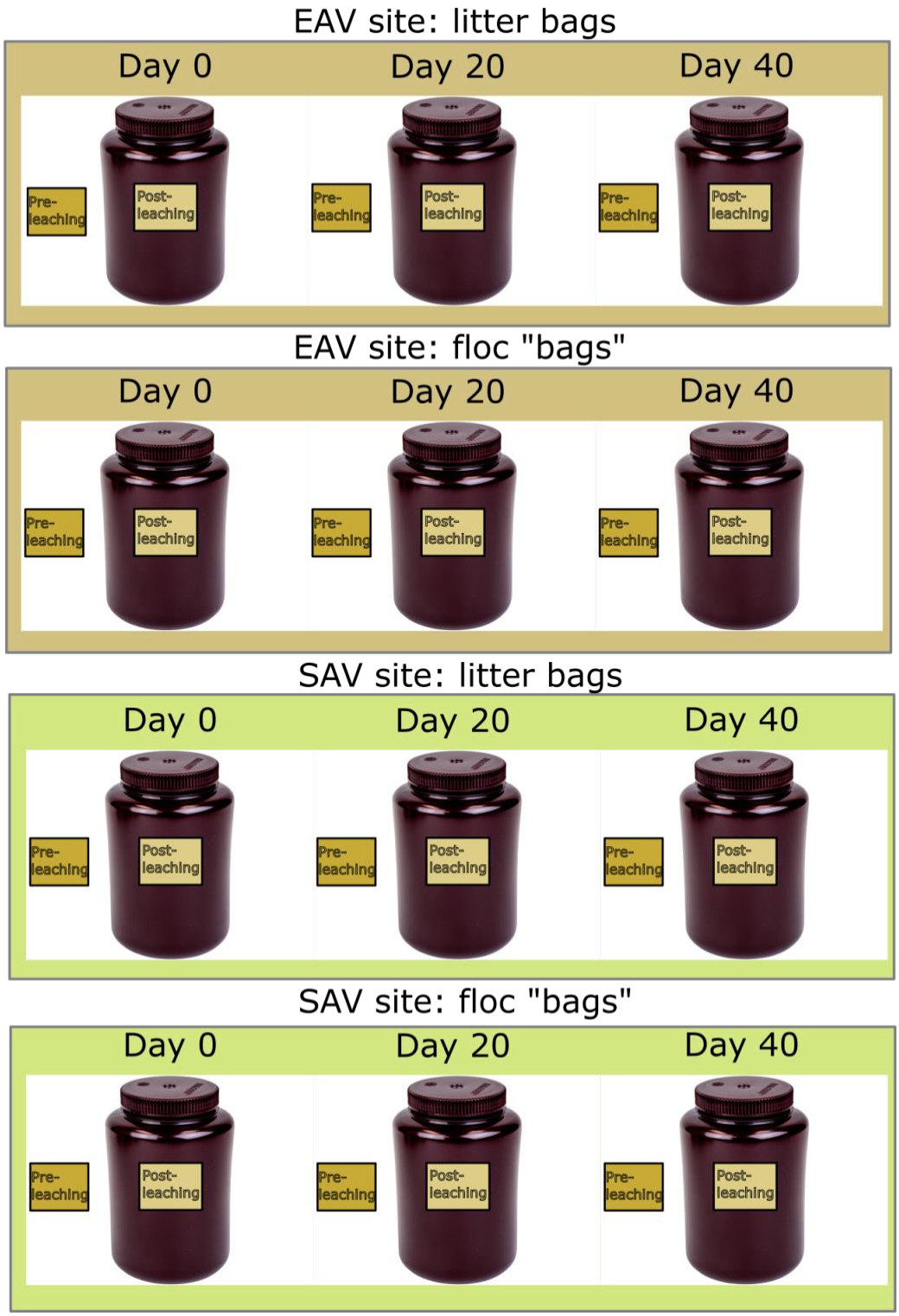


Figure 3. Schematic of Task 5 experimental design.

Table 1. Station codes and descriptions for Task 3.

Station Code	Description
S-354	Lake Okeechobee outflow
G-302	STA-1W
S-319	STA-1E
S-6	STA-2
G-370	STA-3/4
G-508	STA-5/6
G-372	A1 FEB
G-538	L8 FEB

Table 2. Analyses proposed for Task 3.

Hydrologic Unit	SampleType	UF-Bianchi		UF-Judy		UF-Osborne			UF-Inglett			
		Amino acids, stable isotopes	Ultrafiltration	XAS spectra	P-NMR	DOC	TDN	Fluorescence Spectroscopy	SRP	TDP (0.45 um filtrate)	TDP (0.7 um filtrate)	Microbial bioassay, enzyme additions
Lake Okeechobee canal discharge	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 1W (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 1E (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 2 (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 3/4 (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
STA 5/6 (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
A1 FEB (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	3
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
L8 FEB (distribution canals downstream of structure discharge)	Whole Water	-	-	-	-	-	-	-	-	-	-	-
	POM	3	-	1	1	-	-	-	-	-	-	-
	DOM: whole	3	3	1	1	3	3	1	-	-	-	-
	DOM: low MW (< 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	DOM: high MW (> 10 KDa)	-	-	-	-	3	3	1	3	3	3	-
	Number of Samples per Analysis	**48**	**24**	**16**	**16**	**72**	**72**	**24**	**48**	**48**	**48**	**12**
		Amino acids, stable isotopes	Ultrafiltration	XAS spectra	P-NMR	DOC	TDN	Fluorescence Spectroscopy	SRP	TDP (0.45 um filtrate)	TDP (0.7 um filtrate)	Microbial bioassay, enzyme additions
		UF-Bianchi		UF-Judy		UF-Osborne			UF-Inglett			

Table 2. District laboratory services requested for Task 3.

Hydrologic Unit	SampleType	TP (water)	TDP (water)	SRP (water)
Lake Okeechobee canal discharge	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 1W (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 1E (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 2 (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 3/4 (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
STA 5/6 (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
A1 FEB (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
L8 FEB (distribution canals downstream of structure discharge)	Unfiltered water	3	-	-
	DOM (filtered water)	-	3	3
	Number of Samples per Analysis	**24**	**24**	**24**

Table 3. Analyses to be run for Task 4. Samples in the “Trexler lab” column will be collected by Trexler et al. but analyzed by the District.

Experiment Type	Faunal size class or Fish density	Timepoint	SampleType	Analyses and who will run them										
				Trexler Lab					UF-Bianchi					
				TN	NH_4	TP	SRP	TDP	DOC	TDN	Stable isotopes (C, N)	TC, TOC, TN	Fecal sterols	High-res mass spec
Excretion	Large	Initial	Whole water	3		3								
			DOM		3		3	3	3	3			3	1
			POM								3	3	3	
	Large	Final	Whole water	3		3								
			DOM		3		3	3	3	3			3	1
			POM								3	3	3	
Bioturbation	High Density	Initial	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
		Final	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
	Control	Initial	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
		Final	Whole water	3		3								
			DOM		3		3	3	3	3				1
			POM								3	3	3	
			Floc	3		3					3	3	3	
			Soil	3		3					3	3	3	
			Periphyton			3					3	3	3	
		TASK 2	**Total samples**	42	18	54		18	18	18	54	54	60	6
				TN	NH_4	TP	SRP	TDP	DOC	TDN	Stable isotopes (C, N)	TC, TOC, TN	Fecal sterols	High-res mass spec
				Trexler Lab					UF-Bianchi					

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