DELIVERABLE #3B: FINAL WORK PLAN

Assess Feasibility and Benefits of Consolidating Accrued Marl in the Everglades Stormwater Treatment Areas (STAs)

November 18, 2021

To: Odi Villapando, South Florida Water Management District

From: DB Environmental

This report was prepared in fulfilment of Agreement 460004012-WO10

This work plan outlines DB Environmental's (DBE) approach to assessing benefits of consolidating marl soil as a management technique, to increase the physical and chemical stability of these soils in the Everglades Stormwater Treatment Areas (STAs), and to improve STA phosphorus (P) removal performance.

Project Description and Objectives

Background

The Everglades STAs were built south of Lake Okeechobee to reduce total phosphorus (TP) concentration in surface water runoff prior to discharge into the Everglades Protection Area. To achieve and sustain the Water Quality Based Effluent Limit (WQBEL) of 13 µg/L at the STA outflow structures, cost-effective management strategies to control internal loading of phosphorus to the water column are essential. While some recent studies have suggested that water velocities in the STAs are typically lower than the critical shear stress required to resuspend accreted soils (Fugate et al. 2021), resuspension of flocculent marl soils does occur. Turbid conditions have been observed in areas where submerged aquatic vegetation (SAV) coverage has declined, producing bare areas of loose unconsolidated soils. Unstable soil substrates increase the potential for rooted macrophytes to be dislodged by wind/wave energy, while resuspension, bioturbation, and movement of unconsolidated material produce increased turbid conditions and discharge TP concentrations higher than the WQBEL. The objective of this study is to evaluate methods that improve the physical and chemical stability of STA soils to improve STA TP reduction performance.

There are 24 cells within the Everglades STA network that were designed to be predominantly SAV. These cells comprise 32,000 acres, much of which have been occupied by SAV and are located in downstream portions of most flow-ways. In 2013, about 55% of the treatment area within the Everglades STA footprint was occupied by SAV communities (DBE 2018a). SAV communities remove P from the water column by direct uptake of P by the plants and attached periphyton and through indirect removal by pH-mediated coprecipitation with $CaCO_3$ (Dierberg et al. 2002). Marl is produced in these SAV communities when carbon dioxide is removed from the water by photosynthetic activity of both algae and aquatic macrophytes. This activity shifts the

bicarbonate/carbonate equilibrium towards carbonate (Pedersen et al. 2013), and in the abundance of calcium, calcium carbonate is precipitated. Most of the floc and recently accreted soils in STA-2 flow-way (FW) 3, an SAV-dominant flow-way, is calcium carbonate (University of Florida 2019; Reddy et al. 2020). Calcium carbonate encrustations commonly found on SAV plant surfaces may add to marl accretion as these plants die and decompose.

Marl has accumulated to depths of 20 cm or more in some SAV-dominated areas (Dierberg et al. 2021) and is not as physically stable (resistant to resuspension) as the antecedent predominately organic soils (Histosols) upon which many STAs were constructed. This accumulated marl contains lower organic matter content and few aggregates, compared to the Histosols. Soil aggregates are important to P retention and loss mechanisms (Li et al. 2020) and chemical stability of soil P in Histosols (Wright 2009). In the STAs, aggregate formation within newly accreted soils, including marl, is poorly understood, but may be important to increasing the physical and/or chemical stability of these soils for retaining P. On the basis that the underlying highly organic Histosols appear more aggregated and physically stable than the accruing calcareous marls. Therefore, it has been hypothesized that organic matter inputs (and decomposition) could improve marl stability.

Objectives

This project will evaluate the technical feasibility of consolidating/aggregating marl in the SAV cells/flow-ways of the Everglades STAs and will determine if consolidation or improved aggregation of marl has the potential to increase P storage, reduce internal P loading, and reduce P concentrations in water discharged from the downstream regions of the STAs.

Project Components

There are 3 Phases and 12 tasks in this project, with STOP/GO decisions at the conclusion of Phase I and Phase II (**Table 1**). Phase I includes a literature review, benchtop mesocosm-scale studies, and preliminary analysis and reporting of findings. If Phase I results demonstrate technical feasibility and benefits of consolidating marl, field mesocosm-scale studies in Phase II will compare the effectiveness of consolidated marl to amorphous marl and limerock in reducing water column P concentration. If significant water quality benefits from consolidated marl soils are found in Phase II, then Phase III will evaluate the cost-benefits of this technology. If warranted, additional experimentation or field-scale trials will be developed through a revised Work Plan for Phase II.

Within Phase I, the first task (Task 1), the Project Kick-off Meeting, was completed on June 22, 2021. Task 2, the Literature Review, was completed on September 1, 2021. Task 3 is this Work Plan. Task 4 includes three sequential benchtop-scale experiments (**Table 2**), with a STOP/GO decision point after Experiment II. Task 5 will comprise the final summary data analyses and reporting for the Phase I experiments, with a STOP/GO decision for Phase II (**Table 1**).

The first task in Phase II, Task 6, will be an update to the Work Plan based on the information synthesized from Phase I. Task 7 is the outdoor, flow-through mesocosm studies. Task 8 will comprise the final summary data analyses and reporting for Phase II.

The first task in Phase III, Task 9, is a cost-benefit analysis of the methods. Task 10 will be preparation of a draft manuscript for publication, Task 11 is a final project presentation, and Task 12 is the project close-out.

Table 1. Project tasks, deliverables, and submission dates.

Task	Task Description	Milestone Deliverable	Deliverable Date (months after NTP)
Phase I			
1	Kick-off Meeting	1. Memorandum Kick-off Meeting Notes	6/30/2021 (**1**)
2	Literature Review	2a. Draft Literature Review	7/30/2021 (**2**)
		2b. Final Literature Review and Literature Database	8/31/2021 (**3**)
3	Project Work Plan	3a. Draft Project Work Plan	9/30/2021 (**4**)
		3b. Final Project Work Plan	10/29/2021 (**5**)
4	Benchtop-scale Experiments	4a. Letter Report of Activities of Experiments I and II	1/31/2022 (**8**)
		4b. Presentation of Results of Experiments I and II; STOP/GO	4/29/2022 (**11**)
		4c. Letter Report of Activities of Experiment III – 1st quarter for Task 4	7/29/2022 (**14**)
		4d. Letter Report of Activities of Experiment III – 2nd quarter for Task 4	9/30/2022 (**16**)
5	Report	5a. Draft Summary Report for Experiments I, II, and III	1/31/2023 (**20**)
		5b. Final Summary Report for Experiments I, II, and III and Analytical Data Report; STOP/GO	3/31/2023 (**21**)
Phase II STOP/GO Decision Dependent			
6-8	Updated Work Plan		Spring 2023
7-8	Field-scale Experiments and Reports/Presentations of Findings		TBD
Phase III STOP/GO Decision Dependent			
9	Cost-Benefit Analysis		TBD
Project Closeout and Final Reporting			
10	Draft Manuscript	10. Draft manuscript	4/28/2023 (**23**)*
11	Final Project Presentation	11. Final project presentation	4/28/2023 (**23**)*
12	Project Close-out	12. Return of equipment, instruments, keys, and badges; remaining field notes, data, and other project items	5/31/2023 (**24**)*

* Indicates target completion date if STOP after Phase I.

Table 2. The hypotheses and experimental components for each experiment in Phase I, Task 4.

Experiment	Hypotheses	Experiment Components
I	Overlying water-column turbidity and particulate P are inversely related to the organic matter content of the soil.	Physical Stability Test and Soil Analysis
II	The degree of consolidation or aggregation of amorphous marl can be enhanced by addition of organic matter.	Addition of Organic Amendments, Dryout, Physical Stability Test, Soil Analysis
III	The release of P from dried marl soils upon rehydration will be greater for unamended than amended treatments.	Addition of Organic Amendments, Dryout, 6-Floodwater Exchanges, Physical Stability Test, Soil Analysis

List of Project Personnel and Their Responsibilities

Kevin Grace– Project Manager, Principal Investigator
Mike Jerauld – Data Analysis/Principal Investigator
Jessica Vaccare – Project Coordinator/Scientist
Luke Evans – Data Analysis/Scientist
Kimberly Moughon –Scientist/Data Validator
Sam Colios – Field Operations Manager/Scientist
Nichole Carr – Laboratory Supervisor/Scientist
Dawn Sierer Finn – Senior Scientist/Field Quality Officer
Tom Prevratil – Field Scientist/Senior Technician/Field Safety Officer
Karen Hileman – Field Scientist/Senior Technician/Field Safety Officer
Nancy Chan – Laboratory Manager
Michelle Kharbanda – Project Administrator
Tom DeBusk – Program Manager

Schedule of Activities

The project schedule identified below includes forthcoming benchtop experiments and follow-on Phase II activities (**Figure 1**). This Work Plan focuses on tasks, deliverables, and due dates for Phase I of this project, as listed in **Table 1**.

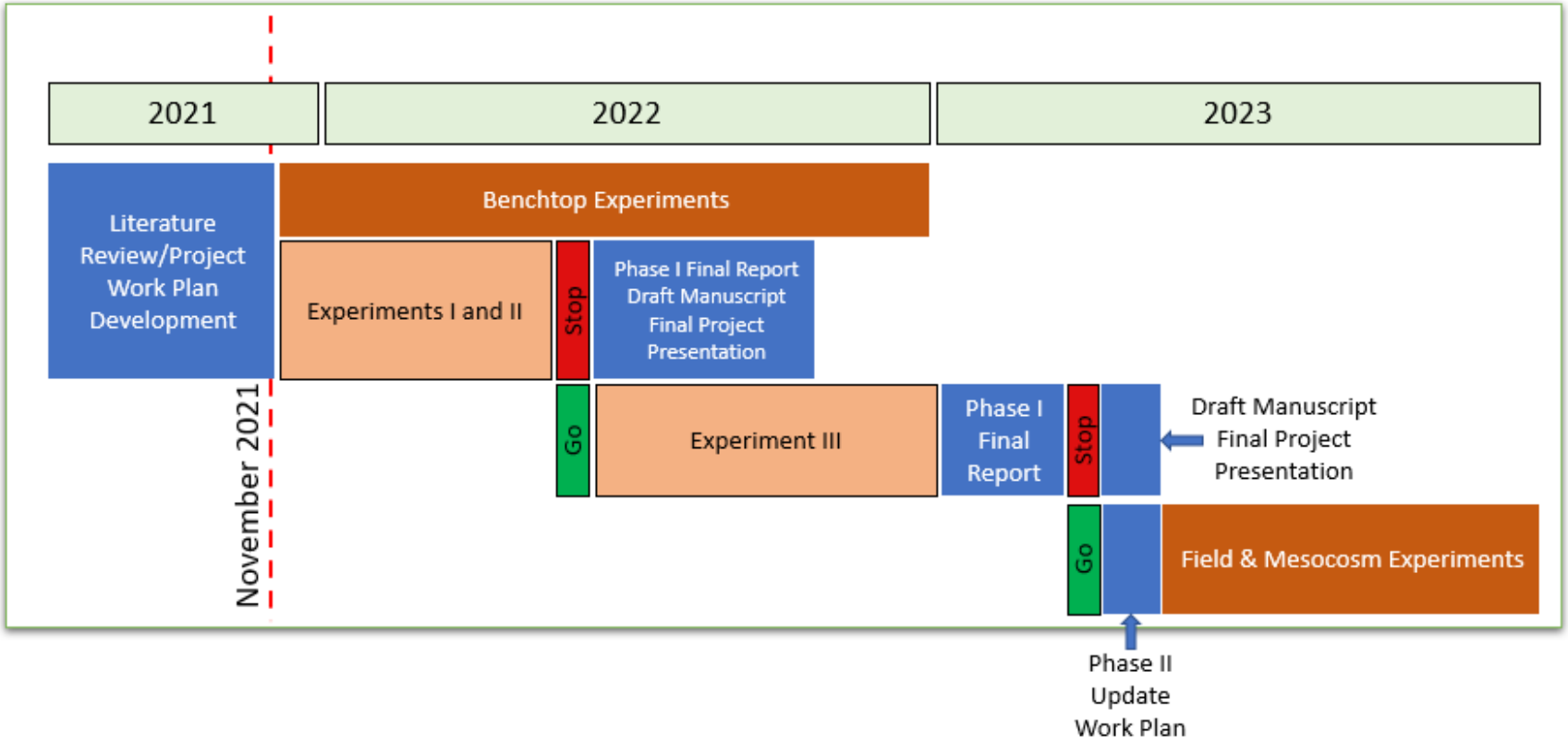

Figure 1. Project schedule

Description of Phase I Experiments

This study will evaluate the physical and chemical stability of dried marl soils that are organically amended or unamended and rehydrated for laboratory suspension tests. These tests will identify soil properties that make them susceptible or resistant to suspension under turbulent conditions. It is hypothesized that the ability of consolidated soil to resist disruption through agitation is directly related to the quality and quantity of organic matter content.

Three sets of experiments will be conducted. The first experiment will be an evaluation of techniques and methods to determine physical stability and soil aggregation in unconsolidated marl soils from historically SAV-designated cells/flow-ways. This will address the questions, "Are marl soils more susceptible to resuspension than other soils?" and "Is there a relationship between settling after resuspension and soil organic matter or aggregation?" The second experiment will compare stability of consolidated marl with and without organic matter amendments applied at two rates for each of the three amendment types and will examine short-term water column P dynamics following resuspension of marl soil. It will address the question, "Can amendments reduce P internal loading following suspension?" The third experiment will assess the physical and chemical stability of soil-P in consolidated/aggregated soils collected from multiple field sites after several months of rehydration ex situ.

Experiment I – Marl Aggregation and Physical Stability

The relationship between soil stability and soil native organic matter (OM) content is currently unknown, both between SAV marls and emergent aquatic vegetation (EAV) organic soils, and even among marls of varying OM contents. Furthermore, the influence of OM additions to marl will likely depend on the OM content already within the marl soil matrix. Similarly, the capacity for an amendment to enhance P chemical stability within the marl soil is predicated on P content within that marl soil type. Therefore, **Experiment I** will measure the physical stability, P content, and other characteristics of existing STA marl soils spanning a range of organic matter contents.

Organic matter content can be approximated by measuring total organic carbon (TOC) or ash free dry weight (AFDW). The relationship between TOC and AFDW is generally very strong (AFDW is approximately twice the TOC content), yet such data are lacking for recently accrued soil from many STA FWs. Our review of available data for STA marl soils indicated that OM contents can range from 6 to 33 % TOC and TP concentrations from <200 to >1200 mg/kg (**Figure 2**). EAV accrued soils had TOC > 25%, while most marl soils were less than that level.

Experiment I will use marl soils from multiple STA locations historically dominated by SAV, and for comparison, emergent aquatic vegetation (EAV) organic soils (**Figure 3**). Ten intact soil cores, each collected from a different STA site, spanning a range of soil conditions (**Table 3**), will be subjected to a physical stability assessment (Section 0, below) and then analyzed for physical and chemical properties (Section 0, below). Bivariate relationships will be examined between soil properties and aggregation and stability metrics across the range of marl soils.

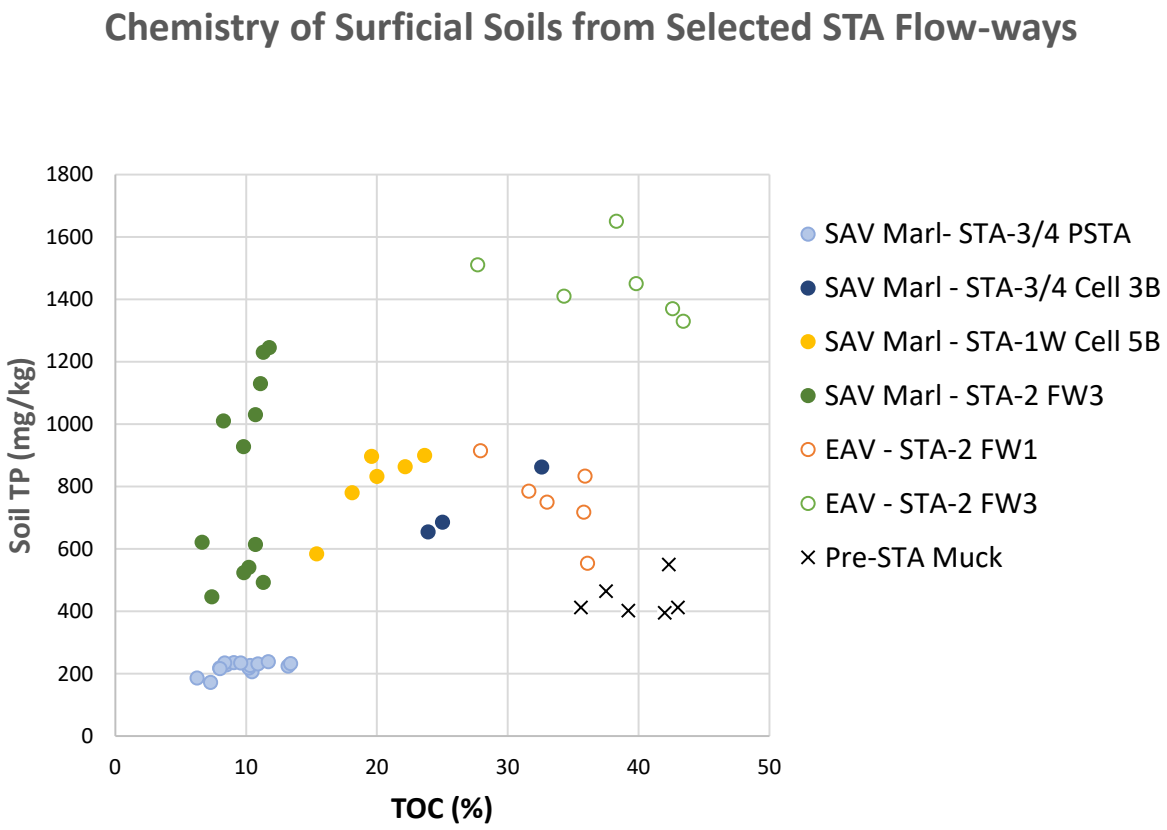

Figure 2. Total organic carbon (TOC) contents and total phosphorus (TP) concentrations in surficial soils of selected Stormwater Treatment Area flow-ways (DB unpublished data). Also shown are samples of "Pre-STA Muck" soils collected from below the accrued soil layer in STA-3/4 Lower SAV Cell.

Table 3. Target conditions for defining classifications.

Classification	Description
P-enriched	TP > 600 mg/kg
P-limited	TP < 600 mg/kg
High Organic Matter	TOC > 15 %
Low Organic Matter	TOC < 15 %

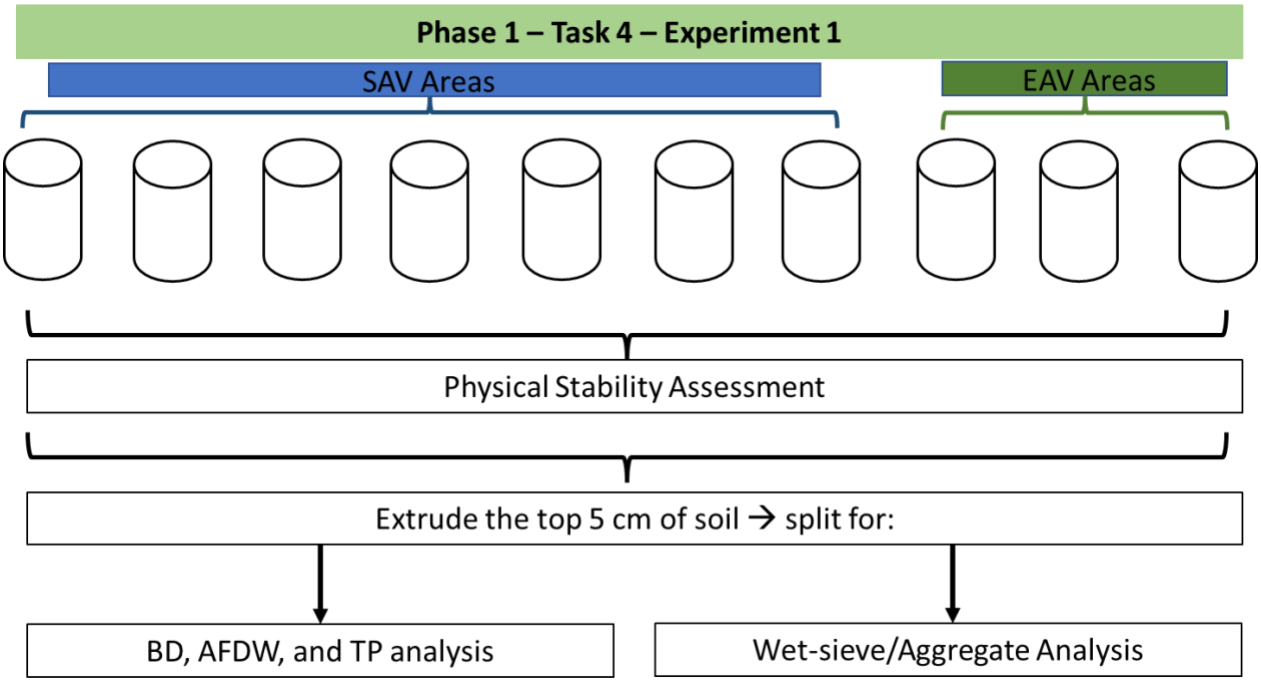

Figure 3. The treatments, experiment structure, and analyses for **Experiment I** in Phase I, Task 4.

Site Selection

The cores collected for **Experiment I** will represent existing STA conditions where marl soils have accumulated and represent unstable, unconsolidated substrates. Intact soil cores will be collected from both P-enriched (inflow or mid-cell) and P-limited (outflow) regions, to capture a range of organic matter content within the soils.

Historical soils data indicate a range of OM contents in SAV marl soils and EAV soils from potential soil source locations for **Experiment I** (**Table 4** and **Figure 2**). However, because current site-specific conditions may differ from the conditions during the most recent soil sampling efforts for which data are available, and (1) some of these FWs/regions might not be readily accessible given other STA activities, (2) existing historical data for OM contents of surficial soils are not available for every FW and (3) other FWs without available historical data could have target soil conditions, a fuller set of cells of potential interest for further consideration is shown in **Figure 4**. For example, many cells (e.g., STA-1W Cell 4) historically dominated by SAV have now become mixed marsh communities with abundant cattail. Some STA cells are likely to have multiple areas of interest. Consultation with District scientists and vegetation management regarding site access, restrictions and field conditions will further refine the final selection of areas of interest for each of the 10 cores required for **Experiment I**. The precise collection locations within pre-identified areas of interest will be determined in the field, based on current vegetation and soil conditions. Once soil cores are collected, the site characteristics and coordinates will be provided in a future progress report.

Table 4. Chemical characteristics of select surficial soils in STAs when organic matter (total organic carbon [TOC]) were measured along with phosphorus contents (DBE unpublished data).

Vegetation	Soil Type	Location	Soil TP (mg/kg)	Organic Matter (TOC %)	Soil Layer and Year
SAV	**P-enriched/High OM**	STA-3/4 Cell 3B outflow	654-862	23.9 - 32.6	Accrued soil layer in 2013
		STA-1W Cell 5B	584-900	15 – 23 *	Soil floc layer in 2017
	P-enriched/Low OM	STA-2 FW 3 inflow and mid SAV region	928 -1245	8.3 - 11.8	Upper 0-4 cm of accrued layer in 2016
	P-limited/Low OM	STA-2 FW 3 back end SAV region	493 – 614	9.8 - 11.3	Accrued soil layer in 2016
		STA-3/4 PSTA	167-496	6.6 – 14.9	Accrued soil layer in 2014
EAV	**P-enriched/High OM**	STA-2 FW 3 mid EAV region	1330-1650	27.7 - 43.4	Upper 0-4 cm of accrued layer in 2016
	P-limited/High OM	STA-2 FW 1 outflow	554-915	31.6 - 35.9	Soil floc layer in 2013

* AFDW values from STA-1W Cell 5B converted to TOC (as AFDW/2).

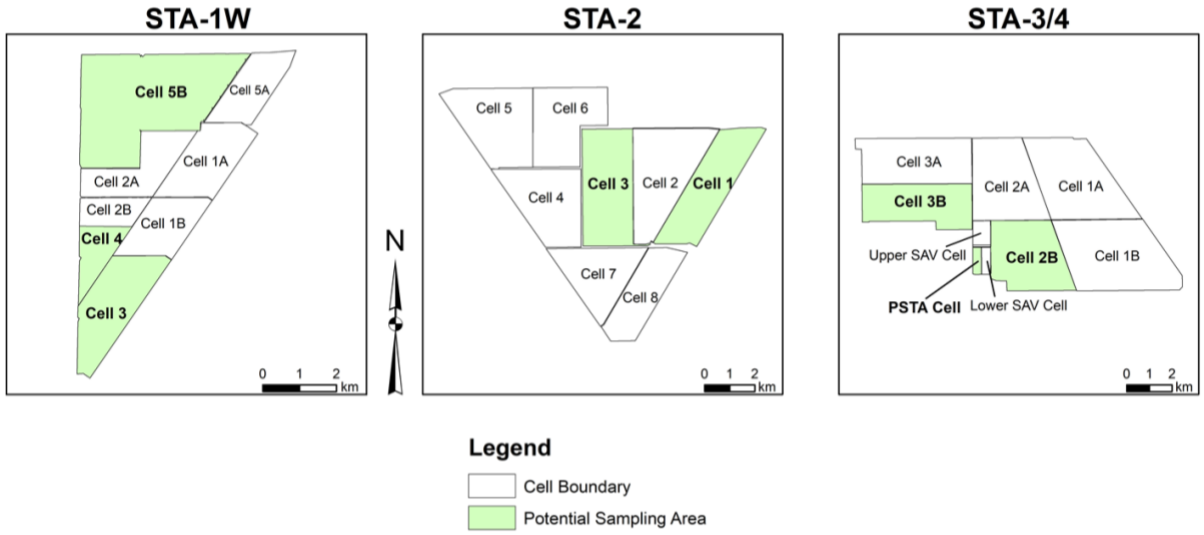

Figure 4. Potential soil sampling areas for **Experiment I**.

Soil Collection

For **Experiment I**, intact soil cores (5.75" (14.6 cm) i.d.) will be retrieved from selected STA locations and will encompass the floc and recently accrued soil (RAS) layers to a minimum depth of 10 cm (**Figure 5**). The cores will be flooded with site water and kept in the shade during transport from the field to the DBE laboratory in Rockledge, FL for the bench-top experiments.

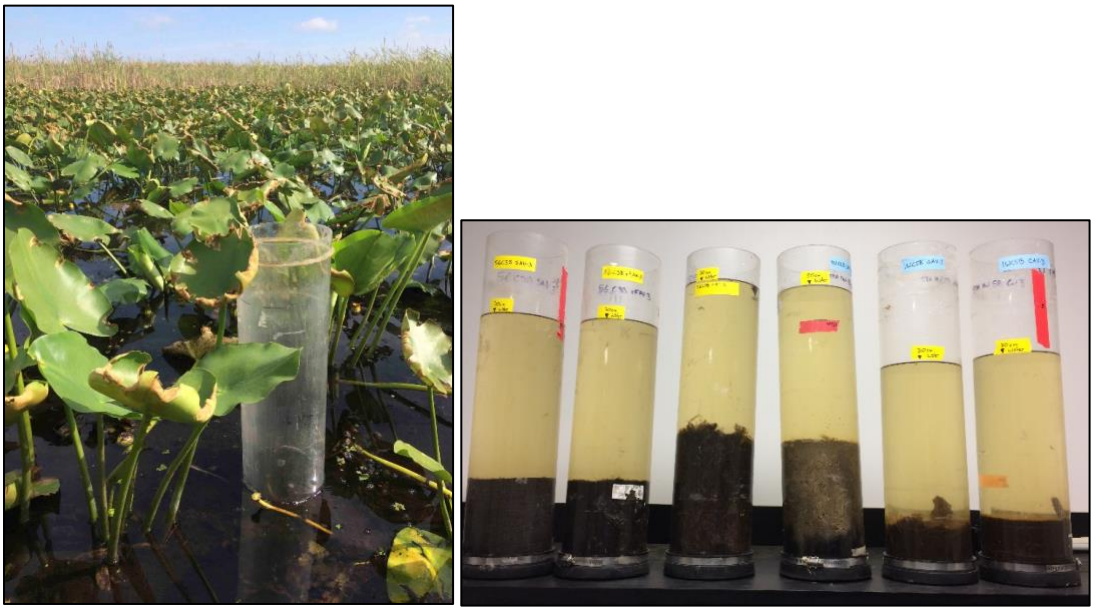

Figure 5. Examples of intact soil cores retrieved from STA flow-ways on May 16, 2018 and used in soil suspension studies (DBE 2018b).

Physical Stability Assessment with Suspension

Soils will be subjected to a physical stability assessment test to investigate whether: marl soils under current conditions are physically less stable than EAV soils or if (1) variation in physical stability is related to organic matter content (**Experiment I**), (2) marl soil physical stability is affected by consolidation and/or OM amendments (**Experiment II**) and (3) enhanced physical stability benefits from soil amendment persist several months after rehydration, or are related to changes in chemical P stability (**Experiment III**). These physical stability tests will use the procedure described in DBE (2018b), which is briefly described below.

In the laboratory, surface water will be drained from each core to the soil surface, then slowly reflooded with low-nutrient STA outflow water until a 30-cm water column is established above the soil surface. The flooded cores will be allowed to sit overnight, and the depth of the soil layer will be measured prior to initiation of the suspension studies. The cores will be agitated for 30 seconds with a paddle attached to a variable speed motor to deliver equivalent energy to each core during which time the surficial soil particles became entrained in the water column (**Figure 6**). Through this simple assessment, the exchange of P from soil particles (and porewater) into the water column will be directly compared between soil types and amendment treatments.

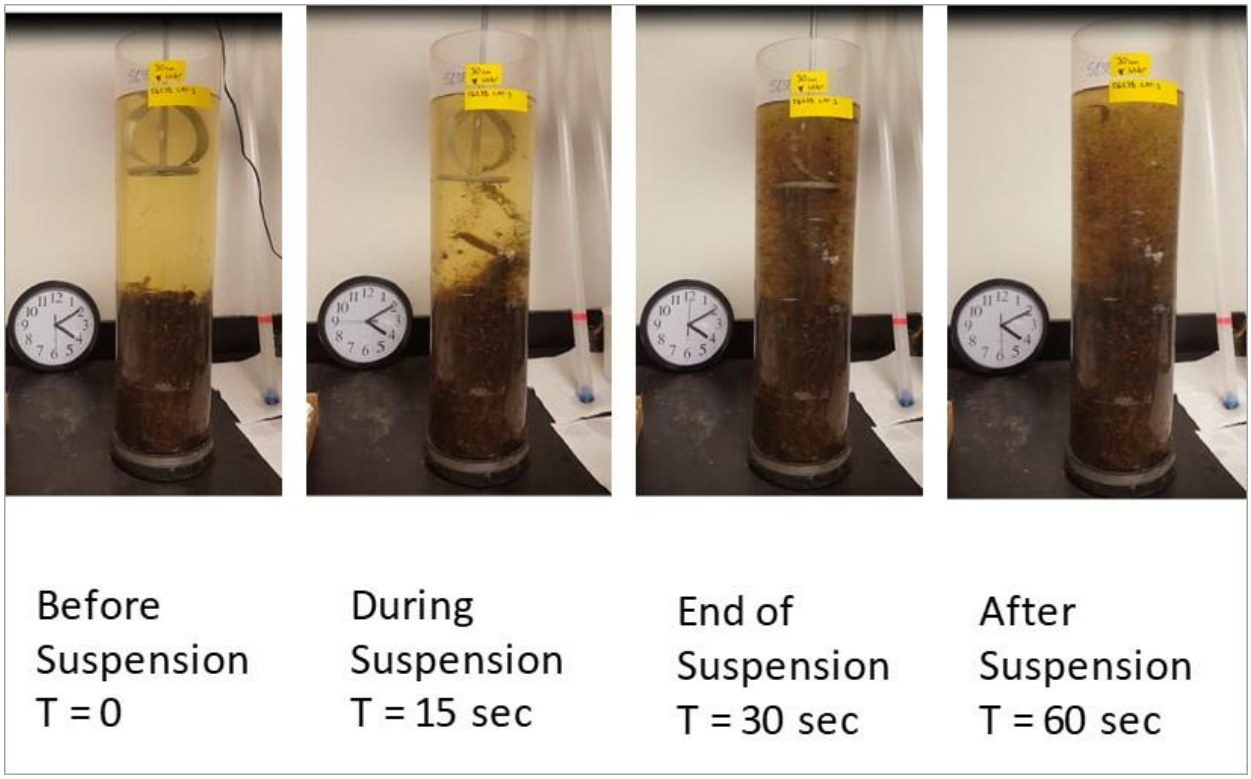

Figure 6. Example of sediment suspension achieved by agitation with a paddle at 48 rpm for 30 seconds in a sediment core collected from a STA-5/6 Cell 3B cattail stand.

Water Sampling

During the Physical Stability Assessment, water samples will be collected using a Coliwasa tube sampler (see double-check valve bailer sampling in FDEP 2017a, section FS2100), inserted to the mid-depth of the overlying water column (15 cm), at 2 minutes, 20 minutes, 60 minutes, and 24 hours after resuspension (**Figure 7**). A sample of the reflood water also will be retained for analysis. The samples will be analyzed for turbidity and P species (TP, total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP). In-situ measurements of pH, DO, and temperature will be taken at 10 cm below the water surface after each sample collection.

Figure 7. Sampling technique using a Coliwasa tube sampler to collect water samples from core tubes.

Soil Analyses

After the physical stability assessment, overlying water will be removed, and the soil will be extruded so the top 5 cm section can be retained for analysis. In **Experiment I**, soil analysis will include bulk density, AFDW, and TP, as well as the aggregated soil fraction.

Soil Aggregation

Soil aggregates will be separated using a wet-sieve method based on Wright 2009. Fifty grams of wet soil will be placed on a 0.25-mm mesh sieve (described as the cut-off between macro- and micro-aggregates (Six et al. 1998; USDA - Soil Quality Institute 2001) and rinsed with DI water while shaking the sieve back and forth. The sample retained on the sieve will be transferred to a weighing boat and dried at 65 °C to a constant weight. The aggregated soil fraction will be calculated as the fraction of initial soil sample dry weight that was retained on the sieve.

Experiment II - Comparing Stability of Organically Amended and Unamended Dried Marl Soils

Experiment II will test the effects of drying and OM amendments of different types and application rates on the consolidation, aggregation, and physical stability of marl soil. Marl soil from a single site will be used. The source soil will be selected to represent a "typical", "problematic" marl soil, based on the results of **Experiment I** (high water column turbidity and TP concentration after suspension). Soil for **Experiment II** will be collected from the selected site following the conclusion of **Experiment I**.

For **Experiment II**, accrued marl soil from the selected location will be collected, separated from underlying muck soils, and homogenized before partitioning into each experimental unit. To avoid differences in marl characteristics between amendment treatments, the marl soil will be kept free of muck soil or large plant fragments. The accrued soil material will be mixed during amendment application.

Twenty-eight experimental units, each a 6" diameter soil core, will be established and separated into 7 groups (treatments) of four soil cores each. Three amendments selected for investigation will be applied at two rates (see Section 0, below), creating a 2 x 3 factorial experimental design. Each treatment will be evaluated in triplicate. The fourth core in each treatment group will be sacrificed for soil analysis after the consolidation phase. An additional set of cores will serve as controls (unamended) during **Experiment II**.

Marl soil will be amended at the prescribed rates for each treatment (controls will receive no amendment), then distributed into replicate cores to be consolidated via drying over a 2-month period. After consolidation, the soils will be reflooded and then subjected to a physical stability test (as described in Section 0) and analyzed for physical and chemical characteristics. The treatments, experiment structure, and analyses are in **Figure 8**.

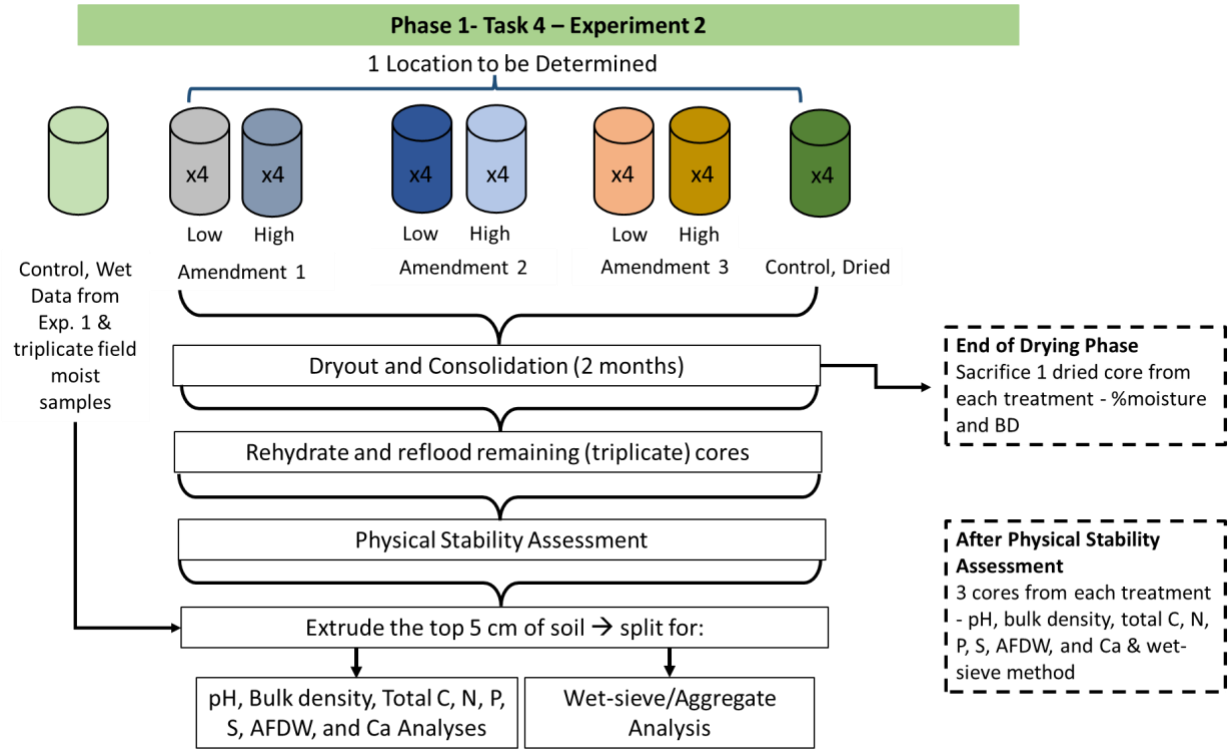

Figure 8. The treatments, experiment structure, and analyses for **Experiment II** in Phase I, Task 4.

Amendments

Organic amendments selected for testing in **Experiment II** during this study include two primary candidates: Cattail leaf litter and sugarcane bagasse. This selection was made based on local availability and chemical composition characteristics (DBE 2021a). Standing dead cattail leaves from above the water were collected from two low-P regions of the STAs: STA-3/4 Cell 2B and STA-3/4 PSTA Cell. Sugarcane bagasse was acquired from a local sugar mill. The third amendment will be selected after further laboratory testing. Potential amendment materials identified during the literature review, including wood chips, cardboard stock, rice hulls, biochar, and humic acid soil amendments were also acquired to determine C, N, and P contents of these materials for consideration as a third amendment type (**Table 5**).

Each of the three selected amendments will be dried, ground, and sorted into plant fragments between 1 mm and 1 cm to normalize the size fraction of the amended material. The dry:wet weight ratio and bulk density of amendments and the homogenized marl soil will be determined so the soil can be amended at a rate of either 10 % or 25 % by volume with the selected organic matter materials, and weight ratio of each amendment rate can be determined.

A clean plastic container (e.g., 20 L) will be used to mix the amendment into the marl soil. Then, a 15 cm deep soil layer will be reconstituted in a clean acrylic 6” diameter core tube (inner diameter

= 5.75" or 14.6 cm, surface area = 167 cm^2). This approach will eliminate confounding factors of differing soil depth between "replicates", as well as the influence of large roots and plant fragments, or varying amounts of muck soil beneath the accrued soil layer. The reconstituted soil columns will then be allowed to air-dry and consolidate before use in subsequent dryout-reflood and resuspension assessments.

Table 5. Chemical composition of organic matter considered as potential amendments for improving marl soil stability characteristics.

Amendment Material	TP (mg/kg)	TN (mg/kg)	TC (mg/kg)	C:N Ratio (weight:weight)
Biochar	869	10170	429000	43
Humic OM	160	22500	472000	21
Rice Hulls	567	4613	376667	83
Sem-Chi Rice Hulls	1030	5383	417000	78
Bagasse Plates	43	<2360*	453000	>192**
Cardboard	47	<2360*	463000	>196**
Woodchips	326	5347	477000	90
Fresh Bagasse	293	4468	460167	103
Cattail - STA-3/4 Cell 2B	166	4680	492333	105
Cattail - PSTA	137	4687	485667	104

* Result is below the method detection limit of 2360 mg/kg
** TN result was below the detection limit.

Dryout and Consolidation

Soil cores with and without OM amendments will be dried to promote consolidation of the flocculent soil, decomposition of the OM, and aggregation within the soil. Consolidation will be evaluated by measuring the soil depth within each core once a week during the drying period. After approximately two months of drying in an outdoor drying chamber, one core for each treatment will be sacrificed, and the upper 5 cm soil extruded from the soil core tube and retained for analysis.

Soil Analyses

In **Experiment II**, soils will be analyzed for the same parameters as **Experiment I** (bulk density, AFDW, TP, as well as the aggregated soil fraction), as well as pH, total carbon (TC), total nitrogen (TN), total sulfur (TS), and total calcium (TCa) contents.

The percent moisture and bulk density will be determined to characterize the extent to which drying achieved changes in bulk density within the marl. The remaining cores of each treatment will be evaluated after rehydration and the physical stability assessment. At that time, the upper 5

cm soil will be extruded from each soil core tube and retained for analysis. The soil sample will be split for chemical analysis, which will be refrigerated until analysis and for soil aggregate fraction determination, which will not be refrigerated, and will be processed as soon as possible (within 48 hr) under wet conditions.

Experiment III - Release of P from Consolidated/Aggregated Soils Upon Rehydration

If **Experiment II** shows that drying and OM amendment have a positive effect on consolidation and the physical stability of STA marl soil, **Experiment III** will apply one OM amendment at a single rate (amendment type and rate to be selected based on findings from **Experiment II**) to marl soils from four different STA locations to test the efficacy of the approach on marl soils of different character.

Locations for soil collection in **Experiment III** will be selected based on the results of **Experiment I.** Soils for **Experiment III** will be collected from the selected sites following the conclusion of **Experiments I** and **II.**

As in **Experiment II**, each soil receiving the amendments will be homogenized and amended, then distributed into replicate cores and consolidated over a period of 2 months. After consolidation, the cores will be reflooded with low-nutrient water and the nutrient flux from the soils to the water will be monitored. The cores will be kept wet for 12 weeks (nutrient flux incubation), and then subjected to a final physical stability assessment, to test the sustained benefit of drying and OM amendment on marl soil stability. It is hypothesized that the release of P from dried marl soils upon rehydration will be greater for unamended than amended treatments. The treatments, experiment structure, and analyses are shown in **Figure 9**. The rehydration nutrient flux incubation, unique to **Experiment III**, is described in Section 0, below.

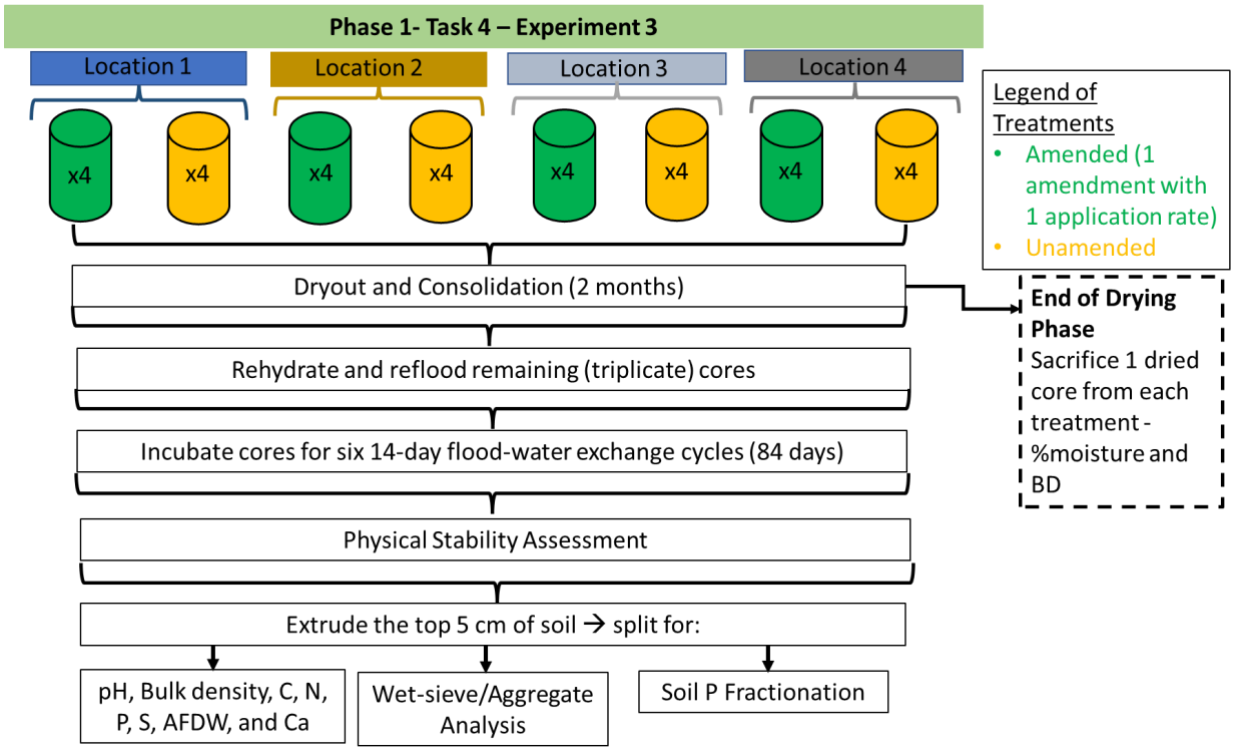

Figure 9. The treatments, experiment structure, and analyses for **Experiment III** in Phase I, Task 4

Rehydration Nutrient Flux Incubation

In **Experiment III**, triplicate soil cores either amended or unamended and subsequently dried will be reflooded with low-nutrient STA water and placed in a temperature-controlled room. The water column in the cores will be kept aerobic by bubbling air into the water column. The cores will be covered with black polyethylene bags to exclude light and prevent algal growth and incubated indoors at room temperature. Thirty milliliters of floodwater will be collected via Coliwasa on days 0, 3, 7, 10 and 14, filtered and analyzed for SRP. A larger volume of sample also will be taken on day 14 of the incubation period for TP and TDP analyses. At the end of the 14-day cycle, the remaining water in the cores will be removed by applying gentle suction to minimize disturbance to the floc layer and refilled with STA site water to re-establish a depth of 30 cm. These steps will be repeated for a second 14-day batch cycle. Cores will remain aerated and flood water exchanged every 14 days for a total of six exchange cycles (6 x 14 days = 84 days), and during the final (sixth) batch cycle, water-column P (SRP, TP and TDP) will again be sampled as described above to determine P flux after an extended period of incubation; water samples will not be collected during the third through fifth 14-day batch cycles. The purpose of this extended period is to allow time for the amendments to affect soil P stability under the flooded conditions typical of the STAs.

In-situ measurements of pH, dissolved oxygen (DO), temperature, and specific conductivity will be recorded on days 0 and 14 of each incubation period. At the conclusion of the 84-day nutrient flux incubation, a Physical Stability Assessment will be performed on each core. Once complete, soil sampling and analysis will be performed to conclude **Experiment III**. Phosphorus flux calculations will be based on the changes in water column SRP concentrations over the first two and last (sixth) 14-day batch cycles. The average P flux will be compared between amended and unamended treatments across all locations.

Soil Analyses

At the conclusion of **Experiment III**, After the physical stability assessment is complete, the upper 5 cm soil will be extruded and retained for analysis. Soil P fractions will be determined on the resulting surface 0-5 cm soil layer, as well as analytes from **Experiment II**.

P Fractionation

Soil P pools will be characterized with sequential chemical extraction according to the organic P fractionation scheme described in Ivanoff et al. (1998) and **Figure 10**. This procedure results in operationally defined pools of different stability: readily labile P, microbial biomass P, moderately labile P and non-labile P pools.

Readily Labile P_o

The readily labile organic P (P_o) and inorganic P (P_i) sorbed onto the soil surface and in soil solution will be removed by a 0.5 M $NaHCO_3$ extraction. The difference between inorganic and organic fractions of this labile pool will be determined by SRP or TP analysis of the extraction solution after 16-hr shake and centrifuge separation of the supernatant (**Figure 10**). The microbial biomass P pool will be determined by chloroform fumigation to release additional P from the microbial biomass pool. Duplicate soil samples will be prepared, with one sample a "Non-fumigated" sample (the soil that had already been extracted for P_i) and the other a "Fumigated" sample. The Fumigated sample will have 2 mL of ethanol-free $CHCl_3$ added to lyse microbial cells. The Fumigated samples will be placed in uncapped tubes loosely covered with paper towels in a chamber with chloroform for 24 hours, to allow the chloroform to interact with the soil samples. After 24 hours, the soils will be extracted with 0.5 M $NaHCO_3$, as described above. The difference in P content between Fumigated and Non-fumigated samples will be attributed to the microbial biomass P pool.

Moderately Labile and Nonlabile P_o

The residue from the Fumigated samples will be put through a 1 M HCl pre-treatment to remove any P_i from the soil. Any P_o extracted at this step, determined as the difference between TP and SRP on the HCl-extract, will be considered part of the moderately labile P_o pool. The next step is a deionized water rinse, which removes the residual acidity in preparation for base extraction but is not expected to remove P as the labile P pool has already been extracted. The residue will then be extracted with 0.5 M NaOH to remove the majority of the moderately labile P_o (aka fulvic acid-

bound P) and some of the nonlabile P_o (aka humic acid-bound P and nonlabile P_i). To separate the fulvic acid-bound P from the humic acid-bound P, an aliquot of the NaOH extract will be acidified to a pH of 0.2 with concentrated HCl. At this pH, the humic acids precipitate and the fulvic acids remain soluble. The remaining residue (highly resistant, nonlabile P_o) will be ashed, dissolved in 1 M H_2SO_4, and analyzed for TP content.

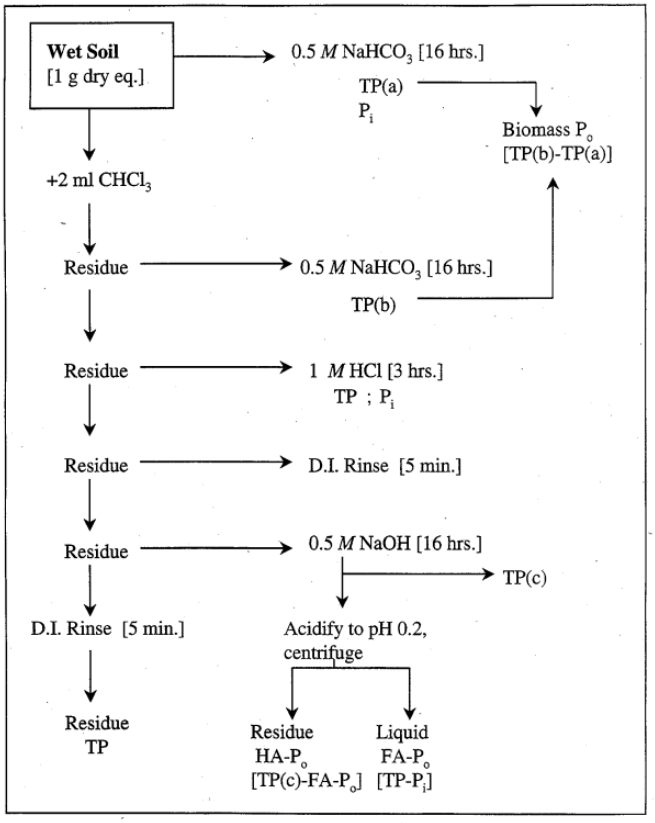

Figure 10. The phosphorus fractionation scheme used to determine the pools of P in Histosols (from Ivanoff et al. 1998). The time lengths in brackets refer to how long the tubes are shaken on a reciprocating mechanical shaker for each step.

Field Sampling

All field sampling and data collection activities and field quality control requirements for this project will be performed in compliance with the DBE Field Quality Manual (DBE 2021b), and project specific monitoring plans (this Work Plan). Staff conducting sampling and/or field measurements will be properly trained prior to working on this project. Sample handling and preservation procedures for the requested parameters will be performed as outlined in DEP-SOP-001/01 (FDEP 2017b).

While every effort must be made to collect field samples and data in accordance with the standard protocols, conditions may arise that require field activities to deviate from the standard protocols

or project requirements under special circumstances. Any deviation from the standard protocols or project requirements will be documented, in detail, in the field notes for the sampling trip.

Analytical Methods

DB Environmental Laboratories, Inc. (Lab ID E83330, "the laboratory") will conduct analysis of water and soil samples collected for this project, and is accredited by The National Environmental Laboratory Accreditation Conference (NELAC) Institute (TNI) National Environmental Laboratory Accreditation Program (NELAP) through the Florida Department of Health (FDOH) for the analysis methods and matrices specified in **Table 6**.

The laboratory shall evaluate the data in accordance with the data quality objectives stated in the Tier 2 approach from DEP EAS 00-01. All laboratory data will be validated in accordance with FDEP's Quality Assurance Rule, 62-160, F.A.C. Reported data are to be qualified in accordance with FDEP's Quality Assurance Rule and any applicable data validation Standard Operation Procedures (SOPs).

Data Analysis

Laboratory and field data will be analyzed for quality control, and appropriate statistical tests will be used to draw conclusions from the data. Hypotheses will be tested against what is considered the background condition (or null hypothesis), through the application of appropriate statistical tests (parametric vs. non-parametric).

The data analysis plan includes statistical testing for a variety of comparisons including, but not limited to, among, and within site comparisons, along with the identification of the relevant field parameters or analytes contributing to the differences. Analysis of variance (ANOVA) will be used to evaluate the significance of treatment factors. The principal statistical test to be employed to evaluate differences between treatments will be the Tukey's honestly significant difference (HSD) test. Matched-pairs t-test may be also applied. When normality assumptions are not met, data may be transformed or non-parametric tests will be applied.

In addition, bivariate analyses (i.e., Pearson's product-moment correlation) may be applied to relate water chemistry observations to physicochemical (pH, DO) parameters. As necessary, the non-parametric Spearman rank order correlation will be used. Differences in water chemistry and in soil characteristics will be examined using ANOVA.

Table 6. Surface water and soil chemical parameters to be analyzed by DB Environmental Laboratories, Inc (Lab ID E83330) for this project along with their associated analytical methods, method detection limits (MDL) and practical quantitation limits (PQL).

Analyte	Method	MDL	PQL
Surface Water			
Total Phosphorus	SM 4500-P F or SM 4500-P E	3 µg/L	12 µg/L
Total Dissolved Phosphorus	SM 4500-P F or SM 4500-P E	3 µg/L	12 µg/L
Soluble Reactive Phosphorus	SM 4500-P F or DBE SOP OPO4	2 µg/L	8 µg/L
Soil			
Bulk Density	ASA 13	0.001 g/cc	0.004 g/cc
Total Phosphorus	DBE SOP TP	10 mg/kg	40 mg/kg
Total Carbon	DBE SOP MVP	0.279 %	0.112 %
Total Nitrogen	DBE SOP MVP	0.850 %	0.340 %
Total Sulfur	ASTM E1915-11*	0.020 %	0.080 %
Total Calcium	EPA/SW 7140	50 mg/kg	200 mg/kg
Ash Free Dry Weight	EPA/COE 3-59	1.35%	5.40%
pH	EPA/COE 3-51	N/A	N/A
Moisture	ASA 21-2	0.01 %	0.04 %
Total Weight	ASA 21-2	0.01 g	0.04 g
%Dry Weight	ASA 21-2	0.01 %	0.04 %

*Analyzed by SVL Analytical Utah (TNI) ID 000192015-1

Even though the data collection and analysis methods may be unbiased, the sample data are subject to random and systematic errors at different stages of acquisition, from field collection to sample analysis. Selecting the correct baseline condition (null hypothesis: H_o) therefore will be important. We can manage potential random and systematic errors through field replication, sampling design (where and when to sample), lab duplicates, and setting an appropriate probability threshold for Type I errors in hypothesis testing. For our study, we will use $\alpha = 0.05$ as the probability criteria for false rejection (i.e., rejecting the null hypothesis when it is really true) while being aware of the possibility of Type II errors (β; not rejecting the data when it is false), and thereby recognizing the importance of the power ($1-\beta$) of the statistical test being applied.

Data Quality Objectives and QA/QC Management

QA/QC Management

Project-specific quality assurance and quality control (QA/QC) protocols specified in this Work Plan will be followed. Relevant samples, including field testing and field quality control (QC) samples, are collected in accordance with DEP-SOP-001/01 (FDEP 2017b). Applicable sections of DEP-SOP-001/01 include, but are not limited to, field sample collection procedures, decontamination procedures, field testing, and quality control requirements. Samples will be analyzed according to the provisions within the laboratory's QA/QC documents (DBE 2021c). These documents are periodically updated, and therefore, the most recent version of the QA/QC documents details the specific laboratory analyses' data quality objectives for this project at the time of sample analysis. Data are verified and qualified when necessary, in accordance with laboratories' QA/QC documents.

DBE shall perform data verification, including performing the following QC checks as part of their routine data validation: reversal checks (for example, verify that TP>SRP), sensitivity checks, matrix interference checks, precision checks, and accuracy checks for applicable chemical analytes. Criteria and recommended corrective actions will be those stated in the chemical analytical laboratories' QA/QC documents or agreed upon with the Project Manager.

DBE shall also review the field QA/QC results according to DEP-SOP-001/01 (FDEP 2017b). All raw data and findings shall be available for District review. A final QA'd data report for Phase I will be provided with Final Summary report for Experiments I, II, and III, Deliverable 5b (see Section 0).

General Quality Control Protocols

The following sections define the quantitative approach to evaluating QC samples for this project. Laboratory QC samples will be analyzed at the frequency defined in the laboratories' Quality Manual or SOP. Acceptance criteria for quality control samples for analyses will follow the laboratories' Quality Manual or SOP. Any required corrective actions for the respective parameter will be performed as outlined in the laboratories' Quality Manual or SOP.

Quality Objectives and Criteria

The Data Quality Objectives for this demonstration project will be expressed by using Data Quality Indicators (DQIs). These DQIs include both quantitative and qualitative indicators. Quantitative indicators for this project are precision, bias, accuracy, and sensitivity. These will be described using the methodology (quality control samples) specified in the list below (**Table 7**). Acceptance criteria for these QC samples are the QC limits stated for the specific analysis.

The qualitative indicators are representativeness, comparability, and completeness. Representativeness will be described by the design and collection procedures for the project. Comparability will be documented by sampling comparisons between the soil selected for

Experiment II and a range of soils examined in Experiment I, then confirmed with multiple soils in Experiment III to determine if the associated quantitative DQIs have been met. Completeness of the data set will be expressed by comparing the final data set with the proposed collection regime. When a corrective action is required for any analytical method the laboratories will follow the procedures described in the laboratory's respective quality manual.

Table 7. Quality control samples used as Data Quality Indicators.

Data Quality Indicator	Determination Methodology (Quality Control Samples)
Precision	Laboratory Duplicates, Experimental Replicates, Field Duplicates, Method Blanks, Field Blanks
Bias	Check Standards, Method Spikes, Method Blanks, Field Blanks
Accuracy	Method Spikes, Check Standards
Sensitivity	Analytical method detection limits (See **Table 6**)

Instrument/Equipment Testing, Inspection and Maintenance

The Field Operations Manager will ensure that all equipment is operational and will resolve any deficiencies. A maintenance log will be kept for each field equipment/instrument to record maintenance and calibration information. Spare parts for any critical equipment will be maintained in inventory. Laboratory equipment and instruments used by DBE laboratories will be tested, inspected, and maintained in accordance with their laboratory manuals.

Documentation and Records Management

Original or master copies of laboratory generated records will be maintained by the originator. DBE will maintain both current and historical method and operating procedures so, at any given time, the conditions that were applied to a sampling event can be evaluated.

Records associated with any laboratory analyses will be maintained in a manner that will protect the physical condition and/or integrity of the records. Storage of records submitted to the District shall be maintained and stored in accordance with the laboratory's quality manual. Corrections of data records shall follow the applicable requirements specified in the FDEP SOPs, DBE's field quality manual, and the laboratories' quality manual and/or the project plan.

DBE shall submit all relevant field and laboratory data collected under this project to the District in an Excel spreadsheet in a format that is compatible with uploading the data to a District database. Laboratory records will be retained for a minimum of five years after the creation date as specified in the FDEP Quality Assurance Rule.

Other Work Plan Components

Field health and Safety Plan

DBE maintains a Safety Plan outlining safety concerns and procedures for DBE employees conducting field research activities in various locations including, but not limited to, the Everglades STAs. DBE's field personnel are trained and instructed to follow this safety plan for personal protection at all times.

The Safety Plan covers preventative and emergency aspects of field-related work including, but not limited to, infrastructure maintenance, weather, vehicle safety, and sampling activities. The plan provides first aid procedures and contact information for nearby emergency services.

Plans for Collaborative Publication

DBE and the District will co-author one scientific manuscript for publication in a peer-reviewed journal. DBE shall take the lead in preparing the draft manuscript, which will be submitted to the District for review and collaborative revision. Any revisions to the manuscript which may be required after peer review by the journal are beyond the scope of this project but will be pursued by DBE in coordination with the District.

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