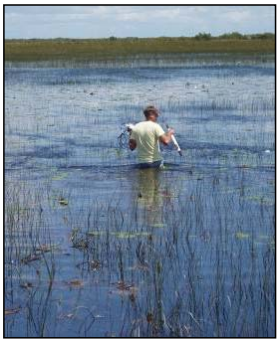
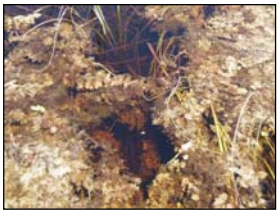




An Evaluation of the Role of Sulfate in South Florida Wetlands

Task 2 Final Report: Laboratory Incubations for Screening the Effects of Sulfate on Organic Matter Decomposition and the Release of Phosphorus

Contract #: 4600001324

October 5, 2009

Prepared for:

South Florida Water Management District

Everglades Agricultural Area – Environmental Protection District

Prepared by:

DB Environmental, Inc
365 Gus Hipp Blvd.
Rockledge, FL 32955

Table of Contents

List of Figures

List of Tables

Executive Summary

This report presents the results of laboratory incubations (Task 2) designed to test the potential effects of elevated water column sulfate levels on microbial respiration and P release for soils collected from unimpacted and impacted wetlands in south Florida. Soils from four separate sites (WCA-3A, WCA-2A site U3, STA-2 and STA-5), ranging from low P and low sulfate to high P and high sulfate environments, were examined. The soils were subjected to anaerobic laboratory incubations to evaluate P release and organic matter decomposition in response to sulfate amendments (0.33 mM [0.32 mg/L] or 1.0 mM [96 mg/L]).

Three processes have been invoked in the literature as to why sulfate enrichment can lead to P release from soils under anaerobic conditions:

- Alkalinization (leads to more favorable pH environment for decomposition)
- Higher electron acceptor concentrations (leads to higher rates of decomposition)
- Formation of FeS_x compounds (mobilizes Fe-associated P)

For the wetland soils examined, alkalinization due to the hydrogen ion-consuming reaction of sulfate reduction was not an overriding process. We found that pH decreased in the incubation vessels, and that increases in alkalinity were more attributable to $CaCO_3$ dissolution than sulfate reduction. Moreover, all the soils exhibited near circum-neutral pH levels, with moderate to high concentrations of native alkalinity.

Amending the soils with sulfate did not result in either more microbial respiration as measured by CO_2 and CH_4 gas emissions, or increased P mobilization. This implies that an addition of 0.33 or 1.0 mM of sulfate did not translate into meaningful enhanced decomposition of organic matter, likely due to limitations imposed by substrate quality and low P in the oligotrophic WCAs. In the more enriched STA sites, where P release did occur during anaerobic incubation, sulfate enrichment still did not result in more net P release because the source of P was likely due to dissolution of an inorganic substrate ($CaCO_3$) that was sensitive to soil solution acidity. Neither oxidation-reduction potential (ORP) nor electron acceptor concentrations, as influenced

by sulfate additions, would be expected to accelerate $CaCO_3$ dissolution and associated P release.

Soils from only one of the study sites (WCA-3A) had iron (Fe) concentrations (0.73 – 1.0%) that would be expected to be high enough to be associated with substantial levels of soil P. Porewater Fe:SRP ratios (> 83:1 wt/wt) observed at this site suggest that Fe theoretically could be controlling the release of P from the soil. However, the available P pools in the soil at this site were too low to result in a measurable response in this P-limited environment.

The lack of a P response to sulfate amendments observed in the incubation studies are in agreement with findings from long-term field monitoring efforts. For example, after a prolonged history (over 40 years) of sulfate enrichment in WCA-2A, surface water and porewater P concentrations are not elevated at U3, a site which historically has been considered relatively pristine, exhibiting little P enrichment and a balanced biological community.

The results from other research platforms, including field-scale mesocosms and chemical gradient analyses in STAs, which are designed to further explore the effects of elevated sulfate levels on P release from soils and to examine the toxicity of sulfide on plant communities and species, will be presented in future reports.

Introduction

Sulfate enrichment in the Everglades Water Conservation Areas (WCAs), Stormwater Treatment Areas (STAs), and northern regions of Everglades Protection Area (EPA) has raised concerns about potential toxic effects to plants, increased soil phosphorus (P) mobilization, and enhanced organic matter decomposition. Bates et al. (2002) reported sulfate concentrations of 24 mg/L for Lake Okeechobee, and even higher values in canals that drain the Everglades Agricultural Area (mean of 68 mg/L). These sulfate levels contrast with the low ambient surface water sulfate levels (< 1 mg/L) in the Everglades National Park (ENP) (Scheidt and Kalla 2007) and the low concentrations (<1 – 5 mg/L) reported for rainfall in south Florida (Bates et al. 2002; Scheidt and Kalla 2007). Porewater sulfide levels over 5 mg/L have been reported in the Everglades WCAs (Scheidt and Kalla 2007), an observation that has led some investigators to speculate that sulfate may be responsible in part for the historic encroachment of cattails into sawgrass stands in the WCAs (Axelrad et al. 2007).

Understanding the biogeochemistry of sulfur (S) in the Everglades is important because elevated sulfate levels may contribute to P release from soils - a process termed "internal eutrophication" (Lamers et al. 2002; Smolders et al. 2006a). Sulfate can act as an electron acceptor in waterlogged soils, and therefore may accelerate the decomposition of organic matter in reduced environments where other electron acceptors (e.g., oxygen, nitrate) are not available (Wright and Reddy 2001). Sulfate reduction to sulfide also produces bicarbonate, which can buffer (neutralize) soil solution acids, creating a pH environment more conducive to organic matter decomposition (Smolders et al. 2006a). In turn, enhanced decomposition of organic matter can result in the release of P into the soil porewater. Finally, sulfides produced as a result of microbial sulfate reduction can reduce the availability of iron (Fe) to sequester phosphate (Zak et al. 2006; Guerts et al. 2008).

These processes were clearly elucidated by a wetland mesocosm study performed in the Netherlands that demonstrated an increase in porewater sulfide, alkalinity, ammonium and soluble reactive P (SRP) in response to increases in surface water sulfate levels (Lamers et al. 1998). Moreover, the increase in concentration of selected porewater constituents (i.e., ammonium, sulfide) in the Lamers et al. study was thought to be responsible for observed

reductions in the growth rates of marsh plants, in particular *Carex nigra*. A thorough review on the internal eutrophication research in the Netherlands can be found in Smolders et al. (2006a).

Calcium (Ca) can also be an important element in mediating P cycling. Elevated daytime water column pH in submerged aquatic vegetation (SAV) communities may facilitate the co-precipitation of phosphorus with calcium carbonate ($CaCO_3$) (Picot et al. 1991; Moutin et al. 1992). Phosphorus removed from the water column in SAV communities may be deposited as relatively stable, high calcium, marl sediment (DB Environmental 2002). However, in areas of high productivity, bacterial respiration in the sediment may lower the pH to solubilize the $CaCO_3$-bound P.

While the Dutch studies point to the importance of sulfate as a precursor for sulfide toxicity and P release, there are several important differences between the wetlands in their studies and the Everglades. Many of their wetlands had significant groundwater intrusion that altered the water chemistry in surface and pore waters. Soil and water in the Dutch wetlands frequently contained high Fe and low Ca concentrations, which contrasts to the low Fe and high calcium concentrations in the Everglades. Lastly, the climate and plant communities are vastly different in the Everglades and wetlands in the Netherlands.

Even though the above processes are well understood from a theoretical standpoint, the extent to which they may occur in south Florida wetlands is unknown, especially the interactions among Ca, Fe, and sulfur (S) in affecting the P retention capacity. This report presents the results of laboratory incubations designed to test the potential effects of elevated water column sulfate levels on microbial respiration and P release for soils collected from un-impacted and impacted wetlands (with respect to both P and S) in south Florida. The results from other research platforms, including field-scale mesocosms and chemical gradient analyses in STAs, which are designed to further explore the effects of elevated sulfate levels on P release from soils and examine the toxicity of sulfide on plant communities and species, will be presented in future reports.

Site Selection and Description

Soils from six separate sites, ranging from low P and low sulfate to high P and high sulfate environments, were examined (Table 1). The location of each sampling site is shown in Figure 1.

Table 1. Site locations, sampling date, and historical mean total phosphorus (TP) and sulfate (SO_4) concentrations in the water column at each of the four sites where soils were retrieved for the sulfate amendment experiments performed as laboratory incubations. See Revised Detailed Project Plan (Appendix A) number of samples that were averaged at each location. n.d. = no data. POR = Period of record.

		GPS coordinates (decimal degrees)		Historical Water Column Concentrations*		
Site	**Sampling Date**	**Latitude**	**Longitude**	**POR**	**SO_4 (mg/L)**	**TP (µg/L)**
WCA-3A slough	9/19/08	26.0432	-80.72742	January 2003 – February 2008	0.8	8
WCA-3A ridge	9/19/08	26.0432	-80.72742	January 2003 – February 2008	0.8	8
WCA-3A Slough	1/19/09	26.0432	-80.72742	January 2003 – February 2008	0.8	8
WCA-2A U3	1/15/09	26.2875	-80.41133	1978 – May 2008	39	7
STA-5 Cell 2A Mid N	8/25/08	26.4427	-80.93117	November 1998 – May 2008	n.d.	50
STA-5 Cell 2B Out S	8/25/08	26.4375	-80.88629	November 1998 – May 2008	33	39
STA-2Cell 1 In	6/2/09	26.4166	-80.48783	January 2003 – December 2007	66	78

* Data from DBHYDRO (WCA-2A U3; STA-2 Cell 1 In) and S. Newman (pers. commun.) (WCA-3A) of the SFWMD.

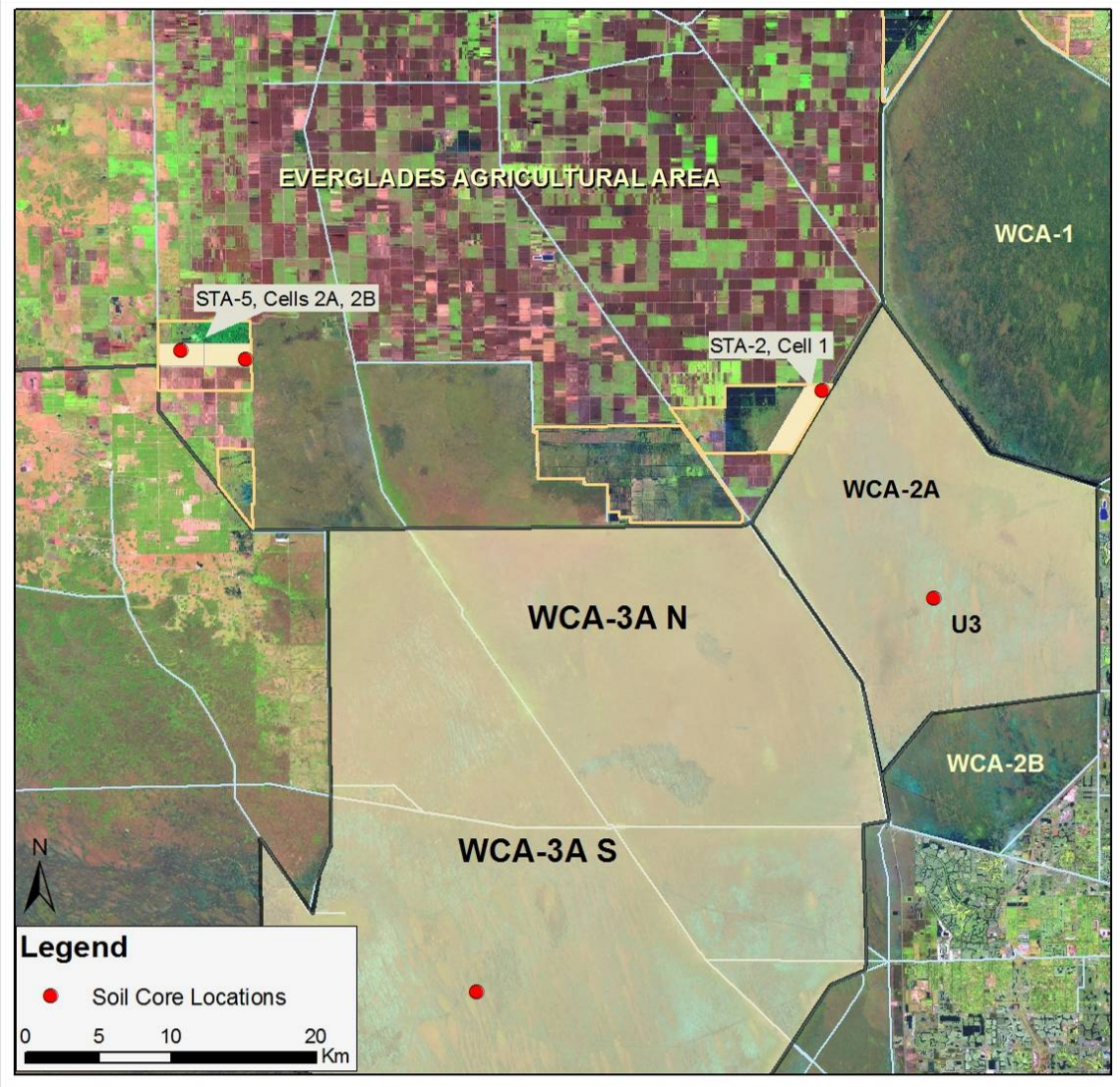


Figure 1. Locations of soil cores retrieved for the lab incubations with and without sulfate enrichment. Two separate sites (ridge and slough) were cored in WCA-3A.

What follows is a brief description of each wetland location where soils were cored and returned to the lab for incubation.

WCA-3A: Low Sulfate and Low P Environment

The landscape in WCA-3A consists of ridges of wet prairie and sloughs at slightly lower elevations. Approximately half of WCA-3A's water inputs originate from rainfall, with general water quality characteristics of the interior areas being more acidic, and lower mineral and

nutrient contents, than the other two sites evaluated in this study (Scheidt and Kalla 2007). Mineral-rich canal water enters WCA-3 at several points around its northern perimeter, giving rise to gradients in concentrations of P and other minerals downstream of the outflows (FDEP 2001; Scheidt and Kalla 2007).

The site located in WCA-3A was sampled on two occasions to discern if there may be seasonal effects on soil P release with sulfate amendments. The first, on September 19, 2008, included both ridge and slough (Figure 2). The ridge community is dominated by sawgrass (*Cladium jamaicense* Crantz), whereas the deeper slough community is characterized by spike rush (*Eleocharis cellulosa* Torr.), bladderwort (*Utricularia* spp.), and calcareous periphyton (cyanobacteria). The soil elevation difference between the two communities at the sampling site (Figure 2) is 23 cm. The second sampling on January 19, 2009 was performed only at the slough site.

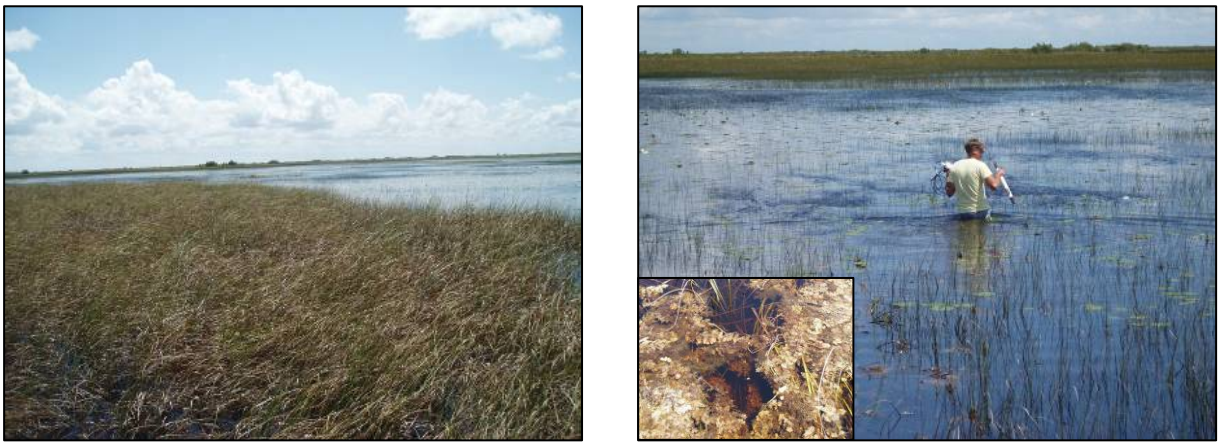


Figure 2. The ridge (left panel) and slough (right panel) vegetation communities where soil, surface water, and pore water samples were collected on September 19, 2008 and January 19, 2009 (slough only). The inset is a photo looking down into the water column showing the calcareous periphyton, bladderwort (*Utricularia purpurea*), and emergent spikerush (*Eleocharis cellulosa*).

WCA-2A U3: High Sulfate and Low P Environment

Site U3 in WCA-2A lies 10-11 km downstream of the S10 control structures, through which agricultural drainage water (ADW) was routed to WCA-2A from the EAA prior to the

construction of STAs. It represents an environment that has been exposed to high sulfate, but low P concentrations (Table 1), for the last 40+ years. Phosphorus was removed by the substrates and biota in upstream areas of WCA-2A prior to reaching U3, but sulfate was not. Site U3 provides an ideal environment for examining the effects of sulfate enrichment in an environment that was sulfate-enriched, but P impoverished. The vegetation communities at this site (Figure 3) are similar to those in WCA-3A.

Figure 3. Pore water sampling in the slough at site U3 on January 15, 2009.

STA-5: Moderate Sulfate and High P Environment

STA-5 is 9 years in existence, and was previously farmed. The central flow path (Cells 2A and 2B), where we sampled at two locations (Figure 1), consists of emergent vegetation (Cell 2A) and SAV or open water (Cell 2B) (Figure 4).

Figure 4. Cattail (*Typha* sp.) and smartweed (*Polygonum*) in STA-5 Cell 2A (top panel) and open water (bottom left panel) and hydrilla (bottom right panel) in Cell 2B.

STA-2 Cell 1 Inflow Region: High Sulfate and High P Environment

STA-2 Cell 1 is dominated by emergent vegetation (mixed cattail and sawgrass). It has existed as an STA for 10 years, and was not farmed prior to flooding.

STA-2 Cell 1 was sampled in the inflow region during June 2009, two weeks after it had been rehydrated. Standing water had been absent in the cell for 76 days due to a prolonged drought (Figure 5).

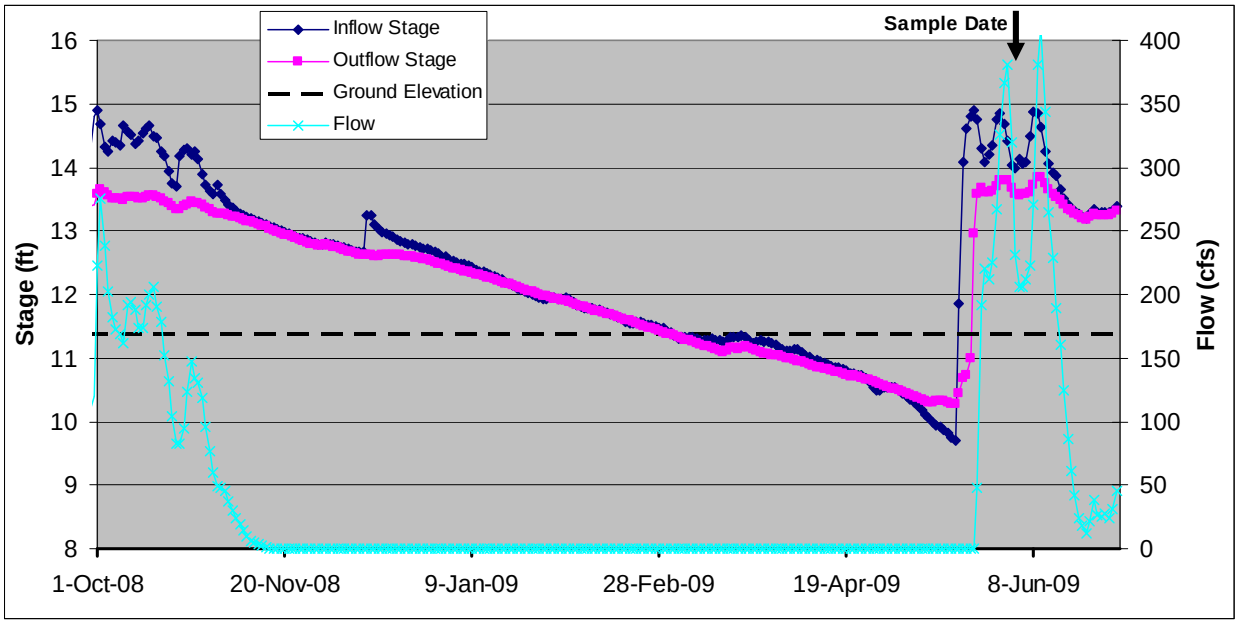


Figure 5. Ground elevation, stage, and flow for STA-2 Cell 1 between October 1, 2008, and June 30, 2009.

Methods

Field Methods

Three or four replicate cores at each location were retrieved in the field with a 10-cm i.d. aluminum corer (Figure 6). After removing the benthic algal layer and any periphyton "sleeves" (present in WCA-2A U3 and WCA-3A), and prior to subsequent soil extrusion in the field, soil redox potential was measured at a depth of 3 – 7 cm below the soil surface (Figure 7). Calibration of the Accumet or Hach Pt electrode (referenced to a Ag/AgCl electrode in saturated KCl) with either a Corning pH103 or a Hach sensION2 pH/ISE meter was accomplished by measuring the potential of a Light's solution (Light 1972). Electrode potentials of the samples were subsequently adjusted to a standard hydrogen electrode and deviations in temperature from 25°C.

Soil within each core was then extruded at 0-4, 4-10, and 10-23 to 30 cm depth intervals, with each depth interval composited among the three or four replicate cores in zip-lock bags. The composited cores were transported on ice to the lab where they received a blanket of N_2 gas and were stored at 4°C in the dark.

Figure 6. Soil core retrieval in WCA-3A on September 19, 2008. The inset shows the top of the marl layer for a soil core retrieved at WCA-2A U2 on January 15, 2009.

Figure 7. Measuring redox potential in a soil core from the inflow region of STA-2 Cell 1 on June 2, 2009.

In addition to coring each site in Table 1 for soil, surface and pore waters were also collected from three nearby locations at each site. A clean 125-mL polypropylene bottle was gently immersed 0.1 m below the water surface for collecting surface water, being certain that particles associated with the nearby vegetation communities were excluded from the sample. Pore waters were sampled by deploying a "sipper" (Figure 8) that penetrated the soil and subsampled the pore water from within the 6-10 cm horizon. The pH and temperature readings were performed with a Hach pH Pt series electrode (model part number 51910-00) and Hach sensION2 pH/ISE meter.

The chemical species (Fe, SRP, sulfide) most sensitive to oxidizing conditions, plus ORP, were subsampled immediately from the 60 mL syringe containing porewater sampled with the "sipper". Sulfide (2.0 mL sample volume) was immediately preserved with 1 drop of 0.5 M ZnAc and 2 drops of 10 N NaOH. Porewaters designated for Fe and SRP were filtered through a 0.45 μm syringe filter (Whatman PES), before either being preserved with concentrated nitric acid to pH < 2 (Fe analysis) or placed on ice (SRP analysis). Sample filtration also occurred for the chemical species less sensitive to oxidation (Ca, ammonia, TSP, and sulfate). Alkalinity

samples were not filtered. The ammonia and TSP samples were preserved with concentrated sulfuric acid to pH < 2; nitric acid was the preservative for Ca. Alkalinity and sulfate samples were not preserved other than being kept cold (4°C).

Figure 8. Deployment of the "sipper" for sampling pore waters at the ridge location in WCA-3A on September 19, 2008.

Analytical Methods and Quality Control

A Quality Assurance Project Plan was completed and authorized by representatives from the SFWMD, EAA-EPD, and DBE in January 2009. It can be found in the Revised Detailed Project Plan (Appendix A). Table 2 lists the method references, method detection limits or reporting limits, and QA acceptance criteria by matrix for the analytes measured in this investigation. Because total oxidizable sulfur (TOS), carbon dioxide (CO_2) and methane (CH_4) quantity analyses were developed "in house" without a Standard Operating Procedure, those analyses are described in more detail below.

We used a modification of the TOS method presented by Smolders et al. (2006b). Wet soil (12.5 mL) from each soil horizon (0-4, 4-10, and 10-30 cm) cored in WCA-3A, WCA-2A U3, and STA-2 Cell 1 was inserted into a 125-mL Erlenmeyer flask and 100 mL of deionized water added. After mixing, the soil slurry was flushed with either lab air or with compressed N_2 gas. The N_2 gas was administered to the incubation flasks either by purging over an initial 24 hours followed by tightly sealing the flasks with rubber stoppers for the remainder of the incubation period (WCA-3A and WCA-2A U3), or daily purging for one hour and then tightly sealing with rubber stoppers (STA-2 Cell 1). The aerated flasks were continuously purged during the entire 29- or 30-day incubation period. Each soil horizon receiving air or N_2 gas was replicated three times. The dissolved sulfate concentrations were determined in filtered (0.45 μm) water at the end of the 29- or 30-day incubation. The concentration of the sulfate reached in the aerated flasks minus the concentration reached in the N_2-flushed flasks was used to calculate the maximum concentration of oxidizable sulfur in units of mg per g dry wt of soil.

Table 2. Analytical method references, detection limits, and acceptance criteria for laboratory spikes and duplicates followed in the chemical analyses of water, soil, and gas. † denotes analytes for which DBE is NELAP certified. †† denotes subcontract lab (SVL Analytical) is NELAP certified.

				Acceptance Criteria			
				Spikes (% Recovery)		Lab Dup (% rsd)	
Analyte	Method	Matrix	MDL	Low Range	High Range	Low Range	High Range
Alkalinity	EPA 310.1	porewater	3 mg $CaCO_3$/L	75-125	85-115	0-30	0-20
Ammonia†	EPA 350.1	porewater	0.015 mg/L	70-130	80-120	0-40	0-25
Calcium (dissolved)†	EPA 215.1	porewater	0.02 mg/L	80-120	90-110	0-30	0-10
Eh	n/a	porewater	n/a	n/a	n/a	n/a	n/a
Iron (dissolved)	Modified Bathophenanthroline	porewater	0.025 mg/L	75-125	85-115	0-40	0-40
pH	EPA 150.1	porewater	n/a	n/a	n/a	±0.2 units	±0.2 units
SRP†	SM 4500P-F/DBE SOP OPO_4	porewater	2 µg/L	75-125	85-115	0-30	0-20
Sulfate†	EPA 375.4/SM 4110 B	porewater	0.5/1.0 mg/L	70-130	80-120	0-40	0-20
Sulfide†	DBE SOP Sulfide	porewater	0.006 mg/L	65-135	75-125	0-50	0-25
TSP†	SM 4500P-F	porewater	3 µg/L	70-130	80-120	0-40	0-20
Alkalinity	EPA 310.1	surface water	3 mg $CaCO_3$/L	75-125	85-115	0-30	0-20
Ammonia†	EPA 350.1	surface water	0.015 mg/L	70-130	80-120	0-40	0-25
Calcium (dissolved) †	EPA 215.1	surface water	0.02 mg/L	80-120	90-110	0-30	0-10
Eh	n/a	surface water	n/a	n/a	n/a	n/a	n/a
Iron (dissolved)	Modified Bathophenanthroline	surface water	0.025 mg/L	75-125	85-115	0-40	0-40
pH	EPA 150.1	surface water	n/a	n/a	n/a	±0.2 units	±0.2 units
SRP†	SM 4500P-F/DBE SOP OPO_4	surface water	2 µg/L	75-125	85-115	0-30	0-20
Sulfate†	EPA 375.4/SM 4110 B	surface water	0.5/1.0 mg/L	70-130	80-120	0-40	0-20
Sulfide†	DBE SOP Sulfide	surface water	0.006 mg/L	65-135	75-125	0-50	0-25
TP†	SM 4500P-F/EPA 365.2	surface water	3 µg/L	70-130	80-120	0-40	0-20
TSP†	SM 4500P-F	surface water	3 µg/L	70-130	80-120	0-40	0-20
DOP†	Calculation (TSP-SRP)	surface water	3 µg/L	n/a	n/a	n/a	n/a
PP†	Calculation (TP-TSP)	surface water	3 µg/L	n/a	n/a	n/a	n/a

				Acceptance Criteria			
				Spikes (% Recovery)		Lab Dup (%rsd)	
Analyte	Method	Matrix	MDL	Low Range	High Range	Low Range	High Range
Dry Wt or % Dry Wt	ASA 21.2	soil	0.01%	n/a	n/a	n/a	n/a
0.1M NaOH-SRP†	DBE SOP OPO_4	soil	*	75-125	85-115	0-30	0-20
0.1M NaOH-TP†	COE 3-227/EPA 365.2	soil	*	70-130	80-120	0-40	0-20
0.5M NaHCO3-SRP†	DBE SOP OPO_4	soil	*	75-125	85-115	0-30	0-20
Bulk Density	ASA 13	soil	0.001 g dry wt/cc	n/a	n/a	n/a	n/a
Bulk Dry or Wet Wt	ASA 21.2	soil	0.01 g	n/a	n/a	n/a	n/a
Cellulose	AOAC 973.18	soil	4.31%	n/a	n/a	0-30	0-30
Core Depth	n/a	soil	n/a	n/a	n/a	n/a	n/a
Eh	n/a	soil	n/a	n/a	n/a	n/a	n/a
Total Recoverable Fe (TFe)†	EPA/ SW 7380	soil	250 mg/kg	75-125	85-115	0-40	0-20
Lignin	AOAC 973.18	soil	1.98%	n/a	n/a	0-30	0-30
Microbial Biomass P†	Calculation	soil	*	n/a	n/a	n/a	n/a
TS††	D423-9 and ASTM E-1915-07	soil	0.0100%	80-120	80-120	0-14	0-14
TC†	DBE SOP MVP	soil	0.12%	75-125	80-120	0-20	0-10
TCa†	EPA/SW 7140	soil	8 mg/kg	75-125	85-115	0-30	0-15
TIC†	DBE SOP MVP/COE 3-73 (Calculation)	soil	0.58%	75-125	75-125	0-20	0-15
TN†	DBE SOP MVP	soil	0.09%	80-120	85-115	0-20	0-20
TOC†	DBE SOP MVP/COE 3-73	soil	0.12%	75-125	80-120	0-20	0-10
TOS†	EPA 375.4 (Sulfate)	soil	*	n/a	n/a	0-40	0-20
TP†	COE 3/227 EPA 365.2	soil	10 µg/g	65-135	75-125	0-40	0-30
Wet Wt	ASA 21.2	soil	0.01 g	n/a	n/a	n/a	n/a
CH_4	Gas chromatrography	gas	40 ppm (vol/vol)**	n/a	n/a	0-30	0-20
CO_2	Gas chromatrography	gas	400 ppm (vol/vol)**	n/a	n/a	0-30	0-20

* MDL's differ per sample based on dilution, dry/wet ratio, and wet weight subsample
** Lower reporting limit

Carbon dioxide and CH_4 concentrations were measured simultaneously by injecting 0.5 mL of sample headspace directly into a Varian 450 gas chromatograph equipped with flame ionization (FID) and thermal conductivity (TCD) detectors. Two 120-cm long x 0.16-cm wide columns (Supelco Hayesep Q 80/100 and Hayesep N 80/100) separated the CO_2 and CH_4 gases prior to detection. Oven temperature was 100°C, and the detector temperatures were 150°C (TCD) and 300°C (FID). The carrier gas was ultra-high purity (UHP) helium (He) (18 cm^3 min^{-1}). Flow rates for the fuel gas (UHP hydrogen or H_2) and the oxidizing gas (ultra-zero air) to the FID flame were 30 and 300 cm^3 min^{-1}, respectively.

Primary and secondary standards (Scott Specialty Gases; Praxair Specialty Gas & Equipment) were made up fresh in Tedlar bags on each day of sub-sampling the incubation vessels. Either UHP N_2 or He served as the diluent gas for the primary standard curve. The r^2-value for the standard curves ranged from 0.985 to 1.00 for either CO_2 or CH_4. The secondary standard, which originated from either a different gas supplier or from a different lot number if from the same supplier that the standard curve was made from, typically was within 80-120% agreement with the known value. Replicate injections deviated no more than 80-120% from each other. A continuing check standard (CCS), which was represented by one of the primary standards, was analyzed at a frequency of once every 20 samples, including QA (blanks, secondary standards). The acceptance criterion for the CCS was 85-115 % recovery.

The purity of UHP N_2 or He gases was measured each day that samples and standards were analyzed on the gas chromatograph. Blank subtractions were carried out on diluted samples if the blank concentrations were greater than the lower reporting limits of 40 ppm (vol/vol) for CH_4 and 400 ppm (vol/vol) for CO_2. This was accomplished by subtracting the difference between the blank value and the lower reporting limit, adjusted for the amount the sample had been diluted. For example, if the sample had been diluted 1:2, then one-half of the difference between the blank and the lower reporting limit would be subtracted from the sample value. Whenever a sample concentration was less than the reporting limit for either CH_4 or CO_2, then the concentration was assigned a value that was one-half the lower reporting limit.

Atmospheric pressure in the serum bottles was determined before sub-sampling the headspace on days 0, 1, 3, 5, 7, 10 and 14 days during the incubation with an Omega Digital Pressure Indicator 705. Production of CO_2 and CH_4 was calculated by Henry's Law constants (Wilhelm et al. 1977), universal gas law, pressure inside the serum bottles, gas concentrations in the headspace, and solution and headspace volumes. The dissociation of dissolved CO_2 to HCO_3^- ions (the major ionic carbonate species at pH < 8) was accounted for by using a modified Henderson-Hasselbach equation (Martens 1987).

The total mass of the CO_2-C and CH_4-C produced though day 10 of the incubation was divided by the initial dry weight of the soil to convert the terminal C flow to a basis of the soil mass incubated. For the percentage of the soil organic C mineralized to CH_4 and CO_2, the sum of the CH_4-C and CO_2-C evolved by day 10 of the incubation was divided by the soil organic C originally present in the serum bottle.

Laboratory Incubation Methodology

Incubations using subsampled 0-4 and 4-10 cm soil horizons from zip-lock bags stored at 4°C were initiated within 25 days of field collection. A 120-mL serum bottle received either 20 g of wet soil followed by 50 mL of low P and low sulfate water (purged with N_2 gas beforehand) from WCA-3A (except for STA-5 incubations, which received outflow water from STA-5) or 70 mL if soil was absent. Soil-free controls (sulfate amended and unamended) were included with every set of soil incubations to account for background CO_2 or CH_4 production, which were negligible compared to the bottles containing soil. Each serum bottle was capped with a butyl serum stopper aluminum-crimped top. Using a 100 mM stock solution, the ambient sulfate concentrations were incremented by either 0.33 mM [32 mg/L] or 1.0 mM [96 mg/l]) prior to rendering the bottle anaerobic by alternately evacuating (5 min) and purging (5 min) with UHP N_2 or He gas 3 times (total 30 minutes). These sulfate additions equaled 1.4-1.9 (0-4 cm) and 0.9-1.2 (4-10 cm) mg SO_4/g dry for the 0.33 mM, and 1.8-5.6 (0-4 cm) and 2.7-3.7 (4-10 cm) mg SO_4/g dry for the 1.0 mM sulfate amendments, respectively.

We chose 1.0 mM as the highest sulfate concentration since it approximates the maximum annual inflow sulfate concentration observed among the STAs. Each treatment and control was

replicated in four (without soil) or five (with soil) serum bottles (Figure 9). Either one or two of the replicates for each treatment or control group was sacrificed for initial concentrations of CO_2, CH_4, and solutes (Figure 9). The remaining three replicate bottles were incubated in the dark and at 25.5°C. Each serum bottle was vigorously shaken once each day for ~ 10 seconds. Soil depth, source of incubation water, sulfate amendment concentrations, and incubation time varied depending on the source of the soil (Table 3).

At times equal to 1, 3, 5, 7, 10, and 14 day, aqueous (2 mL) or headspace (0.5 mL) subsamples were collected from each serum bottle for SRP and gas (CO_2 and CH_4) analyses (subsampling of headspace gases did not occur on day 1). The 2.0 mL for SRP analysis and the 0.5 mL of headspace for gas analyses withdrawn at each time step were replaced with equal volumes of low P and low sulfate WCA-3A surface water and UHP N_2 or He gas. This amounted to a cumulative dilution of 9% of the aqueous volume and 4% of the headspace volume during a 14-day incubation. In addition to SRP and gas (CO_2 and CH_4) analyses, we sampled and analyzed the aqueous phase within each control and treatment group at the beginning and end of the incubation for pH, total soluble P (TSP), alkalinity, sulfate, sulfide ammonia, and dissolved Ca and Fe concentrations. The ORP was read at the end of the incubation only.

Statistical Analyses

The Shapiro-Wilks test for normal distribution of the data was performed with JMP (SAS Institute Inc., Cary, NC), version 4. All other statistical analyses were computed using VassarStats, an online web site (http://faculty.vassar.edu/lowry/VassarStat.html). We mainly tested whether sulfate enrichment yielded higher or lower concentrations of SRP, DOP, sulfide, and Fe within either the 0-4 or 4-10 cm soil depth after 14 days of anaerobic incubation. Whenever we used all data collected from all six subsampling dates (and not just day 14 data) to test for treatment differences in SRP concentration, the data were analyzed as a repeated measure (i.e., correlated samples) design. For the normally distributed data sets, we used a paired t-test for testing significant differences between two means. For testing significant differences among three or more means, we employed a one-way analysis of variance (ANOVA) followed by a *post hoc* multiple comparison test (Tukey HSD) when the overall ANOVA was significant. For non-normal data sets, we used nonparametric tests for testing

significant difference(s) between distributions of two (Mann-Whitney) or three (Kruskal-Wallis) independent samples. The level of significance (α) was 0.05 for all comparisons.

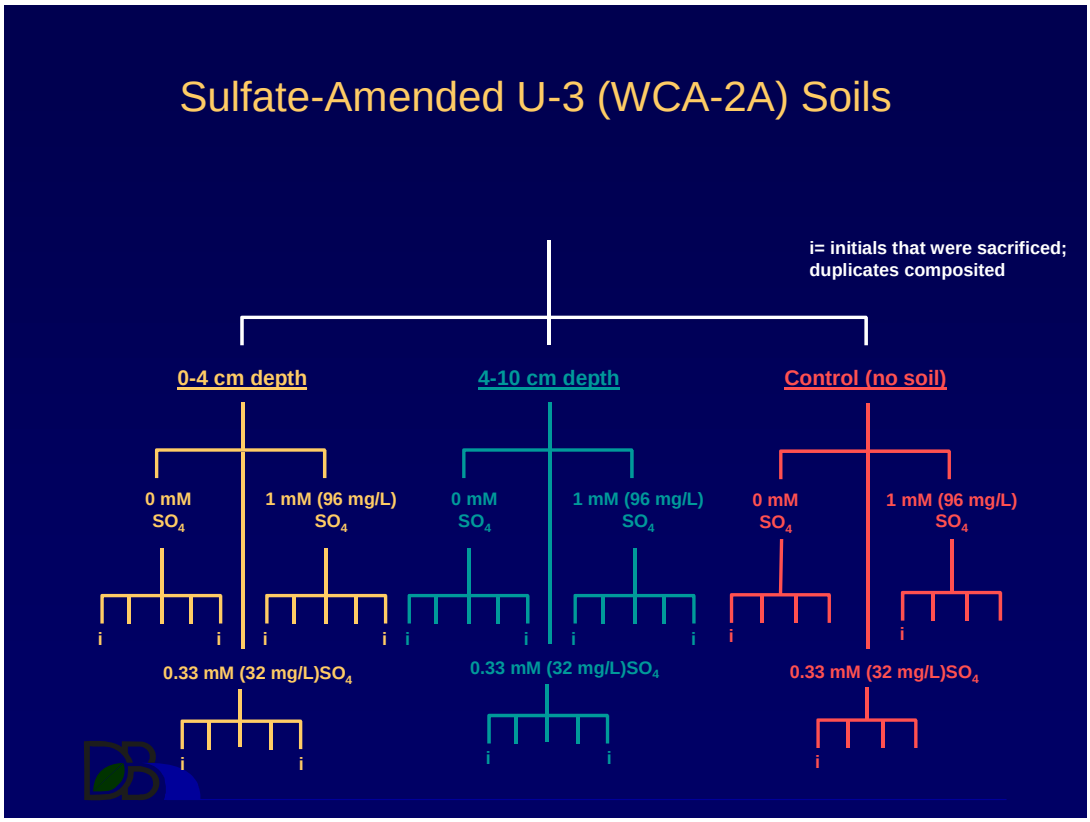


Figure 9. Schematic of experimental design for one of the five soil incubations where two soil depths (0-4 and 4-10 cm) were each exposed to WCA-3A surface waters with and without sulfate amendments of 0.33 and 1.0 mM. i = initials (n=2 for soils and n=1 for water-only controls). The replicate initials for the soils were composited before subsampling for water chemistry.

Table 3. Soil depths, source of incubation water, sulfate amendments and the duration of incubation for each location where anoxic soil incubations were performed in the lab**.** Soil depths, sulfate amended concentrations, and duration of incubation varied depending on whether incubation was part of the Work Plan (see Appendix), or was a preliminary "screening" experiment to determine ranges of analytes produced.

Site	Soil Depth(s) (cm)	Source of Incubation Water	Sulfate Amendments (mM)	Duration of Incubation (days)
WCA-3A slough	0-4	WCA-3A	1.0	14
	4-10		1.0	14
WCA-3A ridge	0-4	WCA-3A	1.0	14
	4-10		1.0	14
WCA-3A slough	0-4	WCA-3A	0.33 and 1.0	14
	4-10		0.33 and 1.0	14
WCA-2A U3 slough	0-4	WCA-3A	0.33 and 1.0	14
	4-10		0.33 and 1.0	14
STA-5 Cell 2A Mid N	0-4	STA-5 Out	1.0	7
STA-5 Cell 2B Out S	0-4	STA-5 Out	1.0	14
STA-2 Cell 1 In	0-4	WCA-3A	0.33 and 1.0	14
	4-10		0.33 and 1.0	14

Results

Soil Characteristics

All soils could be characterized as organic, varying in organic C content between 17.9 and 47.4%, 32.1 and 47.0%, and 37.4 and 48.0%, for the 0-4, 4-10, and 10-30 cm soil horizons (Tables 4 – 6). The N content ranged among the surficial (0-4 cm) wetland soils from 1.83% to 3.66% (Table 4). Nitrogen concentrations increased or remained approximately the same with soil depth at most of the wetland sites (Tables 5 and 6). Total recoverable iron (TFe) concentrations in the 0-4 cm depth layer ranged from 0.08% at WCA-2A U3 to 1.00% at WCA-3A sampled on Jan. 19, 2009. Decreases in TFe in the 4-10 cm soil depth occurred at all sites except WCA-2A U3 (Table 5). However, TFe concentrations increased again in the 10-30 cm soil layer at all sites except STA-2 Cell 1 (Table 6). The uppermost 0-4 cm soil layer was enriched with Ca at all sites, with the soil from WCA-2A U3 exhibiting the highest Ca content (16.4%). The Ca concentrations decreased with soil depth at all stations (Tables 4 – 6).

Total S content in the upper 0-4 cm of soil was between 0.44% and 0.76% (dry wt) for the four sites (WCA-3A Ridge, WCA-3A Slough, WCA-2A U3, and STA-2 Cell 1 Table 4). The soil TS concentrations in the 4-10 cm soil depth horizon increased at most of the sites (0.63% to 0.91% by dry wt). The range of the TS content in the 10-30 cm soil stratum widened to 0.46% - 1.2% (Table 6). The greatest increase in sulfur content with depth was in the WCA-2A U3 soil, where the TS content increased from 0.44% to 0.91% to 1.2% in the 0-4 cm, 4-10 cm, and 10-30 cm depth horizons, respectively.

Total oxidizable S, which is a measure of the reduced sulfur in organic and inorganic matrices that can be oxidized within 29-30 days of continuous aeration, comprised only a small percentage (0.4-11.8%) of the TS. Concentrations of TOS and the percentage composition of the soil consisting of TS were generally higher in the 0-4 cm soil layer, and decreased with soil depth (Tables 4 – 6). WCA-2A U3 soil contained higher TOS concentrations than the WCA-3A soil at all depth strata.

Total P concentrations in the soils varied widely; the slough soils in the WCAs contained less P in the 0-4 and 4-10 cm soil depths than the ridge station in WCA-3A or the STAs (Tables 4 and

5). However, the differences in soil P between the WCAs and STA-2 Cell 1 narrowed at the 10-30 cm depth (187-401 μg/g dry for the WCAs vs. 248 μg/g dry for STA-2 Cell 1).

The distribution of P among the different pools varied across locations (Tables 4 – 6). Concentrations of the porewater + NH_4-Cl extractable P pool (the labile pool) were low in the two WCA soils at all soil depths (0.05 – 0.6 μg/g dry; less than 0.3% of the TP), compared to STA-2 Cell 1 at all three sampled soil depths (20-52 μg/g dry; 0.3 to 8.0% of TP).

A larger proportion of the TP in the soil, but nevertheless still minor, consisted of Fe- and Al-bound P (Figure 10). Concentrations in this pool increased with soil depth in the WCAs, and decreased with soil depth in STA-2 Cell 1 (Tables 4 – 6). However, the concentration differences between the WCA soils and STA-2 Cell 1, albeit large, narrowed with soil depth. STA-2 Cell 1 soil contained 12-20 times more Fe-and Al-bound P in the 0-4 cm soil horizon (59 μg P/g dry) compared to the same horizon from either WCA-3A (5 μg P/g dry) or WCA-2A U3 (3 μg P/g dry); the differences narrowed to 4-7 times and 4-5 times at the 4-10 and 10-30 cm soil horizons, respectively.

Biogenic P, which is the difference between the NaOH-extracted SRP and TP and represents organic P and polyphosphates (Poly-P), constituted a higher proportion of the TP than the Fe- and Al-bound P in the three soil horizons collected in the WCAs (Tables 4 – 6). The biogenic P concentration was approximately the same as the Fe- and Al-bound P concentration for the three soil depths in STA-2 Cell 1 (Figure 10). Biogenic P concentrations increased with soil depth in the WCA-3A soil from 10 μg P/g dry (0-4 cm) to 18 μg P/g dry (10-30 cm). Concentrations decreased in the soils from STA-2 Cell 1 from 51 μg P/g dry in the surficial soil to 20 μg P/g dry in the 10-30 cm depth layer. Biogenic P concentrations were unchanged in the WCA-2A U3 soil depth intervals at 12-13 μg P/g dry.

The $NaHCO_3$-extractable SRP, a measure of the plant available P, was over 400 times higher (102 vs. <0.25 μg P/g dry) in the 0-4 cm soil layer at STA-2 Cell 1 than the same soil horizon of either of the slough soils (Figure 10; Table 4). The range between STA-2 Cell 1 and the WCA soils narrowed considerably with depth (Tables 4 – 6).

Table 4. Chemical characteristics of the 0-4 cm soil depth from locations within WCA-3A, WCA-2A U3, STA-5, and STA-2 Cell 1 In where soils were collected for anaerobic laboratory incubations. n.d. indicate where analyses were not performed. Each value represents a composite of 3 or 4 soil cores collected from each location.

	WCA-3A Ridge 9/19/2008	**WCA-3A Slough** 9/19/2008	**WCA-3A Slough** 1/19/2009	**WCA-2A U3 Slough** 1/15/2009	**STA-5 Cell 2A Mid N** 8/25/2009	**STA-5 Cell 2B Out S** 8/25/2009	**STA-2 Cell 1 In** 6/2/2009
TP (µg/g)	746	385	400	190	1030	1450	1570
TN (%)	3.40	3.36	3.66	1.83	3.44	3.22	2.13
TC (%)	47.6	40.1	42.4	25.7	47.5	38.5	34.2
TOC (%)	47.4	35.7	40.5	17.9	45.6	39.9	29.2
TIC (%)	<0.3	4.4	1.9	7.8	1.9	<0.3	5.0
TS (%)	0.67	0.64	0.76	0.44	n.d.	n.d.	0.50
TOS (mg/g)	n.d.	n.d.	0.32	0.52	n.d.	n.d.	0.4
Total Recoverable Fe (%)	0.83	0.80	1.00	0.077	0.53	0.79	0.14
Total Ca (%)	2.8	9.4	3.9	16.4	3.6	2.9	10.7
Lignocellulose Index (LCI)	n.d.	n.d.	0.71	0.68	n.d.	n.d.	0.65
Microbial Biomass P (µg/g)	n.d.	n.d.	53	41	n.d.	n.d.	116
1M NH_4Cl SRP (Readily Available) (µg/g)	n.d.	n.d.	<0.1	<0.1	n.d.	n.d.	52
0.1M NaOH TP (µg/g)	n.d.	n.d.	15	14	n.d.	n.d.	111
0.1M NaOH SRP (Fe- and Al-P) (µg/g)	n.d.	n.d.	5	3	n.d.	n.d.	59
0.1 M NaOH Organic P (Biogenic) (µg/g)	n.d.	n.d.	10	12	n.d.	n.d.	51
0.5M $NaHCO_3$ TP (µg/g)	n.d.	n.d.	9	6	n.d.	n.d.	165
0.5M $NaHCO_3$ SRP (Plant Available) (µg/g)	n.d.	n.d.	<0.5	<0.5	n.d.	n.d.	102
0.5M $NaHCO_3$ Organic P (µg/g)	n.d.	n.d.	8.4	5.5	n.d.	n.d.	62
C/N	14.0	11.9	11.6	14.0	13.8	12.0	16.1
C/P	638	1042	1060	1353	461	266	218
N/P	46	87	92	96	33	22	14
Fe/P	11	21	25	4.1	5.1	5.4	0.9
Bulk Density (g/cc)	0.024	0.029	0.054	0.064	0.19	0.22	0.081

Table 5. Chemical characteristics of the 4-10 cm soil depth from locations within WCA-3A, WCA-2A U3, and STA-2 Cell 1 In where soils were collected for anaerobic laboratory incubations. n.d. indicate where analyses were not performed. The redox values represent the mean and standard error of quadruplicate cores taken at each location. Each value represents a composite of 3 or 4 soil cores collected from each location.

	WCA-3A Ridge 9/19/2008	**WCA-3A Slough 9/19/2008**	**WCA-3A Slough 1/19/2009**	**WCA-2A U3 Slough 1/15/2009**	**STA-2 Cell 1 In 6/2/2009**
TP (µg/g)	595	452	311	234	883
TN (%)	2.83	4.07	3.34	2.62	2.35
TC (%)	44.5	45.9	38.9	36.3	40.1
TOC (%)	47.0	43.8	39.2	32.1	36.5
TIC (%)	<0.3	2.1	<0.3	4.2	3.6
TS (%)	0.65	0.77	0.63	0.91	0.73
TOS (mg/g)	n.d.	n.d.	0.17	0.41	0.8
Total Recoverable Fe (%)	0.62	0.69	0.71	0.16	0.22
Total Ca (%)	2.7	5.0	2.7	9.9	7.8
Lignocellulose Index (LCI)	n.d.	n.d.	0.76	0.74	0.69
Microbial Biomass P (µg/g)	n.d.	n.d.	58	31	57
1M NH_4Cl SRP (Readily Available) (µg/g)	n.d.	n.d.	0.2	0.3	27
0.1M NaOH TP (µg/g)	n.d.	n.d.	30	20	99
0.1M NaOH SRP (Fe- and Al-P) (µg/g)	n.d.	n.d.	12	7	48
0.1 M NaOH Organic P (Biogenic) (µg/g)	n.d.	n.d.	18	13	51
0.5M $NaHCO_3$ TP (µg/g)	n.d.	n.d.	15	13	81
0.5M $NaHCO_3$ SRP (Plant Available) (µg/g)	n.d.	n.d.	<0.5	<0.5	37
0.5M $NaHCO_3$ Organic P (µg/g)	n.d.	n.d.	14.5	13	45
C/N	15.7	11.3	11.6	13.9	17
C/P	748	1015	1251	1551	454
N/P	48	90	107	112	27
Fe/P	10	15	23	6.8	2.5
Bulk Density (g/cc)	0.091	0.13	0.116	0.092	0.111
Redox (mV)	n.d.	n.d.	178 ± 12.9	-50 ± 13.8	-126± 9.8

Table 6. Chemical characteristics of the 10-30 cm soil depth from locations within WCA-3A, WCA-2A U3, and STA-2 Cell 1 In where soils were collected for anaerobic laboratory incubations. n.d. indicate where analyses were not performed. Each value represents a composite of 3 or 4 soil cores collected from each location.

	WCA-3A Ridge 9/19/2008	**WCA-3A Slough 9/19/2008**	**WCA-3A Slough 1/19/2009**	**WCA-2A U3 Slough 1/15/2009**	**STA-2 Cell 1 In 6/2/2009**
TP (µg/g)	401	294	243	187	248
TN (%)	2.81	3.65	3.27	3.71	2.77
TC (%)	38.1	42.9	37.6	48.1	46.1
TOC (%)	38.7	41.7	37.4	48.0	46.6
TIC (%)	<0.3	1.2	<0.3	<0.3	<0.3
TS (%)	0.46	0.61	0.52	1.19	0.50
TOS (mg/g)	n.d.	n.d.	<0.02	0.37	0.05
Total Recoverable Fe (%)	0.73	0.88	0.82	0.24	0.13
Total Ca (%)	2.8	2.1	1.8	1.6	3.9
Lignocellulose Index (LCI)	n.d.	n.d.	0.79	0.81	0.73
Microbial Biomass P (µg/g)	n.d.	n.d.	16	10	16
1M NH_4Cl SRP (Readily Available) (µg/g)	n.d.	n.d.	<0.1	0.6	2.8
0.1M NaOH TP (µg/g)	n.d.	n.d.	21	17	40
0.1M NaOH SRP (Fe- and Al-P) (µg/g)	n.d.	n.d.	3.4	5	20
0.1 M NaOH Organic P (Biogenic) (µg/g)	n.d.	n.d.	18	12	20
0.5M $NaHCO_3$ TP (µg/g)	n.d.	n.d.	19.7	15	24
0.5M $NaHCO_3$ SRP (Plant Available) (µg/g)	n.d.	n.d.	1.2	<0.5	7
0.5M $NaHCO_3$ Organic P (µg/g)	n.d.	n.d.	18.6	15	17
C/N	13.6	11.8	11.5	13.0	17
C/P	950	1459	1547	2572	1859
N/P	70	124	135	198	112
Fe/P	18	30	34	13	5.2
Bulk Density (g/cc)	0.13	0.099	0.101	0.075	0.183

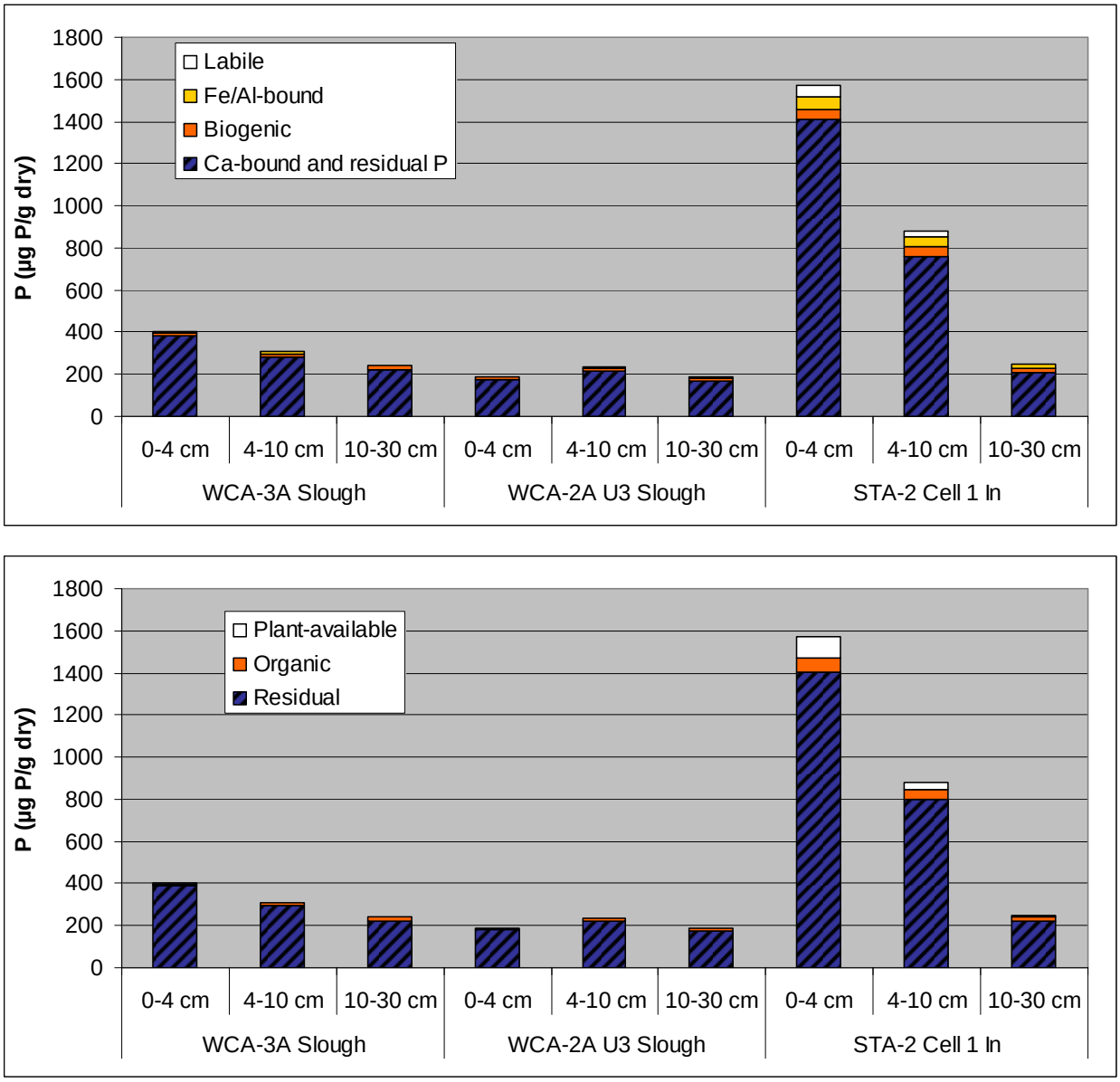


Figure 10. Initial phosphorus (P) concentrations in three soils at three horizons (0-4, 4-10, and 10-30 cm) after sequential extraction with 1.0 M NH_4Cl (labile) followed by 0.1 M NaOH (Fe/Al-bound and Biogenic) (top panel) or only 0.5 M $NaHCO_3$ (Plant-available and Organic) (bottom panel). Each value represents a composite of 3 or 4 soils cores collected at each location.

Surface Water

The pH of the surface waters was slightly alkaline (7.65-8.04) at the wetland sites (Table 7). The alkalinity varied by more than two-fold, from 119 mg $CaCO_3$/L at the ridge site in WCA-3A to 297 mg $CaCO_3$/L in the inflow region of STA-2 Cell 1. Soluble reactive P, dissolved organic P

(DOP=TSP-SRP), and total soluble P (TSP=SRP+DOP) concentrations were low (<8 µg/L) at the P-unimpacted sites in the WCAs, and were higher for the STAs (Table 7). Total P concentrations, which include all soluble P forms as well as particulate P, ranged from < 3 to 13 µg/L at the WCA sites, and 29-300 µg/L at the STA locations. Ammonia concentrations in the WCAs and STA-2 Cell 1 were low (<0.02 to 0.11 mg N/L). Sulfate concentrations in WCA-3A were less than the detection limit of 0.1 mg/L. The surface waters in WCA-2A U3, STA-5, and STA-2 Cell 1 exhibited sulfate concentrations that were greater than 6.4 mg/L, with the highest sulfate level of 84 mg/L measured at STA-2 Cell 1, which corresponded to the highest sulfide concentration (0.056 mg/L) found at the same site. The lowest sulfide concentrations were reported for WCA-3A (>0.006 to 0.010 mg/L), which correlated with the lowest sulfate concentrations (Table 7). Dissolved Ca concentrations varied within a tight range (45-57 mg/L) for WCA-3A, WCA-2A U3, and STA-5 out, but were nearly twice as high (101 mg/L) in Cell 1 of STA-2. Dissolved Fe concentrations were higher (0.18-0.20 mg/L) during the first sampling in WCA-3A than for the second sampling (0.05 mg/L) four months later; concentrations were <0.074 mg/L for the remaining wetland locations (WCA-2A U3 and STA-2 Cell 1). Water temperatures varied according to the season when samples were collected (Table 7), and dissolved oxygen (DO) concentrations were >5.08 mg/L for all surface waters.

Porewater

Porewater collected at a soil depth of 6-10 cm had a circumneutral pH and ranged from 6.76 to 7.42. Due to microbial respiration in the soil, porewater alkalinity and Ca concentrations more than doubled (> 260 mg $CaCO_3$/L and > 91 mg Ca/L) from surface water levels at the slough stations in WCA-3A and WCA-2A U3. Alkalinity increases were also noted in porewaters collected at STA-2 Cell 1 (Table 8). Porewater TSP concentrations were more than twice as high in comparison to the surface water concentrations at the WCA locations, yet porewater concentrations still remained very low (<8 µg/L). Porewater TSP concentrations in the STAs also were twice as high as respective surface water concentrations. Additionally, STA porewater SRP levels were 20-46 times higher than porewaters in WCA soils (Table 8). Levels of SRP in pore water were similar for the WCAs, averaging 6 µg/L and comprising approximately 40% of the TSP at these locations. . Mean pore water SRP concentrations in the STAs ranged from 290-700 µg/L and comprised 74-93% of the TSP. Ammonia concentrations exceeded 1.0 mg N/L in

all porewaters except at the ridge station in WCA-3A, which was 0.18 mg N/L. Porewater ammonia concentrations increased >20 times compared to surface water levels for all sites except STA-2 Cell 1, which exhibited a 13-fold increase. Sulfate concentrations measured in porewaters from WCA-3A were below method detection limits. Samples from WCA-2A U3, STA-5, and STA-2 Cell 1 exhibited higher sulfate concentrations in surface than in pore waters (Tables 7 and 8). Porewater sulfate concentration was highest (40 mg/L) at STA-2 Cell 1. Due to reducing conditions in the soils (Table 5), porewater sulfide concentrations increased at all sites relative to their respective surface water concentrations (Tables 7 and 8). Sulfide concentrations in the porewaters at WCA-3A were low (0.035-0.064 mg/L), probably because of high Fe concentrations reported for that WCA. These conditions lead to the tendency of sulfide to precipitate with reduced Fe to form FeS_x. For the other wetland sites (WCA-2A U3 and STA-2 Cell 1), which had dissolved Fe concentrations of 0.03 and 0.08, the sulfide concentrations reached as high as 5.5 and 4.6 mg/L in the porewaters.

Table 7. Chemical characteristics of the surface waters within WCA-3A, WCA-2A U3, STA-5, and STA-2 Cell 1 In where soils were collected for laboratory incubations. n.d. indicate where analyses were not performed. The values represent the mean and standard error of triplicate samples taken at each location except STA-5 where only one sample was collected.

	WCA-3A Ridge	**WCA-3A Slough**	**WCA-3A Slough**	**WCA-2A U3 Slough**	**STA-5 Cell 2A Mid-N**	**STA-5 2B-S**	**STA-2 Cell 1 In**
	9/19/2008	**9/19/2008**	**1/19/2009**	**1/15/2009**	**8/25/2008**	**8/25/2008**	**6/2/2009**
pH	7.86 ± 0.012	7.84 ± 0.012	7.66 ± 0.047	8.04 ± 0.022	n.d.	n.d.	7.65 ± 0.015
Alkalinity (mg $CaCO_3$/L)	119 ± 0.7	122 ± 1.2	151 ± 5.8	204 ± 14.4	n.d.	n.d.	297 ± 2.4
TP (µg/L)	13 ± 5.2	5 ± 0.6	<3	8 ± 1	300	n.d.	153 ± 2.2
TSP (µg/L)	5 ± 0.0	7 ± 1.5	4 ± 0.3	6 ± 0.3	177	n.d.	117 ± 1.0
SRP (µg/L)	3 ± 1.0	2 ± 0.6	<2	3 ± 0.6	139	n.d.	92 ± 6.7
DOP (µg/L)	2 ± 1.0	5 ± 1.0	3 ± 0.3	3 ± 0.9	38	n.d.	25 ± 7.7
Ammonia-N (mg/L)	<0.020	<0.020	0.042 ± 0.006	0.112 ± 0.016	n.d.	n.d.	0.079 ± 0.005
Sulfate (mg/L)	<1	<1	<0.5	16 ± 0.3	6.36	n.d.	84 ± 0.6
Sulfide (mg/L)	0.010 ± 0.000	0.009 ± 0.001	<0.006	0.031 ± 0.007	n.d.	n.d.	0.056 ± 0.007
Dissolved Ca (mg/L)	45 ± 0.3	45 ± 0.3	57 ± 0.3	47 ± 0.6	n.d.	n.d.	101 ± 1.2
Dissolved Fe (mg/L)	0.202 ± 0.036	0.183 ± 0.003	0.053 ± 0.006	0.034 ± 0.000	n.d.	n.d.	0.073 ± 0.003
Temperature (°C)	30.8 ± 0.03	30.3 ± 0.29	15.9 ± 0.30	13.8 ± 0.00	n.d.	n.d.	29.7 ± 0.20
DO (mg/L)	5.09 ± 0.105	5.51 ± 0.103	6.3 ± 0.31	8.22 ± 0.344	n.d.	n.d.	n.d.

Table 8. Chemical characteristics of the porewaters collected at the 6 – 10 cm soil depth within WCA-3A, WCA-2A U3, STA-5, and STA-2 Cell 1 In where soils were collected for laboratory incubations. n.d. indicate where analyses were not performed. The values represent the mean and standard error of triplicate samples taken at each location where only one sample was collected.

	WCA-3A Ridge	**WCA-3A Slough**	**WCA-3A Slough**	**WCA-2A U3 Slough**	**STA-5 Cell 2A Mid-N**	**STA-5 2B-S**	**STA-2 Cell 1 In**
	9/19/2008	**9/19/2008**	**1/19/2009**	**1/15/2009**	**8/25/2008**	**8/25/2008**	**6/2/2009**
pH	7.30 ± 0.04	6.91 ± 0.05	6.76 ± 0.07	7.12 ± 0.02	n.d.	n.d.	7.42 ± 0.01
Alkalinity (mg $CaCO_3$/L)	144 ± 7.0	260 ± 26.5	320 ± 15.3	432 ± 51.9	n.d.	n.d.	373 ± 33
TSP (µg/L)	17 ± 5.1	14 ± 1.5	15 ± 4.1	14 ± 2.1	750	790	344 ± 67.1
SRP (µg/L)	6 ± 1.7	6 ± 1.2	6 ± 0.3	6 ± 0.7	700	586	290 ± 59.3
DOP (µg/L)	11 ± 3.5	8 ± 0.3	10 ± 3.8	8 ± 1.7	50	204	54 ± 114.7
Ammonia-N (mg/L)	0.181 ± 0.061	2.16 ± 0.80	1.13 ± 0.21	4.05 ± 1.19	n.d.	n.d.	1.03 ± 0.34
Sulfate (mg/L)	<1	<1	<0.5	1.2 ± 0.173	3.4	0.5	40 ± 12.8
Sulfide (mg/L)	0.036 ± 0.012	0.035 ± 0.010	0.064 ± 0.008	5.5 ± 0.67	0.117	0.017	4.62 ± 2.83
Dissolved Ca (mg/L)	47 ± 1.7	91 ± 9.5	116 ± 3.0	106 ± 11.8	61	87	106 ± 3.3
Dissolved Fe (mg/L)	0.592 ± 0.044	1.17 ± 0.15	0.50 ± 0.04	0.030 ± 0.002	n.d.	n.d.	0.082 ± 0.008
Temperature (°C)	33.5 ± 0.35	31.3 ± 0.17	19.2 ± 0.38	17.7 ± 0.18	n.d.	n.d.	29.3 ± 0.20

SRP Releases During Anaerobic Incubations

This section presents the time course for SRP release from sulfate-amended and unamended soils incubated anaerobically in the dark at 25.5°C, for either 7 days (STA-5) or 14 days (WCA-3A, WCA-2A U3, and STA-2 Cell 1). Also included are data on the accumulation or depletion of key water quality constituents that help explain the SRP response to added sulfate.

Low Sulfate and Low P Environment: WCA-3A (sampled Sept. 19, 2008)

There were no significant differences ($p>0.05$)observed in SRP release in the unamended and amended (1.0 mM of sulfate) soil-less (control) slough water, or between the unamended and amended 0-4 cm deep ridge and slough soils during the 14-day incubation (Figure 11). There was an early release of SRP from the 4-10 cm horizon of the ridge soils in both the amended and unamended treatments, which dissipated by the end of the incubation. However, the SRP release was higher ($p<0.05$) by an average of 5 µg/L in the unamended than amended vessels, indicating no enhanced SRP release from ridge soils due to the 1.0 mM sulfate addition.

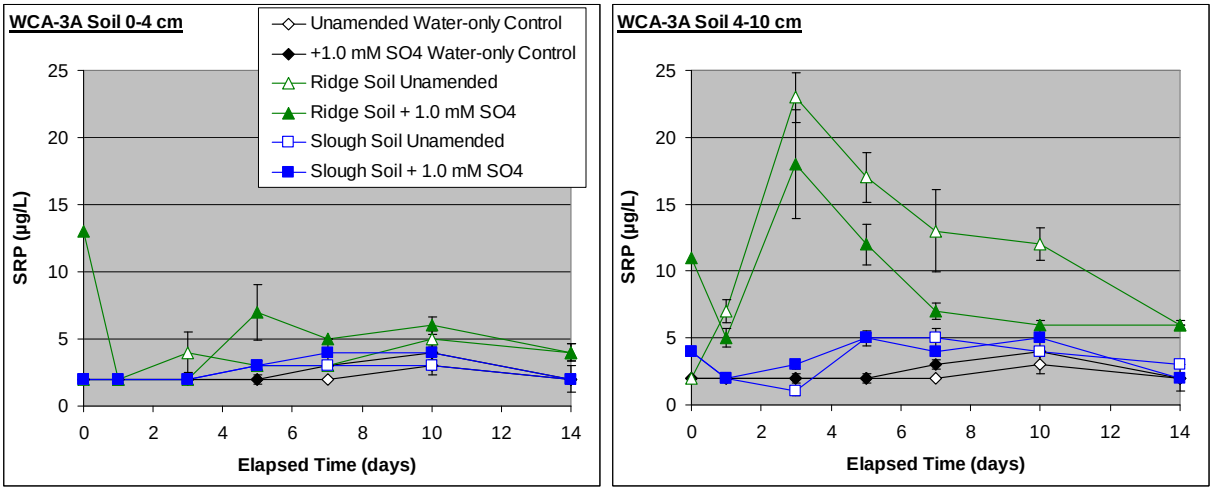


Figure 11. Release of soluble reactive phosphorus (SRP) during a 14-day anaerobic incubation of 0-4 cm (left panel) and 4-10 cm (right panel) homogenized soil layers retrieved from ridge and slough communities in WCA-3A on September 19, 2008. The incubation vessels containing water-only (control) and soils were exposed to unamended and sulfate-amended (1.0 mM (96 mg/L)) low P and low sulfate surface water from WCA-3A. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

Ammonia concentrations increased in all amended and unamended soils during the 14-day incubation (Figure 12). Beginning with an initial ammonia N concentration of ~ 0.4 to 1.0 mg/L, the vessels containing soil increased two- to five time in ammonia concentration by the end of the incubation. The ammonia concentration increments were unrelated to the sulfate enrichment, soil source (ridge or slough) and soil depth (0-4 and 4-10 cm). The water-only controls, which contained less ammonia than the vessels receiving soil, remained unchanged throughout the incubation.

The ridge and slough soils contributed to an increase in DOP concentrations in the incubation water; a higher contribution originated in the 4-10 cm than 0-4 cm soil depth (Figure 12). The ridge soil released slightly more DOP than did the slough soil. However, no sulfate treatment effect was observed. Soluble reactive P concentrations within the vessels containing slough soil remained low and undifferentiated from the water-only controls during the incubation (Figure 12). The ridge soils from the 0-4 and 4-10 cm soil depths contributed to a slight increase in SRP concentrations by the end of the incubation (Figures 11 and 12).

Adding 1.0 mM (96 mg/L) of sulfate vastly increased the concentration above the ambient (unamended) concentration of <1.0 – 3 mg/L (Figure 13). Sulfate reduction was nearly complete by the end of the incubation in the amended slough soil, but not in the amended ridge soil (Table 9). The sulfate concentration remaining after 14 days in the amended soil-less (water-only) controls vessels was 73 mg/L, which represented a decrease of only 12 mg/L from the initial concentration. None of the unamended water-only or soil vessels had detectable sulfate concentrations at the end of the incubation.

Sulfide levels in the sulfate-amended soils indicated sulfate reduction had occurred, and that some of the end products resulted in sulfide accumulation (Figure 13). Sulfide concentrations were higher in the 0-4 cm sulfate-amended ridge and slough soils (1.31 and 1.26 mg/L, respectively) than the sulfate-amended 4-10 cm ridge (1.08 mg/L) and slough (0.82 mg/L) soils at the end of incubation. However, there were no significant differences ($p>0.05$) between sulfate-amended slough or ridge 0-4 cm soils, or between the 4-10 cm soils from the same sites, indicating sulfide production occurred about the same extent in the soils from both

communities. Although not as extensive as in the sulfate-amended soils, sulfide production also was observed in the unamended soils, indicating that endogenous soil processes were producing a small amount of sulfide. Sulfide was not detected in the water-only control vessels.

Table 9. The percentage increase or decrease in the sulfate concentration between the initial and final day of anaerobic laboratory incubations of sulfate-amended (0.33 and 1.0 mM) and unamended soil from the 0-4 and 4-10 cm soil horizons from locations within WCA-3A, WCA-2A U3, STA-5 Cell 2, and STA-2 Cell 1. Positive or negative values indicate a percentage increase or decrease, respectively, in the initial sulfate concentration.

	Water-Only Control			**Soil Depth: 0-4 cm**			**Soil depth: 4-10 cm**		
Location	**No Amend**	**+0.33 mM**	**+1.0 mM**	**No Amend**	**+0.33 mM**	**+1.0 mM**	**No Amend**	**+0.33 mM**	**+1.0 mM**
WCA-3A Slough (1st)	0		-14	-67		-98	0		-99
WCA-3A Ridge (1st)	0		-14	0		-94	0		-78
WCA-3A Slough (2nd)	0	-22	-18	-92	-99	-99	-90	-99	-61
WCA-2A U3 Slough	0	-21	-20	-93	-95	-84	-91	-94	-50
STA-5 Cell 2A N	33		-11	-96		-40			
STA-5 Cell 2B Out	33		-11	-48		-12			
Sta-2 Cell 1 In	8	-14	-18	-97	-96	-95	-96	-98	-82

Under the reducing conditions of the incubation (ORP = +11 to –162 mV), the dissolved Fe concentrations increased in all of the incubated soils, amended and unamended, and from both horizons. The unamended slough soils yielded high levels of dissolved Fe, more so for the 0-4 than 4-10 cm soil depths (Figure 13). This was not observed to the same extent for the slough soil that received sulfate, suggesting that some of the sulfide produced may have reacted with the dissolved Fe to form insoluble FeS_x compounds. The saturation index (SI), which is the ion activity product (IAP) divided by the solubility product constant (K_{sp}) for FeS, was three times more saturated in the 1.0 mM sulfate-amended than the unamended 0-4 cm slough soil. This indicates that the 1.0 mM sulfate treatment is more likely to have the Fe and sulfide concentrations controlled by FeS under equilibrium conditions, although the SI for the unamended soil also indicated eventual regulation of Fe and sulfide by FeS. The same comparison between amended and unamended 0-4 and 4-10 cm deep ridge soil was not as obvious as it was in the slough soil, likely because of lower sulfate reduction. The water-only controls showed negligible changes in dissolved Fe during the incubation.

The pH values were similar across all soils and treatment at the end of the incubation, and were 0.5-1.0 units lower than the water-only controls (Figure 14). The reduction from the initial pH was greatest in the unamended and amended 0-4 cm deep slough soil; the pH changes were negligible for the 4-10 cm slough depth and the soil from the ridge station. The dissolved Ca concentrations decreased, while the alkalinity levels increased, in the 1.0 mM amended 0-4 cm and 4-10 cm soil layers from both ridge and slough sites compared to the corresponding unamended soils at the end of the incubation (Figure 14). Dissolved Ca concentrations increased during the soil incubations, but more so in the unamended than 1.0 mM amended soils. Levels of dissolved Ca remained the same in the water-only controls before and after the incubation.

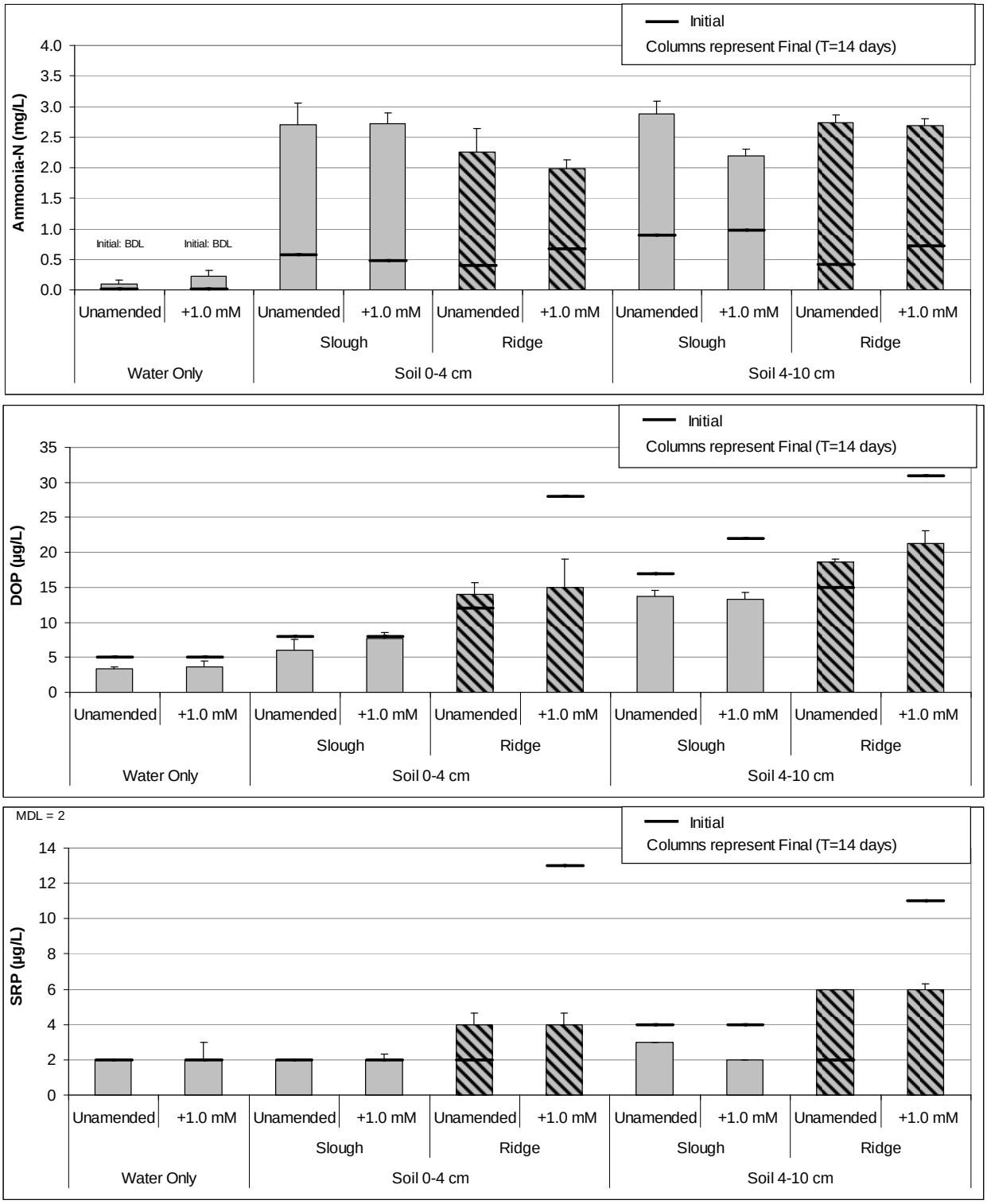


Figure 12. Ammonia-N (top panel), dissolved organic phosphorus (DOP) (middle panel), and soluble reactive phosphorus (SRP) (bottom panel) concentrations before and after a 14-day anaerobic incubation of ridge and slough soils from WCA-3A with sulfate-amended (1.0 mM) and unamended WCA-3A surface water. The columns depict the means while error bars represent +1 standard error.

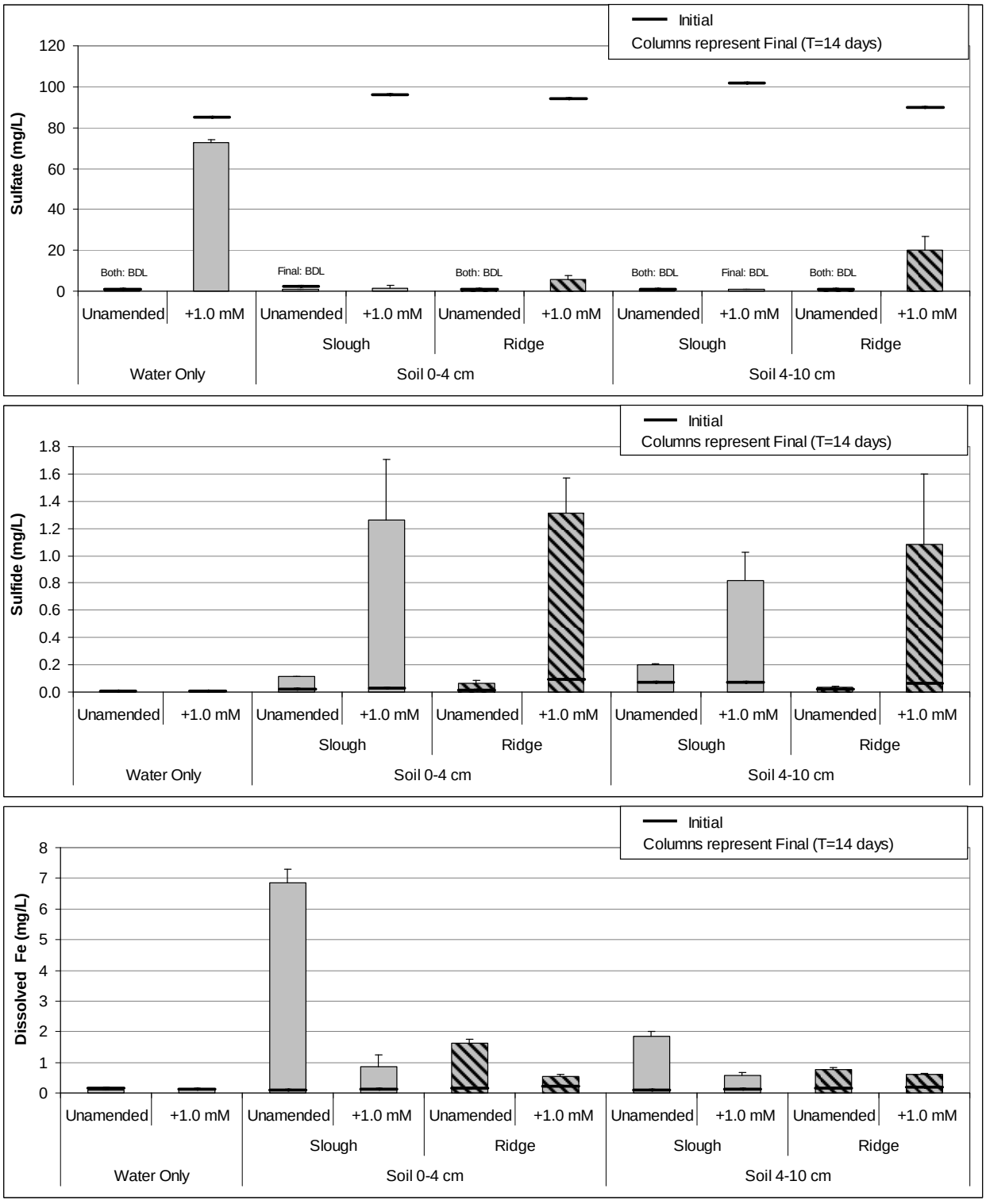


Figure 13. Sulfate (top panel), sulfide (middle panel), and dissolved iron (Fe) (bottom panel) concentrations before and after a 14-day anaerobic incubation of ridge and slough soils from WCA-3A with sulfate-amended (1.0 mM) and unamended WCA-3A surface water. The columns depict the means while error bars represent +1 standard error.

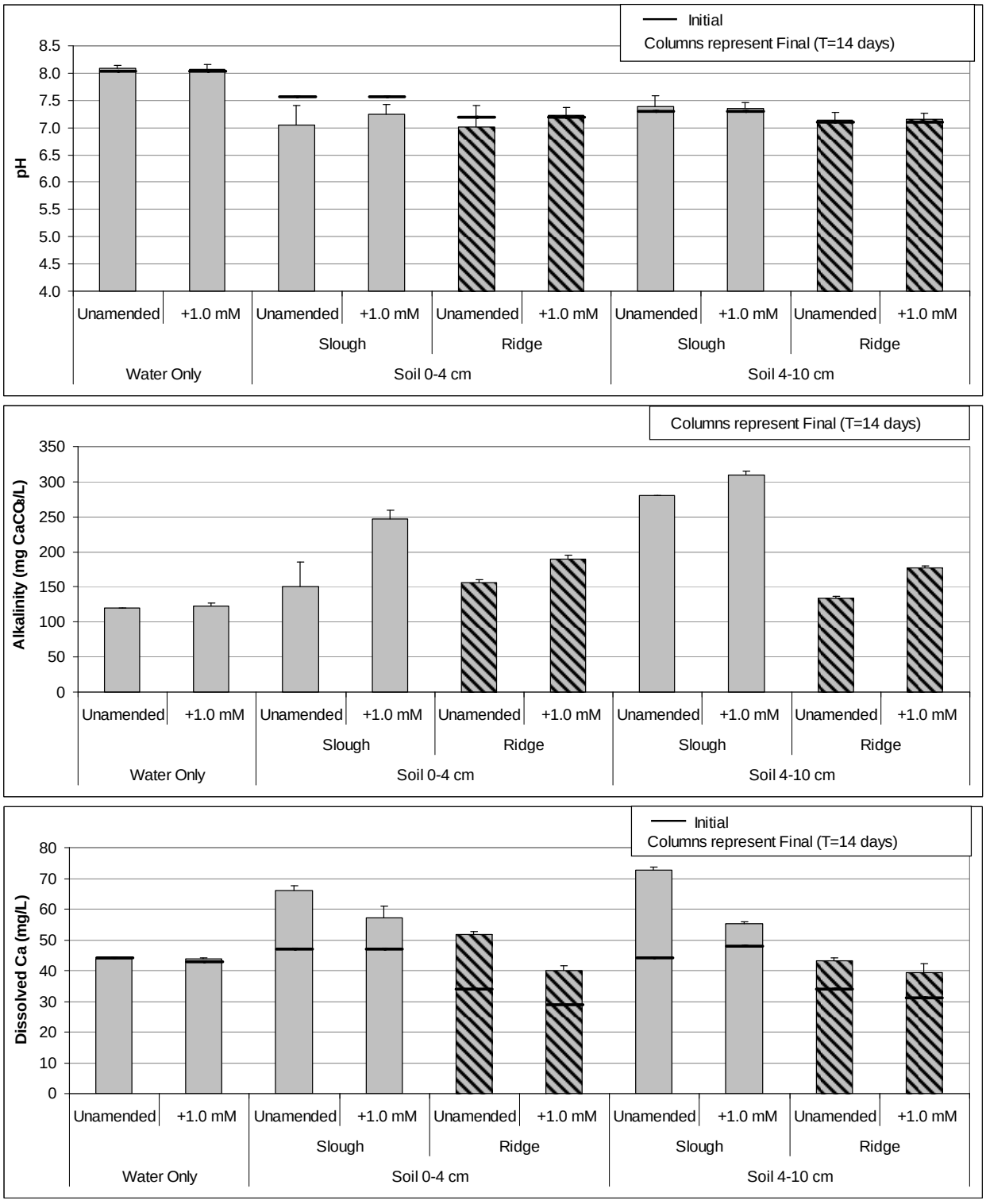


Figure 14. pH (top panel), alkalinity (middle panel), and dissolved calcium (Ca) (bottom panel) concentrations before and after a 14-day anaerobic incubation of ridge and slough soils from WCA-3A with sulfate-amended (1.0 mM) and unamended WCA-3A surface water. The columns depict the means while error bars represent +1 standard error.

Low Sulfate and Low P Environment: WCA-3A (sampled Jan. 19, 2009)

The release of SRP during the second batch incubation on soils collected only from the slough in WCA-3A on January 19, 2009 mimicked the pattern observed during the first incubation of slough soil (*cf*. Figures 11 and 15). That is, SRP releases were negligible for the sulfate unamended and amended water-only controls and the 0-4 cm soil layer during the incubation period using slough soil from WCA-3A (Figure 15). An initial SRP concentration of 18 μg/L was observed for the 1.0 mM sulfate-amended treatment of the 4-10 cm soil layer, but the SRP levels decreased to the detection limit (2 μg/L) after 5 days. This was likely due to carry-over of SRP originally contained in the soil.

Ammonia concentrations increased in all of the sulfate-amended soils during the second experiment using WCA-3A slough soil, an observation similar to the response exhibited during the first experiment (*cf*. Figures 15 and 16). Overall, there was a two-to-four times increase in ammonia during the 14-day incubation. There appeared to be a slight decrease in the ammonia concentrations, which was more pronounced in the 0-4 than 4-10 cm soil depth, with higher sulfate amendments in this second experiment (Figure 16). Ammonification was independent of the soil depth.

Although the soil collected in the 4-10 cm stratum was associated with higher DOP concentrations than the 0-4 cm soil layer, there was no sulfate treatment effect for either horizon (Figure 16). Overall, DOP concentrations before and after the incubations were low (< 15 μg/L), indicating DOP was not mobilized by microbial processes such as was observed for ammonia. Soluble reactive P concentrations were basically unchanged and at or near the detection limit during the incubation, except for a higher initial SRP concentration in the 4-10 cm soil amended with 1.0 mM sulfate.

Due to the reducing conditions during the incubation (+84 to +30 mV), sulfate amendments to the 0-4 cm soil all but disappeared during the incubation (Figure 17). Sulfate also became exhausted in the 0.33 mM, but not in the 1.0 mM, amended 4-10 cm soil layer (Table 9). In contrast, the water-only controls retained most of the sulfate added after two weeks of incubation.

By the end of 14-day incubation, sulfide concentrations had increased in proportion to the amount of sulfate initially added, but levels never reached as high as in the first incubation (*cf*. Figures 13 and 17). The highest mean sulfide concentration at the end of the incubation period during this incubation was 0.27 mg/L in the 1.0 mM amended 0-4 cm deep soils. The controls without soil did not respond to sulfate amendments.

The unamended soil cored from the 0-4 cm horizon released the most dissolved Fe during the incubation, attaining a concentration of 3.0 mg/L. This was more than two times lower than the dissolved Fe concentration measured in the first incubation (*cf*. Figures 13 and 17). Dissolved Fe concentrations in the 0.33 mM amended 0-4 cm soils were significantly higher ($p<0.05$) than the 1.0 mM amendment (Figure 17) because of the greater FeS_x binding at the higher sulfide concentrations produced by the 1.0 mM sulfate amendment. The SI for FeS was twice as high for the 1.0 mM sulfate-amended than unamended 0-4 cm soil horizon (SI=200 vs. 398), suggesting the higher sulfide concentrations in the 1.0 mM amended soil was more oversaturated with respect to FeS than the unamended soil. The dissolved Fe concentrations were unaffected between the two sulfate amendments in the 4-10 cm soil interval, and were considerably lower at the end of the incubation than their respective treatment levels in the 0-4 cm soil layer. This indicates that relative to the 0-4 cm soil, the Fe-reducible pool was considerably less in the 4-10 cm soil layer.

The pH, and alkalinity and dissolved Ca concentrations remained unchanged in the unamended and amended water-only controls during the incubation (Figure 18). Adding soil incremented the initial alkalinity and dissolved Ca concentrations, and lowered the pH, relative to control (soil-less) vessels. Higher alkalinity and Ca concentrations were measured at the end of the incubation in soils from both horizons, a consequence of soil microbial processes lowering the pH (Figure 18). There appeared to be a slight increase in alkalinity, coinciding with a slight decrease in dissolved Ca concentrations, with increasing sulfate amendments in the 0-4 cm soil layer. This was not observed for the 4-10 cm soil layer.

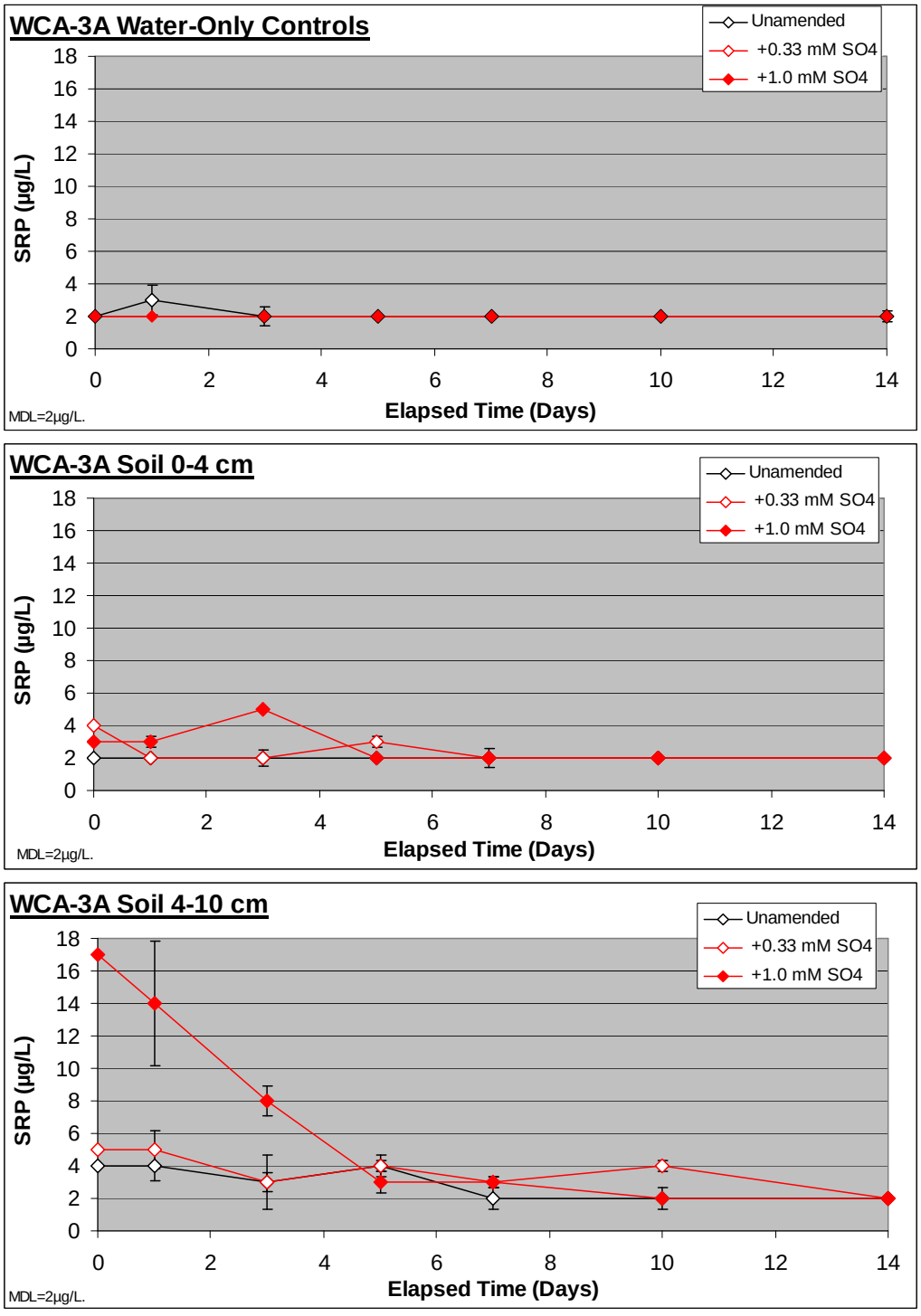


Figure 15. Release of soluble reactive phosphorus (SRP) during a 14-day anaerobic incubation of water-only controls (top panel), and 0-4 cm (middle panel) and 4-10 cm (bottom panel) homogenized soil layers retrieved from slough communities in WCA-3A on January 19, 2009. The soils were exposed to unamended and sulfate-amended (0.33 mM (32 mg/L) or 1.0 mM (96 mg/L)) low P and low sulfate surface water from WCA-3A. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

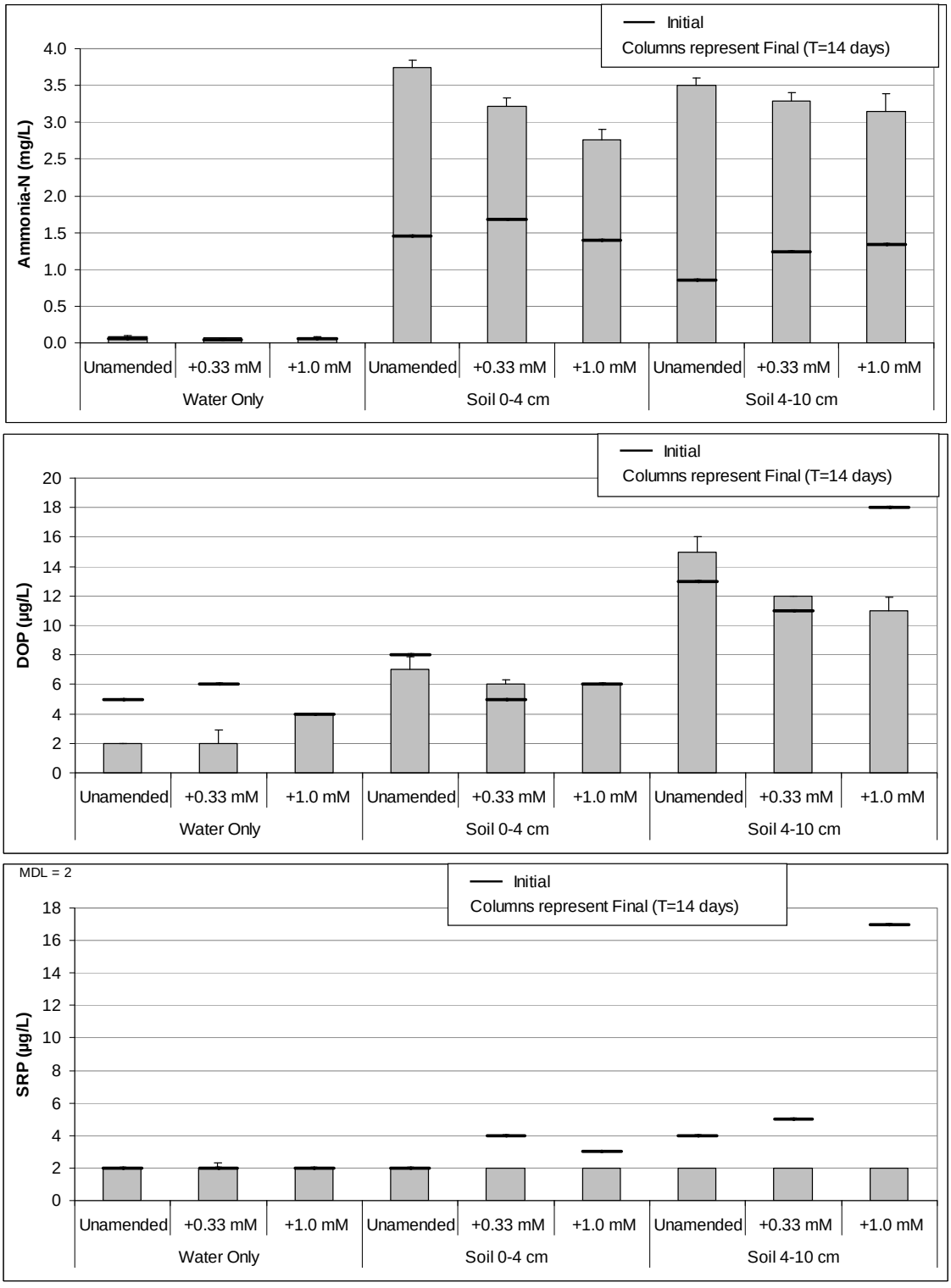


Figure 16. Ammonia-N (top panel), dissolved organic phosphorus (DOP) (middle panel), and soluble reactive phosphorus (SRP) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from WCA-3A with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. The columns depict the means while the error bars represent + 1 S.E.

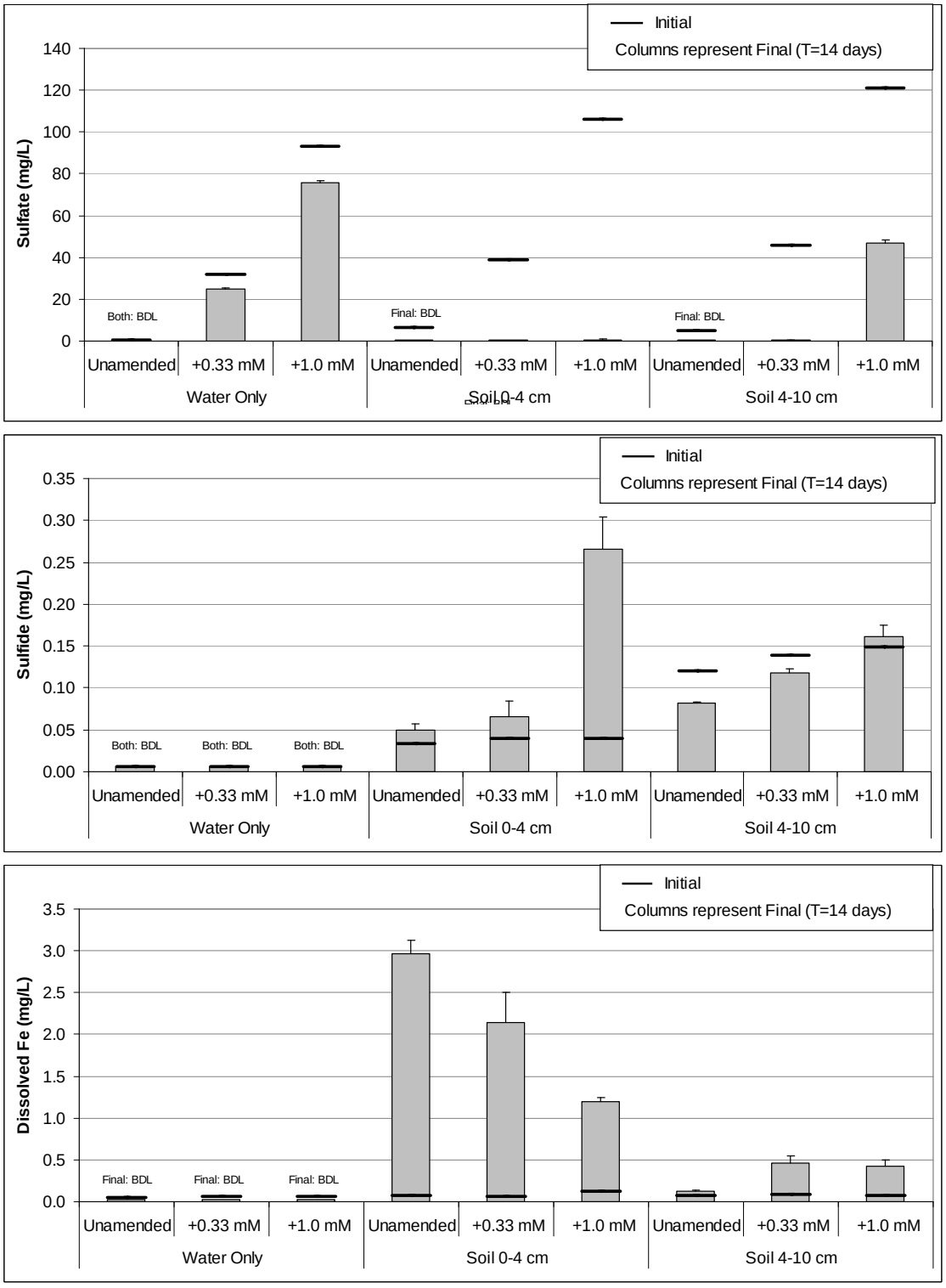


Figure 17. Sulfate (top panel), sulfide (middle panel), and dissolved iron (Fe) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from WCA-3A with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

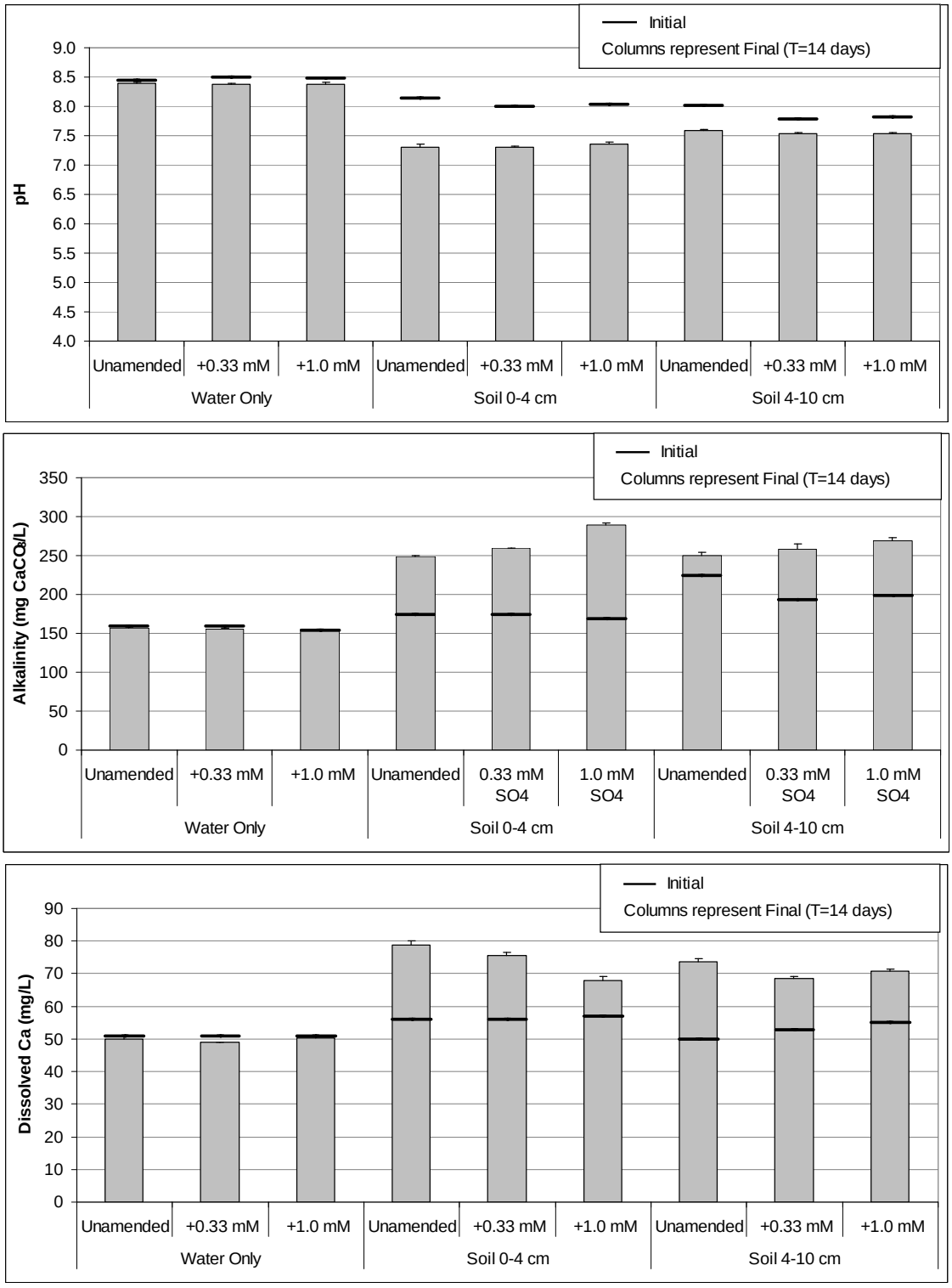


Figure 18. pH (top panel), alkalinity (middle panel), and dissolved calcium (Ca) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from WCA-3A with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

High Sulfate and Low P Environment: WCA-2A U3

With the exception of a slight elevation in SRP concentration in the unamended and amended water-only controls on day 5 of the incubation, SRP concentrations remained below 7 μg/L during the incubation period for all treatments and controls (Figure 19). More importantly, there was no evidence that amending with the two sulfate levels (0.33 and 1.0 mM) enhanced the mobilization of SRP from the soils.

Consistent with the ammonia increases observed in the two incubations of WCA-3A soils, ammonia concentrations increased during the incubation of soils collected at 0-4 and 4-10 cm depth intervals in WCA-2A U3 (Figure 20). More ammonification occurred in the 0-4 cm (between three to four times the initial ammonia concentrations) than the 4-10 cm (two-fold or less) soil depths. There was no relationship between added sulfate and ammonia produced for either soil depth. Negligible concentrations of ammonia were present in the water-only controls, which were unchanged at the end of the incubation.

The ending mean concentrations of DOP were <12 μg/L for all soil treatments. The initial DOP concentrations in the unamended soils from both horizons were reduced after the 14-day incubation, as was the water-only controls (Figure 20). There was a slight positive, but nevertheless significant ($p<0.05$), relationship between added sulfate and ending DOP concentrations in the 4-10 cm soil depth, but not in the 0-4 cm soil depth.

Soluble reactive P concentrations were frequently near or at detection limits in the soils cored from both horizons at the end of the incubation (Figure 20). The water-only controls ranged from 5-7 μg SRP/L at the end of the incubation, compared to the initial SRP concentrations of 2 μg/L.

Although all sulfate-treated soils had measurable sulfate concentrations remaining, most of the added sulfate was reduced during the incubation period (Table 9; Figure 21) under the reducing conditions present in the incubation vessels containing soil (-61 to –132 mV). The exception was the 1.0 mM treated soil from the 4-10 cm horizon where approximately one-half of the added sulfate still remained 14 days later. The unamended soils, which initially had 7.5 mg/L (0-4 cm

layer) and 5.4 mg/L (4-10 cm layer) in the soil slurry, completely reduced the native sulfate to levels below detection (< 0.5 mg/L).

Sulfide concentrations were proportional to the amount of sulfate reduced (Figure 21). The highest sulfide concentration (23 mg/L) measured in any of the five incubations on the four separate wetland soils occurred in the 0-4 cm soil layer of WCA-2A U3 that was amended with a 1.0 mM final sulfate concentration. For the 4-10 cm soil layer, sulfide concentrations were the same in the 0.33 mM and 1.0 mM sulfate treatments. The initial and final sulfide concentrations in the water-only controls were below detection limit (<0.006 mg/L). The high sulfide concentrations measured in the sulfate-amended soils at the end of the incubation was due to the low concentrations (<0.032 mg/L) of dissolved Fe found in the post-incubated unamended soils (Figure 21). Dissolved Fe reacts with sulfide under low ORP to form insoluble FeS_x. The dissolved Fe concentrations were low initially, and most treatment and control vessels ended with dissolved Fe concentrations below the detection limit of 0.025 mg/L.

Compared to the pH (8.45) in the water-only controls, the pH decreased to 7.6-7.9 after the 14-day incubation in the soil-containing vessels (Figure 22). There was no sulfate-treatment effect, indicating a well-buffered system.

Alkalinity levels during the incubation increased by the same amount in the amended and unamended soil collected from the 0-4 cm horizon (Figure 22). Higher alkalinity increases occurred in the unamended and 0.33 mM sulfate amended soil, but not in the 1.0 mM sulfate treated soil, from the 4-10 cm horizon. Alkalinity levels remained unchanged in the water-only controls.

Dissolved Ca concentrations increased during the incubation period in the unamended and amended 0-4 cm soil depth, with the 1.0 mM sulfate amended soil exhibiting the least response (Figure 22). Dissolved Ca concentration increases were not as obvious in the 4-10 soil depth incubation as they were for the 0-4 cm layer. Ca levels decreased slightly during the incubation in the water-only controls.

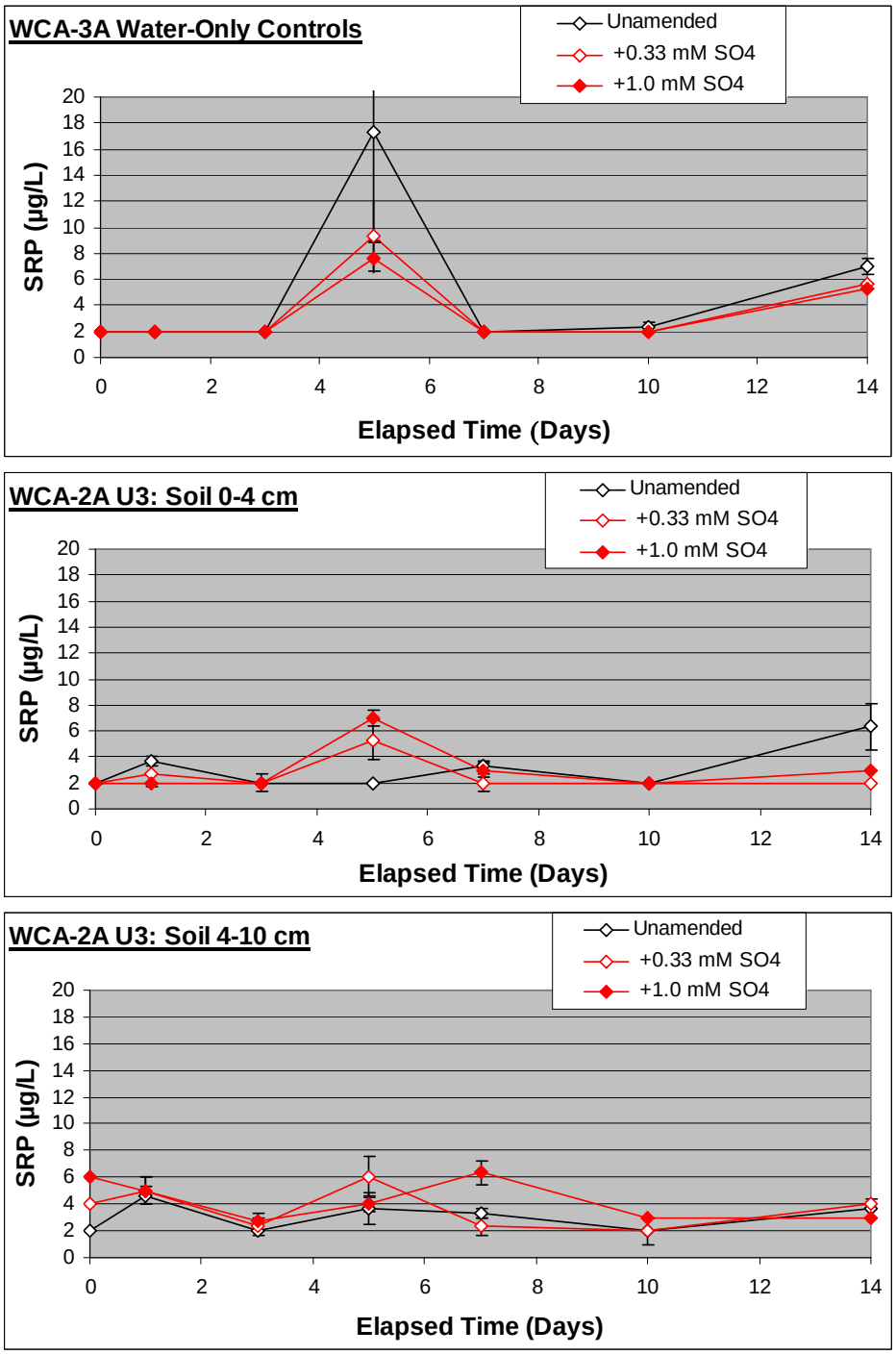


Figure 19. Release of soluble reactive phosphorus (SRP) during a 14-day anaerobic incubation of water-only controls (top panel), and 0-4 cm (middle panel) and 4-10 cm (bottom panel) homogenized soil layers retrieved from WCA-2A U3 on January 15, 2009. The soils were exposed to unamended and sulfate-amended (0.33 mM (32 mg/L) or 1.0 mM (96 mg/L)) low P and low sulfate surface water from WCA-3A. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

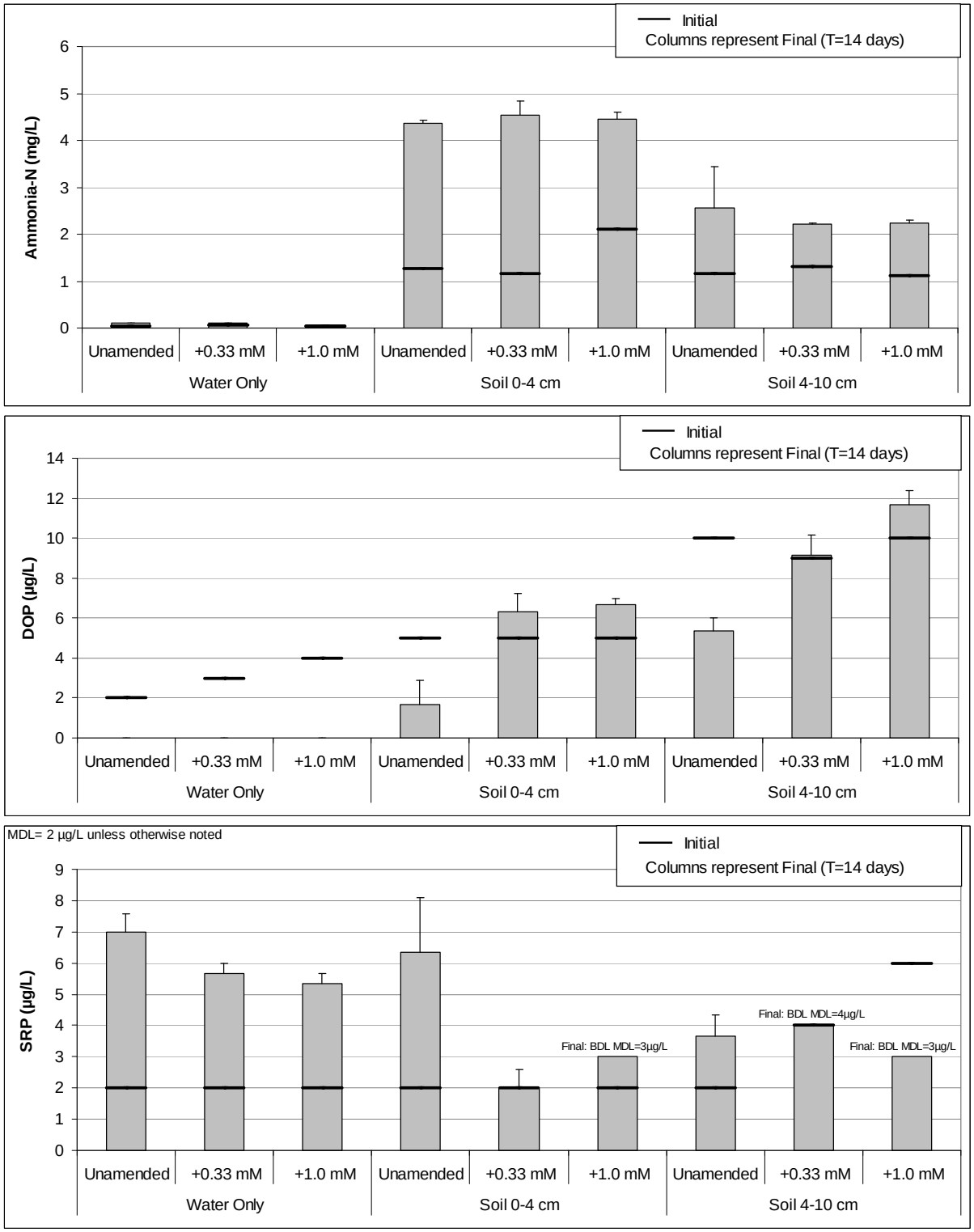


Figure 20. Ammonia-N (top panel), dissolved organic phosphorus (DOP) (middle panel), and soluble reactive phosphorus (SRP) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from WCA-2A U3 with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

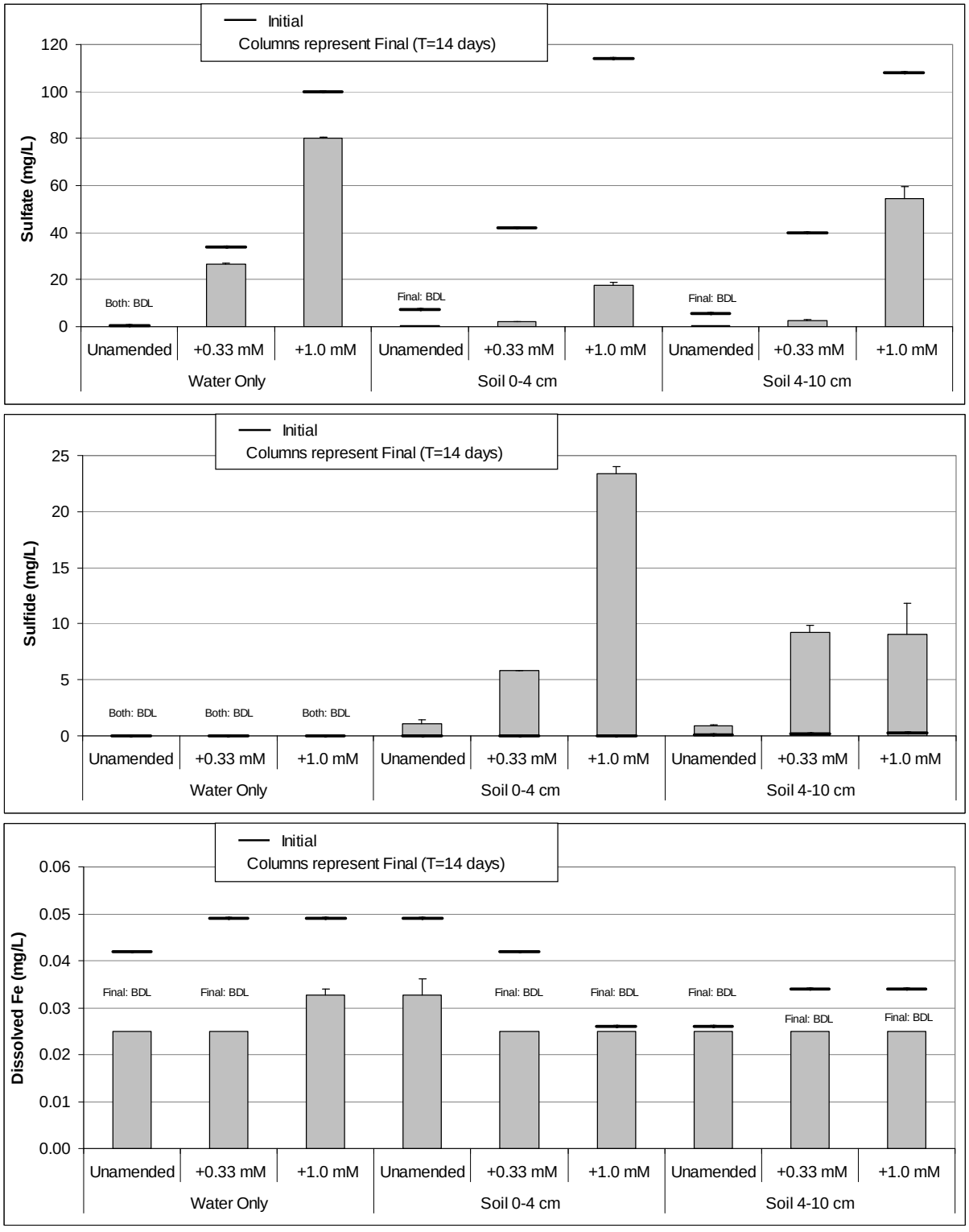


Figure 21. Sulfate (top panel), sulfide (middle panel), and dissolved iron (Fe) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from WCA-2A U3 with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

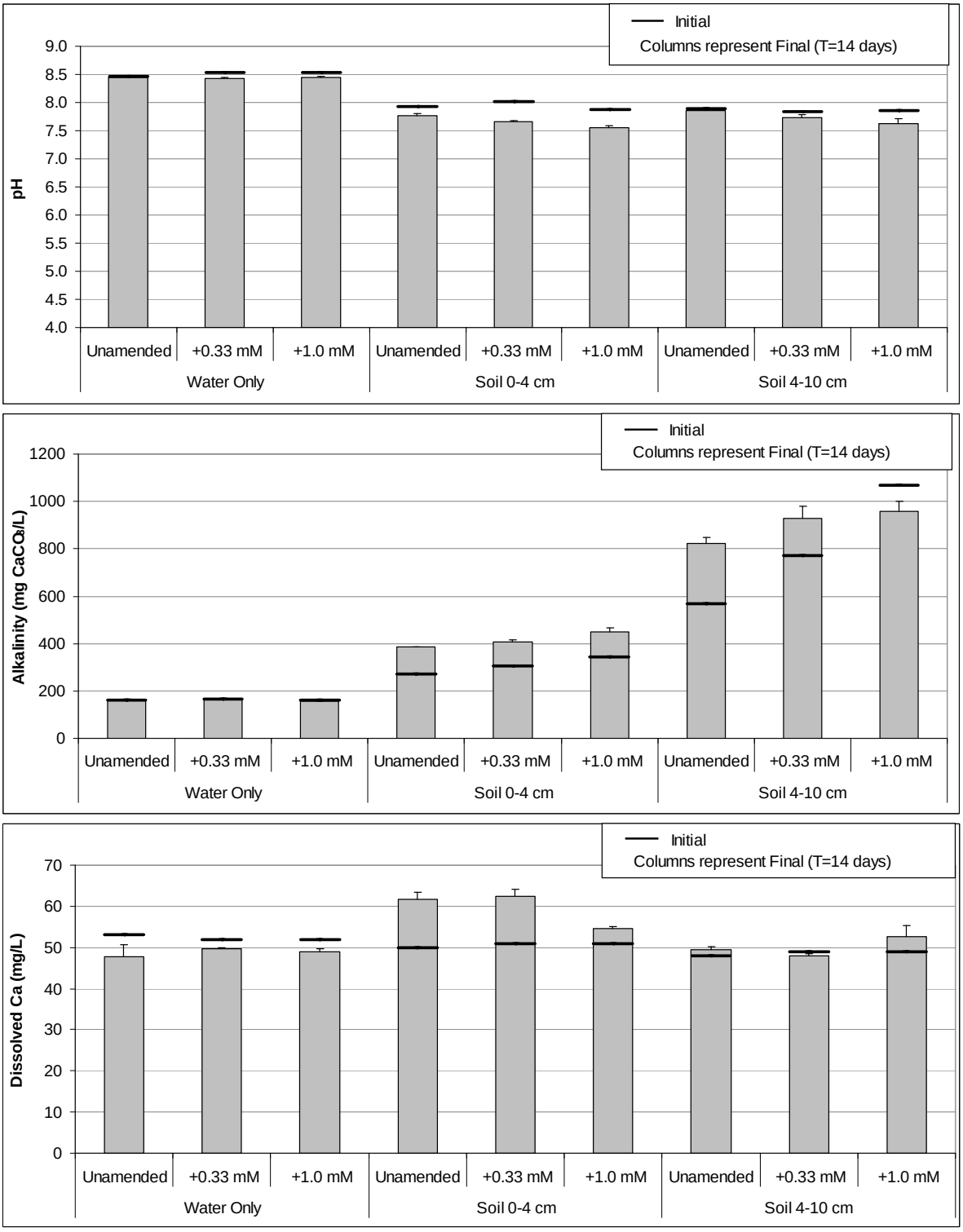


Figure 22. pH (top panel), alkalinity (middle panel), and dissolved calcium (Ca) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from WCA-2A U3 with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

Moderate Sulfate and High P Environment: STA-5

The incubation period for soils sampled from two locations in STA-5 was limited to 7 days, rather than the 14-day duration practiced for the other wetland soils. Soils from this P-enriched environment (Table 7) exhibited a consistent rate of SRP release during the 7-day incubation compared to the soil-less incubation of unamended and sulfate-amended water collected at the outflow of the STA (Figure 23). Higher rates of release were observed for the soils collected in the middle of the flow path than at the outflow region. There was no heightened SRP release ($p>0.05$) associated with the addition of 1.0 mM (96 mg/L) of sulfate for either soil.

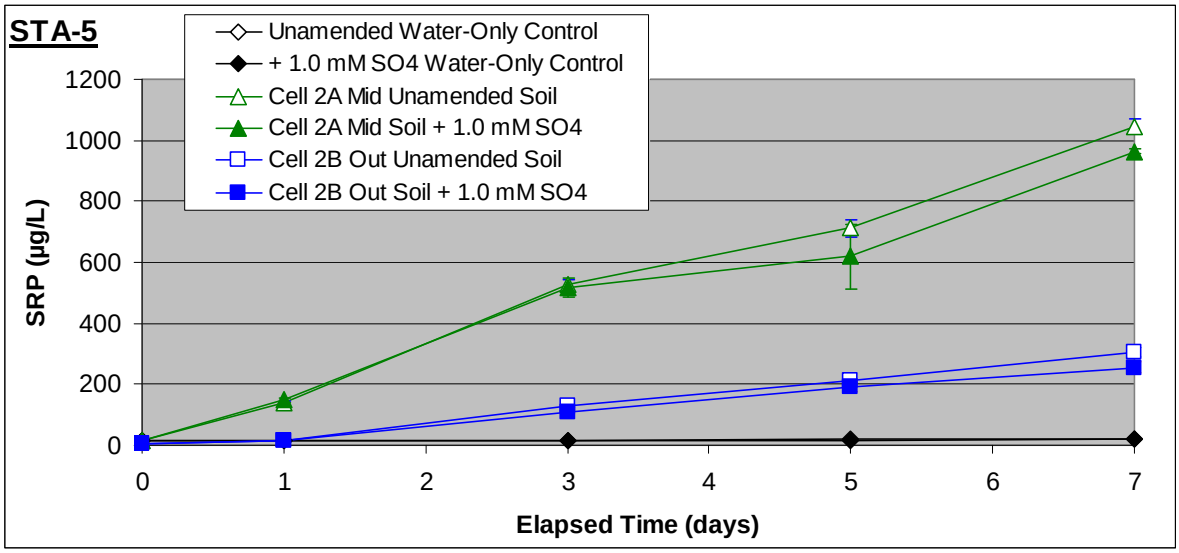


Figure 23. Release of soluble reactive phosphorus (SRP) during a 7-day anaerobic incubation of water-only controls and the 0-4 cm homogenized soil layer retrieved from two locations in STA-5 on August 25, 2008. The soils were exposed to unamended and sulfate-amended (1.0 mM (96 mg/L)) surface water from the outflow at G-344 of the STA. Only the 0-4 cm soil layer from each location was incubated. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

Ammonia concentrations in the unamended and amended soils increased during the 7-day incubation for the 0-4 cm soil layer cored in the middle of Cell 2A and in the outflow region of Cell 2B (Figure 24). The soil near the outflow had lower initial and final concentrations of ammonia than did the more interior soil collected in Cell 2A. There appeared to be a slight inhibition effect on ammonification by the 1.0 mM sulfate amendment to the outflow region soil. The water-only controls, which consisted of outflow water from Cell 2B (and not WCA-3A surface water), had negligible amounts of ammonia before and after the incubation.

Dissolve organic P and SRP concentrations increased at the end of the incubation for soils taken at both sites, but more so for the Cell 2A soil (Figure 24). For example, SRP concentrations increased from an initial 14 and 5 μg/L to final concentrations of > 900 and > 200 μg/L at the end of the incubation for Cell 2A and 2B soils, respectively. There was no distinction between the unamended and 1.0 mM amended treatments with respect to SRP or DOP releases for either soil source. Water-only controls exhibited negligible SRP and DOP concentrations.

Due to the low ORP in the incubation vessels (-29 to –122 mV), sulfate concentrations in both the unamended and 1.0 mM amended soils from both sites decreased during the 7-day incubation (Table 9; Figure 25). Less of a reduction occurred in the amended water-only control. Notwithstanding the sulfate decreases, there were still high sulfate concentrations remaining in the unamended and amended soils from both sites, and in the amended water-only control.

Soil from Cell 2A in STA-5 exhibited a higher sulfide concentration in response to the 1.0 mM sulfate amendment, but this did not occur in the Cell 2B soil (Figure 25). However, the response to sulfate enrichment was not statistically significant ($p>0.05$) for soils from both sites. An incubation time longer than 7 days would likely have widened the differences in sulfate reduction and sulfide production between unamended and amended soils from the same site. Sulfide concentrations were negligible in the water-only controls.

Dissolved Fe concentrations increased substantially during the incubation in the unamended and amended Cell 2A and Cell 2B soils: concentrations increased from 0.08 – 0.18 mg/L to 0.48 – 0.59 mg/L (Figure 25). There was only a slightly higher ending level of dissolved Fe in the 1.0 mM sulfate amended soils than unamended soils. No differences were observed between initial and ending Fe concentrations in the water-only controls.

The beginning and ending pH values between the two soil sources were indistinguishable from each other and within treatment groups (unamended and amended) at the end of incubation (Figure 26). Exposing the soils to the outflow water from Cell 2B decreased the pH by 1.0 unit compared to the water-only controls.

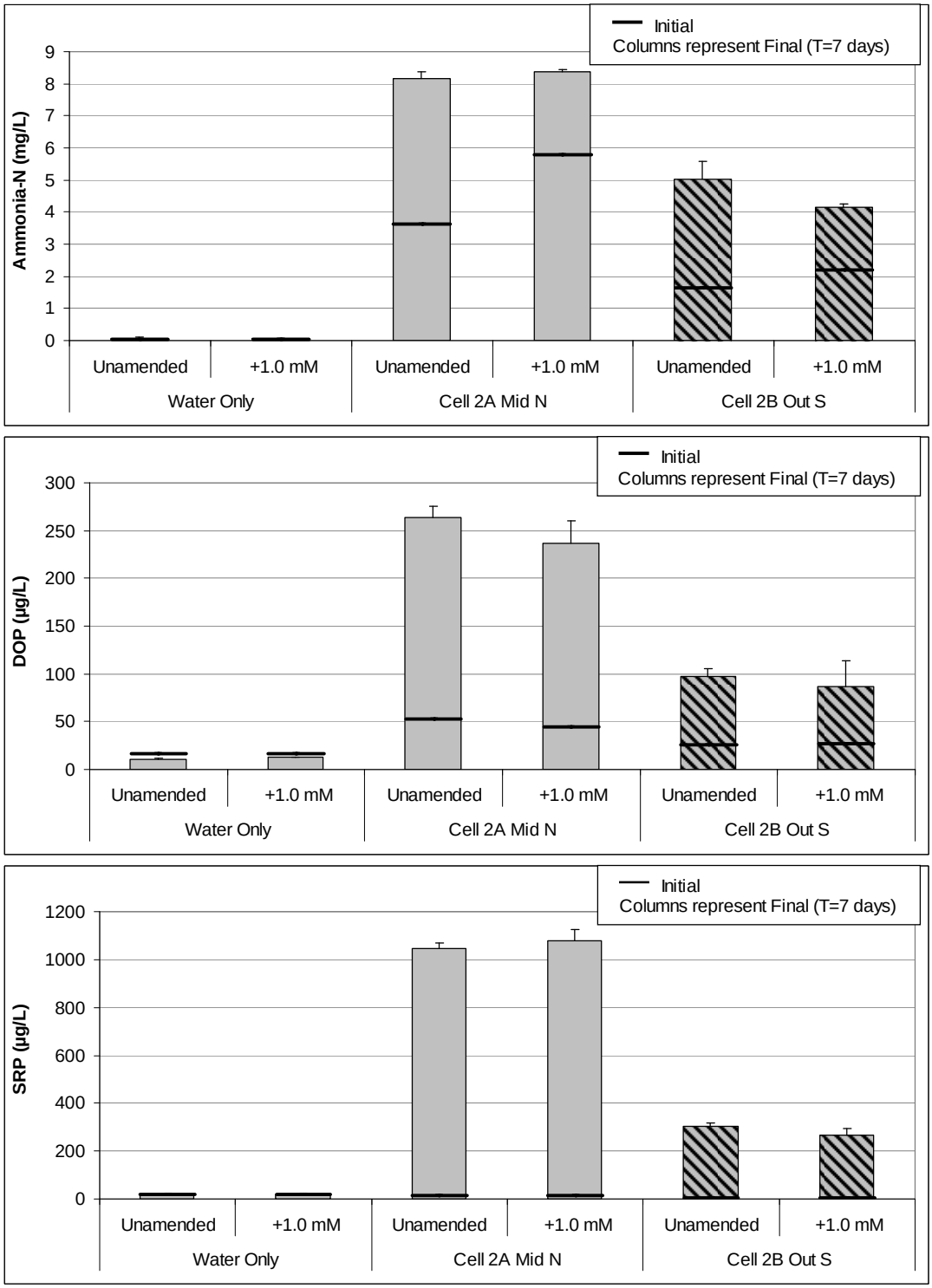


Figure 24. Ammonia-N (top panel), dissolved organic phosphorus (DOP) (middle panel), and soluble reactive phosphorus (SRP) (bottom panel) concentrations before and after a 7-day anaerobic incubation of Cell 2A Mid N and Cell 2B Out S soils from STA-5 with sulfate-amended (1.0 mM) and unamended STA-5 Out surface water. Only the 0-4 cm soil layer from each location was incubated. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

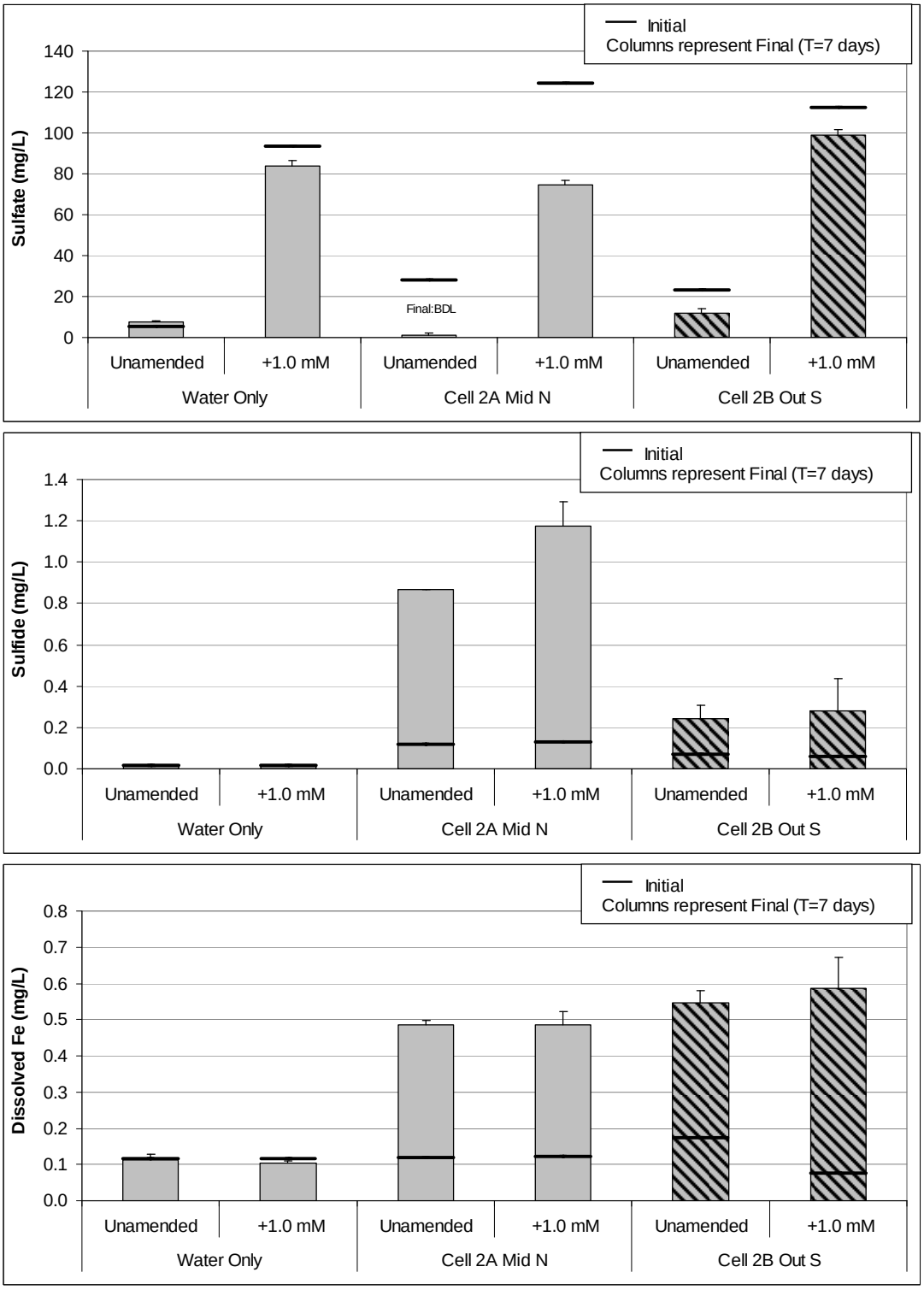


Figure 25. Sulfate (top panel), sulfide (middle panel), and dissolved iron (Fe) (bottom panel) concentrations before and after a 7-day anaerobic incubation of Cell 2A Mid N and Cell 2B Out S soils from STA-5 with sulfate-amended (1.0 mM) and unamended STA-5 Out surface water. Only the 0-4 cm soil layer from each location was incubated. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

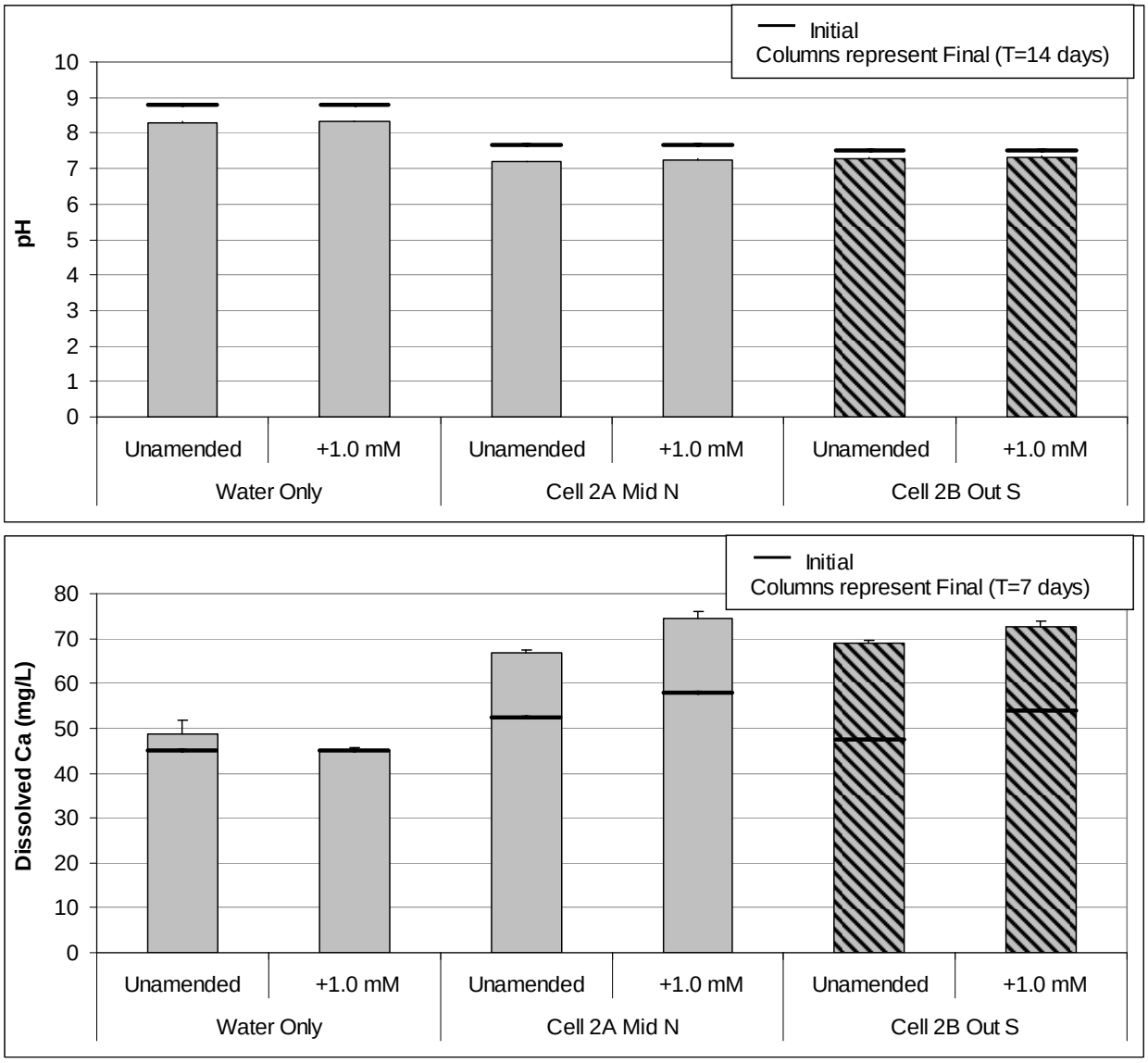


Figure 26. pH (top panel) and dissolved calcium (Ca) (bottom panel) concentrations before and after a 7-day anaerobic incubation of Cell 2A Mid N and Cell 2B Out S soils from STA-5 with sulfate-amended (1.0 mM) and unamended STA-5 Out surface water. Only the 0-4 cm soil layer from each location was incubated. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

As a result of the anaerobic incubation, dissolved Ca concentrations in both soils increased between 14 – 22 mg/L (Figure 26). This increase was not affected by a 1.0 mM sulfate amendment.

High Sulfate and High P Environment: STA-2 Cell 1

The initial SRP concentration of 200 μg/L at the onset of the incubation was unexpectedly high given the low-SRP (2 μg/L) water from WCA-3A that was added to the soil (Figure 27: top panel). The high labile soil P concentrations in STA-2 Cell 1 (Figure 10: Tables 4 and 5) was the source of elevated SRP concentration immediately after flooding the soil. As a result of the high labile P pools, five and three times the mass of the initial SRP amount were released from the 0-4 and 4-10 cm soil layers, respectively (Figure 27). As observed in the previous incubations on soils collected from WCAs and STA-5, there was no effect ($p>0.05$) of adding sulfate in the release of SRP.

Ammonia levels increased dramatically during the 14-day incubation in the soils cored from the inflow region of STA-2 Cell 1 (Figure 28). Ammonification was highest in the 0-4 cm soil where increases of greater than 10 times (to between 15 and 17 mg/L) of the initial concentrations occurred. Ammonia concentrations in the water-only controls were negligible, both before and after incubation. There was no effect of sulfate amendments in measured releases of ammonia.

Besides SRP, DOP concentrations also increased during the incubation (Figure 28). However, the extent of DOP release was not as great as for SRP release, but the same pattern of less DOP release associated with the 4-10 than 0-4 cm soil layers was observed. The DOP and SRP releases were independent ($p>0.05$) of the sulfate treatment ($p>0.05$). Concentrations of SRP were at or less than the detection limit (2 μg/L) in the water-only controls.

Sulfate concentrations decreased significantly in the sulfate amended soils (Table 9); smaller quantities were reduced in the water-only controls (Figure 29). Sulfide concentrations varied directly with the sulfate treatment for both the 0-4 cm and 4-10 cm soil layers. For a given initial

sulfate concentration, more sulfide production occurred in the 0-4 cm than 4-10 cm layer (Figure 29).

Because of the low redox potentials (-116 mV to –156 mV) during the incubation, dissolved Fe concentrations increased in the soil slurries during the incubation, but concentrations never exceeded 0.12 mg/L at the end of the incubation (Figure 29). The highest dissolved Fe concentrations originated within the unamended soils from both depth intervals.

The pH decreased during the incubation across both water-only controls and soil treatments (Figure 30). However, the magnitude of the decrease was minor, never more than 0.5 units lower than the initial pH.

Alkalinity levels decreased significantly in the post-incubated sulfate amended soils collected from the 4-10 cm soil horizon (Figure 30), which may have been due to titrating fine $CaCO_3$ particles in the initials. Such decreases were not observed during the incubation of the 0-4 cm soil layer.

Dissolved Ca concentrations increased in all the vessels containing soil at the end of the incubation, whereas Ca levels remained the same in the water-only controls (Figure 30). More Ca was released in the 0-4 cm than 4-10 cm soil layer. The release was independent of the sulfate treatment, except for the 1.0 mM sulfate amendment to the 0-4 cm soil layer, where Ca mobilization was slightly less than the unamended and 0.33 mM sulfate amended soils from the same horizon.

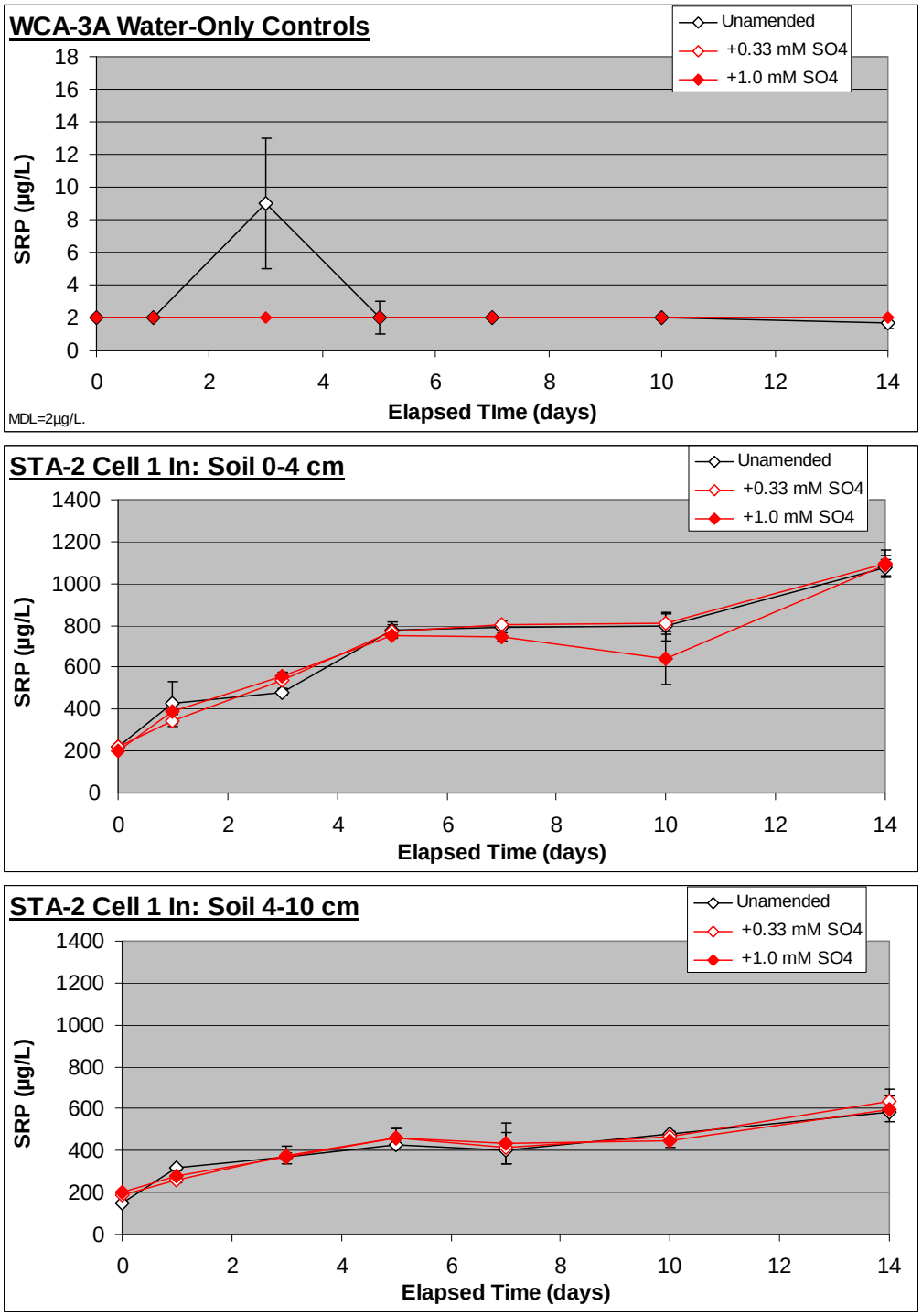


Figure 27. Release of soluble reactive phosphorus (SRP) during a 14-day anaerobic incubation of water-only controls (top panel), and 0-4 cm (middle panel) and 4-10 cm (bottom panel) homogenized soil layers retrieved from the inflow region of STA-2 Cell 1 on June 2, 2009. The soils were exposed to unamended and sulfate-amended (0.33 mM (32 mg/L) or 1.0 mM (96 mg/L)) low P and low sulfate surface water from WCA-3A. Note the differences in the Y-axis scales. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

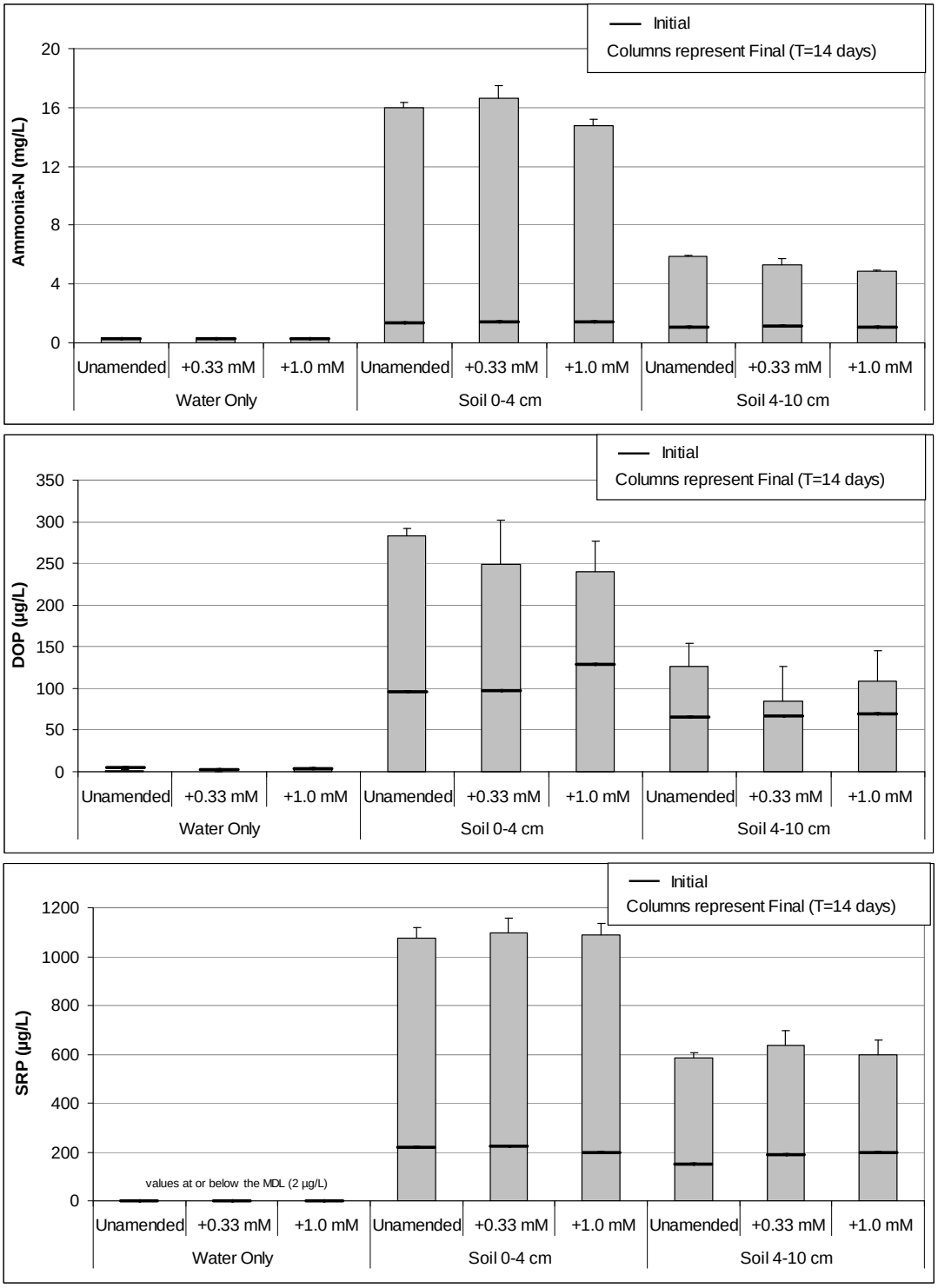


Figure 28. Ammonia-N (top panel), dissolved organic phosphorus (DOP) (middle panel), and soluble reactive phosphorus (SRP) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from STA-2 Cell 1 with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

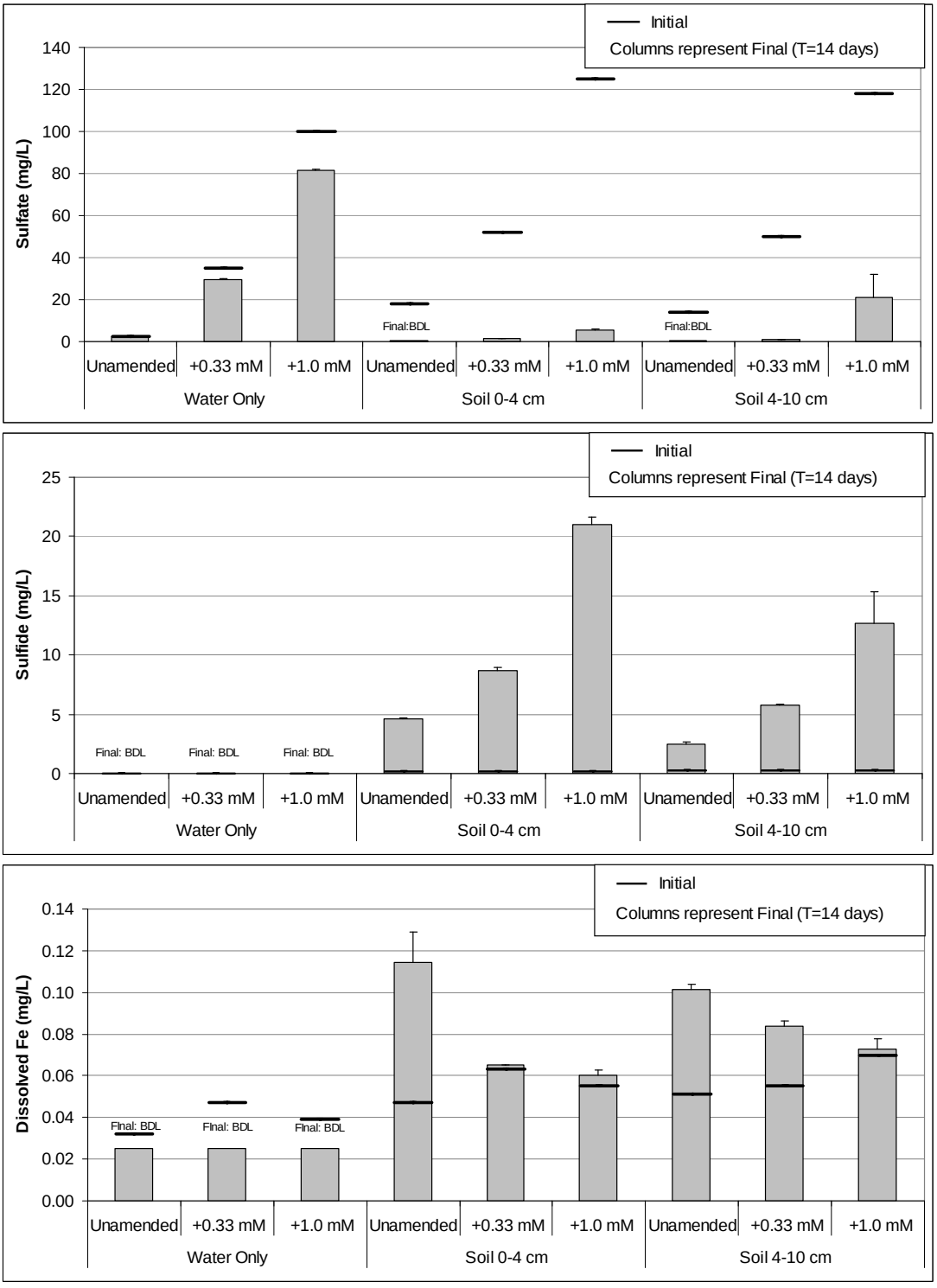


Figure 29. Sulfate (top panel), sulfide (middle panel), and dissolved iron (Fe) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from STA-2 Cell 1 with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

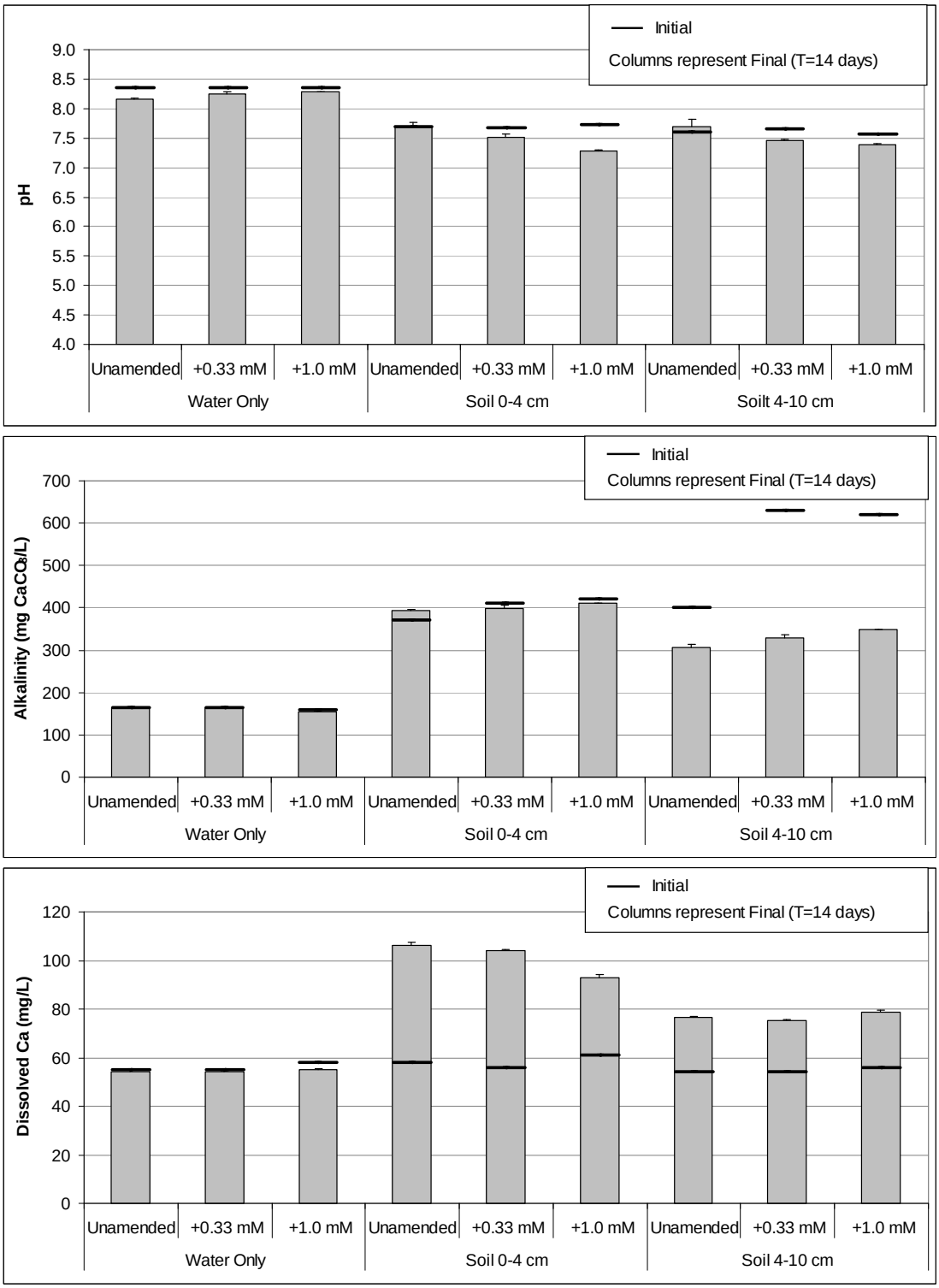


Figure 30. pH (top panel), alkalinity (middle panel), and dissolved calcium (Ca) (bottom panel) concentrations before and after a 14-day anaerobic incubation of soils from STA-2 Cell 1 with sulfate-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water. Each data point represents the mean (n=3) ± 1 S.E. except for T=0 where a composite of two replicates were analyzed.

Heterotrophic Microbial Respiration and Methanogenesis During Anaerobic Incubations

The CH_4 concentrations emitted to the headspace of the incubation vessels varied among sites, depths and sulfate treatment (Figures 31 – 33). Increasing sulfate additions resulted in less methane production in all three of the incubated soils, except for the 0-4 cm STA-2 Cell 1 soil layer where the unamended and 0.33 mM sulfate-amended soils produced approximately the same methane concentrations. Headspace CH_4 concentrations for the unamended 0-4 cm depth soils reached 2.9 – 3.4% on the final day of incubation in all three wetland soils (Figures 31 – 33). On the other hand, the 1.0 mM sulfate-amended 0-4 cm deep soils were considerably lower, ranging from 0.7 (WCA-3A) to 1.5 (WCA-2A U3) and 2.0% (STA-2 Cell 1). Methane concentrations in the 0.33 mM sulfate-amended bottles were usually in-between the unamended and 1.0 mM amended soils from the same location. The STA2 Cell 1 soil exhibited the least CH_4 response to different sulfate amendments for the 0-4 cm depth layer compared to the soils from the WCAs, likely due to the high ambient sulfate concentration (18 mg/L).

Methane production was lower in the 4-10 cm than the 0-4 cm soil strata for all respective treatments, especially for the WCA soils. Methane production in the STA-2 Cell 1 soil was more sensitive to sulfate amendments in the 4-10 cm than 0-4 cm horizon.

The duration of heterotrophic microbial CO_2 activity also varied among the three wetland soils and depth horizons. For WCA-3A soil, CO_2 emissions lasted only 5 days and 3 days in the 0-4 and 4-10 cm depth horizons, respectively, regardless of the level of sulfate enrichment (Figure 31). On the other hand, CO_2 emissions occurred somewhat continuously in all the amended and unamended soils representing the 0-4 soil horizon in WCA-2A U3 and STA-2 Cell 1, where headspace CO_2 concentrations reached 3.5% (Figures 32 and 33). The only effect of sulfate treatment in the soil slurries originating from the 0-4 cm soil depth was observed for WCA-2A U3, where adding 1.0 mM sulfate resulted in a lower CO_2 emission (Figure 32).

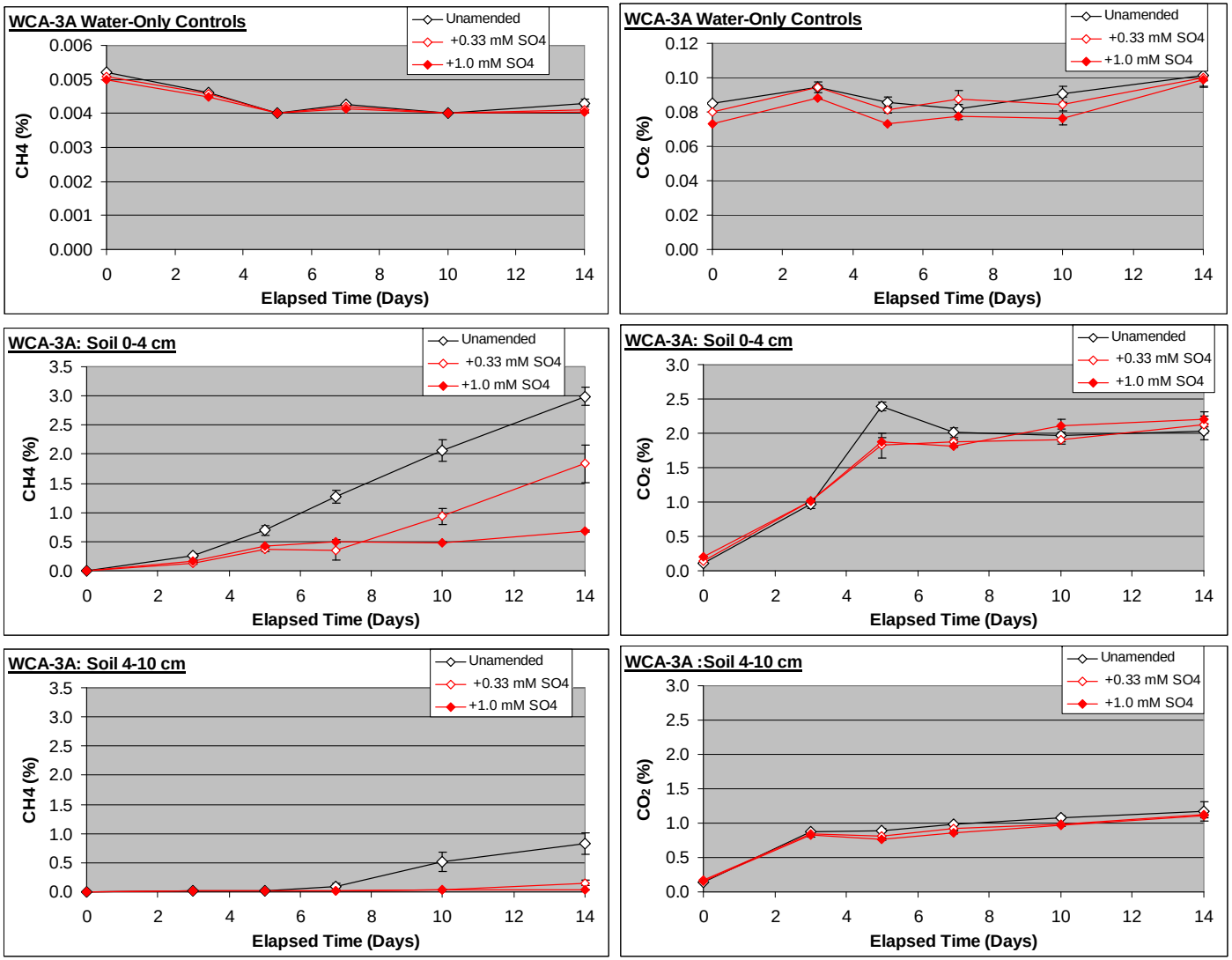


Figure 31. Time course of methane (CH_4) and carbon dioxide (CO_2) mean (n=3 ± 1 S.E.) concentrations in the headspace during anaerobic incubations of soil slurries from two horizons (0-4 and 4-10 cm) in WCA-3A. Each soil-less (water only) and soil slurry vessel received either 0.33 or 1.0 mM sulfate, or was not amended. Note the different Y-axis scales.

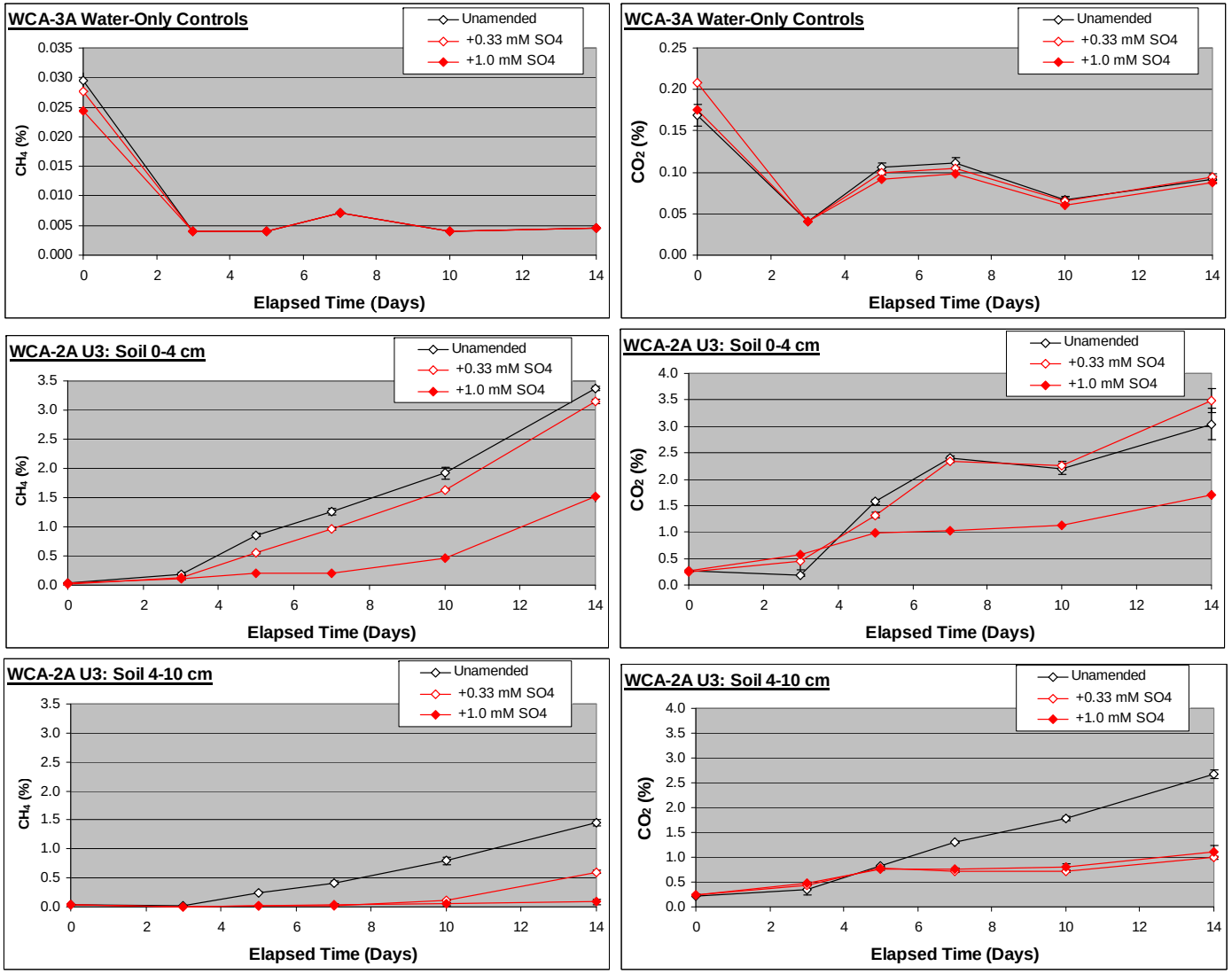


Figure 32. Time course of methane (CH_4) and carbon dioxide (CO_2) mean (n=3 ± 1 S.E.) concentrations in the headspace during anaerobic incubations of soil slurries from two horizons (0-4 and 4-10 cm) in WCA-2A U3. Each soil-less (water only) and soil slurry vessel received either 0.33 or 1.0 mM sulfate, or was not amended. Note the different Y-axis scales.

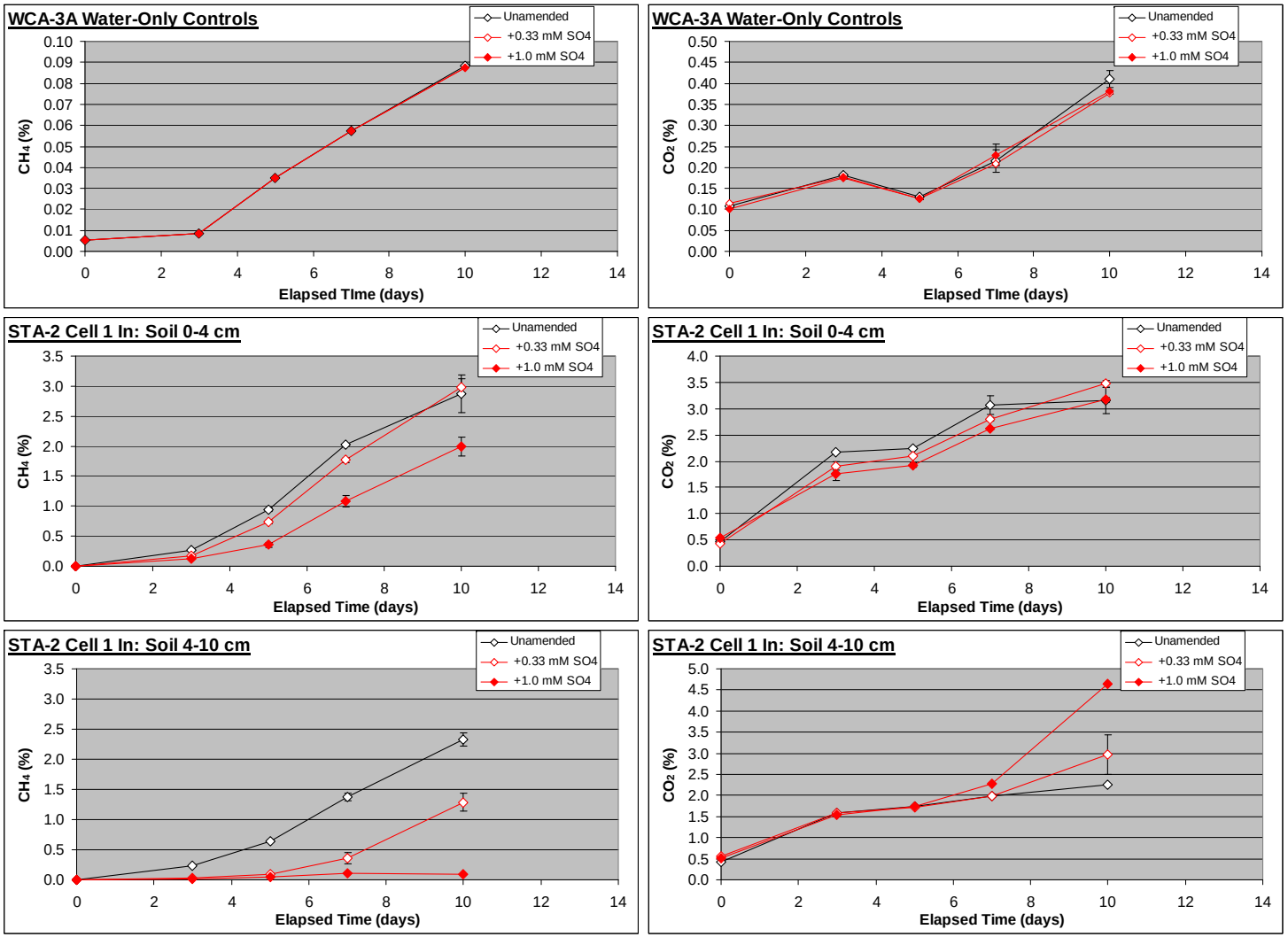


Figure 33. Time course of methane (CH_4) and carbon dioxide (CO_2) mean (n=3 ± 1 S.E.) concentrations in the headspace during anaerobic incubations of soil slurries from two horizons (0-4 and 4-10 cm) in STA-2 Cell 1 In. Each soil-less (water only) and soil slurry vessel received either 0.33 or 1.0 mM sulfate, or was not amended. The sampling period covered only 10 instead of 14 days due to a technical difficulty. Note the different Y-axis scales.

As was observed for the CH_4 concentrations, the CO_2 concentrations measured in the headspace were lower in the 4-10 than 0-4 cm soil layers within each wetland soil (Figures 31 – 33). The CO_2 emission in the 4-10 cm soil layer from WCA-2A U3 was less responsive to sulfate treatment than in the 0-4 cm soil horizon. Both the 0.33 and 1.0 mM sulfate additions inhibited heterotrophic microbial activity relative to the unamended soil in the 4-10 cm soil layer (Figure 32). Such treatment inhibition did not exist in the 4-10 cm soil layer collected from the other two wetlands (Figures 31 and 33). The only indication of a positive effect on heterotrophic microbial respiration of adding sulfate was on day 10 of the 4-10 cm soil layer from STA-2 Cell 1 (Figure 33).

Due to the higher solubility of CO_2 than CH_4 in water (24-fold), and the hydration of dissolved CO_2 and subsequent dissociation to HCO_3^- depending on pH, the mass of CO_2-C generated in each of the incubation vessels was typically > than 10 times the mass of CH_4-C released in the same control or treated vessel (Tables 10 – 12). The soil-less (water only) controls produced negligible quantities of CH_4-C compared to the vessels containing soils, indicating that potential methanogens in the soil-less controls were substrate-limited.

The amounts of C emitted as either CH_4 or CO_2 per initial dry weight and organic carbon content of soil are plotted for each soil and treatment (Figures 34 and 35) after 10 days of incubation. Since organic C in each soil comprised a large amount of the dry weight (Tables 4 and 5), the relationship among the soil types and treatments were similar using dry weight or organic C as the unit of measure.

CO_2-C accounted for up to 9.1% of the soil carbon mineralized in 10 days for the unamended 0-4 cm soil layer from WCA-2A U3. The lowest amount (1.3%) of initial organic soil C mineralized in 10 days for the same soil horizon was the unamended control at WCA-3A (Figure 35). For the 4-10 cm soil depth, the unamended WCA-2A U3 was still the highest evolving CO_2-C soil with 3.3% of the soil organic C mineralized after 10 days. The lowest mineralized soil was WCA-3A, where unamended and amended treatments resulted in only 0.6-0.7% of the soil organic C mineralized as CO_2-C.

The combined CO_2-C + CH_4-C emission, which represents the sum of the respiratory and methanogenic microbial processes, clearly demonstrate higher mineralization rates in the 0-4 than 4-10 soil horizons for all three wetland soils (Figure 36). The inhibition in the CO_2 production rates by sulfate for the WCA-2A U3 and STA-2 Cell 1 soils, particularly in the 0-4 cm horizon, is contrary to the original hypothesis that mineralization of organic matter is amplified with increasing sulfate concentrations.

Table 10. Mass (mg) of CH_4-C and CO_2-C emitted from soil-less (water only) and slurries representing the 0-4 and 4-10 cm soil depths of WCA-3A after 14 days of anaerobic incubation in the lab. Each value represents the mean of three replicates.

		Sulfate Amendment Concentration		
Treatment	**Gas**	**None**	**+0.33 mM**	**+1.0 mM**
Water only	CH_4-C	0.002	0.001	0.001
0-4 cm soil	CH_4-C	0.84	0.48	0.18
4-10 cm soil	CH_4-C	0.22	0.04	0.01
Water only	CO_2-C	2.27	2.26	2.19
0-4 cm soil	CO_2-C	6.68	6.48	7.43
4-10 cm soil	CO_2-C	6.22	5.44	5.32

Table 11. Mass (mg) of CH_4-C and CO_2-C emitted from soil-less (water only) and slurries representing the 0-4 and 4-10 cm soil depths of WCA-2A U3 after 14 days of anaerobic incubation in the lab. Each value represents the mean of three replicates.

		Sulfate Amendment Concentration		
Treatment	**Gas**	**None**	**+0.33 mM**	**+1.0 mM**
Water only	CH_4-C	0.002	0.002	0.002
0-4 cm soil	CH_4-C	1.00	0.94	0.44
4-10 cm soil	CH_4-C	0.44	0.18	0.02
Water only	CO_2-C	2.43	2.41	2.46
0-4 cm soil	CO_2-C	28.1	24.7	9.44
4-10 cm soil	CO_2-C	28.9	8.15	6.85

Table 12. Mass (mg) of CH_4-C and CO_2-C emitted from soil-less (water only) and slurries representing the 0-4 and 4-10 cm soil depths of STA-2 Cell 1 Inflow Regions after 10 days of anaerobic incubation in the lab. Each value represents the mean of three replicates.

		Sulfate Amendment Concentration		
Treatment	**Gas**	**None**	**+0.33 mM**	**+1.0 mM**
Water only	CH_4-C	0.032	0.032	0.032
0-4 cm soil	CH_4-C	0.86	0.88	0.58
4-10 cm soil	CH_4-C	0.66	0.37	0.02
Water only	CO_2-C	5.67	6.45	7.01
0-4 cm soil	CO_2-C	22.8	17.8	10.0
4-10 cm soil	CO_2-C	16.9	12.7	16.8

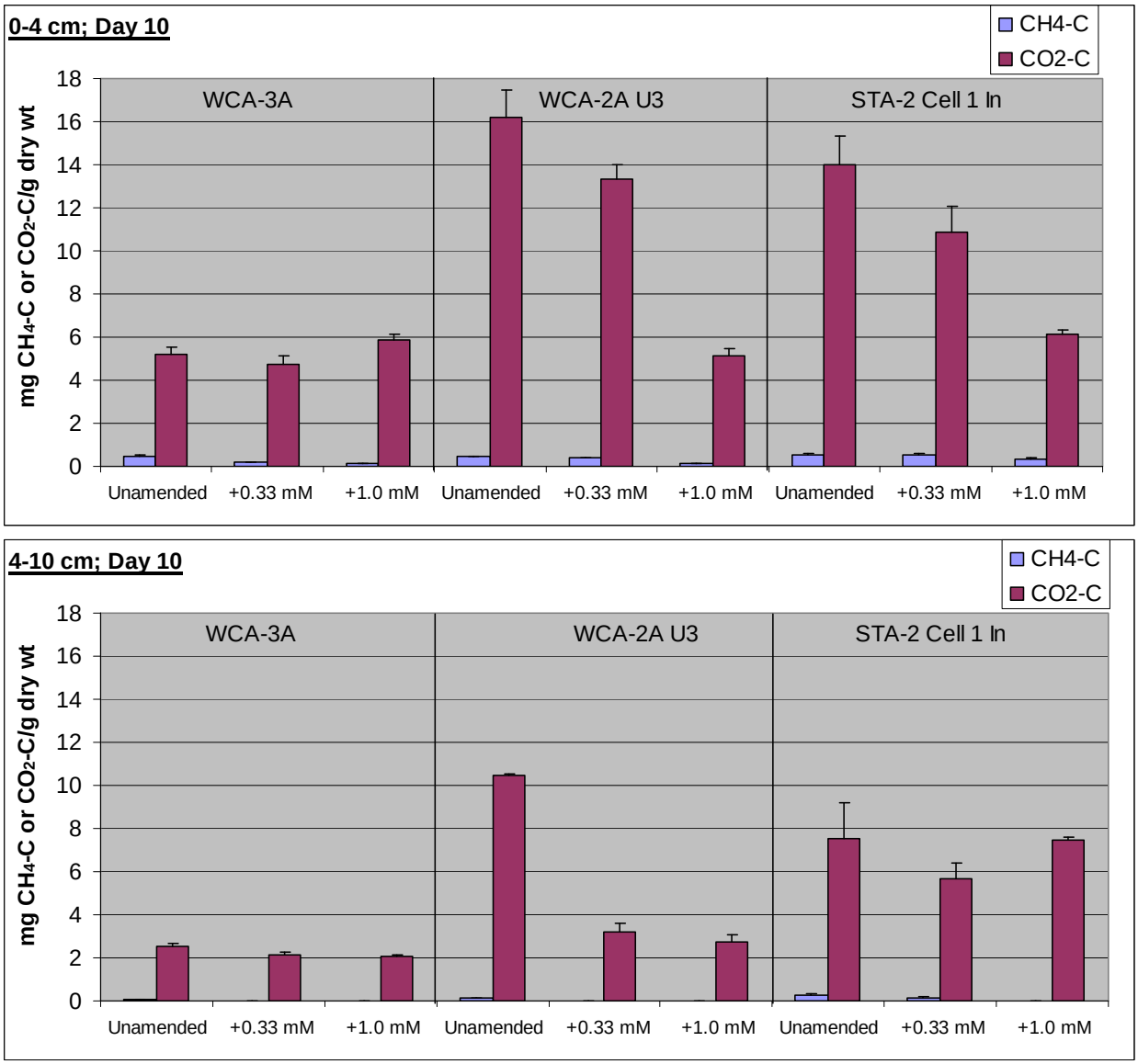


Figure 34. The mass of methane (CH_4) and carbon dioxide (CO_2) mineralized per initial dry weight of soil in the 0-4 cm (top panel) and 4-10 cm (bottom panel) soil layers of WCA-3A, WCA-2A U3, and STA-2 Cell 1 In after 10 days of anaerobic incubation. Each soil layer was either unamended or amended with 0.33 mM (32 mg/L) or 1.0 mM (96 mg/L) sulfate. Each column represents the mean (n=3) and 1 S.E.

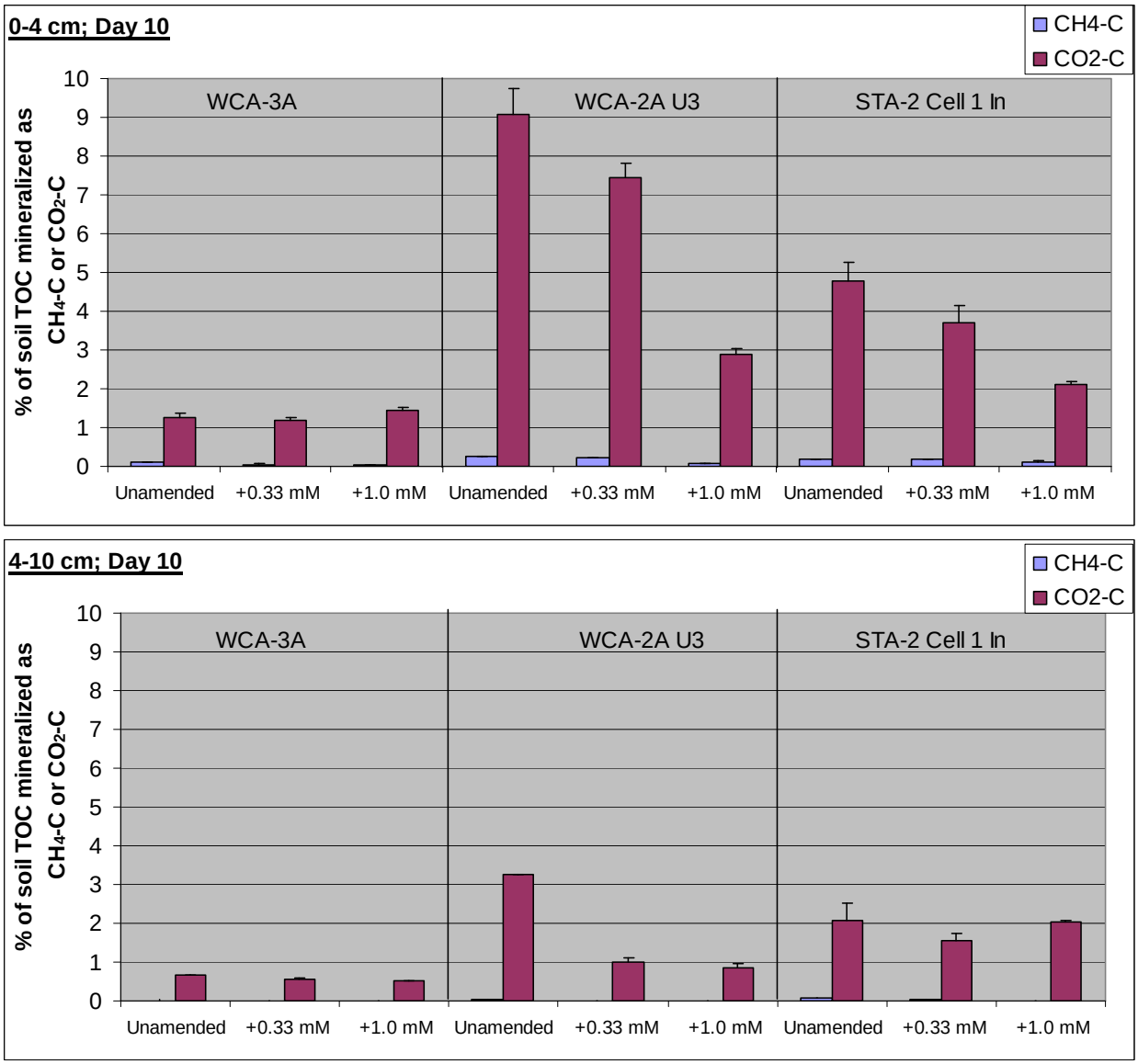


Figure 35. The percent of initial soil organic carbon (TOC) mineralized as methane (CH_4) and carbon dioxide (CO_2) in the 0-4cm (top panel) and 4-10 cm (bottom panel) soil layers of WCA-3A, WCA-2A U3, and STA-2 Cell 1 In after 10 days of anaerobic incubation. Each soil layer was either unamended or amended with 0.33 mM (32 mg/L) or 1.0 mM (96 mg/L) sulfate. Each column represents the mean (n=3) and 1 S.E.

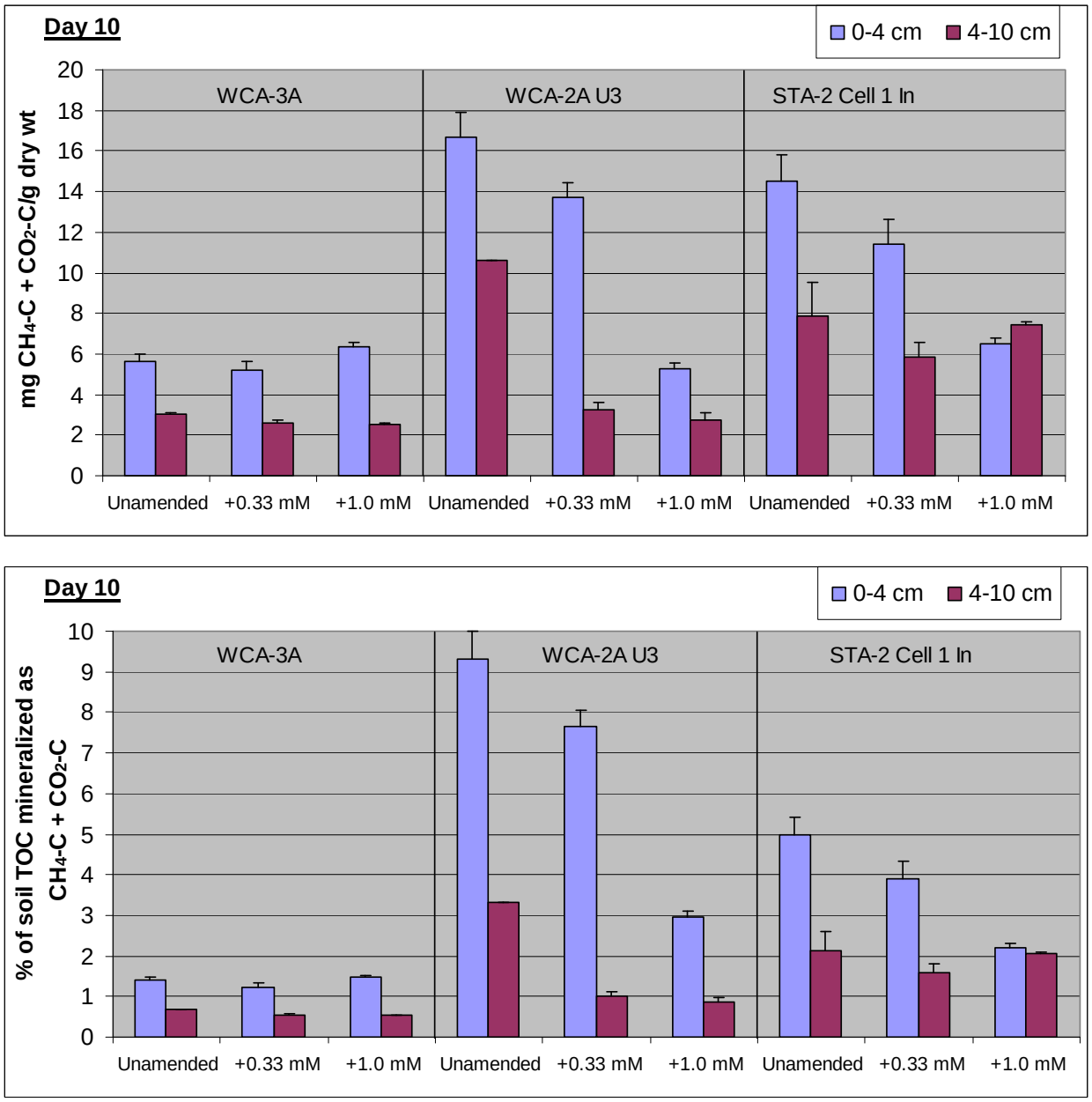


Figure 36. The mass of methane (CH_4) plus carbon dioxide (CO_2) mineralized per initial dry weight of soil (top panel) or as a percentage of initial soil total organic carbon (TOC) (bottom panel from the 0-4cm and 4-10 cm soil layers of WCA-3A, WCA-2A U3, and STA-2 Cell 1 In after 10 days of incubation. Each soil layer was either unamended or amended with 0.33 mM (32 mg/L) or 1.0 mM (96 mg/L) sulfate. Each column represents the mean (n=3) and 1 S.E.

Discussion

Soil Characteristics and Porewater Phosphorus

Overall, soil TFe concentrations at all sites were low compared to wetland soil TFe values reported in the literature (Loeb et al. 2008; Guerts et al. 2008). Futher, the Fe-P fraction was low in the WCA-3A soil, even though the TFe concentration was highest in WCA-3A among the three sites (Tables 4 – 6). Only 0.05, 0.17, and 0.04% of the TFe was associated with P in the 0-4, 4-10, and 10-30 cm soil horizons in P-limited WCA-3A.

The high S concentration (1.19%) in the 10-30 cm soil at WCA-2A U3 represents a value that is close to the original S content of the soil in that region prior to agricultural drainage water (ADW) inputs, that began in the late 1960s or early 1970s. Bates et al. (2002) reported TS concentrations greater than 2% below 122 cm soil depth in the EAA. In WCA-2A U3, the accrued soil has become more enriched with $CaCO_3$, which has diluted the TS content in those accrued layers. However, other processes, such as enhanced oxidation of organic sulfur (with subsequent leaching of the sulfate) due to altered hydroperiods or a more favorable chemical milieu (e.g., a higher pH), may also have influenced soil S concentrations. Apparently the increased sulfate concentrations in the ADW at WCA-2A U3 did not result in an increase in the S content of the soil, indicating plant uptake and adsorption were not prominent pathways in retaining sulfate. If plant uptake and adsorption of the elevated sulfate in the ADWs were prominent processes at WCA-2A U3, then one would expect an increase (and not a decrease) in the S content of the soils. Due to the reduced conditions in the soil, the likely fate of most of the introduced sulfate was conversion to sulfide.

The observed concentrations of P in the soil porewater depended on the location with respect to distance from the inflow of P-laden canal waters. By their design, STAs receive higher P concentration waters than downstream WCAs. After operating for 7-8 years, the soil and porewater P concentrations in STA-5 and STA-2 Cell 1 inflow regions are much higher than those at the WCA sites that are remotely located from canal discharges.

Organic Matter Mineralization and Methanogenesis

Mineralization

We found no evidence that increasing concentrations of sulfate (electron acceptor) resulted in enhanced microbial respiration at any of the wetland sites. Although organic matter mineralization and sulfate reduction were clearly occurring in all of the wetland soil incubations, several independent lines of evidence demonstrate that adding sulfate to the soils did not enhance mineralization or P release.

We view ammonia as a proxy for organic matter mineralization in these P-limited soils. Even though ammonia concentrations increased significantly during all of the soil incubations, the increases were usually unaffected by sulfate additions (Figures 12, 16, 24, 28). This indicates that sulfate amendments had no effect on ammonification, the process where nitrogenous compounds in organic matter are deaminated.

Sulfate reduction to oxidize organic matter consumes H^+ ions, thereby increasing the pH and alkalinity. However, the pH decreased during the incubations of all wetland soils (Figures 14, 18, 22, 26, and 30), indicating that microbial processes other than organic matter oxidation - with sulfate serving as the terminal electron acceptor - were occurring. The alkalinity concentrations increased in most of the soils during the incubation (Figures 14, 18, and 22), and at times higher alkalinities were measured for the sulfate-amended than unamended soils at the end of the incubation (Figures 14 and 18). However, the increases in alkalinity were more likely due to the dissolution of $CaCO_3$ (Ca concentrations increased during all of the soil incubations (Figures 14, 18, 22, 26, and 30)) caused by the lowered pH values than by the action of sulfate-reducing prokaryotes.

A more direct way to determine anaerobic organic matter mineralization is by measuring the gases (CO_2 and CH_4) that represent the end products of anaerobic respiration. Based on the emission rates of those gases, we found no evidence of enhanced organic matter mineralization with 0.33 mM or 1.0 mM sulfate amendments to soils from WCA-3A, WCA-2A U3, or STA-2 Cell 1 (Figure 35). In fact, sulfate amendments had an apparent inhibitory effect on the emission of CO_2 and CH_4 in the WCA-2A U3 soil (Figures 32 and 35).

WCA-2A U3 soils were a consistent outlier group compared to the other two wetland soils when soil P properties are plotted versus the percentage of soil organic carbon mineralized to CO_2 and CH_4 during the incubations (Figure 37). Correlations were found between the soil P pools (i.e., TP, MBP, Fe/Al-bound P, TFe:TP (wt/wt), Fe/Al-bound P:TP, labile P) and the percent of organic carbon mineralized to CO_2 and CH_4 in the WCA-3A and STA-2 Cell 1 soils, but not for the WCA-2A U3 soil (Figure 37). This indicates that the soil P pools may limit the mineralization of organic matter in WCA-3A and STA-2 Cell 1, but not in WCA-2A U3.

Acetate normally is an intermediate product used by sulfate-reducing prokaryotes and methanogens as a substrate to produce CO_2 and CH_4. However, detailed studies into the structure and function of soil communities present along the trophic gradient in WCA-2A indicated a dominance of incomplete acetate oxidizers in the sulfate-reducing guilds in U3 soil (Castro et al. 2002; Chauhan and Ogram 2006). This would mean that sulfate-reducing prokaryotes may be limited in their ability to metabolize acetate in U3 soil, which would lead to the accumulation of acetate as a carbon-containing end product of anaerobic mineralization at the expense of other carbon-containing end products such as of CO_2 and CH_4. Hines et al. (2008) found acetate was a major end product of anaerobic metabolism in many northern (boreal) wetland sites, exceeding CH_4 production in most cases and even CO_2 production in some.

We did not measure acetate in this study, and therefore cannot substantiate whether acetate accumulated in the amended U3 soils. If it did, as suggested by other investigators, then the mineralization of organic matter may have been actually enhanced by sulfate amendments, which went undetected by measuring only CO_2 and CH_4 (and not acetate) as the end products. If acetate did accumulate in significant quantities during the incubation, then our calculated values of organic matter mineralization based on gaseous C evolution represent underestimates.

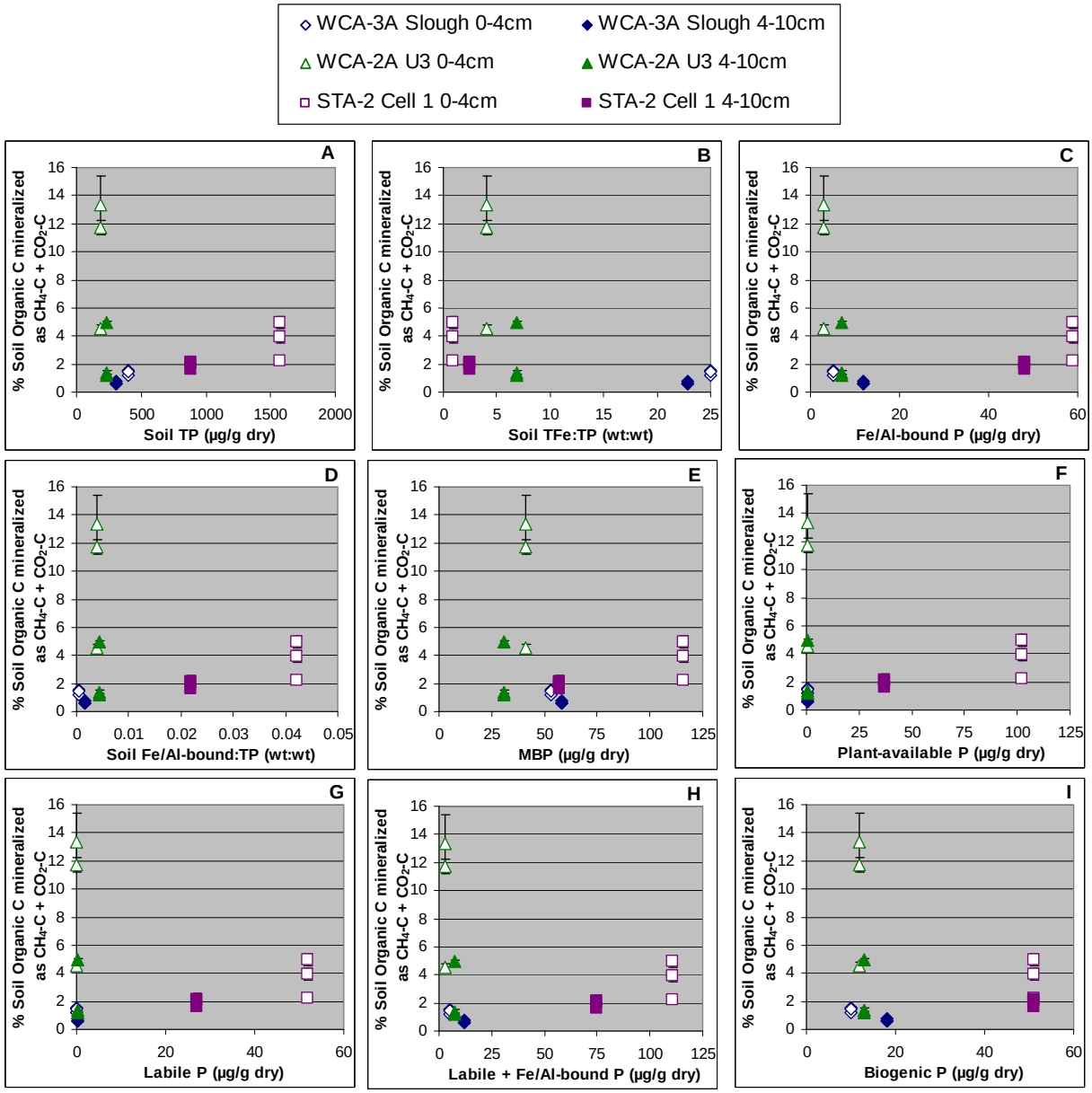


Figure 37. The relationship between initial soil phosphorus pools and the amount of organic carbon mineralized as carbon dioxide (CO_2) and methane (CH_4) for two soil depths (0-4 and 4-10 cm) from each of three wetland soils that received two (+0.33 mM, and 1.0 mM) sulfate amendments and one unamended treatment. Anaerobic incubations were performed for 10 (STA-2 Cell 1) and 14 (WCA-3A and WCA-2A U3) days. MBP = Microbial Biomass P; Plant-available P = 0.5 M $NaHCO_3$ extracted SRP; Labile P = Porewater + 1.0 M NH_4Cl extracted SRP; Fe/Al-bound P = 0.1 M NaOH extracted SRP; Biogenic P = 0.1 M NaOH organic P. Each data point represents the mean (n=3) ± 1 S.E.

Notwithstanding the possible underestimates of organic matter mineralization with sulfate enrichment, mineralization in the WCA-2A U3 soils was the highest for any of the three wetland soils in terms of g dry weight and soil organic carbon (Figure 36). Our measured CO_2 production rates at WCA-2A U3 were two times higher than rates reported by Wright and Reddy (2001), who also measured CO_2 rates in an unimpacted area of WCA-2A in 1997. They reported a CO_2 production of 2.5 and 1.3 mg CO_2-C/g dry for the detrital and 0-10 cm soil layers under sulfate-reducing conditions during a 10-day incubation, compared to 5.2 and 2.7 mg CO_2-C/g dry for the 0-4 (includes the detritus layer) and 4-10 cm soil depth in our study. Their initial sulfate amendment was 6.97 mg SO_4/g dry, which was higher than the 1.8-5.6 (0-4 cm) and 2.7-3.7 (4-10 cm) mg SO_4/g dry we added as a 1.0 mM amendment.

A variety of environmental factors may affect the composition and activities of syntrophic consortia, including the availability of carbon (Chauhan and Ogram 2006). We attribute the lack of a response by sulfate-reducing prokaryotes in WCA-3A to sulfate enrichment as due to substrate limitation, which caused the early decline in CO_2 emissions in the 0-4 and 4-10 cm soil layers (Figure 31). The CO_2 production ceased after five days in the 0-4 cm and 3 days in the 4-10 cm soil depth incubations regardless of the sulfate treatment. This indicates that organic substrates (the electron donor) became exhausted early in the incubation and limited further decomposition. Whether this was due to the recalcitrant nature of the carbon bonds in the substrate or to P limitation inherent in the carbon compounds is uncertain. Both reasons are plausible given the high LCIs (0.71 and 0.76), TC:TP ratios (1060 and 1251, wt/wt), and TFe:TP ratios (25 and 23, wt/wt) for the 0-4 and 4-10 cm soils from that location (Figures 37B; 38A and B). Although all of the initial sulfate concentrations were reduced by the end of the 14-day incubation period in the unamended and amended soil slurries from the 0-4 cm layer (Figure 17), the rate of sulfate production may have been rapid enough to have removed the initial sulfate concentrations by the fifth day, corresponding to when the production of carbon-containing end products was arrested (Figure 31).

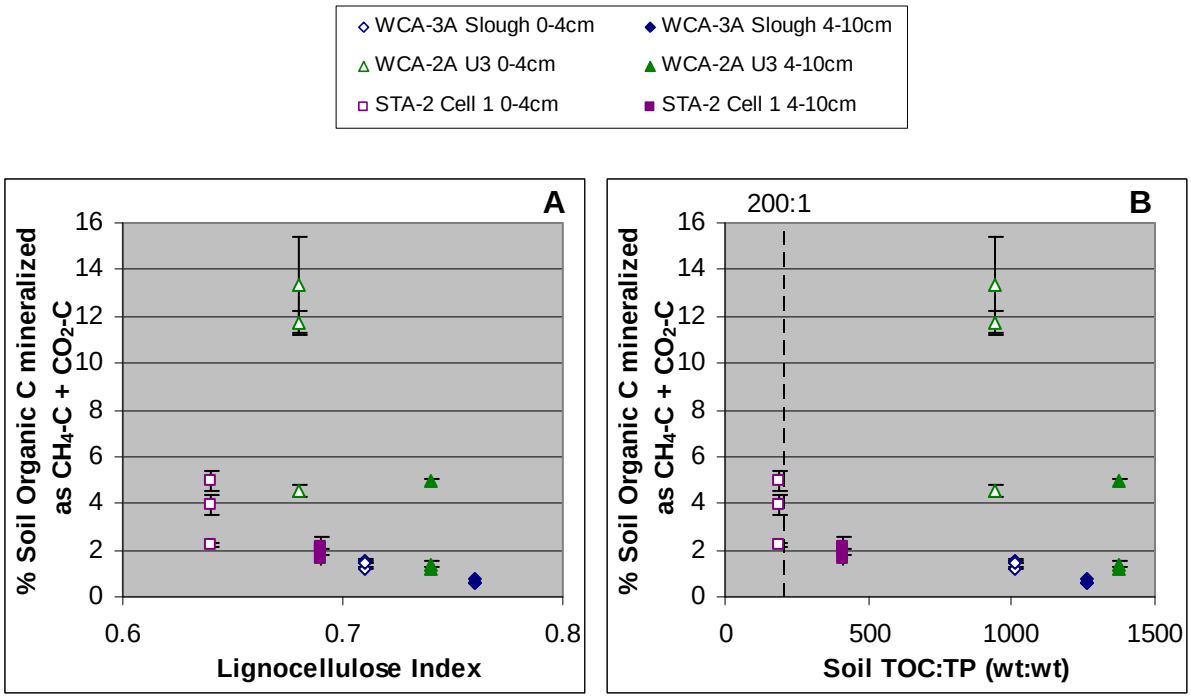


Figure 38. The relationship between the lignocellulose index (A) and soil total organic carbon (TOC) to total phosphorus (TP) ratio (B) and the amount of organic carbon mineralized as carbon dioxide (CO_2) and methane (CH_4) for two soil depths (0-4 and 4-10 cm) from each of three wetland soils that received two (+0.33 mM and 1.0 mM) sulfate amendments and one unamended treatment. Anaerobic incubations were performed for 10 (STA-2 Cell 1) and 14 (WCA-3A and WCA-2A U3) days. The higher the lignocellulose index, the more recalcitrant the substrate. The vertical dashed line in panel B marks the ratio or soil TOC:TP above which P can limit decomposition (Brinson 1977). Each data point represents the mean (n=3) ± 1 S.E.

Soils from the more enriched sites (STA-5 and STA-2 Cell 1) did release SRP during the incubations, but still without an effect of added sulfate (Figures 23 and 27). The substrates in these environments were not as limiting since the organic matter was more labile and contained higher labile P pools and total P concentrations (Figure 37 and Figure 38). Yet, sulfate amendments did not increase mineralization rates in the STA-2 Cell 1 soil (Figure 36). For these soils, there may have been an initial adequate supply of electron acceptors (nitrate and sulfate) in the unamended soils to cause indistinguishable effects of added sulfate. We did not measure nitrate concentrations, but the initial sulfate concentrations were 23 – 28 mg/L in STA-5 and 14 – 18 mg/L in STA-2 soils due to the carry-over within the soils when they were transferred and mixed with the low P and low sulfate incubation waters.

Higher CO_2 production occurred within the 0-4 than the 4-10 cm soil strata in all three of the soils (Figures 34 and 35), indicating substrate quality is less desirable as an electron donor during decomposition of the 4-10 cm soils. This is supported by the differences in the LCI, TC:TP ratio, and TFe:TP ratio among and within the soils (Tables 4 and 5). For all three soils, the LCI was higher in the 4-10 than 0-4 cm soil depths. A higher LCI indicates more lignin present than the more easily degradable cellulose component of organic matter. The C:P ratio (wt/wt) also increased with soil depth for all soils, indicating that decomposition of organic matter for both soil depths was likely P limited since most of the ratios were > 200, a cutoff point where decomposition of organic matter can be P limited (Brinson 1977). The MBP, a P pool that can readily contribute labile P, decreased in the 4-10 cm compared to the 0-4 cm soil depth in two of the three soils (Table 13). Other investigators (Wright and Reddy 2001) have also reported less organic mineralization with soil depth, which corresponded to lower substrate quality. Thus substrate quality is likely to be a primary controlling factor of heterotrophic microbial respiration in subsurface soils.

Table 13. The lignocellulose index (LCI), total carbon (TC) to total phosphorus (TP) ratio (wt/wt), and the microbial biomass phosphorus (MBP, μg/g dry) for the soils collected at the 0-4 and 4-10 cm horizons in WCA-3A, WCA-2A U3, and STA-2 Cell 1 In. Each value represent a composite of 3 or 4 soil cores collected from each site.

	WCA-3A		WCA-2A U3		STA-2 Cell 1 In	
	0-4 cm	4-10 cm	0-4 cm	4-10 cm	0-4 cm	4-10 cm
LCI	0.71	0.76	0.68	0.74	0.65	0.69
TC:TP	1060	1251	1353	1551	218	454
MBP	53	58	41	31	116	57

Methanogenesis

Methane in nature can be produced by two different groups of methanogenic archaea:

1. Acetate Fermentators: acetate $\rightarrow CO_2 + CH_4$ acetoclastic methanogenesis
2. Hydrogenotrophs: $4H_2 + CO_2 \rightarrow 2H_2O + CH_4$ hydrogenotrophic methanogenesis

Both groups are strictly anaerobic, and are suppressed by sulfate-reducing prokaryotes (Lovley and Klung 1983). The electron donors used by sulfate-reducing prokaryotes are primarily low-molecular-weight compounds and most of these are known to be fermentation products of bacterial degradation of carbohydrates, proteins and other detritus compounds. However, sulfate-reducing prokaryotes often out-compete fermenting and methanogenic bacteria because of a high affinity for the most common substrates in submerged soils and sediments, such as acetate and H_2 (Holmer and Storkholm 2001). This likely occurred in our anaerobic soil slurries where methane production for the three soils was inhibited with increased sulfate additions at both the 0-4 and 4-10 cm depths (Figures 31 – 33).

Methanogenesis was minor compared to other fermentative processes and sulfate-reduction in the terminal carbon flow (Figures 34 and 35). This was likely due in large measure to the added sulfate, where methane emission rates clearly decreased with sulfate addition (Figures 34 and 35). The short incubation times chosen for this study of 10 and 14 days may have also contributed to the low CH_4 emission rates, as the sulfate amendments would have remained

inhibitory to methanogenesis during most, if not all, of the incubation period. The CH_4 generation profiles shown in Figures 31 – 33 typically show higher CH_4 generation rates towards the end of the incubations when the amended sulfate concentrations would have been reduced to very low levels as shown in Figures 17, 21, and 29. Nevertheless, methane generation was also low in the unamended soils. This may have been partially due to the initial sulfate concentrations that were carried over with the soils inserted into the serum bottles: WCA-3A, WCA-2A U3 and STA-2 Cell 1 soils had initial sulfate concentrations of 6.5, 7.5, and 18 mg/L, respectively. Another possible reason for low methane production, particularly in the oligotrophic WCA sites, was the dominance of incomplete acetate oxidizing sulfate-reducing prokaryotes (Castro et al. 2002; Chauhan and Ogram 2006).

Soluble Reactive P Release

We never observed a sustained net increase in SRP due to sulfate enrichment compared to unamended soils, which included two separate incubations for WCA-3A soil, and one incubation for each of WCA-2A-U3, STA-5, and STA-2 Cell 1 soils (Figures 11, 15, 19, 23, and 27). Although there is ample evidence that sulfate enrichment leads to enhanced soil P release (Wright and Reddy 2001; Smolders et al. 2006a; Zak et al. 2006; Guerts et al. 2008), there are notable exceptions reported in the literature (Lamers et al. 2001, 2002).

Across all three wetland soils (WCA-3A, WCA-2A U3, and STA-2 Cell 1), two soil depths (0-4 and 4-10 cm), and three sulfate treatments (unamended, +0.33 mM, and +1.0 mM), there was no relationship between the amount of soil organic carbon mineralized and SRP released during the incubations (Figure 39). The wetland soil that deviated the greatest was WCA-2A U3, where the highest mineralization rates were measured (Figure 36) along with very low P release (Figure 19).

The soil P pools did correlate well with ending SRP concentrations in the incubation waters for all wetland soils and depth intervals (Figure 40). The implication is that the sizes of the readily available soil P pools (i.e., MBP, Fe/Al-bound, labile, plant-available) affect the release of SRP into low P and low sulfate incubation water during anaerobic incubations. A similar correlation also was observed, but to a lesser extent, for soil TP content (Figure 40A). Other investigators

(Zak et al. 2006; Loeb et al. 2008; Guerts et al. 2008) have reported a correlation of P release with the soil Fe-P fractions, amorphous Fe:Fe-P fractions, or TFe:TP concentrations.

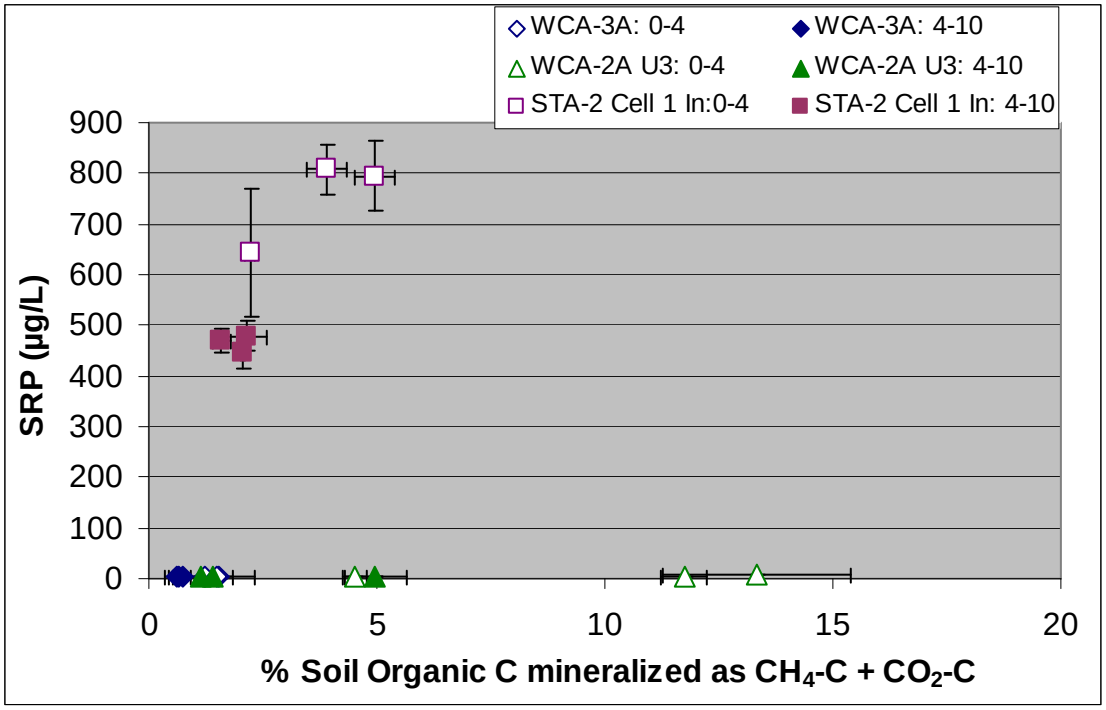


Figure 39. The relationship between the percentage of initial organic carbon (C) mineralized as carbon dioxide (CO_2) and methane (CH_4) with the soluble reactive phosphorus (SRP) concentration in the overlying water of soil slurries from the 0-4 and 4-10 cm soil horizons after 10 (STA-2 Cell 1 In) or 14 (WCA-3A, WCA-2A U3) days of incubation. Each soil layer (0-4 and 4-10 cm depth strata) was either unamended or amended with 0.33 mM (32 mg/L) or 1.0 mM (96 mg/L) sulfate. The error bars represent ± 1 S.E. of the mean (n=3).

In addition to the size of the available P pools, the TFe content in the soils may also play a role in the release of SRP (Figure 40B). Guerts et al. (2008) suggested the pore water Fe:PO_4 and soil Fe:TP ratios could be a valuable prognostic tool for the restoration of water quality and biodiversity in fen waters. Based on a cross-section study of 145 fen waters in the Netherlands, Ireland, and Poland, they found SRP and TP concentrations in the water layer increased markedly at SRP concentrations above 155 to 310 µg/L in sediment pore water. High surface water SRP and TP concentrations appeared to be sulfate-induced below threshold values of pore water Fe:SRP of 6.3:1 and total soil Fe:P of 18.1:1 by weight.

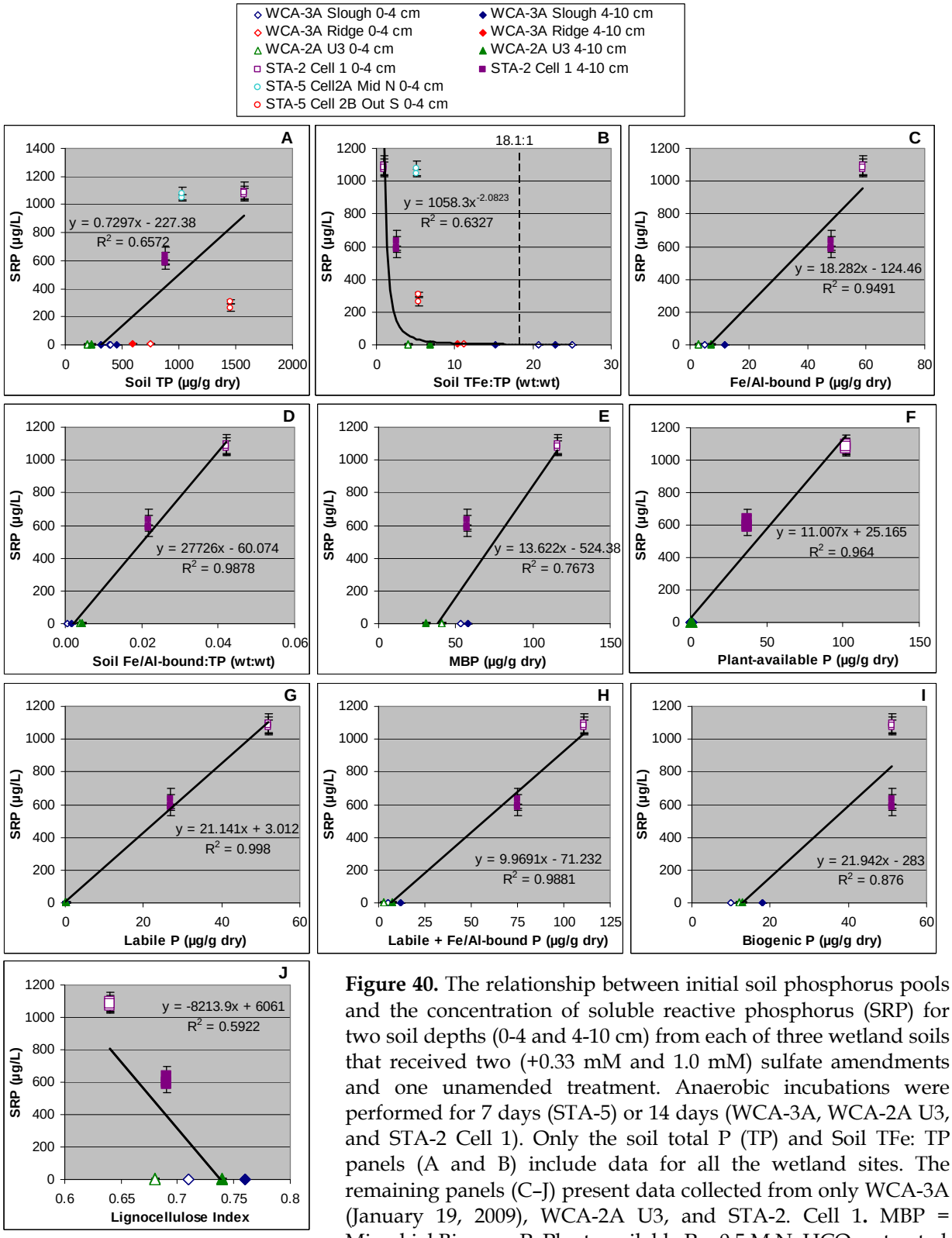


Figure 40. The relationship between initial soil phosphorus pools and the concentration of soluble reactive phosphorus (SRP) for two soil depths (0-4 and 4-10 cm) from each of three wetland soils that received two (+0.33 mM and 1.0 mM) sulfate amendments and one unamended treatment. Anaerobic incubations were performed for 7 days (STA-5) or 14 days (WCA-3A, WCA-2A U3, and STA-2 Cell 1). Only the soil total P (TP) and Soil TFe: TP panels (A and B) include data for all the wetland sites. The remaining panels (C–J) present data collected from only WCA-3A (January 19, 2009), WCA-2A U3, and STA-2. Cell 1. MBP = Microbial Biomass P; Plant-available P = 0.5 M $NaHCO_3$ extracted SRP; Labile P = Porewater + 1.0 M NH_4Cl extracted SRP; Fe/Al-bound P = 0.1 M NaOH extracted SRP; Biogenic P = 0.1 M NaOH organic P. The vertical dashed line in panel B marks the ratio of soil TFe:TP

above which Fe can immobilize SRP according to Guerts et al. (2008). Each data point represents the mean (n=3) ± 1 S.E.

Our data from the Everglades WCAs and STAs substantiate the relationship between porewater SRP concentrations and surface water SRP and TP concentrations (Figure 41). The higher soil and porewater Fe concentrations at WCA-3A points to the possibility that Fe minerals may be important in regulating surface water P concentrations. Porewater Fe:SRP and soil TFe:TP ratios (wt/wt) ranged from 83:1 to 195:1, and 11:1 to 25:1, respectively, for the ridge and slough soils in WCA-3A. However, we found two to three orders of magnitude lower measured phosphate concentrations compared to the theoretical concentrations based on thermodynamic equilibria relationships among four solid phase Fe minerals [$Fe(OH)_2$, $FeCO_3$, FeS, and $Fe_3(PO_4)_2$] for the ridge and slough porewaters in WCA-3A. This suggests that $Fe_3(PO_4)_2$ is not controlling the solubility of inorganic P in the porewaters of WCA-3A soils. For WCA-2A U3, STA-5, and STA-2 Cell 1, the porewater Fe:SRP and soil TFe:TP ratios (wt/wt) were < 6.3: and < 18.1:1, respectively, suggesting that Fe also does not control P solubility and release to overlying waters (Guerts et al. 2008).

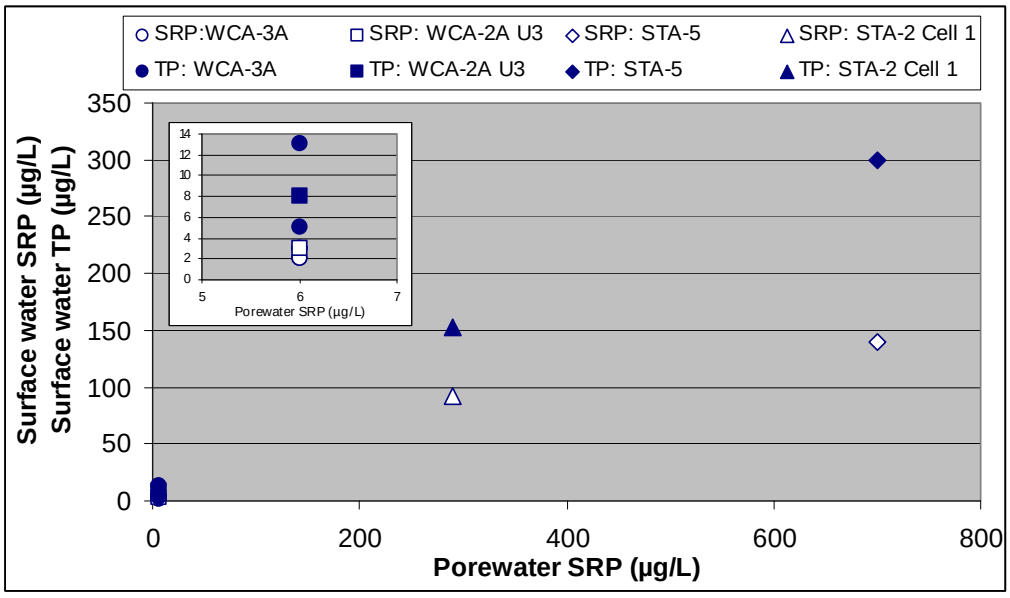


Figure 41. The relationship between porewater soluble reactive phosphorus (SRP) concentration, and surface water SRP and total phosphorus (TP) concentrations for the wetland soils where cores were retrieved for anaerobic incubations in the lab. Each data represent the mean of three replicate samples collected at each site.

Phosphorus can also be released when Ca compounds in soil are being dissolved, which depends on pH changes. Whereas aerobic respiration results in a decrease of pH from CO_2 production, the anaerobic respiration pathways increase the pH until the fermentative stage is reached when organic acids and CO_2 are produced. The pH decreased, and dissolved Ca concentrations increased, during all the incubations (Figures 14, 18, 22, 26, and 30), suggesting that acid-generating processes, such as fermentation, were more prominent than acid-consuming processes, such as sulfate reduction.

It may appear that the build-up of CO_2 in the incubation vessels may be an artifact due to the closed system of the gas-tight vessels, which would result in greater decrease in pH and dissolution of $CaCO_3$ than what would be expected to occur in soils under field conditions. However, in most instances, decreases in pH and increases in Ca and alkalinity concentrations that were observed between *in situ* porewaters and overlying surface waters (Tables 7 and 8) were greater than the recorded changes in the incubation vessels between days 0 and 14 (Figures 14, 18, 22, 26, and 30). This indicates that the conditions during incubation did not result in lower pH values than those observed for porewaters of the corresponding soil in the field.

Conclusions

The lack of a soil P mobilization response with sulfate enrichment under anaerobic conditions during the incubations is due to a combination of biogeochemical factors present in the northern Everglades marsh soils. These include high alkalinity, P-limited (and to a lesser extent, C-limited) substrates, low Fe-associated P pools, and dissolution of an inorganic substrate ($CaCO_3$) associated with P. Results from the laboratory incubations are consistent with the historical record collected at sulfate-enriched WCA-2A U3 and recent pore water analyses in STAs and WCAs. Further investigations using controlled mesocosms in the field will provide a more definitive resolution as to whether sulfate enrichment enhances the mobilization of soil P in northern Everglades soils.

References

Axelrad, D., T. Atkeson, T. Lange, C. Pollman, C. Gilmour, W. Orem, I. Mendelssohn, P. Frederick, D. Krabbenhoft, G. Aiken, D. Rumbold, D. Scheidt and P. Kalla. 2007. Chapter 3B: Mercury monitoring, research and environmental assessment in South Florida. Chapter 3B in 2007 South Florida Environment Report. South Florida Water Management District and Florida Dept. of Environmental Protection. ,http://www.sfwmd.gov/sfer/SFER_2007/> West Palm Beach, FL 57 pages.

Bates, A.L., W.H. Orem, J.W. Harvey, and E.C. Spiker. 2002. Tracing sources of sulfur in the Florida Everglades. J. Environ. Qual. 31:287-299.

Brinson, M.M. 1977. Decomposition and nutrient exchange of litter in an alluvial swamp forest. Ecology 58:601-609.

Castro, H., K.R. Reddy, and A. Ogram. 2002. Composition and function of sulfate-reducing prokaryotes in eutrophic and pristine areas of the Florida Everglades. Appl. Environ. Microbiol. 68(12):6129-6137.

Chauhan, A., and A. Ogram. 2006. Fatty-acid oxidizing consortia along a nutrient gradient in the Florida Everglades. Appl. Envr. Micobiol. 72(4):2400-2406.

DB Environmental. 2002. Demonstration of submerged aquatic vegetation/limerock treatment technology for phosphorus removal from Everglades Agricultural Area waters. Prepared for the South Florida Water Management District and the Florida Department of Environmental Protection.

Florida Department of Environmental Protection (FDEP). 2001. Everglades Phosphorus Criterion Technical Support Document. Staff Draft Part III. Water Conservation Area 3 and Everglades National Park. Everglades Technical Support Section, Tallahassee, FL.

Hines, M.E., K.N. Duddleston, J.N. Rooney-Varga, D. Fields, and J.P. Chanton. 2008. Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution. Global Biogeochem. Cycles 22, GB2017, doi:10.1029/2006GB002903

Guerts, J.J.M., A.J.P. Smolders, J.T.A. Verhoeven, J.G.M. Roelofs, and L.P.M Lamers. 2008. Sediment Fe:PO_4 ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. Fresh. Biol. 53:2101-2116.

Holmer, M., and P. Storkholm. 2001. Sulphate reduction and sulphur cycling in lake sediments: a review. Freshwater Biology 46:431-451.

Lamers, L.M., H.M. Tomassen, and J.M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environ. Sci. Technol. 32:199-205.

Lamers, L.P.M., G.E.T. Dolle, S.T.G. van den Berg, S.P.J. van Delft, and J.G.M. Roelofs. 2001. Differential responses of freshwater wetland soils to sulphate pollution. Biogeochemistry 55:87-102.

Lamers, L.P.M., S.-J. Falla, E.M. Samborska, I.A.R. van Dulken, G. van Henstum, and J.G. M. Roelofs. 2002. Factors controlling the extent of eutrophication and toxicity in sufate-polluted freshwater wetlands. Limnol. Ocanogr. 47(2):585-593.

Light, T. S. 1972. Standard solution for redox potential measurements. Analytical Chem. 44:1038-1039.

Loeb, R., L.P.M. Lamers, and J.G.M. Roelofs. 2008. Prediction of phosphorus mobilization in inundated floodplain soils. Environ. Pollut. 156:325-331.

Lovley, D.R., and M.J. Klug. 1983. Appl. Environ. Microbiol. 45:187-192.

Martens, R. 1987. Estimation of microbial biomass in soil by the respiration method: importance of soil pH and flushing methods for the measurement of respired CO_2. Soil Biol. Biochem. 19(1):77-81.

Mendelssohn, I.A., S. Li, H. Chen, and W.H. Orem. 2006. Is sawgrass more sensitive than cattail to sulfate loading and resultant hydrogen sulfide Presentation documents. South Florida Environmental Report Peer Review Panel, South Florida Water Management District, West Palm Beach, FL.

Moutin, T., J.Y. Gal, H. Halouani, B. Picot and J. Bontoux. 1992. Decrease of phosphate concentration in a high rate pond by precipitation of calcium phosphate: theoretical and experimental results. Water Research: 26(11):1445-1450.

Picot, B, H. Halouani, C. Casellas, S. Moersidik and J. Bontoux. 1991. Nutrient removal by high rate pond system in Mediterranean climate (France). Water Science and Technology 23:1535-1541.

Scheidt, D.J., and P.I. Kalla. 2007. Everglades ecosystem assessment: water management and quality, eutrophication, mercury contamination, soils and habitat: monitoring for adaptive management: a R-EMAP status report. USEPA Region 4, Athens, GA. EPA 904-R-07-001. 98 pp.

Smolders, A.J.P., L.P.M. Lamers, E.C.H.E.T. Lucasseen, G. Van der Velde, and J.G.M. Roelofs. 2006a. Internal eutrophication: How it works and what to do about it – a review. Chemistry and Ecology 22:93-111.

Smolders, A.J.P, M. Moonen, K. Zwaga, E.C.H.E.T. Lucassen, L.P.M. Lamers, and J.G.M. Roelofs. 200b. Changes in pore water chemistry of desiccating freshwater sediments with different sulfur contents. Geoderma 132:372-383.

Wilhelm, E., R. Battino, and R.J. Wilcock. 1977. Low-pressure solubility of gases in liquid water. Chemical Reviews 77(2):219-262.

Wright, A. L., and K.R. Reddy. 2001. Heterotrophic microbial activity in northern Everglades wetland soils. Soil Sci. Soc. Am. J. 65:1856-1864.

Zak, D., A. Kleeberg, and M. Hupfer. 2006. Sulphate-mediated phosphorus mobilization in riverine sediments at increasing sulphate concentration, River Spree, NE Germany. Biogeochemistry 80:109-119.

DETAILED PROJECT PLAN

AN EVALUATION OF THE ROLE OF SULFATE IN SOUTH FLORIDA WETLANDS

Prepared by:

DB Environmental, Inc.
365 Gus Hipp Blvd.
Rockledge, Florida 32955

For the:

The South Florida Water Management District
and
Everglades Agricultural Area-Environmental Protection District

Mark Gabriel– Grant Manager (SFWMD)	8/4/08 Date
Charles Wilson- Grant Manager (EAA-EPD)	08-29-08 Date
Tom DeBusk – Project Director (DBE)	7/31/08 Date
Forrest Dierberg – Project Manager (DBE)	08/01/08 Date
Michelle Kharbanda - Project QA Manager (DBE)	7/31/08 Date

A. TABLE OF CONTENTS

B. PROBLEM DEFINITION/BACKGROUND

The Everglades is an internationally recognized ecosystem that covers approximately two million acres in south Florida and represents the largest subtropical wetland in the United States. During recent decades, the biotic integrity of the Everglades ecosystem has been endangered by the alteration of hydrologic and nutrient regimes due to urban and agricultural development. The 1994 Everglades Forever Act (EFA) mandates that waters discharged to the Everglades Protection Area (EPA) must achieve water quality standards. This goal is to be achieved through a combination of best management practices (BMPs), stormwater treatment areas (STAs), advanced treatment technologies, and regulatory programs.

Reduction of external phosphorus has been an integral part of the Everglades pollution reduction strategy. In the first phase of Everglades' restoration, the primary focus is placed on the institution of agricultural BMPs, and the testing, design and construction of > 50,000 acres of macrophyte-based STAs. The BMPs are intended to reduce phosphorus in agricultural runoff by 25 percent. The STAs are designed to reduce concentrations of phosphorus (P) in Everglades Agriculture Area (EAA) waters released to the Water Conservations Areas (WCAs) for compliance with an interim standard of 50 parts per billion (ppb).

Recently, concerns have been raised over the effects that elevated concentrations of sulfate in the WCAs, STAs, and northern regions of EPA may have on plant physiology, sediment P mobilization, and decomposition. Surface water sulfate levels are elevated in the northern Water Conservation Areas relative to observed concentrations in the Everglades National Park (ENP). The USGS reported average sulfate concentrations of 28.6 mg/L for Lake Okeechobee, and even higher values in canals that drain the Everglades Agricultural Area (72.8, 55.8 and 65.6 mg/L for the Hillsborough, North New River and Miami Canals, respectively). These sulfate levels contrast with the low ambient surface water sulfate levels in the ENP (< 1 mg/L) and the low concentrations (1 - 5 mg/L) reported for rainfall in South Florida.

Adverse ecological effects of sulfate are related to its potential for being reduced to sulfide, a chemical species that can be toxic to marsh flora and that also can inhibit soil P retention (Lamers et al. 1998). For example, a recent laboratory hydroponic study demonstrated adverse

effects of sulfide to sawgrass at solution sulfide concentrations in the range of 7 to 15 mg/L. Cattail proved slightly more resistant to solution sulfide levels, with adverse effects occurring at concentrations of 22 – 29 mg/L (Mendelssohn et al. 2006). Porewater sulfide levels as high as 12 mg/L have been reported in the Everglades water conservation areas (WCAs), an observation that has led some investigators to speculate that sulfate may be responsible in part for the historic encroachment of cattails into sawgrass stands in the WCAs (Axelrad et al. 2007).

Elevated sulfate levels also may contribute to P release from soils - a process termed "internal eutrophication". Sulfate can act as an electron acceptor in waterlogged soils, and therefore may accelerate the decomposition of organic matter in reduced environments where other electron acceptors (e.g., oxygen, nitrate) are not available. Sulfate reduction to sulfide also produces bicarbonate, which can buffer (neutralize) soil solution acids, creating a pH environment more conducive to organic matter decomposition. In turn, enhanced decomposition of organic matter can result in the release of P into the soil porewater. Finally, sulfides produced as a result of microbial sulfate reduction can reduce the availability of iron (Fe) (a necessary micronutrient) to sequester phosphate. These processes were clearly elucidated by a wetland mesocosm study performed in the Netherlands that demonstrated an increase in porewater sulfide, alkalinity, ammonium and soluble reactive P (SRP) in response to increases in surface water sulfate levels (Lamers et al. 1998). Moreover, the increase in concentration of selected porewater constituents (i.e., ammonium, sulfide) in this study was thought to be responsible for observed reductions in the growth rates of marsh plants, in particular *Carex nigra*. A thorough review on the internal eutrophication research in the Netherlands can be found in Smolders et al. (2006).

Calcium can also be an important element in mediating P cycling. Elevated daytime water column pH in submerged aquatic vegetation (SAV) communities may facilitate the co-precipitation of phosphorus with $CaCO_3$ (Picot et al. 1991, Moutin et al. 1992). Phosphorus removed from the water column in SAV communities may be deposited as relatively stable, high calcium, marl sediment (DB Environmental 2002). However, in areas of high productivity, bacterial respiration in the sediment may lower the pH to solubilize the Ca-bound P.

While the Dutch studies point to the importance of sulfate as a precursor for sulfide toxicity and P release, there are several important differences between the wetlands in their studies and the Everglades. Many of their wetlands had significant groundwater intrusion that altered the water chemistry in surface and pore waters. Soil and water in the Dutch wetlands frequently contained high Fe and low Ca concentrations. Lastly, the plant communities are vastly different in the Everglades and wetlands in the Netherlands.

Even though the above processes are well understood from a theoretical standpoint, the extent to which they may occur in south Florida wetlands is unknown, especially the interactions among Ca, Fe, and S in affecting the P retention capacity.

C. PROJECT APPROACH

To elucidate the role of sulfur in P release, organic matter decomposition and phytotoxicity, we propose to conduct:

1) Laboratory screening trials to determine effects of elevated water column sulfate levels on microbial respiration and P release, using soils collected from un-impacted and impacted wetlands (with respect to both P and S) in south Florida.
2) Mesocosm studies to evaluate plant toxicity and P cycling effects for a number of water, vegetation and soil types.
3) Field monitoring to assess spatial and temporal variations in surface water and sediment porewater P and S chemistry, and effects on wetland vegetation.

D. DATA QUALITY OBJECTIVES

Figure 1 is an overview of the quality system structure that DBE plans to follow in pursuit of this project's objectives. The flow chart in Figure 1 begins with the Data Quality Objectives (DQOs). The DQOs are part of a systematic planning process for developing an appropriate data collection system to support decision-making. The data collected for characterization of environmental processes and conditions need to be of the appropriate type and quality for their intended use. The data collected during the laboratory incubations, mesocosm experiments, and monitoring phases of this project need to support the main objectives of the study: S toxicity to aquatic plants, internal eutrophication, and organic matter decomposition.

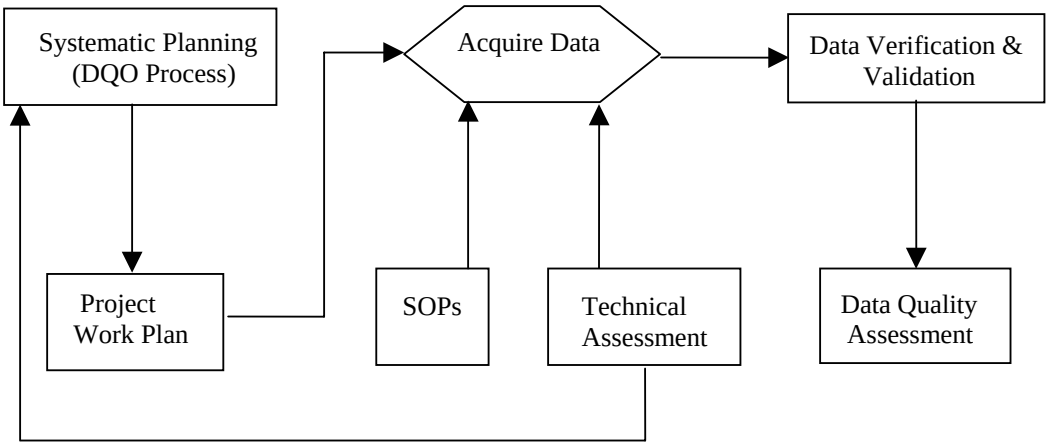


Figure 1. DBE quality system components for research on the Role of Sulfate in South Florida Wetlands**.**

The DQOs will be met by:

- Selection of pertinent field and lab parameters for monitoring
- Statistically comparing the water quality and sediment results within and among lab and mesocosms experiments, and the three designated field monitoring locations (reflective of a range of P, sulfate, and Ca concentrations)
- Judicious site selection and transect location to obtain chemical gradients of sufficient spatial resolution
- Replication of incubation vessels, mesocosms, and cores
- Field QA/QC (e.g., field duplicates, blanks, appropriate preservation, etc.)
- Frequent sampling over time
- Lab QA/QC including Data Quality Indicators (DQIs)
- Basing future experimental manipulations (e.g., amendments) on results obtained in earlier studies (i.e., an iterative process shown in Figure 1)
- Monthly or bi-monthly progress meetings and reports

The DQOs include the identification of the project team, objectives of the research, resources and deadlines, sampling and analysis methods, population parameters, tolerance limits, and the data collection design. In addition, the spatial and temporal boundaries of the DQOs and operational requirements for implementing the data collection design need to be defined and documented. Each of the components of the DQOs is presented below.

D.1 Project Management

D.1.1 Project Organization

Figure 2 depicts the organization for this project. The responsibilities of the personnel listed in the organizational chart are summarized below.

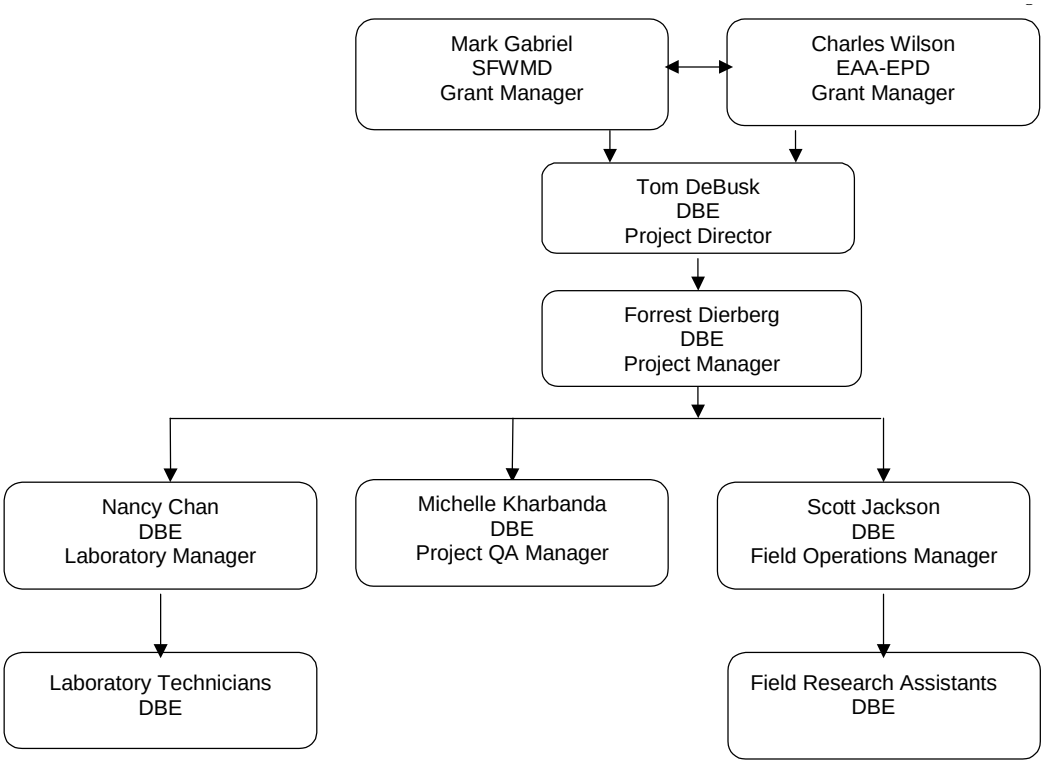


Figure 2. Organizational Chart.

Project Director

Mr. Tom DeBusk will be the Project Director for this effort. He will be responsible for the overall operation of the project, including fiscal resources, personnel, and client deliverables.

Project Manager

Dr. Forrest Dierberg will be the Project Manger for DBE during this project. He will oversee DBE's involvement with this project, and supervise all technical aspects of this project and provide data interpretation for project deliverables. He will also assist Mr. DeBusk with client deliverables.

Project QA Manager

The QA Manager for this project will be Ms. Michelle Kharbanda. Ms. Kharbanda will objectively evaluate all the analyses and accompanying quality control data and will respond with the appropriate corrective action if the data fail to meet any of the QA criteria outlined within the Detailed Project Work Plan. The QA Manager will initiate system audits and supervise the updating of the quality control tables and method detection limit determinations. Ms. Kharbanda will also be responsible for maintaining the Project Work Plan and distributing any necessary revisions.

Field Operations Manager

Mr. Scott Jackson will be the field operations manager for this project. He will oversee all field operations including personnel and equipment (maintenance and decontamination) as outlined in the Project Work Plan. The field operations manager is responsible for training field technicians and ensuring that all samples collected follow the QC requirements as outlined in the Project Work Plan. He will also work in conjunction with the Laboratory Manager to coordinate the scheduling of project samples in both the field and laboratory to ensure that all the deadlines are met as outlined within the Project Work Plan.

Laboratory Manager

The Laboratory Manager, Ms. Nancy Chan, will work in conjunction with the Field Operations Manager to coordinate the scheduling of project samples in both the field and laboratory to ensure that all the deadlines are met as outlined within the Project Work Plan. She is responsible for training laboratory technicians and ensuring that all samples analyzed follow the QC requirements as outlined in the Project Work Plan.

Field Research Assistants and Technicians

Field Research Assistants and Technicians are trained to collect samples and perform field measurements for a variety of parameters. They are responsible for following the standard operating procedure outlined by the lab and for collecting and preserving samples prior to analysis. They will maintain all QC requirements as outlined in the Project Work Plan.

Lab Technician/Analyst

Lab technicians/analysts are trained on a particular analysis. They are responsible for following the standard operating procedures outlined by the lab for an analysis, maintaining all QC requirements throughout that analysis, and constructing a results report to be submitted for approval by the QA Manager. In addition, the analyst is required to follow all safety and hazardous regulations, maintain all paperwork regarding quality control, sample processing, equipment calibration, and reagent and standard preparation requirements. If there are any problems throughout an analysis, the analyst is responsible for reporting them to the Laboratory Manager or QA Manager for corrective action to be taken.

D.1.2 Distribution Lists

Individuals who will be provided copies of the approved Detailed Work Plan and any subsequent revisions are as follows:

Person	**Title**	**Organization**
Mark Gabriel	Grant Manager	South Florida Water Management District (SFWMD or District)
Charles Wilson	Grant Manager	Everglades Agricultural Area – Environmental Protection District (EAA – EPD)
Tom DeBusk	Project Director	DB Environmental, Inc. (DBE)
Forrest Dierberg	Project Manager	DBE
Scott Jackson	Field Operations Manager	DBE
Nancy Chan	Laboratory Manager	DBE
Michelle Kharbanda	Project QA Manager	DBE

D.2. Special Training/Certification

The field manager for this project has over 10 years experience designing, operating and collecting samples from the field and from mesocosms in South Florida wetlands. In addition, the field manager has completed the Florida Department of Environmental Protection (FDEP or DEP) Quality Assurance (QA) Program. The field Research Assistants and Technicians have all been fully trained using FDEP-SOP-001/01 guidelines and have over 1 – 10 years experience performing this type of work.

The laboratory manager has 8 years experience in a supervisory capacity. She has attended many workshops pertaining to laboratory management and operations. She has also guided the laboratory through 9 successful accreditation inspections by NELAP and client auditors.

The project QA Manager is familiar with the requirements for a successful implementation of a QA plan. She has implemented numerous QA plans related to wetlands research in south Florida in the past.

The project manager has over 30 years of experience in biogeochemistry, which includes 15 years in academia. He is familiar with all aspects of the technical analyses that this project requires.

The project director has over 30 years of experience in directing large-scale projects of this nature for both private and government clients. He has specialized experience in south Florida, having directed numerous Everglades research projects.

D.3. Schedule

A schedule of the work to be performed within the designated three-year project period, from April 2008 through April 2011, is provided below.

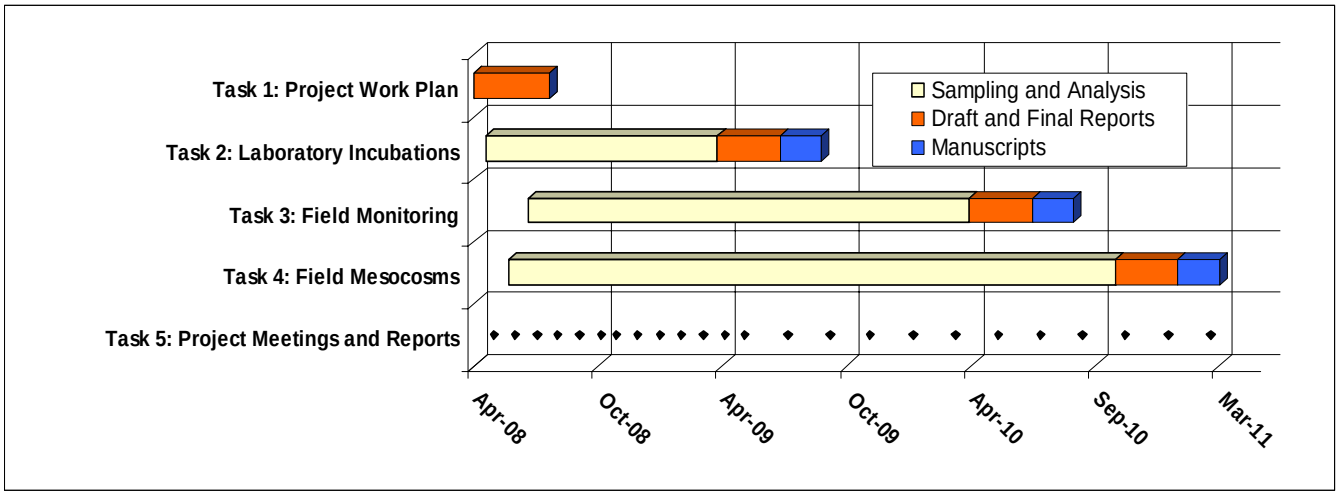


Figure 3. Project schedule from April 2008 to March 2011. Note that for Task 4, several months will be devoted to mesocosm facility construction during the project period.

D.4. Documents and Records

The Project QA Manager will be responsible for distributing the most current approved Detailed Work Plan to individuals identified in Section D.1.2. Documentation that provides the date that the original and any subsequent revisions were sent to each individual will be kept with project files. The Detailed Work Plan will be distributed in either hard copy or electronic format.

All records for field activities, which include calibration, verification, decontamination, and maintenance of field equipment, will be documented in accordance with DEP-SOP-001/01 (2004).

Additional documents to be produced during this project are as follows:

- Records related to sample collection, chain-of-custody, and analysis as described in the National Environmental Laboratory Accreditation Conference (NELAC) Chapter 5, Quality Systems.
- Monthly and Bi-monthly Progress Reports and Meetings
- Final Project Reports and Manuscripts

All electronic records will be backed-up weekly onto external storage media. The final data report will be archived onto a CD and kept with the project files for easy retrieval. All project documentation and records will be stored at DBE headquarters in Rockledge, Florida for a period of no less than five years after project completion.

D.5. Site Locations

The project is located throughout the northern and middle Everglades (Figure 4), with one site on the east side of Lake Okeechobee at Port Mayaca (26°59'N, 80°36'W). The Port Mayaca site is one of two mesocosm locations. The source water will come from Lake Okeechobee.

Water Conservation Area – 2A (WCA – 2A) is in the northern part of the Everglades Protection Area (EPA). WCA – 2A is located 25 km west of West Palm Beach (26°18'N, 80°25'W), and is a large (42,706 ha), shallow, diked impoundment managed for water storage and wildlife. Vegetation is characterized by marsh, slough and tree islands. Cattail (*Typha domingensis*) occupies about 8,100 ha of northeast WCA – 2A (Davis and Ogden 1994), an area previously impacted by agricultural drainage waters. An unimpacted site (U3) has been selected as one of the three sediment sources for the Laboratory Incubation Study (Phase 2). This site has been monitored frequently, and consequently the background levels of key elements are well known (Table 2).

For the field-monitoring phase of the research program (Phase 3), flow paths within each of the three Stormwater Treatment Areas (STAs) have been selected for sampling and analysis along flow and vegetation gradients (Figure 4). A brief description of each of the flow paths to be monitored follows.

STA-2 Cell 1 is located at 80°30'W; 26°24'N and is dominated by emergent vegetation (mixed cattail and sawgrass). It has existed as an STA for 8 years, and was not farmed prior to flooding.

STA-3/4 (80°37'03"W; 26°22'04N") was flooded 4.5 years ago. The central flow way has cattail in Cell 2A and SAV in Cell 2B. It was previously farmed.

STA-5 (80°54’ 56”W; 26°26’24”N) is 8 years in existence. The central flow path (Cells 2a and 2b) consists of emergent vegetation (2a) and SAV (2b). It was previously farmed.

A site located within a 10-km diameter circle in WCA-3 (239,274 ha) will be accessed for two of the research components (Laboratory Incubation Study [Phase 1] and the Mesocosm Study [Phase 2]) undertaken in this study. The exact location within the circled area shown in Figure 4 has yet to be determined, pending a survey by airboat of the area. Approximately half of WCA-3’s water budget originates from rainfall with general water quality characteristics of the interior areas being more acidic, and lower mineral and nutrient contents, than the other sites to be evaluated in this study. Mineral rich canal water enters WCA-3 at several points around its northern perimeter giving rise to gradients in concentrations of phosphorus and other minerals downstream of the outflows (FDEP 2001). The landscape consists of ridges of wet prairie and sloughs at slightly lower elevation.

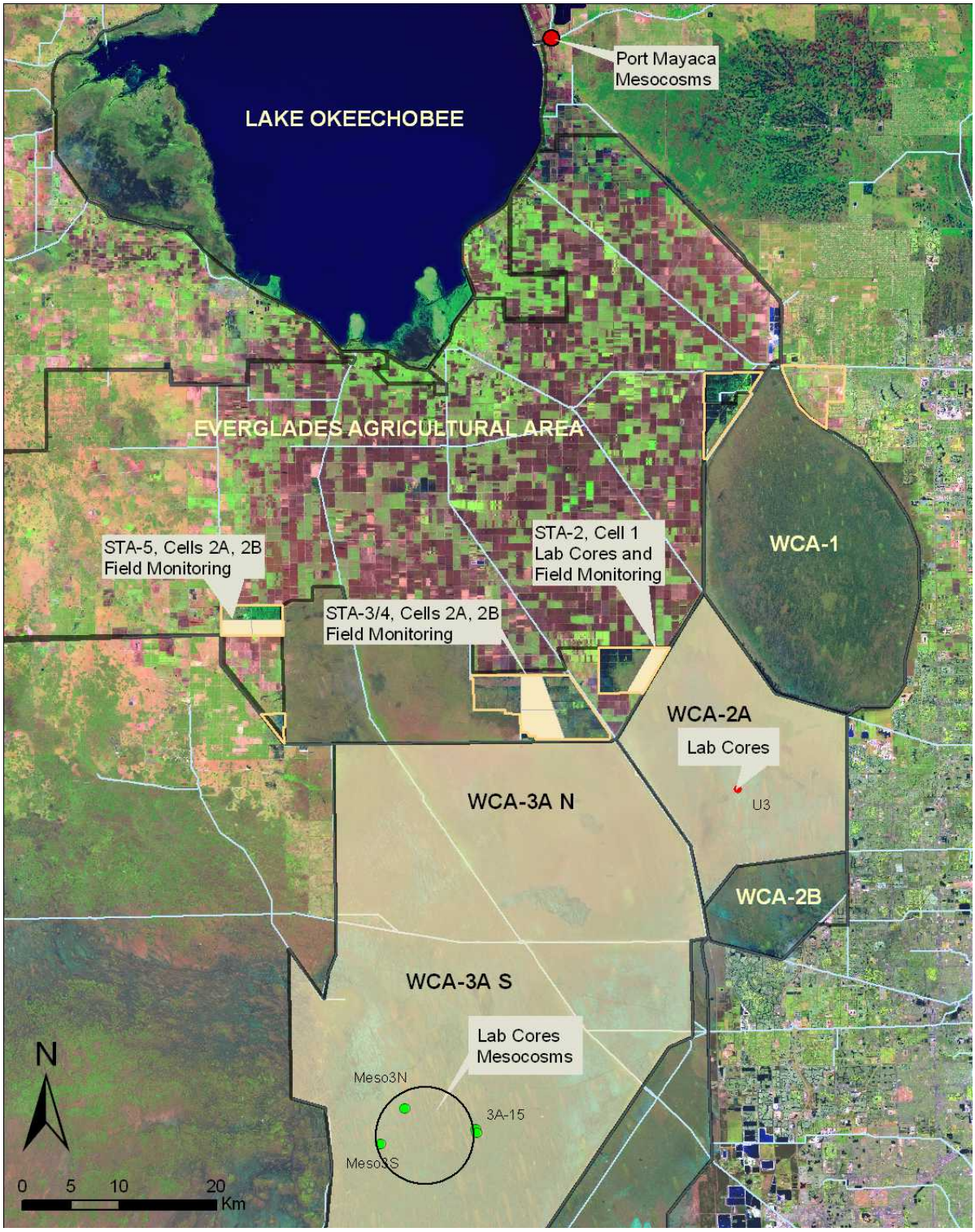


Figure 4. Locations of the core retrieval, mesocosm and field monitoring sites. The location of the sediment cores to be used in the Lab Incubation Study (Phase 2) and mesocosms in Phase 4 will be within the 10-km diameter circle in WCA-3A S. Meso 3N and Meso 3S are former District sampling stations; 3A-15 is where USGS has performed numerous mesocosm studies.

D.6. Project Description

We propose to first evaluate phosphorus release and organic matter decomposition in laboratory-incubated sediment cores collected from STAs and the Everglades that have been exposed to long periods of low, moderate, and high concentrations of P, sulfate, and Ca. Sulfate amendments of various levels will be incorporated as part of this study. Second, field monitoring of surface waters, pore waters, and sediments along P gradients within flow paths in three STAs will be conducted to examine sulfide and SRP relationships and whether sulfate/sulfide concentrations correlate to STA performance and vegetation health. In the case of STA-2 Cell 1, *in situ* data can be compared to the results from the initial laboratory incubation of cores (Phase 2). Finally, we plan to deploy a series of mesocosms at two sites, each with a different history of surface water levels of P, sulfate, and Ca. Mesocosms provide a platform where experiments can be conducted using controlled flow, vegetation type, sediment type, and amendments under realistic field conditions. The mesocosm experiments will be key for testing the toxicity effects of sulfide on various plant populations, in addition to testing the effects of sulfate on P mobilization.

This project is defined by the following tasks:

- Task 1 involves the development of a Project Work Plan.
- Task 2 will encompass laboratory incubations using soil cores collected from the inflow region of Cell 1 of STA-2, station U-3 of WCA-2A, and WCA-3. These locations represent a range of low to high concentrations of P, sulfate, and Ca. A final report and a manuscript will be prepared at the end of the task.
- Task 3 will evaluate the chemical concentration gradients in the surface waters and soil porewaters along flow paths in three STAs. A final report and a manuscript will be prepared at the end of the task.
- Task 4 will include controlled amendments of sulfate and Ca, with and without the removal of P, to outdoor flow-through mesocosms at two sites: Port Mayaca and WCA-3. A final report and a manuscript will be prepared at the end of the task.
- Task 5 comprises the monthly (first year) and bi-monthly (second and third years) project meetings and reports.

D.7 Data Quality Indicators (DQIs)

Part of the DQO process is to specify tolerable levels of errors in the quality of the data. These are expressed by using Data Quality Indicators (DQIs), which include quantitative and qualitative indicators. Quantitative indicators are precision, bias, accuracy, sensitivity, and completeness. The qualitative indicators are representativeness, comparability, and completeness.

D.7.1 Quantitative DQIs

The quantitative indicators are shown in Table 1, and are evaluated using the methodology (quality control samples) listed in the table. A performance criterion for each can be found in Section H, Table 8 of this document.

Table 1. Quantitative data quality indicators and methodologies.

Data Quality Indicator	Determination Methodology (QC Samples)
Precision	Laboratory and Field Duplicates, Digestion Blanks, Field Blanks
Bias	Reference Standards, Method Spikes, Digestion Blanks, Field Blanks
Accuracy	Continuing Check Standards, Method Spikes, Reference Standards, Digestion Blanks, Field Blanks
Sensitivity	Analytical Method Detection Limits

The tolerance limits placed on decision errors rely on bias being kept to a minimum. Six major causes of bias have been identified for environmental sampling and analysis:

1. non-representative sampling;
2. instability of samples between sampling and analysis;
3. interferences and matrix effects in analysis;
4. inability to determine the relevant forms of the parameter being measured;
5. calibration;
6. failure to blank-correct.

DBE addresses these inherent biases in the following manner. Non-representative sampling bias will be reduced by having adequate number of sampling stations, compositing, and stratification.

Instability of samples between sampling and analysis is controlled by adequate preservation techniques and adhering to holding times. Interferences and matrix effects during the analysis is tested by laboratory spikes on every 20 samples. The ability to determine the relevant form of the parameter being measured is realized by the appropriate selection of the methodology. Calibration bias is gauged by the correlation coefficients and slopes of standard curves, and by the performance of second-source standards at high and low concentrations. Field and laboratory blanks are analyzed routinely at the beginning of each sample project. Countering these sources of bias is included in the laboratory accreditation process and by training of personnel.

D.7.2 Qualitative DQIs

Qualitative DQIs such as representativeness will be described by the design and collection procedures for the project. All treatment compartments will be either in duplicate or triplicate. In addition, field personnel will be collecting field replicates that number from two to as many as six. Another qualitative DQI, comparability, will be documented by performing temporal sampling comparisons and determining if the associated quantitative DQIs have been met. Completeness of the data set will be expressed by the number of missing data due to inclement weather, equipment malfunction, or for analytical reasons.

D.8 Defining the Boundaries

The target populations are ions, molecules, and compounds containing C, N, P, and S in surface and pore waters, soils and sediments, and plant tissues. Spatial boundaries define the physical areas to be studied and where samples will be collected (Figure 4), while the temporal boundaries describe the time frame that the study will represent and when the samples will be taken (provided in Section O).

D.9 Data Collection Design

The Data Collection Design is a critical component in the DQO process. It encompasses the Field Sampling and QA Plans, and includes the following elements:

- number of samples
- sample type (grab vs. composite)
- collection techniques

- volume or quantity of sample
- sample locations (surface coordinates and depth) and how locations were selected
- timing issues for sample collection, handling and analysis
- analytical methods
- sampling scheme

Each of the above aspects to the Data Collection Design will be discussed in more detail in the subsequent sections devoted to each task.

Part of the Data Collection Design also includes identifying statistical tests to confirm or disprove hypotheses and reviewing existing data.

D.9.1 Statistical Testing

Statistical testing consists of testing hypotheses by appropriate selection of what is considered the background condition (or null hypothesis), and then applying the appropriate statistical tests (parametric vs. non-parametric).

Establishing Background Conditions and Testing Hypotheses

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 appropriate probability cut-offs for Type I and Type II errors in hypothesis testing. For our study, we will use $\alpha = 0.05$ and $\beta = 0.20$ as probability criteria for false rejection (i.e., rejecting the null hypothesis when it is really true) and false acceptance (i.e., failure to reject the null hypothesis when it is really false) decision errors, respectively, of the null hypothesis.

Statistical Analyses

A data analysis plan includes statistical test for significant differences among sites and within transects, along with the identification of the relevant parameters contributing to the differences. A completely randomized experimental design will be used with factors such as soil depth,

transect location, season (wet or dry), redox potential, and vegetation type. We expect to use multiple step-wise regression, analysis of variance (ANOVA), Pearson's product-moment correlation, and least significant difference tests for the parameters measured among and within STAs, Port Mayaca, WCA-2A, and WCA-3 sites. In the case where normality or equal variance (homoscedacity) assumptions are violated in the data sets, we will resort to non-parametric statistical methods such as Spearman or Kendall-Tau correlation methods and Wilcoxon rank-sums or Kruskal-Wallis tests for significance if the data cannot be transformed (e.g., log-transformation) to fit a normal distribution.

D.9.2 Existing Data

Many of the proposed study sites have historical data, but there are some gaps with respect to parameters and matrices. Existing data reported for the sites are presented in Tables 2-5. These historical data justify the selection of the locations for core retrieval, field monitoring, and mesocosm placement, which will be discussed in more detail in each of the subsequent task descriptions.

Table 2. Historical arithmetic means and ranges (n=number of samples) of surface water constituents reported at the proposed sites for core retrieval. All units in mg/L. n.d.= not determined.

	Location		
Parameter	STA-2, Cell 1 (Inflow) [a]	WCA-2A, Site U3 [b]	WCA-3AS [c]
TP	0.078 (0.014-0.302) n=276	0.007 (<0.002-0.114) n=370	0.008 (0.004-0.053) n = 118
SRP	0.042 (0.002-0.223) n=267	0.003 (<0.001-0.10) n=344	0.005 (0.002-0.020) n = 119
SO_4	66 (28-183) n=133	39 (2.9-180) n=284	0.77 (0.10-8.7) n = 121
S^{2-}	n.d.	1.4 n=1	n.d.
Diss Ca	95 (45-134) n=130	63 (36-120) n=333	53.9 ((37.6-75.7) n = 121
Total Fe	n.d.	0.025 (<0.003-0.590) n=187	118 (14-366) n = 119 *
NH_4^+-N	0.269 (0.009-0.955) n=174	0.126 (<0.01-15) n=322	0.036 (0.005-0.480) n = 108

References:
a: DBHYDRO database, downloaded 4/18/08, station S6, various sampling frequencies. Data from Jan 03 – Feb 08.
b: DBHYDRO database, downloaded 6/16/08. Weighted means from five stations (U3, WC2U3SW, WCA2AU3, 217WQ, and CA215) within 1000 ft of each other. Data from 1978-May 2008.
c: S. Newman. Weighted means for two stations (3ANMESO and 3ASMESO) in WCA-3AS. Data from Jan 21, 2003-Dec 11, 2007.
* Dissolved Fe (filtered)

Table 3. Historical arithmetic mean and range (n= number of samples) for surface waters collected from selected transects along the flow path of Cell 1 of STA-2. All units are in mg/L. n.d. = not determined.

		Transect (distance from inflow in km)		
Matrix	Parameter	A (0.5)	F (3)	J (5)
sw	TP	0.089 (0.061-0.117) n=2[a]	0.031 (0.023-0.038) n=2 [a]	0.012 (0.010-0.014) n=2 [a]
sw	SRP	0.034 (0.016-0.051) n=2[a]	0.005 (0.002-0.008) n=2[a]	0.004 (0.003-0.004) n=2[a]
sw	SO_4	n.d.	n.d.	n.d.
sw	S^{2-}	n.d.	n.d.	n.d.
sw	Diss Ca	n.d.	n.d.	n.d.
sw	Diss Fe	n.d.	n.d.	n.d.
sw	NH_4^+-N	n.d.	n.d.	n.d.

[a] Collected by DBE on 6/21/07 and 9/28/07.

Table 4. Historical arithmetic mean and range (n=number of samples) for inflow and outflow surface waters of selected flow paths in STA-2 Cell 1, STA ¾ (Cell 2A-2B), and STA 5 (Central Flow Path).

STA 2 Cell 1. Data from 12/8/99-5/14/08.

	Cell 1 Inflow	**Cell 1 Outflow**
Parameter	**G329B**	**G330**
TP	0.062 (0.011 – 0.281) n=312	0.016 (0.005 – 0.338) n=332
SRP	0.029 (< 0.002 – 0.232) n=228	0.003 (< 0.002 – 0.011) n=229
SO_4	n.d.	55 (5.2 – 89) n=44
S^{2-}	n.d.	n.d.
Diss Ca	93 (40 – 133) n=149	87 (50 – 113) n=149
Total Fe	n.d.	n.d.
NH_4-N	0.341 (0.02 – 0.832) n=148	0.034 (< 0.009 – 0.216) n=145
Alkalinity	335 (136-399) n=149	305 (171 – 406) n=149

STA3-4 Cell 2A-2B. Data from 6/24/04-5/19/08.

	Cell 2A Inflow	**Cell 2B Inflow**	**Cell 2B Outflow**
Parameter	**G377B and D**	**G378B, D and E**	**G379ABCD and E**
TP	0.063 (0.008 – 0.245) n=511	0.037 (0.009 – 0.362) n=503	0.029 (0.007 – 0.151) n=704
SRP	0.023 (<0.002 – 0.154) n=303	0.007 (<0.002 – 0.273) n=371	0.003 (<0.002 – 0.030) n=440
SO_4	n.d.	33 (19 – 45) n=6	33 (3.3 – 71) n=192
S^{2-}	n.d.	n.d.	n.d.
Diss Ca	101 (41 – 135) n=146	89 (53 – 123) n=173	64 (25 – 114) n=190
Total Fe	n.d.	n.d.	n.d.
NH_4-N	0.136 (0.009 – 0.555) n=146	0.096 (< 0.009 – 0.936) n=167	0.136 (<0.009 – 1.91) n=208

	Cell 2A Inflow	**Cell 2B Inflow**	**Cell 2B Outflow**
Parameter	**G377B and D**	**G378B, D and E**	**G379ABCD and E**
Alkalinity	273 (120 – 364) n=146	246 (142 – 334) n=152	186 (75 – 301) n=193

STA 5 Central Flow Path. Data from 11/30/98-5/15/08.

	Cell 2A Inflow	**Cell 2B Inflow**	**Cell 2B Outflow**
Parameter	**G342C and D**	**G343F and G**	**G344 C and DB**
TP	0.169 (0.024 – 1.298) n=1299	0.132 (0.023 – 1.130) n=772	0.130 (0.017 – 1.560) n=1103
SRP	0.080 (0.002 – 0.826) n=714	0.097 (<0.002 – 0.939) n=571	0.072 (<0.002 – 0.380) n=659
SO_4	11 (1.7 – 43) n=440	n.d.	7.0 (0.4 – 41) n=393
S^{2-}	n.d.	n.d.	n.d.
Diss Ca	70 (30 – 98) n=238	69 (36 – 123) n=295	58 (29 – 89) n=233
Total Fe	n.d.	n.d.	n.d.
NH_4-N	0.060 (<0.005 – 0.309) n=439	0.136 (<0.009 – 3.110) n=265	0.071 (<0.009 – 0.876) n=394
Alkalinity	203 (80 – 277) n=442	203 (102 – 365) n=292	191 (98 – 327) n=396

Table 5. Historical arithmetic mean and range (n=number of samples) of surface water, porewater, and sediment chemistries collected at the proposed site at Port Mayaca for mesocosm placement. The background chemistry for the mesocosms to be placed in WCA-3 is provided in Table 6. "sw" denotes surface waters with units of mg/L; "pw" denotes porewaters with units of mg/L; "sed" denotes sediments as percentages; "n.d."= not determined.

Matrix	Parameter	Port Mayaca (Lake Okeechobee waters)	
sw	TP	0.265 (0.092-0.678) n=128 [a]	
sw	SRP	0.097 (0.048-0.394) n=57 [a]	
sw	SO_4	43 (28-93) n=20 [d]	
sw	S^{2-}	0.044 n=1 [a], Oct 07	
sw	Diss Ca	51 (25-146) n=126 [a]	
sw	Total Fe	n.d.	
sw	NH_4^+-N	0.208 n=1 [a], Oct 07	
		Cattail	SAV
pw	SRP	0.293 [b]	0.005 [b]
pw	SO_4	<5 [b]	5.2 [b]
pw	S^{2-}	n.d. [b]	n.d. [b]
pw	Diss Ca	171 [b]	118 [b]
pw	Diss Fe	n.d. [b]	n.d. [b]
pw	NH_4^+-N	1.01 [b]	1.09 [b]
sed	TP	0.126 (0.122-0.129) [c]	0.105 (0.095-0.115) [c]
sed	TN	1.95 (1.93-1.98) [c]	1.81 (1.46-2.16) [c]
sed	TC	28.7 (28.4-29.0) [c]	22.2 (19.8-24.5) [c]
sed	TOC	29.7 (27.5-28.4) [c]	19.0 (15.7-22.3) [c]
sed	TS	n.d.	n.d.
sed	TCa	n.d.	n.d.
sed	TFe	n.d	n.d

References:

a: Port Mayaca Ca #2 experiment. No Ca Inf A station; May 05-Feb 08.

b: Port Mayaca Ca #2 experiment: Cattail/SAV mesocosms from the unamended calcium treatment replicate A. n=1, peepers, avg of depths 0-12 cm, n=4, 10/9/07

c: Port Mayaca Ca #2 experiment: Cattail/SAV mesocosms from the unamended calcium treatment, n=2, 10/26/07.

d: DBE data, 11/24/07-2/8/08.

Table 6. Arithmentic mean (n=number of samples) ± 1 s.d. for porewater and sediment concentrations reported in the entireWCA-3 for 1992 (Reddy et al. 1994) and 2005 (Scheidt and Kalla 2007). Data reported from Dr. Sue Newman specific for the 3ANMESO and 3ASMESO District sites in WCA-3AS. n.d. = no data

	Reddy et al.	Reddy et al.	Scheidt and Kalla (R-EMAP)	S. Newman (unpublished)
Year sampled	1992 (n = 188)	1992 (n = 188)	2005 (n = 100)	2000-01 (n=3)
	0-10 cm depth	*10-20 cm depth*	*0-10 cm depth*	*0-10 cm depth*
Porewater (mg/L)				
SRP	0.012 ± 0.001	0.012 ± 0.001	0.017 ± 0.004 (0.002 – 0.360) n= 98	<0.01
SO_4	n.d.	n.d.	1.6 ± 0.42 (0.09 – 26.0) n = 97	n.d.
S^{2-}	n.d.	n.d.	0.308 ±0.051 (0.026 – 4.0) N = 97	n.d.
Diss Ca	n.d.	n.d.	n.d.	~ 60 *
Total Fe	n.d.	n.d.	n.d.	~ 2.5 **
NH_4-N	1.82 ± 0.067	1.87 ± 0.373	0.815 ± 0.094 (0.019 – 5.1) n = 98	~ 2.0 *
Sediments (%)				Nov 2004 (n=6)
				0-2 cm depth
TP	0.0456 ± 0.0014	0.0275 ± 0.0008	0.0461 ± 0.0018 (0.0160 – 0.1400) n = 100	0.034 (0.028-0.042)
TN	2.91 ± 0.05	2.71 ± 0.06	3.09 ± 0.07 (0.94 – 4.7) n = 100	4.2 (4.0-4.4) n
TC	41.2 ± 0.64	40.5 ± 0.89	42.1 ± 0.85 (13.0 – 68.0) n= 100	48.3 (46.5-50.6)
TOC	n.d.	n.d.	n.d.	n.d.
TS	n.d.	n.d.	n.d.	n.d.
Total Ca	n.d.	n.d.	n.d.	1.9 (1.8-1.9)
Total Fe	n.d.	n.d.	n.d.	0.58 (0.47-0.71)
HCl-extractable Ca	4.23 ±0.235	3.61 ± 0.204	n.d.	n.d.
HCl-extractable Fe	0.233 ±0.018	0.262 ± 0.0213	n.d.	n.d.

* Extrapolated from figure presented in District report entitled Effects of Changes in Phosphorus Levels on the Central and Southern Everglades.
** Filtered

E. SAMPLING METHODS

The Field Operations Manager will ensure that all sampling protocols stated below are followed and take appropriate corrective action if problems occur. Any problems and the corrective actions will be documented in the project field records.

Surface Water

All surface water sample collection and handling will follow the procedures outlined in the FDEP SOPs (DEP-SOP-001/01, Revision Date: February 1, 2004). Inflow samples to the mesocosms will be collected by inserting a sample bottle below the delivery pipe. Outflow samples are to be taken from each mesocosm and field enclosure at the exit end by dipping the sample container 2.5 cm below the surface of the water. The same technique will be practiced when sampling surface waters in the field.

Immediately upon collection, the sample water will be distributed into plastic sample containers prepared in accordance with DEP-SOP-001/01 (2004) Table FS1000-4 for TP, SRP, TSP, Ca, SO_4, S^{2-}, alkalinity, Fe, and NH_4-N. Sample volumes, holding times, and preservation techniques required for the parameters collected during this project will follow guidelines listed in DEP-SOP-001/01 (2004) Table FS1000-4.

Filtered samples for SRP analysis will be frozen if they cannot be analyzed within 48 hours after collection. For past projects, DBE has petitioned, and received approval, from FDEP to freeze SRPs at 4 °C in lieu of the 48-hour holding time after we demonstrated no differences in SRP concentrations between the two storage methods (Appendix 6). When freezing the filtered samples for SRP analyses, a self-imposed holding time of two weeks will be adhered to by DBE.

Porewater

Most of the porewater sampling will be accomplished by deploying either sippers or porewater equilibrators ("peepers") within the mesocosms and in the field. A recent porewater methodology comparison study performed by DBE found "peepers" to be superior in characterizing the vertical profile, especially for sulfide. Sippers were found to be useful for integrating porewater constituents over larger vertical depths in the soil. Porewater equilibrators

are wedges of acrylic (Plexiglass or Lucite) with vertically arranged, equidistant (e.g., 1 cm apart) chambers. Each chamber is equipped with a dialysis membrane of 0.2 µm pore size, and is filled with distilled water after being purged with nitrogen. The entire equilibrator is inserted into the sediment such that some of the vertically arranged chambers are below the sediment surface, and some extend upward into the water column. After 14 -21 days, the equilibrators are retrieved and samples rapidly collected from each chamber. For the present assessment, analyses of redox potential, pH, SRP, SO_4, S^{2-}, NH_4-N, Fe and Ca will be performed on aliquots from selected "sippers" or equilibrators.

Vegetation

Since all the mesocosms will be stocked with dominant species of either submerged aquatic vegetation (SAV) or emergent aquatic vegetation (EAV), we will measure the plant stocking densities at the beginning, and then again at the end of the experimental period. Above-ground tissues will be analyzed for TP, TN, TS, and TC content. For measuring the toxic effects of sulfide on plants, we will monitor several plant stress indicators at the Port Mayaca and WCA-3 mesocosm sites as suggested by Dr. Shili Miao of the District. These plant stress indicators represent toxic effects, if present, at the physiological (i.e., chlorophyll and tissue contents), growth and morphological (leaf length; leaf and root biomass), and mortality (leaf and plant death) levels.

Sediments

All sediment sample collection and handling will follow the procedures outlined in the FDEP SOPs (DEP-SOP-001/01, Revision Date: February 1, 2004). Sediment cores will be retrieved with a 10-cm i.d. aluminum core barrel, and extruded in the field into cleaned polyethylene bags and stored on ice. The bottom of the core barrel is beveled to facilitate cutting underground rhizomes and plant roots, as well as large plant fragments common in the peat soils. Rhizomes and large roots, if present, will be removed from the soil sample. An additional benefit of a large core barrel is that it reduces sediment compaction within the core. Accurate representation of the soil characteristics at a particular site will be insured by retrieving six replicate cores prior to homogenizing common depth intervals, and by the large-diameter core barrel (10 cm i.d.).

For sediment sampling within mesocosms where the enclosed area does not permit multiple corings with a large-diameter core barrel (e.g., enclosures in WCA-3), smaller diameter (4 cm) cores will be used.

Core samples will be extruded in the field and will be partitioned into three depth increments, surface to 4 cm, 4-10 cm, and 10-30 cm, for analytical processing and analyses. Analyses to be performed on the sediments may include TP, TN, TC, TOC, TS, TCa, NaOH-extractable SRP and TP, and microbial P biomass.

F. SAMPLING HANDLING AND CUSTODY

After the samples have been collected and preserved as described in section E above, they will be transported to the lab by a DBE field technician and received by a lab technician in the sample receipt department. All documentation of the samples' collection, preservation and transportation will be recorded on the chain-of-custody as outlined in DBE's Quality Manual (Section 7.1). The sample will also be given a unique ID to assist in tracking within the laboratory.

The Field Operations Manager will be responsible for maintaining the field notebooks and initiating the chain-of-custody. The Laboratory Manager will be responsible for the chain-of-custody up until final sample disposal.

G. ANALYTICAL METHODS

Table 7 below lists the analytical methods to be used during this project.

Table 7. List of analytes and associated methods.

Analyte	Matrix	Analysis Method
pH (field)	Surface Water	DEP-SOP-001/01 (2004) FT 1100
Temperature (field)	Surface Water	DEP-SOP-001/01 (2004) FT 1400
Diss.Oxygen (field)	Surface Water	DEP-SOP-001/01 (2004) FT 1500
SRP	Surface & Pore Waters	SM4500-P F
TSP	Surface & Pore Waters	SM4500-P F
TP	Surface & Pore Waters	SM4500-P F
Sulfate	Surface & Pore Waters	EPA 375.4; EPA 300.0
Sulfide	Surface & Pore Waters	EPA 376.1; SM4500 S^{2-} G
Ca	Surface & Pore Waters	EPA 215.1
Fe	Surface & Pore Waters	Modified Bathophenanthroline

Analyte	Matrix	Analysis Method
Ammonium	Surface & Pore Waters	EPA 350.1
DOC	Surface & Pore Waters	EPA 415.1
Alkalinity	Surface & Pore Waters	EPA 310.1
Carbon Dioxide	Surface & Pore Waters	GC/TCD
Methane	Surface & Pore Waters	GC/FID
Total S	Soil & Plant	ASTM D4239/ASTM E1915-05
TP	Soil & Plant	EPA 365.2
TN	Soil & Plant	DBE SOP MVP
TC	Soil & Plant	DBE SOP MVP
TOC	Soil & Plant	DBE SOP MVP/COE 3-73
TFe	Soil & Plant	EPA/SW 7380
TCa	Soil & Plant	EPA/SW 7140
Lignin & Cellulose	Soil & Plant	AOAC 973.18
NaOH-Extractable SRP	Soil	DBE SOP OPO_4
NaOH-Extractable TP	Soil	EPA 365.2
Oxalate-Extractable Fe	Soil	EPA 236.1
Microbial Biomass P	Soil	DBE SOP OPO_4

When a corrective action is required for any analytical method, DBE will follow DBE's CompQAP (Section 9.6).

After analysis, all samples will be stored until the chemical results have been approved by the QA Manager. Once determined that repeat analysis is not required for a sample, it will be disposed down the sanitary sewer. The date of sample disposal will be recorded on the laboratory chain-of- custody.

H. QUALITY CONTROL

DBE is currently a NELAC-certified laboratory, with updated quality manual (CompQAP) and SOPs for analyzing SRP (water), total soluble P (TSP) (water), TP (water and solids), sulfate (water), sulfide (water), Ca (water and solids), total carbon (TC) (solids), total organic carbon (TOC) (solids), and total nitrogen (TN) (solids). The laboratory is pursuing additional NELAC certifications for low volume analyses of ammonia, sulfate, and sulfide, which will be used for this project. These additional certifications should be completed within 6 months.

Section D.7.1 of this document defines the quantitative DQIs (precision, bias, accuracy and sensitivity) and the associated QC samples for this project. Laboratory QC samples will be analyzed at the frequency defined in DBE's QC CompQAP. Acceptance criteria for quality control samples for analyses are provided in Table 8. Any required corrective actions for the respective parameter will be performed as outlined in DBE's QC CompQAP.

Table 8. Acceptance criteria for quality control samples.

Low Level (L) = Concentrations from 0-20% of the linear curve, or concentrations ranging from the MDL to 5 times the MDL.
Mid-High Level (M-H) = Concentrations ranging from >20-100% of the linear curve, or concentrations ranging from >5 times the MDL.

Parameter: Ortho-P						
Method: SM 4500-P F						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 0.002 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 75-125% M-H: 85-115%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-30% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TSP						
Method: SM 4500-P F						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 0.003 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 70-130% M-H: 80-120%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-40% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TP						
Method: SM 4500-P F						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 0.003 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 70-130% M-H: 80-120%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-40% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: Sulfate						
Method: EPA 375.4						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 1 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 70-130% M-H: 80-120%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-40% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 4	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: Ammonium						
Method: EPA 350.1						
Matrix: Surface Water/Porewater						
Method Detection Limit (MDL): 0.012 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	1) Re-pour and rerun, 2) replace with new and rerun	Project Manager	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 70-130% M-H: 80-120%	Remake spike, rerun	Project Manager	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-40% M-H: 0-20%	Change tubing on sampler, rerun	Project Manager	Precision	Same as QA Limits
Lab Control Sample	1 per 20	90-110%	1) Re-pour and rerun, 2) replace LCS with new and rerun	Project Manager	Accuracy	Same as QA Limits

Parameter: Sulfide						
Method: SM 4500-S^{2-} G						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 0.012 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 65-135% M-H: 75-125%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-50% M-H: 0-25%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: Ca						
Method: EPA 215.1						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 0.06 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 80-120% M-H: 90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-30% M-H: 0-10%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 10	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: DOC						
Method: EPA 415.1						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 0.32 mg/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	every 20 samples	<PQL	re-prep batch	bench analyst	Bias	Same as QA Limits
Lab Matrix Spike	every 20 samples	80-120% recovery	re-run MS	bench analyst	Accuracy	Same as QA Limits
Lab Duplicate	every 20 samples	20% RPD	re-run Dup	bench analyst	Precision	Same as QA Limits
Laboratory Control Standard	every batch	90-110% recovery	re-prep batch	bench analyst	Accuracy	Same as QA Limits

Parameter: Alkalinity						
Method: EPA 310.1						
Matrix: Surface Water /Pore Water						
Minimum Detection Limit (MDL): 3 mg CaCO3/L						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 75-125% M-H: 85-115%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-30% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: Total S						
Method: ASTM D4239						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): 0.01%						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 every 20	<0.01%	Rerun boat	QA Officer, Project Manager	Bias	Same as QA Limits
Lab Matrix Spike	N/A	N/A	N/A	N/A	Accuracy	Same as QA Limits
Lab Duplicate	1 every 10	20% RPD	Rerun, recalibrate sample	QA Officer, Project Manager	Precision	Same as QA Limits
Laboratory Control Standard	1 every 20	Recovery 85-115% of true value	Rerun, recalibrate sample	QA Officer, Project Manager	Accuracy	Same as QA Limits

Parameter: TP						
Method: EPA 365.2						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): Soil: 11 mg/kg; Plant: 4 mg/kg						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 65-135% M-H: 75-125%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-40% M-H: 0-30%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TN						
Method: DBE SOP MVP						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): Soil: 590 mg/kg; Plant: 420 mg/kg						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 80-120% M-H: 85-115%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-20% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TC						
Method: DBE SOP MVP						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): Soil: 2090 mg/kg; Plant: 1740 mg/kg						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 75-125% M-H: 80-120%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-20% M-H: 0-10%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TOC						
Method: DBE SOP MVP/COE 3-73						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): Soil: 5010 mg/kg; Plant: 2680 mg/kg						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 75-125% M-H: 75-125%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-20% M-H: 0-15%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TFe						
Method: EPA/SW 7380						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): Soil: 250 mg/kg; Plant: 510 mg/kg						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 75-115% M-H: 85-115%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-40% M-H: 0-20%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 10	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: TCa						
Method: EPA/SW 7140						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): Soil: 7 mg/kg; Plant: 17 mg/kg						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	1 per 20	L: 75-125% M-H: 85-115%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-30% M-H: 0-15%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Continuing Check Standard	1 per 10	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: Lignin						
Method: AOAC 973.18						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): 1.63 %						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	N/A	N/A	N/A	N/A	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-30% M-H: 0-30%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Laboratory Control Standard	1 per 20	N/A	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Parameter: Cellulose						
Method: AOAC 973.18 (Calculation)						
Matrix: Soil / Plant						
Minimum Detection Limit (MDL): 3.20 %						
Laboratory QC	**Frequency (%)/Number**	**Method/SOP QA Acceptable Limits***	**Corrective Action (CA)**	**Person(s) Responsible for CA**	**Data Quality Indicator (DQI)**	**Measurement Quality Objectives**
Method Blank	1 per 20	<MDL	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Bias	Same as QA Limits
Lab Matrix Spike	N/A	N/A	N/A	N/A	Accuracy	Same as QA Limits
Lab Duplicate	1 per 20	L: 0-30% M-H: 0-30%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Precision	Same as QA Limits
Laboratory Control Standard	1 per 20	90-110%	DBE's CompQAP Section 9.6	DBE's CompQAP Section 9.6	Accuracy	Same as QA Limits

Table 9 defines field quality control measures. These samples will be collected in accordance with DEP-SOP-001/01 (2004). Any required corrective actions for the respective parameter will be performed as outlined in DEP-SOP-001/01 (2004).

Table 9. Field quality control (QC) measures to be used during the project.

QC Measure	Purpose	Frequency	Acceptance Criteria
Field Blank (FB): water matrices only	determine if contamination occurred from cleaning, collection, processing and transport	5%	< 5 times the lowest concentration of the associated samples collected
Field Duplicate (FD)	assess sampling and spatial variability at a given sampling site	once every 20 samples	0 – 80 %RSD

I. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Field Equipment/Instrument

A list of the field equipment to be used during this project is provided in Table 10. All field equipment testing, inspection and maintenance will be performed in accordance with DEP-SOP-001/01 (2004): FT1000 and DEP Field SOP for each specific parameter. The Field Operations Manager will ensure that all equipment is operational and will resolve any deficiencies. Spare parts for any critical equipment will be kept in inventory.

Table 10. Field equipment to be used for this project.

EQUIPMENT DESCRIPTION	USE
YSI Model 550	Dissolved oxygen measurements
Hach Sension 2	
Probe:Model: 51910, platinum series pH electrode	pH and temperature measurements
Probe: Model 51937-00, combination ORP electrode	Redox

EQUIPMENT DESCRIPTION	USE
Syringe & 0.45 µm filter	Water samples (SRP; TSP)
Core barrel	Sediment
"Sipper"	Porewater
Porewater equilibrators	Porewater

Laboratory Equipment/Instrument

Laboratory equipment used by DBE will be tested, inspected and maintained in accordance with DBE's CompQAP.

J. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

Field Equipment

Calibration/frequency and verification for all field equipment identified in Table 10 above will be performed in accordance with DEP-SOP-001/01 (2004): FT1000 and DEP Field SOP for each specific parameter.

Laboratory Equipment

Calibration for laboratory equipment used by DBE will be performed in accordance with DBE's QA CompQAP.

K. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All field/laboratory supplies will be purchased from reputable companies. Reagents come with a certificate of analysis that is reviewed and kept on file. All reagents are logged in upon arrival and tracked using the protocol described in DBE's CompQAP (section 8.2 and 8.3).

L. DATA MANAGEMENT

The data management process begins with development of field notes and the production of a chain-of-custody. Field notes and the chain-of-custody are prepared by the field technician and reviewed by the field operations manager. The chain-of-custody will provide a record of samples collected for a specific event and any notes related to those samples. Each sample bottle is given

a unique laboratory ID number, which is also recorded in the Sample Log Book, to assist in tracking within the laboratory.

Once the sample has been analyzed, the laboratory technicians will produce analytical reports that are reviewed by the Project QA Manager. Any necessary corrective actions deemed by the Project QA Manager will be taken.

All field notes and analytical reports are entered into a project specific MS Excel workbook by a data analyst. Data verification will be performed in accordance with DBE's CompQAP Section 9.0. Once the data is verified the data analyst will process, compile and analyze the project data and forward to the Project Director and Project Manager. The Project Director and Principal Scientist will be responsible for reviewing and interpreting the data for final project reporting.

All data is backed-up weekly onto external storage media. The final data report will be archived onto a CD and kept with the project files for easy retrieval.

M. VERIFICATION AND VALIDATION: CRITERIA

Throughout the project, data will be verified in accordance with DBE's CompQAP Section 9.0 using the field/analytical records produced by personnel. Any discrepancy discovered in the records will be researched by the employee performing the verification. The outcome will be recorded on the respective document, the employee will sign and date, and the project manager notified immediately (i.e., discrepancy of sample date on the collection bottles and chain-of-custody).

All data will be reviewed by using the analyte-specific criteria provided earlier in this document. Collection and analytical protocol will be verified by performing the audits described in section M. If deemed necessary, the appropriate corrective actions will be taken (i.e., revision of protocol or re-training) will be performed.

Before a deliverable is provided, data verification for that period will include data entry verification, spot-checking calculations performed by the software and verification of all graphs/table generated for the deliverable in accordance with DBE's CompQAP Section 9.0.

Data validation will be performed before each deliverable is produced. The data set will be reviewed in its entirety using QA/QC criteria, sample verification, historical or unexpected values, and professional judgment to determine the analytical quality of the dataset. Any deficiencies in the dataset and how those deficiencies will be handled will be clearly described in the deliverable.

N. DATA VERIFICATION AND VALIDATION METHODS

Data verification will be performed by all field and laboratory personnel. Each employee will verify his or her own work and that work will again be verified by a second employee when information is passed along to the next level (i.e., the sample receipt department will verify the information provided by the field personnel).

The Project QA Manager will continuously verify data throughout the project as the chain-of-custodies and analytical/field reports are produced. The criteria for data acceptance will be the QC control limits for each parameter as outlined in this document. Any data not meeting the above criteria will be qualified appropriately.

Before each deliverable is produced, the Project QA Manager will review all data collected for the reporting period. This will include data entry verification, spot-checking calculations performed by the software and verification of all graphs/table generated for the deliverable.

The Project Manager and Project Director will be responsible for all data validation. This will be performed throughout the project as deliverables are produced.

O. SCOPE OF WORK BY TASK NUMBER

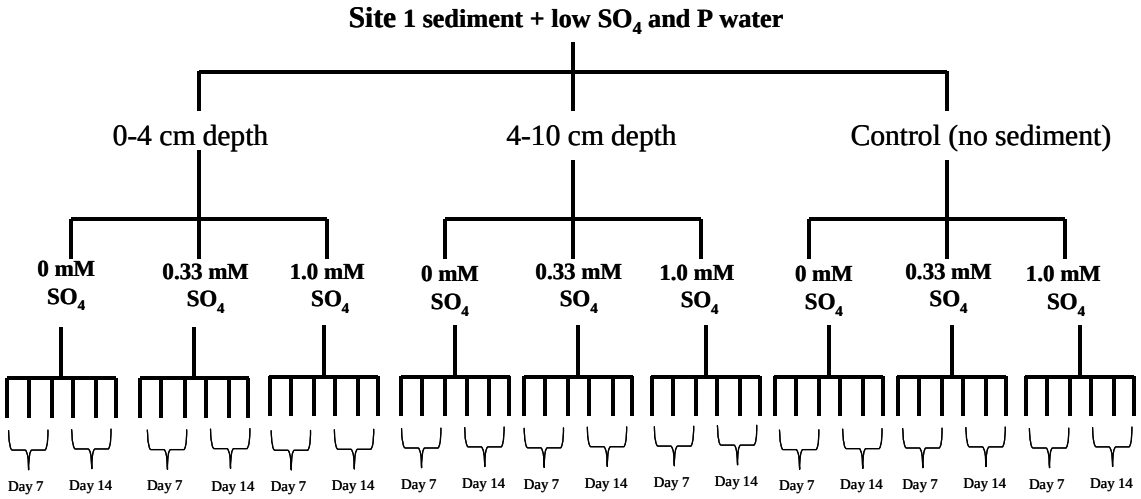
Task 2. Laboratory Incubations for Screening the Effects of Sulfate on Organic Matter Decomposition and the Release of Phosphorus

The purpose of Task 2 is to identify the potential role of sulfate enrichment on organic matter decomposition rates and the release of P from sediments collected using a range of phosphorus (P), sulfate (SO_4^{2-}), and calcium (Ca) regimes within south Florida wetlands.

Sulfate reduction lowers the proton concentration (increases pH). This can lead to an increase in the mineralization of organic matter through alkalinization, which would increase microbial decomposition if the pH is initially low. By serving as an electron acceptor in the oxidation of organic matter, sulfate can also increase organic matter decomposition in anaerobic environments. If either of these two processes occurs in our laboratory incubations, then we should detect "significant" increases in the CH_4 and CO_2 concentrations in the vessel headspace. Organic matter mineralization is quantified by the sum of the CH_4-C and CO_2-C production rates per kg of soil. Statistical analyses will be performed to determine if treatment effects differ from controls.

Substrate quality, as measured by the lignocellulose index (LCI), has been implicated in regulating organic matter degradation. Thus substrate quality, rather than oxygen or sulfate electron acceptors, may be the primary controlling factor of heterotrophic microbial decomposition in areas where the lignin content is high, and TP is low, in the plants and soils. For example, the WCA-3 soil is likely to have a high LCI and low TP contents.

Schematic of Laboratory Incubations



Sediment sources: STA-2, Cell 1 inflow region; WCA-2A, site U3; and WCA-3, site to be determined. Six replicate cores from each source.

Water source: WCA-3. Overlying water in flasks will be amended with SO_4 as noted above.

Days 0, 7, and 14 parameters: CO_2, CH_4, SRP, Ca, Fe, sulfate, sulfide, pH, redox potential

Days 0 and 14 parameters: TP, TSP, ammonia, and microbial biomass P

Figure 5. Generalized schematic of the lab incubations of sediment slurries. Only one of three sites where core retrieval and lab incubations will occur is presented. Cores from the other two sites will follow this same proposed investigation route.

Six sediment cores from each of the following three locations within south Florida wetlands (Figure 4) will be sectioned into three horizons: the 0-4, 4-10 cm, and 10-30 cm depths.

- STA-2, Cell 1 inflow region (represents an environment with a history of high concentration inputs of P, sulfate, and Ca)
- WCA-2A site U3 (represents an environment with a history of low concentration inputs of P and high to moderate levels of sulfate and Ca)
- WCA-3 (represents an environment with a history of low concentration inputs of P, sulfate, and Ca)

Existing data (Table 2) justify the selection of these sites as representing environments receiving low to high inputs of P, Ca, and sulfate. Nevertheless, a reconnaissance trip to each of the proposed sites, which will include preliminary sample collection and analysis, will be made prior to final core collections for the incubation experiment.

Common depth fractions from each of the six core sections within each of the three sites will be composited. Sediment collected from the three field sites at two depth intervals (0-4 cm and 4-10 cm) will be subjected to laboratory incubations in separate replicate bottles (Figure 5). Approximately 2.5 g dry wt. equivalent of homogenized wet sediment will be added to serum bottles, along with ~25 mL of surface water collected from WCA-3. The WCA-3 surface water used for all incubations will be spiked with sulfate (as Na_2SO_4) at three concentrations (for three levels of treatment): none, 32 mg/L (0.33mM) and 96 mg/L (1.0 mM). Each level of SO_4^{2-} - spiked water will be combined with sediment from each site and depth interval. Three replicates of each combination will be prepared for each of two sampling times (6 replicates total). Bottles containing only WCA-3A waters (no sediments) will serve as "controls".

Following addition of sediment and water, the serum bottles will be capped and sealed, then purged with 0.03% CO_2 balance nitrogen gas and maintained at a constant temperature of 20 - 25˚C. Three replicate bottles from each treatment combination will be sampled after 7 days and the three remaining replicates will be sampled after 14 days. We selected the 7- and 14-day incubation times based on similar studies in WCA-2A (White and Reddy 2001; Wright and Reddy 2001). Incubation times of days rather than hours will be required to reach methanogenic conditions, especially at the higher sulfate amendments.

Aliquots (filtered or unfiltered, as appropriate) of the sediment-water slurry withdrawn on days 0, 7, and 14 will be analyzed for SRP, dissolved Ca and iron (Fe), sulfate, and sulfide. Total and soluble P, and ammonium will be analyzed before and at the end of the 14-day incubation period. Redox potential and pH will be measured at day 7 and 14. Microbial biomass P will be analyzed on day 14. Bottle headspace will be sampled periodically for CO_2 and CH_4 analyses. Selected treatments of this study will be repeated, as appropriate, in order to further define/refine the relationships (dose-response) between sulfate levels and P release and organic matter mineralization.

Due to the need for a fast-response time in charting the course of the CO_2 and CH_4 emissions in the incubations (i.e., to insure that the correct sampling times have been selected for linearity of

response; that sample concentrations are above the MDL; that the integrity of the sample is not compromised from storage and preservation drawbacks), analysis of the gases will be performed at DB Environmental's laboratory within a matter of hours to a couple of days. The CO_2 and CH_4 in the headspace of the incubation vessels will be analyzed by gas chromatography. For CO_2 analysis, detection is by thermal conductivity (30°C) after separation by a 0.3 cm x 2 m Poropak N column with He as the carrier gas. Methane detection is by flame ionization (160°C) following separation by a 0.3 cm x 2 m Carboxyn 1000 column (160°C). Nitrogen is the carrier gas.

It should be noted that constituents such as Ca and Fe can exert a strong influence on P cycling, and therefore may interact with (and influence) potential sulfate effects. Calcium concentrations in particular vary spatially in south Florida wetland waters and sediments, so we intend to perform several additional experiments in which we vary the aqueous Ca and Fe concentrations as part of the sulfate incubation trials. For example, if significant quantities of sulfide and P are produced in the sulfate-amended soil slurries compared to the unamended control, then we may decide to add Ca and alkalinity salts to determine whether elevated concentrations of these constituents affect the mobilization of P. Iron would be another candidate amendment, given its preference to bind with sulfide, which theoretically may reduce the P release.

Since part of the DQO process is to clarify objectives and define the appropriate type of data, future incubations beyond the one shown in Figure 5 that may include iron and calcium amendments will remain undefined at this point. We will determine the exact design of these future experiments as data are gathered from the first planned experiment (see Figure 5). Thus, this will be an iterative process where future directions in follow-on experiments are predicated on results from previous experiments. This is shown in the DQO process by the feedback arrow from the Technical Assessments to the Systematic Planning boxes in Figure 1. The frequent (monthly) progress reports and meetings with District/EPD representatives and other stakeholders will facilitate a thorough review of recent data, consensus on future directions, and a timely execution of additional experiments going forward.

For the remaining (non-incubated) sediments from the 0 - 4 cm, 4 - 10 cm and 10 - 30 cm depth layers, the following analyses will be performed: total S (TS), total oxidizable S (TOS), total P

(TP), total N (TN), total C (TC), total organic C (TOC), total iron (TFe), total calcium (TCa), NaOH-extractable SRP and TP, and lignocellulose index (LCI). This latter metric is used as a measure of substrate quality. Porewater analyses on freshly collected sediments will include sulfate, sulfide, Fe, Ca, SRP, and ammonium.

Soil characteristics and microbial processes will be statistically related using Pearson's product-moment correlation and regression analysis. Data will be checked for normal distribution and homogeneity of variances (homoscedacity). If neither normality nor equal variances apply to a data set, then either the data will be transformed (e.g., log transformed) or non-parametric statistical analyses will be employed. Analysis of variance (ANOVA) and least significant difference tests will be used to make comparisons between treatments after pooling the data from the same locations.

These laboratory experiments will:

1. help characterize the role of sulfate/sulfide on the release of sediment P (i.e., internal eutrophication) in south Florida wetlands.
2. define the importance that sulfate/sulfide may or may not have on organic matter mineralization in south Florida wetlands.
3. further our understanding of interactions between sulfur, calcium and iron in wetland environments.
4. assist in screening of treatments (e.g., appropriate chemical amendments and dosages) to be applied to subsequent mesocosm-scale experiments (see below).
5. define accrual rates and diagenesis of S, P, Fe, and Ca in impacted and non-impacted sediments.

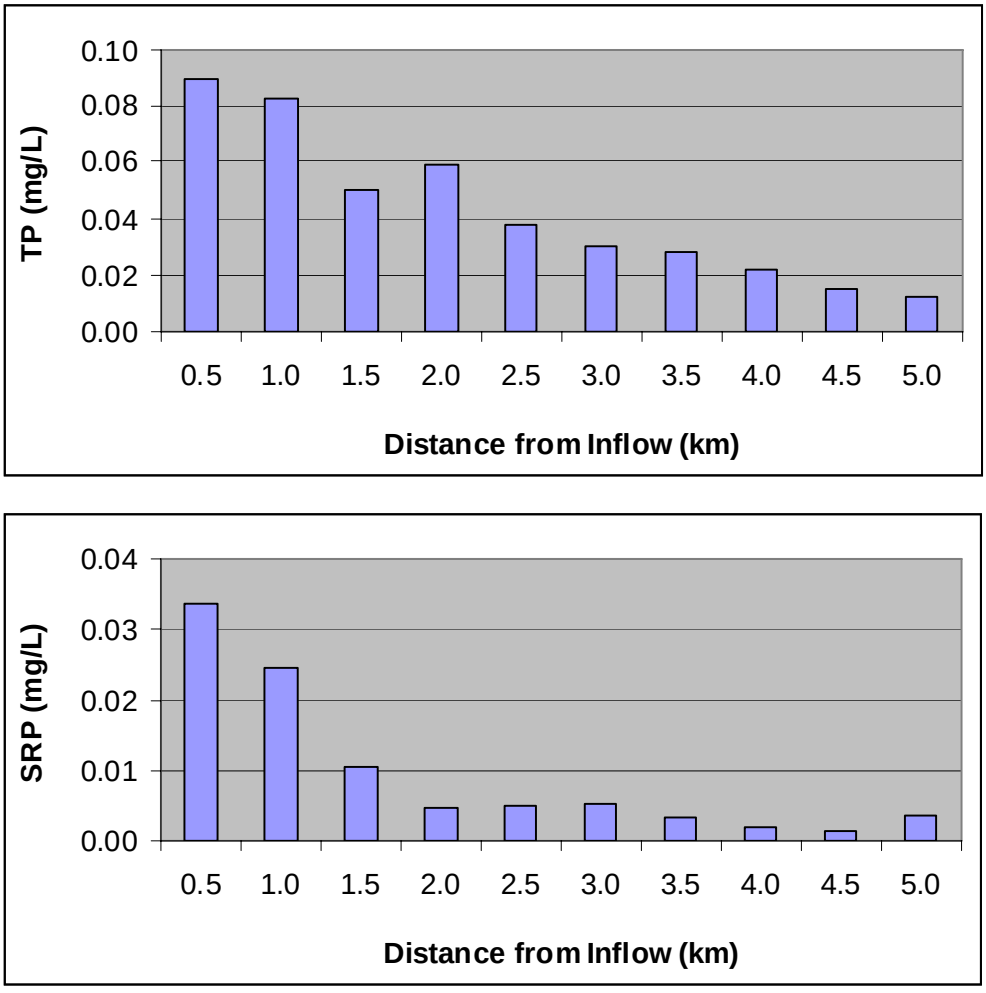
Task 3. Field Monitoring of Surface Water and Sediment Porewater Along Sulfate and Phosphorus Gradients, and Among Vegetation Communities, within STA Flow Paths

Historically, the STAs, and their individual flow paths, have differed considerably in their P removal efficiencies (Juston and DeBusk 2006). The purpose for field monitoring of the surface and porewaters along P gradients within flow paths at each of the three STAs in Task 3 is to understand the nature and extent that critical biogeochemical processes (e.g., sulfate reduction, organic matter decomposition, Ca-P coprecipitation) participate in recycling P in "enriched" and "unenriched" environments. According to the hypothesis that sulfate reduction enhances

sediment P mobilization, one would expect to find higher sulfate and sulfide concentrations in porewaters of under-performing STAs.

Although sulfate concentrations in surface waters do not usually exhibit a pronounced concentration profile within an individual flow path, concentrations do vary among flow paths (Table 4). Phosphorus concentrations, on the other hand, frequently follow a sharp gradient profile in STAs and WCA-2A as shown in Figures 6-7.

STA-2 Cell 1



.

Figure 6. Total (TP) and soluble reactive phosphorus (SRP) concentration gradients along the flow-path in STA-2 Cell 1. The values represent the mean of two sampling events (6/21/07 and 9/28/07).

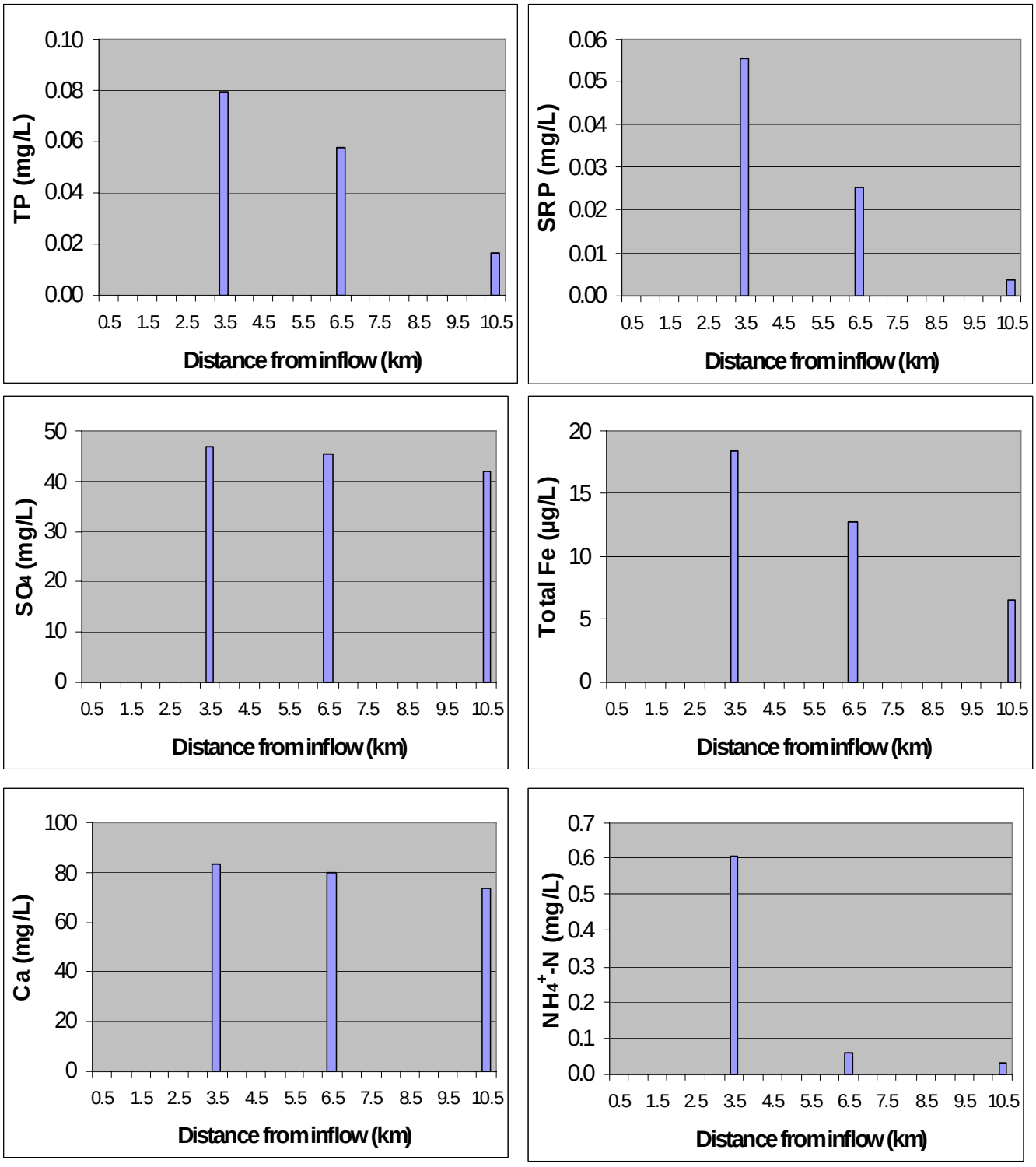


Figure 7. Concentration gradients for nutrients and major ions in the surface water at three stations along the "F" transect in WCA-2A (April 1994-March 2008).

Koch-Rose et al. (1994) reported that porewater SRP and ammonia concentrations increased more than 8 times at eutrophic sites in WCA-2A during the summer compared to the spring. We will also examine whether the porewater concentration seasonality reported for WCA-2A applies to STAs, which do not have the extreme fluctuations in water levels as in WCA-2A, by seasonally monitoring Cell 1 of STA-2. For the stations positioned along the transects in Cell 1 within STA-2, surface and soil pore water sampling will occur every six months over an eighteen-month period. Surface and porewater samples from the remaining two flow paths in STA-3/4 and STA-5 will be sampled only once, during the summer of 2009.

Approximate locations of transects and sampling sites are shown in Figures 8-10. Sampling stations typically will be situated across a range of P and sulfur enrichment conditions along the flow path, as well as within a range of vegetative community types (e.g., cattail and sawgrass). Relationships between porewater chemistry and the vegetation community may provide insights into possible inhibiting effects that sulfide exerts on the flow within STAs.

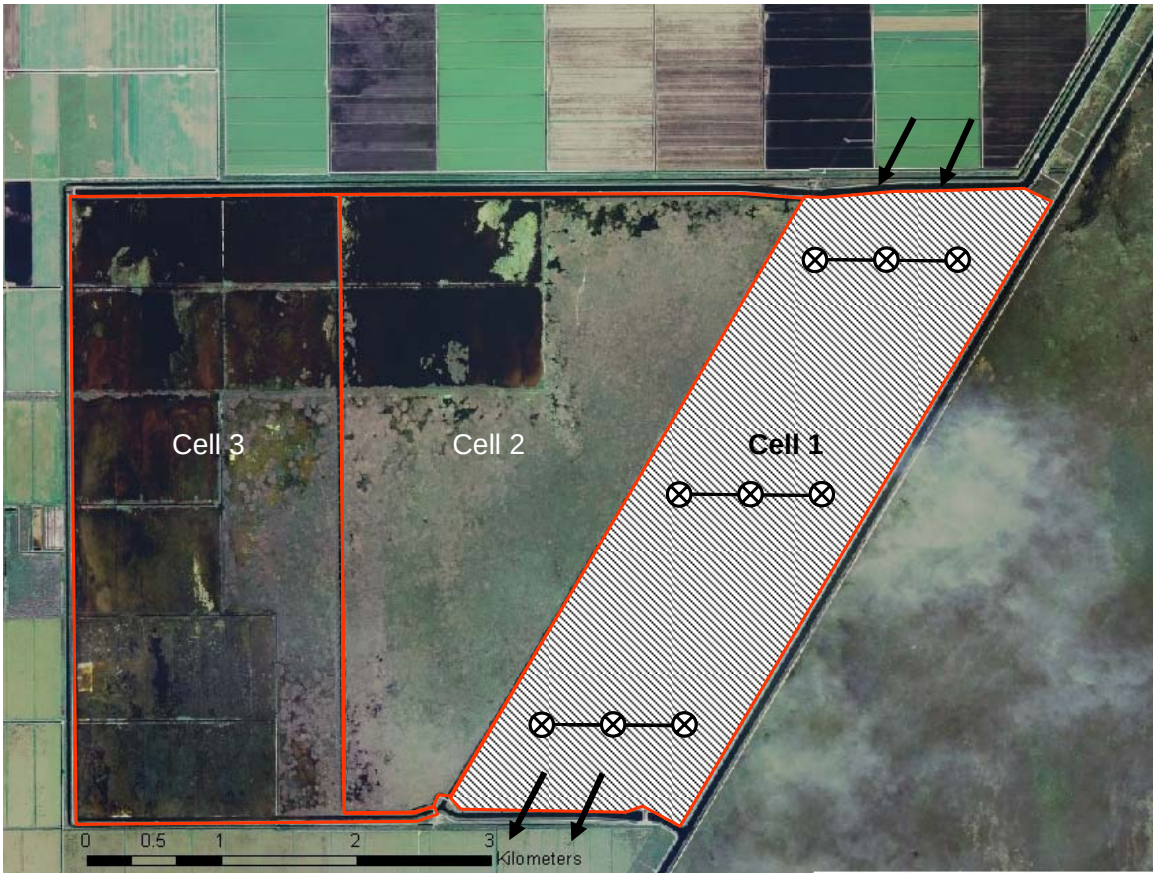


Figure 8. Transect locations for surface water, porewater, and sediment characterization in STA-2 Cell 1.

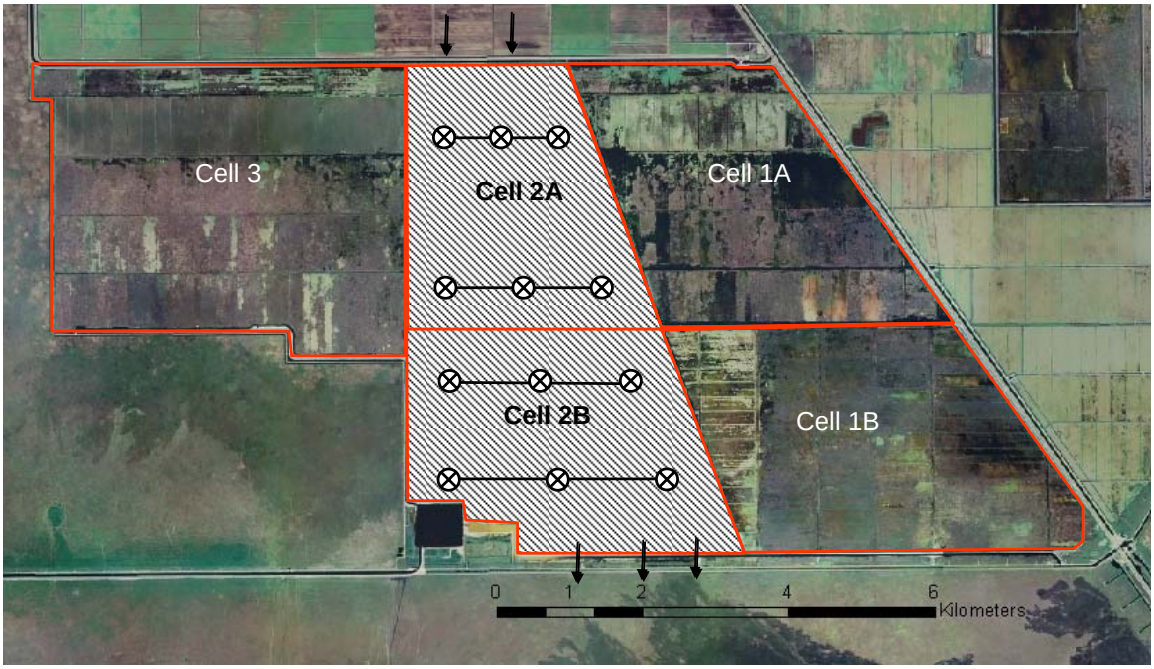


Figure 9. Transects selected for surface water, porewater, and sediment characterization in the STA-3/4 central flow path.

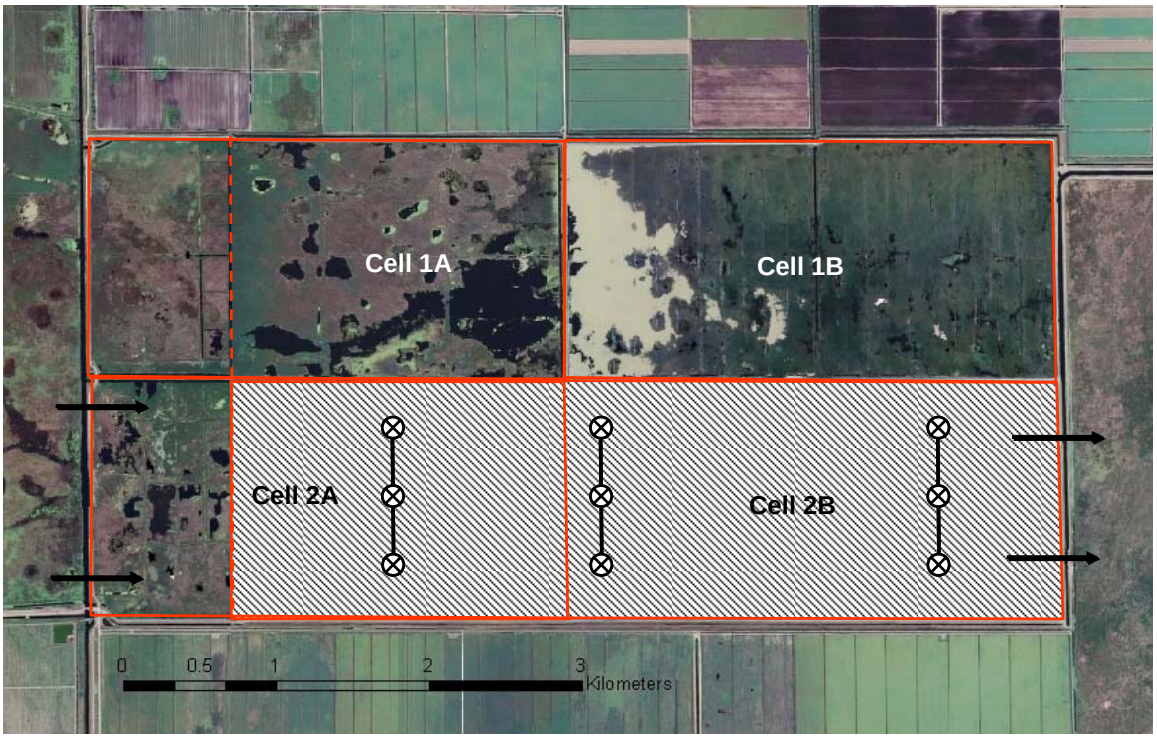


Figure 10. Transect locations for surface water, porewater, and sediment characterization in the STA-5 central flow path.

Surface waters will be collected by grab sample, either by direct collection into sample bottles or, where site disturbance is a potential problem, by collection with a portable peristaltic pump. Sediment porewater will be collected at 2 cm depth intervals using porewater equilibrators.

In situ measurement of surface water temperature, conductivity, pH, and dissolved oxygen will be performed at all sites. Sediment redox levels at 2-cm depth intervals will be measured directly on porewaters retrieved from the porewater equilibrators.

Concomitant with field sampling for surface and porewaters in the selected flow paths in STAs 3/4 and 5, sediment cores will also be retrieved from each station. Sediment cores will also be retrieved on one occasion in STA-2 Cell 1, where multiple pore water collections over time are planned. The cores (3.2 cm i.d.) with approximately 10 cm of intact sediment will be incubated in the lab with low P and S overlying water collected from WCA-3. The anoxic incubations will be performed in the dark and at room temperature. We have performed a similar P release study for sediments collected in STA-2 Cell 3, and found a significant correlation between sediment P release and porewater SRP + sediment P along the flow path ($R^2 = 0.74$). Sulfide concentrations were not measured in that study. In this study, we will measure sulfate, sulfide, and SRP concentrations in the initial and at the end of the 14-day incubation period as a means of relating sulfide levels to SRP release.

Laboratory analyses of *in situ* surface water will include SRP, TSP, TP, sulfate, sulfide, alkalinity, Ca, Fe, DOC, and NH_4^+. Porewater sampled via peepers will be analyzed for pH, SRP, NH_4^+, sulfate, sulfide, alkalinity, Ca and Fe.

The *in situ* surface and pore water concentrations of P, Ca, Fe, ammonium, sulfate, and sulfide in the field will be essential in identifying the critical biogeochemical processes (microbial P uptake and release, sulfate reduction, organic matter decomposition, and Ca-P coprecipitation) that occurs in P-enriched and unenriched areas. The field data will also be compared to the concentrations found in the more controlled phases of the study (lab core incubations and mesocosms) that may have toxic effects on plants, P release, and organic matter decomposition.

Such comparisons will be requisite if laboratory and mesocosm results are to be extrapolated to the larger scale.

Specifically, the results obtained from the field monitoring and laboratory sediment P release studies will:

1. Provide necessary background information on the *in situ* concentrations of key elements and ions influenced by biogeochemical processes responsible for the sequestration/release of P.
2. Examine the spatial variability of elements and ions along sulfur and phosphorus gradients.
3. Characterize vertical concentration gradients within sediment and overlying water for calculating vertical diffusive fluxes.
4. Further an understanding of the role played by sulfate reduction in regulating P cycling.
5. Provide insight into potential toxic effects of porewater sulfide on marsh flora.

Task 4. Field Mesocosms for Relating Sulfur Dynamics to Internal Eutrophication, Organic Matter Decomposition, and Phytotoxicity

Using field mesocosms, the effects of S on plant toxicity, internal eutrophication, and organic matter decomposition can be determined under more controlled conditions than by simply monitoring plant communities and the chemical milieu in the field. Conclusions reached will also have a higher level of realism to *in situ* conditions than those based on laboratory experiments.

We propose two field sites for mesocosm studies, situated in environments that represent a range of historical sulfate and phosphorus impacts. Port Mayaca receives high P waters (Table 5), and is therefore useful for evaluating impacts of sulfate levels on P cycling and plant toxicity under conditions prevalent in the STAs.

There is some concern that high levels of sulfate can influence STA phosphorus removal performance. Farm runoff and Lake Okeechobee waters represent the two dominant sources of inflow water to the Everglades STAs. The Port Mayaca experimental site therefore is ideal for determining potential sulfate impacts under the moderate-to-high concentrations likely to be experienced by the STAs in the foreseeable future.

The second proposed experimental site within WCA-3 contains waters low in P, sulfate and calcium. Because of relatively pristine waters, this site will be ideal for dosing trials, using sulfate alone, or sulfate in combination with calcium, to assess potential impacts to flora and wetland P retention.

Effects of Sulfate on Plant Health: Port Mayaca

Using small outdoor mesocosms at Port Mayaca, we will establish a series of trials to assess the effects of high sulfate levels on P cycling and plant health. We will install small, replicate mesocosms containing pots of cattail (*Typha domingensis*), sawgrass (*Cladium jamaicense*) and a submerged macrophyte (*Najas guadalupensis*). These will be fed ambient site (Lake Okeechobee) water on a continuous basis, which likely will average about 0.15-0.25 mg TP/L. Additionally, a second group of mesocosms will receive "pre-treated" water, in which TP levels are reduced to the range of 0.05 – 0.08 mg TP/L (Figure 11). In previous mesocosm studies conducted at STA-1W, we have been able to manipulate P concentrations of inflow waters in this manner by providing a "pre-treatment" step, which simply consists of a large "front-end" wetland (in this case dominated by cattail) that feeds waters to downstream mesocosms. By using both pre-treated, as well as "raw" inflow waters, we therefore will have the capability at the Port Mayaca site to evaluate sulfur effects on P cycling and phytotoxicity under differing P and S regimes. These conditions would be comparable to those found in the inflow and outflow (or middle-) regions of an STA.

Besides testing two levels of P loading (untreated and pre-treated) inflow water, we will also add sulfate (as Na_2SO_4) to one of each pair of untreated and pre-treated mesocosms that contain the plant containers (Figure 11). Initially, the increment of sulfate will be 48 mg/L (0.5 mM), which will bring the ambient lake water sulfate concentration to 80-90 mg/L, approximately equal to mean EAA runoff concentrations. Therefore the final design at Port Mayaca will consist of replicate pots of each of three species of plants placed in four flow-through mesocosms that receive lake water, that have either been untreated and unamended (ambient), pre-treated (to remove P), untreated but amended with sulfate, or pre-treated and unamended with sulfate (Figure 11).

We plan to sacrifice triplicate pots of each plant species in each of the four mesocosms after 2, 6, and 12 months of treatment exposure. Plant stress indicators listed in Table 11 will be measured.

Table 11. Indicators of plant stress to be characterized.

1. Physiological
a. Chlorophyll
b**.** Nutrient concentration
2. **Growth and Morphology**
a. Architecture
b. Root mass
c. Leaf size
3. Mortality
a. Death and Senescence

Surface water will be assessed every two weeks at the sampling locations depicted in Figure 11. Laboratory analysis of surface water will include SRP, TSP, TP, sulfate, sulfide, alkalinity, Ca, Fe, and NH_4.Additionally, the internal distribution of sulfate concentrations within the mesocosms will be determined periodically. Immediately prior to sacrificing the potted plants after 2, 6, and 12 months, porewaters will be collected by sipper and analyzed for pH, SRP, sulfate, sulfide, alkalinity, Ca, Fe, and NH_4^+.

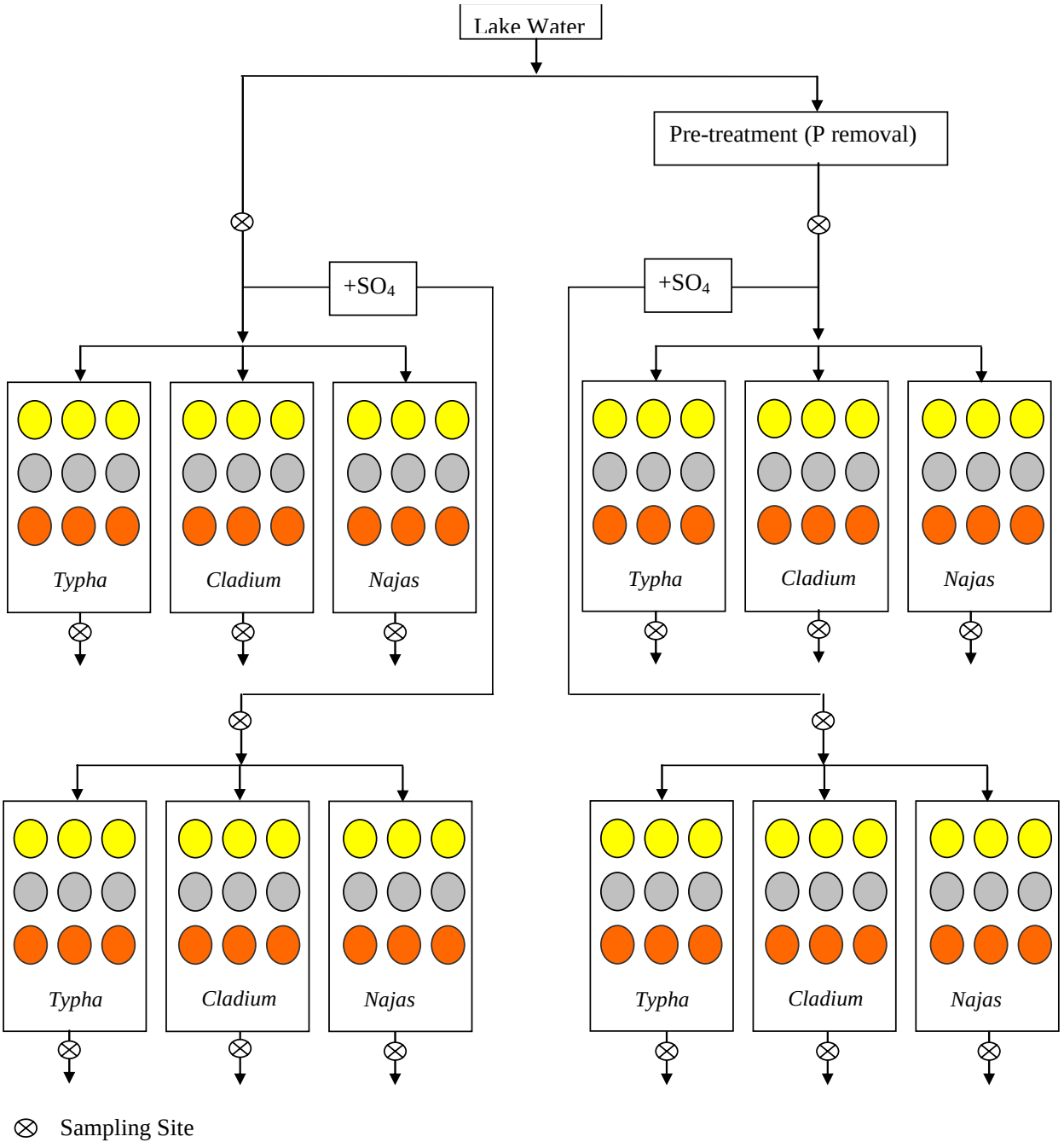


Figure 11. Schematic of the "toxicity" study at Port Mayaca. The plants will be grown in containers and each triplicate set will be sacrificed after 2 (top row-yellow), 6 (middle row-gray), and 12 (bottom row-red) months from start-up for physiological, morphological, and growth effects.

Effects of Sulfate and Calcium on Phosphorus Removal and Containment: Port Mayaca Platform

A second set of larger flow-through mesocosms will be utilized to assess the effects of moderate sulfate + calcium levels vs. high sulfate + calcium levels on P removal and retention in an STA. We will establish triplicate sequential mesocosms, with the front-end mesocosms containing cattail and the back-end mesocosms containing a submerged macrophyte, such as *Najas guadalupensis* (Figure 12). This is the typical "emergent – SAV" community sequence anticipated for most of the STA flow paths. The vegetation will be stocked in typical farm-field soil, obtained from either the compartment "B" or "C" STA expansion sites.

Along with the triplicate set of mesocosms receiving only lakewater, two triplicate sets of mesocosms will receive only sulfate (48 mg/L) or sulfate (48 mg/L) plus Ca (40 mg/L [1 mM]) amendments (Figure 12). Both sulfate and Ca spike levels should bring the concentrations of each to approximate those of EAA runoff.

We propose monitoring inflows and outflows from both "front-end" and "back-end" mesocosms on a bi-weekly basis for pH, SRP, TP, TSP, Ca, and sulfate. Sulfide, NH_4^+, alkalinity and Fe will be measured on inflow and outflow samples collected every four weeks. See Figure 12 for sampling locations. Soil porewaters (sampled by sippers) will be analyzed for the above constituents (except TP) at the beginning of the study, and every six months thereafter. Upon completion of the study, a complete sediment characterization will be performed, which will include P and Fe extractions, total oxidizable sulfur (TOS), microbial biomass P, LCI, and the elements listed above under the Task 2 section.

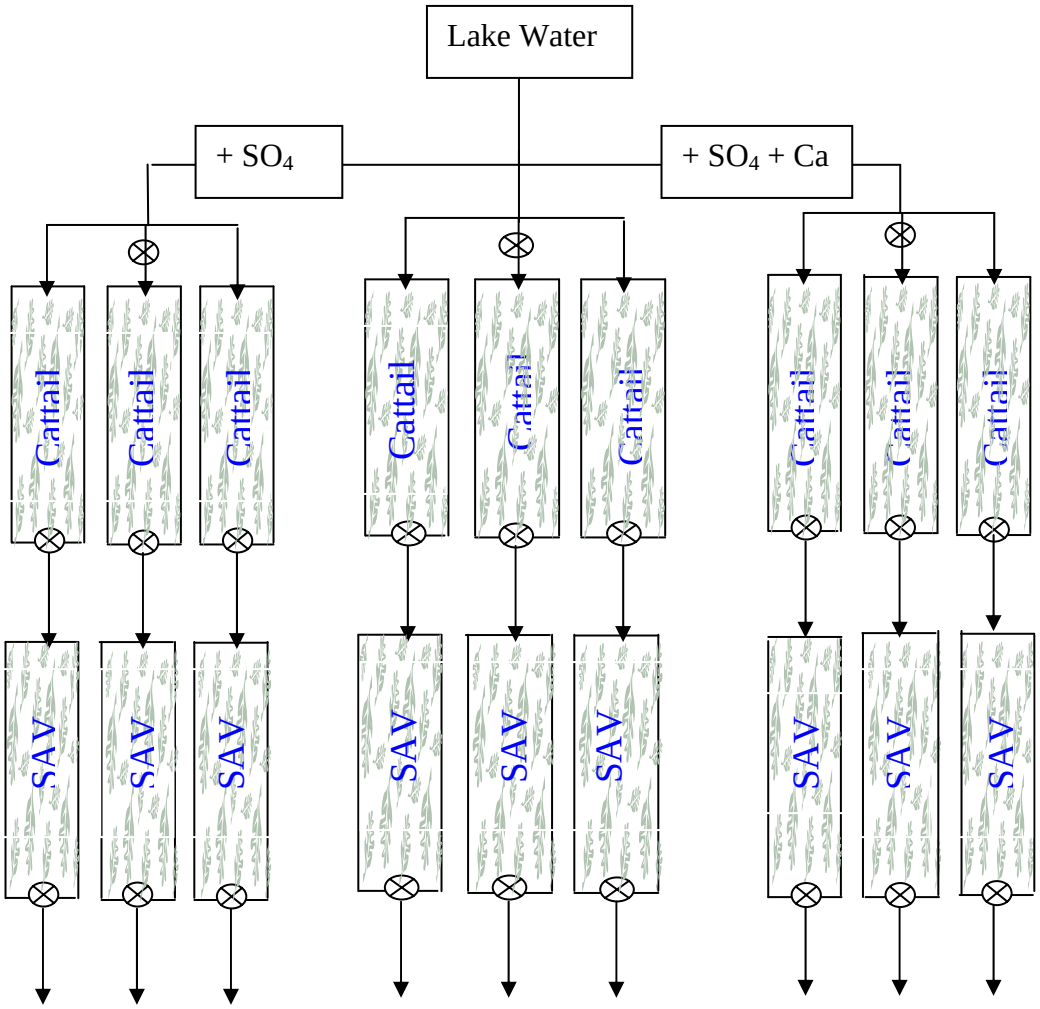


Figure 12. Port Mayaca two tanks-in-series flow-through mesocosms for testing effects of sulfate and calcium on phosphorus removal.

Effects of Sulfate and Calcium Concentrations on Phosphorus Immobilization and Plant Health: WCA-3

Waters throughout portions of WCA-3 contain low concentrations of P, sulfate, and Ca. We intend to construct a remote mesocosm dosing facility to evaluate the effects of sulfate and Ca on surface water and porewater chemistry, and potential phytotoxicity. We anticipate establishing this facility during year two of the study.

Vegetation to be evaluated at this site will include spike rush, bladderwort and calcareous periphyton. We will use *in situ* cylinders operated in batch mode with replacement of water inside the cylinders by ambient outside water every two weeks, followed by spiking sulfate and Ca at levels shown in Figure 13. Both control (no-dose) and dosed (sulfate alone, or sulfate in conjunction with Ca) units will be established in triplicate.

Surface water sampling will occur before and after the bi-weekly exchange of the inside water with ambient outside water and the sulfate and Ca spikes. Constituents analyzed will include pH, SRP, TSP, TP, Ca, alkalinity, sulfate and sulfide. Porewaters will be collected by “peepers” at the end of the wet season, and analyzed for the same constituents as the surface waters (except for TP). Sediment cores will be retrieved from inside and outside the enclosures prior to the site experiencing natural drydown. The 0 - 4 and 4 - 10 cm sections will be analyzed for TP, TN, TS, TCa, TC, TOC, TOS, NaOH-extractable P, and microbial P biomass.

Changes in the percent coverage of the major plant species within the cylinders will be reported throughout the study period on a monthly basis. At the close-out of the experiment, which will coincide with the natural drydown cycle, plants inside and outside the cylinders will be harvested and analyzed for stress according to the indicators listed in Table 11.

The mesocosms studies will:

1. Quantify the direct effects, if any, of sulfide and/or ammonium toxicity to native south Florida wetland flora.
2. Examine the effect that sediment history (e.g., high vs. low P loadings) has on internal P cycling and sulfur interactions.
3. Using flow-through, outdoor platforms, confirm results of laboratory incubations on the effects of varying Ca, sulfate, and P concentrations on P cycling.

4. Characterize effects of inflow sulfate concentrations on P removal effectiveness of STAs.

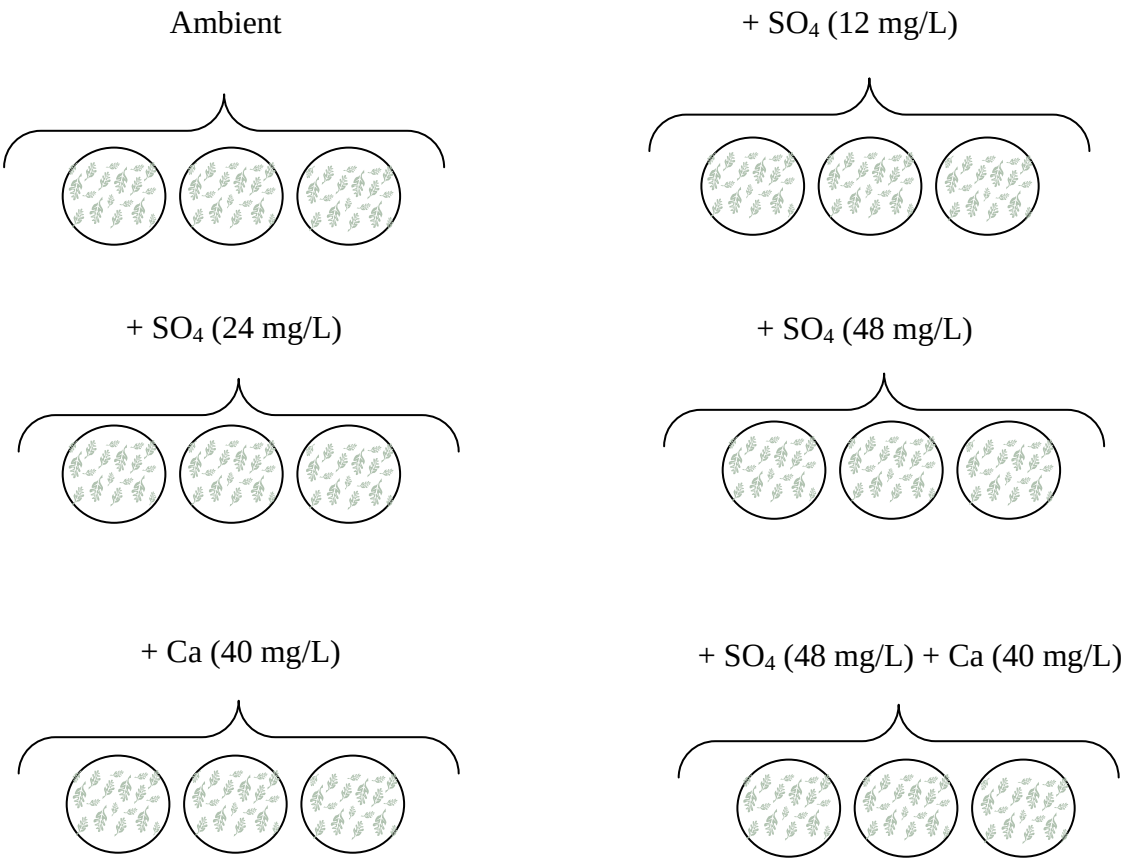


Figure 13. *In situ* cylinders circumscribing native spike rush, bladderwort, and calcareous periphyton for toxicity and phosphorus mobilization studies in WCA-3.

P. MEETINGS, REPORTS AND DELIVERABLES

Meetings will be held monthly during FY08, then bi-monthly from FY09 to FY11. These meetings will be structured to provide updates on each task and to present results. The contractor will submit a progress report in electronic format prior to each progress meeting. The progress reports will detail overall contract status, new data sets, activities, and results associated with past meeting issues. Appendix 1 provides a progress meeting report template and Appendix 2 provides a progress meeting agenda template. The contractor will also electronically submit a meeting summary document within 2 weeks after each meeting. The meeting summaries will serve as a vehicle to transfer results to District staff and will be used to evaluate contractor performance. Appendix 3 provides a progress meeting summary template.

Within 30 days of the end of each fiscal year, the contractor will submit an annual report that summarizes past fiscal year activities. These activities will include, but are not limited to, field activities (e.g. number of sampling trips, number of samples, field issues), data results and major findings. The annual reports will be submitted in electronic draft with five hard copies in Microsoft Office format.

Within 30 days of the FY11 end, the contractor will submit a final report. This report will compile all deliverables into one package, summarize the project, provide conclusions, and a look ahead. The contractor will submit 5 hard-bound copies of the Final report.

When major project milestones (e.g., completion of a manuscript or deliverable) are reached, public workshops will be held to discuss results and provide feedback.

Deliverable 1.1- Draft Work Plan (Deliverable for Task 1):

- Within 60 days of the official project kick-off date (4/1/08), a detailed draft Work Plan will be submitted by the contractor. This draft Work Plan will describe field sampling and laboratory analysis methods, QA/QC protocols, and also discuss how appropriate quality control measures will be incorporated for those unique analyses (e.g., selected low volume chemical analyses of porewaters) where NELAC-approved methodologies for sulfate and

sulfide haven't been applied. Additionally, the Work Plan will address review comments previously provided by SFWMD research scientists, and incorporate recommendations, as appropriate. As part of the Work Plan development, suitable sites for sediment collection (Task 2 sediment cores) and routine field monitoring (Task 3) will be screened and selected. This report will be supplied in electronic draft with five hard copies in Microsoft Office format. The work plan will also provide a cost structure break down. Following submission of Deliverable 1.1 a public workshop will also be held to provide project feedback.

Deliverable 1.2 – Final Work Plan (Deliverable for Task 1):

- Within 60 days of the Deliverable 1.1 meeting, the contractor will submit the final Work Plan. This final Work Plan will be supplied in electronic draft with five hard copies in Microsoft Office format. Spreadsheets detailing all data sets will be attached.

Deliverable 2.1 – Laboratory Incubation Study Report (Deliverable for Task 2):

- Within 60 days of completing Task 2 (middle of FY09), the contractor will provide a draft report for District review on the laboratory incubation study in electronic draft. This report including tables, figures and data summaries will be readable in Microsoft Office (Word or Excel). After review by District staff, the contractor will, within 30 days, submit the final report in electronic draft with five hard copies in Microsoft Office format. Spreadsheets detailing all data sets will be provided as an attachment.

Deliverable 2.2 – Manuscript on the Laboratory Incubation Study Report

- Within 60 days of the final Deliverable 2.1 submission, the contractor will submit a draft manuscript to the District project manager (Mark Gabriel). The project manager will work with the contractor on finalizing the manuscript for journal submission. The project manager will be a co-author along with participating scientists.

Deliverable 3.1 – Field Monitoring Report (Deliverable for Task 3):

- Within 60 days of completing Task 3 (middle of FY10), the contractor will provide a draft report for District review on the field monitoring study in electronic draft. This report

including tables, figures and data summaries will be readable in Microsoft Office (Word or Excel). After review by District staff the contractor will, within 30 days, submit the final report in electronic draft with five hard copies in Microsoft Office format. Spreadsheets detailing all data sets will be provided as an attachment.

Deliverable 3.2 – Manuscript on the Field Monitoring Report

- Within 60 days of the final Deliverable 3.1 submission, the contractor will submit a draft manuscript to the District project manager (Mark Gabriel). The project manager will work with the contractor on finalizing the manuscript for journal submission. The project manager will be a co-author along with participating scientists.

Deliverable 4.1 – Field Mesocosm Report (Deliverable for Task 4):

- Within 90 days of completing Task 4 (end of FY11), the contractor will provide a draft report for District review on the field mesocosm studies in electronic draft. This report including tables, figures and data summaries will be readable in Microsoft Office (Word or Excel). After review by District staff the contractor will, within 30 days, submit the final report in electronic draft with five hard copies in Microsoft Office format. Spreadsheets detailing all data sets will be provided as an attachment.

Deliverable 4.2 – Manuscript on the Field Mesocosm Report

- Within 60 days of the final Deliverable 4.2 submission, the contractor will submit a draft manuscript to the District project manager (Mark Gabriel). The project manager will work with the contractor on finalizing the manuscript for journal submission. The project manager will be a co-author along with participating scientists.

Q. PROJECT FUNDING AND PAYMENT

This is a 3-year cost-share agreement for $1,500,000, of which the District's total contribution is $900,000, for which dedicated sources (Everglades Restoration Trust Fund) in the amount of $125,000 are budgeted. The remainder is subject to Governing Board approval of the FY09-FY11 budgets. The Everglades Agricultural Area Environmental Protection District (EAA EPD) will contribute $600,000 as a cost-share partner. Payments will be invoiced on a bi-monthly

basis. Active MOA status will be contingent on the satisfactory performance on each deliverable and meeting report (see section 5.0). Table 1 in the Appendix 4 shows available funds per fiscal year and Table 2 in the Appendix 4 shows the deliverable and payment schedule. On a bi-monthly basis, the contractor will submit an invoice and payment will follow. The cost structure breakdown is shown in Table 12.

R. PERFORMANCE

The consultant's performance for this agreement shall be evaluated following receipt of each Task deliverable and meeting progress report. Successful completion of Tasks 1 - 4 will be evidenced by the judgment of District staff that the materials produced by the Contractor are understandable, clear, and performed in a timely and satisfactory manner. The Contractor's technical evaluation must be thoughtful, scientifically accurate, and must satisfy the projects' objectives.

COST STRUCTURE BREAKDOWN

Table 12. Cost Structure Breakdown (SFWMD Funding)

	Deliverables According to Task Number								
Cost Category	**1.1**	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**	***Total $***
Principal Scientist	$10,050	5,550	30,150	15,000	30,000	15,000	18,750	10,500	**135,000**
Field Assistants	20,100	11,100	60,300	30,000	60,000	30,000	37,500	21,000	**270,000**
Laboratory Analyses	20,100	11,100	60,300	30,000	60,000	30,000	37,500	21,000	**270,000**
Administration	6,700	3,700	20,100	10,000	20,000	10,000	12,500	7,000	**90,000**
Travel & Airboat	3,350	1,850	10,050	5,000	10,000	5,000	6,250	3,500	**45,000**
Equipment & Supplies	6,700	3,700	20,100	10,000	20,000	10,000	12,500	7,000	**90,000**
Total $	67,000	37,000	201,000	100,000	200,000	100,000	125,000	70,000	**$900,000**

S. REFERENCES

ASA. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. #9 Agronomy, 1982.

Axelrad, D.M., T.D. Atkeson, T. Lange, C.D. Pollman, CC. Gilmour, W.H. Orem, I.A. Mendelssohn, P.C. Frederick, D.B. Krabbenhoft, G.R. Aiken, D.G. Rumbold, D.J. Scheidt, and P.I. Kalla. 2007. Appendix 3B-3: Mercury, Monitoring, Research and Environmental Assessment in South Florida. In: South Florida Environmental Report – Volume 1, South Florida Water Management District, West Palm Beach, FL.

COE. Report EPA/COE-81-1. Procedures for Handling and Chemical Analysis of Sediment and Water Samples. EPA/Corps of Engineers Technical Comm. on Criteria for Dredged and Fill Material. 1981.

Davis, S.M. and J.C. Ogden. 1994. Everglades: The Ecosystem and Its Restoration. St. Lucie Press, Boca Raton, Florida. 826 pp.

DB Environmental. 2002. Demonstration of submerged aquatic vegetation/limerock treatment technology for phosphorus removal from Everglades Agricultural Area waters. Prepared for the South Florida Water Management District and the Florida Department of Environmental Protection.

DEP-SOP-001/01. Department of Environmental Protection Standard Operating Procedures for Field Activities. FDEP Tallahassee, FL Bureau of Laboratories Environmental Assessment Section. February 1, 2004.

EPA. Methods for Chemical Analysis of Water and Wastes. 1979. EPA 600/4-79-020.

Everglades Forever Act. 1994. Section 373.4592, Florida Statutes.

Florida Department of Environmental Protection (FDEP). 2001. Everglades Phosphorus Criterion Technical Support Document. Staff Draft Part III. Water Conservation Area 3 and Everglades National Park. Everglades Technical Support Section, Tallahassee, FL.

Juston, J., and T.A. DeBusk. 2006. Phosphorus mass load and outflow concentration relationships in stormwater treatment areas for Everglades restoration. Ecol. Eng. 26:206-223.

Koch-Rose, M.S., K.R. Reddy, and J.P. Chanton. 1994. Factors controlling seasonal nutrient profiles in a subtropical peatland of the Florida Everglades. J. Environ. Qual. 23:526-533.

Lamers, L.M., H.M. Tomassen, and J.M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environ. Sci. Technol. 32:199-205.

Medelssohn, I.A., S. Li, H. Chen, and W.H. Orem. 2006. Is sawgrass more sensitive than cattail to sulfate loading and resultant hydrogen sulfide? Presentation documents. South Florida Environmental Report Peer Review Panel, South Florida Water Management District, West Palm Beach, FL.

Moutin, T., J.Y. Gal, H. Halouani, B. Picot and J. Bontoux. 1992. Decrease of phosphate concentration in a high rate pond by precipitation of calcium phosphate: theoretical and experimental results. Water Research: 26(11):1445-1450.

NELAC, National Environmental Laboratory Accreditation Conference Standards. EPA/600/R-04/003, 2003.

Picot, B, H. Halouani, C. Casellas, S. Moersidik and J. Bontoux. 1991. Nutrient removal by high rate pond system in Mediterranean climate (France). Water Science and Technology 23:1535-1541.

Reddy, K. R., Y. Wnag, W. F. DeBusk, and S. Newman. 1994. Physico-Chemical properties of Soils in the Water Conservation Area 3 (WCA-3) of the Everglades. Final Report to the South Florida Water Management District. West Palm Beach, FL.

Scheidt, D.J., and P.I. Kalla. 2007. Everglades ecosystem assessment: water management and quality, eutrophication, mercury contamination, soils and habitat. Monitoring for adaptive management: A R-EMAP status report. USEPA Region 4, Athens, GA 904-R-07-001. 98 pp.

Smolders, A.J.P., L.P.M. Lamers, E.C.H.E.T. Lucasseen, G. Van der Velde, and J.G.M. Roelofs. 2006. Internal eutrophication: How it works and what to do about it – a review. Chemistry and Ecology 22:93-111.

SM. Standard Methods for the Examination of Water and Wastewater, 20th ed. 1998. American Public Health Assoc., American Water Works Assoc., and Water Environmental Federation, Washington, D.C.

White, J.R., and K. R. Reddy. 2001. Influence of selected inorganic electron acceptors on organic nitrogen mineralization in Everglades soils. Soil Sci. Soc. Am. J.65:941-948.

Wright, A.L., and K.R. Reddy. 2001. Heterotrophic microbial activity in northern Everglades wetland soils. Soil Sci. Soc. Am. J. 65:1856-1864.

APPENDIX 1

PROGRESS REPORT TEMPLATE

TO:

CC:

FROM:

DATE:

CONTRACT #

SUBJECT: Progress Report # (Time Period:)

Overall Contract Status

Scope

Schedule

Budget

Task Status

Task Status	Name	Work Completed Through (date)	Work Planned Next Period	Future Work Planned
1				
2				
3				
4				

Work completed this period

Work planned next period

Results (Spreadsheet attached)

Problems/Issues

Summary

APPENDIX 2

PROGRESS MEETING AGENDA TEMPLATE

- Introductions
- Work Completed this Period
- Work Planned Next Period
- Discussion of Results
- Issues Encountered
- Schedule Status
- Budget Status
- Other Issues
- Action Items

APPENDIX 3

MEETING SUMMARY TEMPLATE

TO:

CC:

FROM:

DATE:

SUBJECT: Contract #XXX; Progress meeting summary #X on (date)

Agenda

Attendee Contact info

Name	Affiliation	Address	Email	Phone
1				
2				
3				

Discussion Highlights

Work Completed

Work Planned

Results

Issues Encountered

Schedule

Budget

APPENDIX 3 (continued)

SOW Requirements

Other Issues

Action Items

Action Items	Description	Person(s) Responsible	Target Completion Data	Status
1				
2				
3				

Next Meeting

Appendix 4.

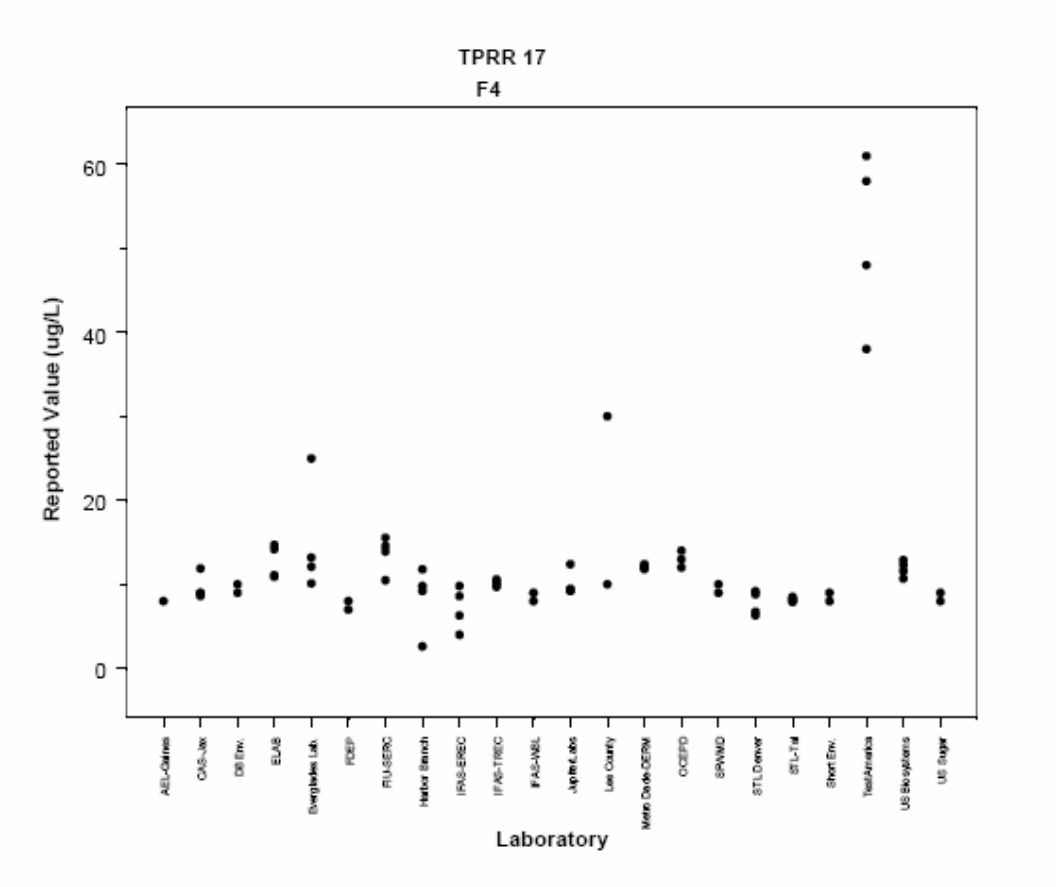
Table 1: Available funds by each fiscal year

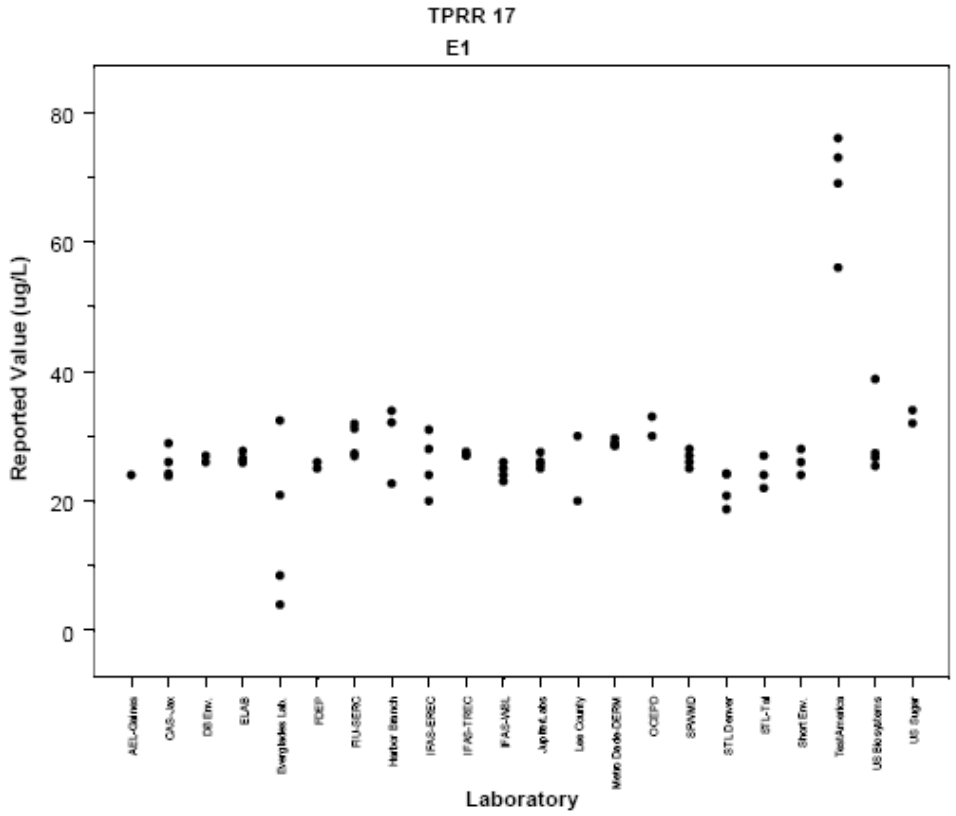
	FY08		FY09		FY10		FY11		Total
Source	9/08	10/08	9/09	10/09	9/10	10/10	9/11	
EAA-EPD	$100K	$100K	$100K	$100K	$100K	$100K		$600K
District	$125K	$150K	$150K	$150K	$150K	$175K		$900K
						Grand total		$1.5M

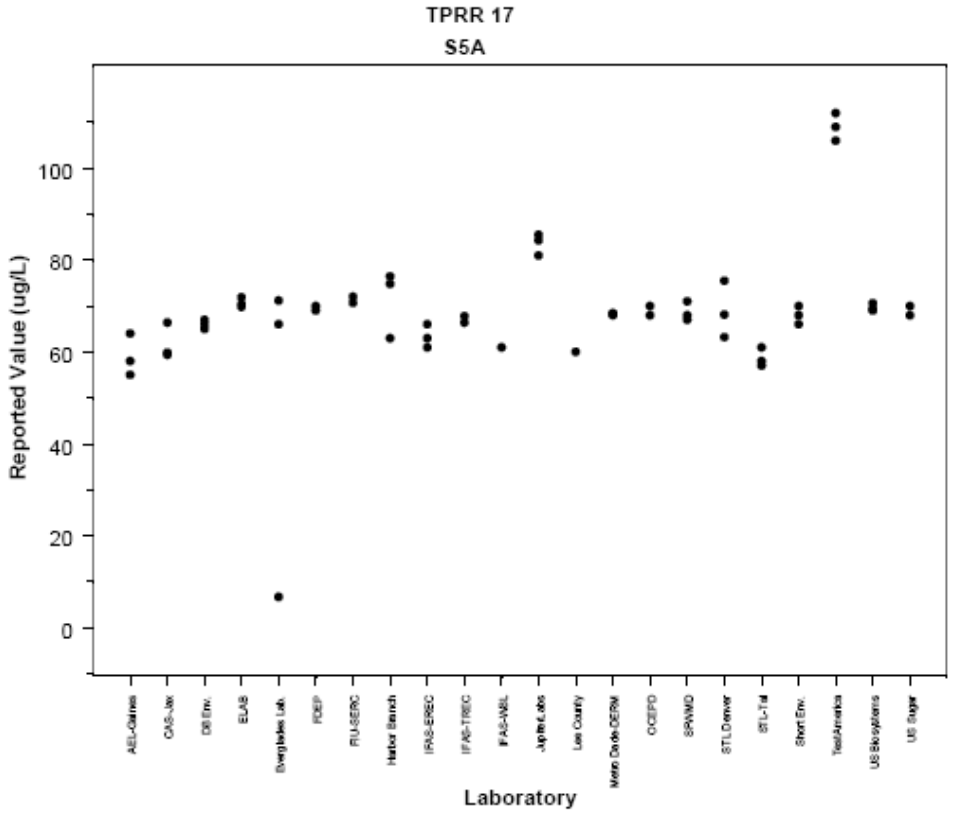
Table 2: Project submission and payment schedule (District Funding) due dates

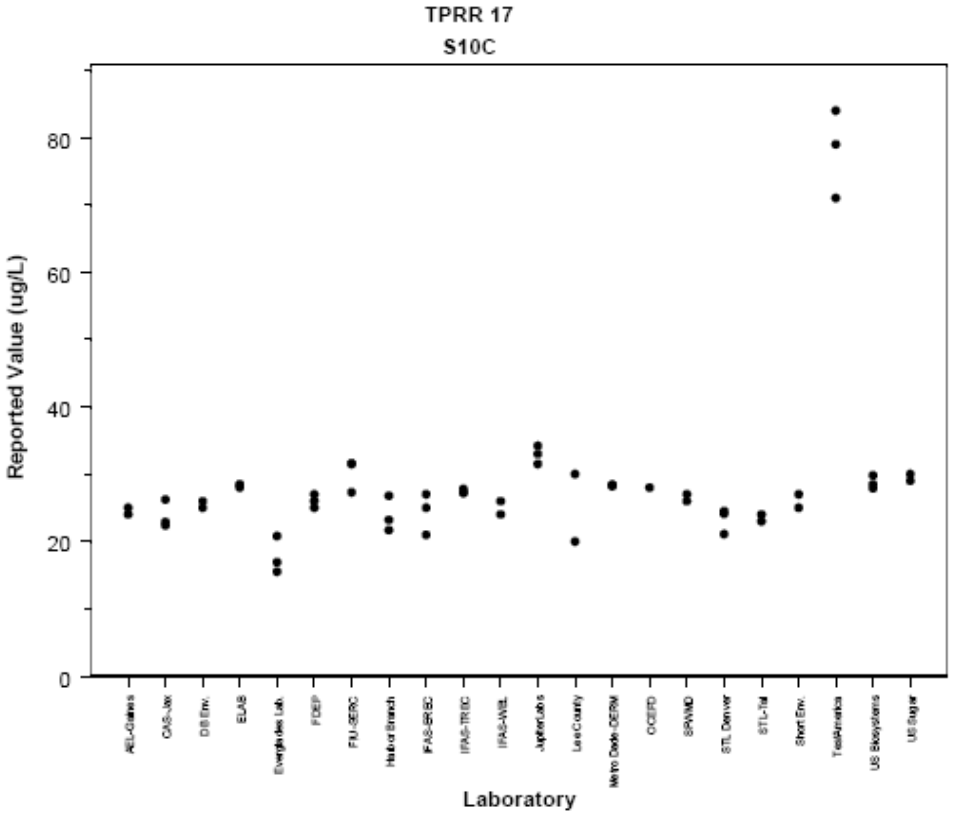
	Month	Deliverable submission	Meeting	Meeting Summary	Report	Amount to be Invoiced
FY08	4		Meeting	Summary	Progress Report	$8,500
	5		Meeting	Summary	Progress Report	$8,500
		Deliverable 1.1				$50,000
	6		Meeting	Summary	Progress Report	$8,500
	7		Meeting	Summary	Progress Report	$8,500
		Deliverable 1.2				$20,000
	8		Meeting	Summary	Progress Report	$8,500
	9		Meeting	Summary	Annual Report	$12,500
	Total					**$125,000**
FY09	10					
	11		Meeting	Summary	Progress Report	$15,000
	12					
	1		Meeting	Summary	Progress Report	$15,000
	2					
	3		Meeting	Summary	Progress Report	$15,000
	4					
	5		Meeting	Summary	Progress Report	$15,000
	6	Deliverable 2.1				$120,000
	7		Meeting	Summary	Progress Report	$15,000
	8	Deliverable 2.2				$85,000
	9		Meeting	Summary	Annual Report	$20,000
	Total					**$300,000**
FY10	10					
	11		Meeting	Summary	Progress Report	$15,000
	12					
	1		Meeting	Summary	Progress Report	$15,000
	2					
	3		Meeting	Summary	Progress Report	$15,000
	4					
	5		Meeting	Summary	Progress Report	$15,000
	6	Deliverable 3.1				$120,000
	7		Meeting	Summary	Progress Report	$15,000
	8	Deliverable 3.2				$85,000
	9		Meeting	Summary	Annual Report	$20,000
	Total					**$300,000**
FY11	10					
	11		Meeting	Summary	Progress Report	$15,000
	12					
	1		Meeting	Summary	Progress Report	$15,000
		Deliverable 4.1				$75,000
	2					
	3		Meeting	Summary	Final Report	$15,000
		Deliverable 4.2				$55,000
	Total					**$175,000**
					Grand Total	**$900,000**

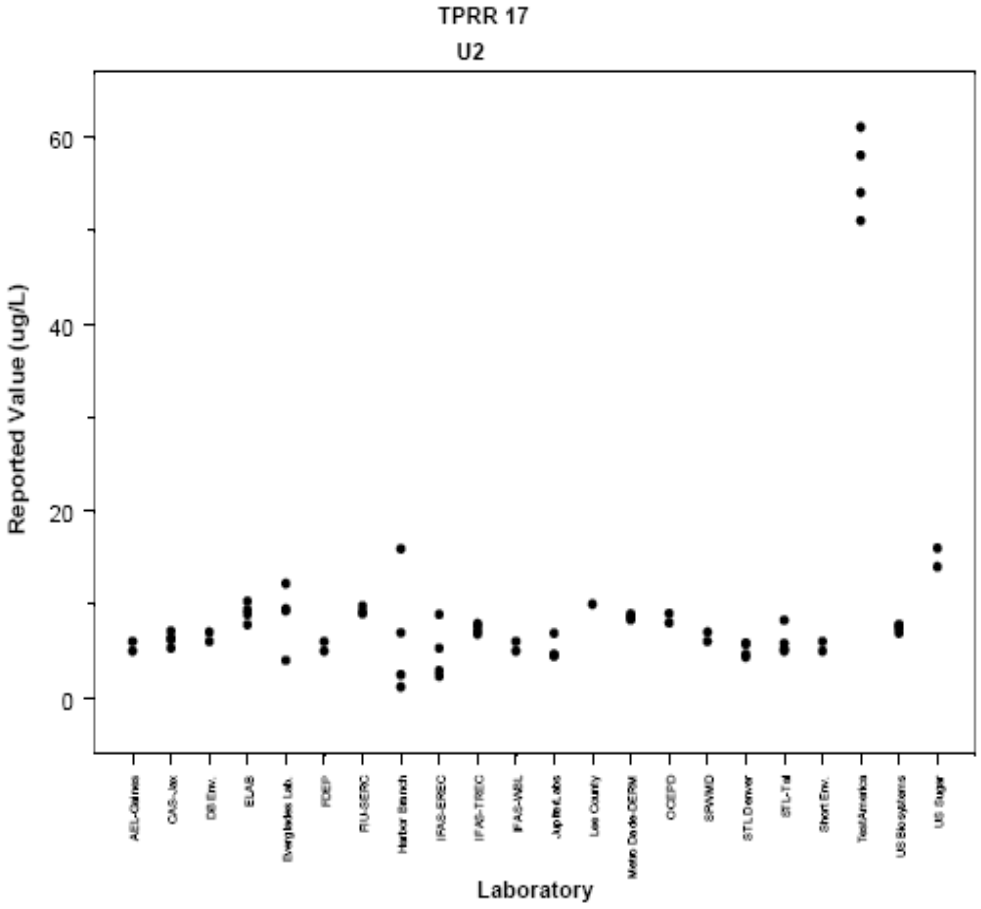
Appendix 5. Results of DBE's performance in the latest FDEP Everglades round-robin laboratory assessment.




TPRR 17
E1
Reported Value (ug/L)
80
60
40
20
0
AEL-Gaines
CAS-Jax
DEEnv.
EUAS
Everglades Lab.
FDEP
FIU-SERC
Harbor Branch
IFAS-EREC
IFAS-TREC
IFAS-WEL
Jupiter Labs
Lee County
Miami Dade-DERM
OCEPD
SFWMD
STL Denver
STL-Tal
Short Env.
TestAmerica
US Sky Waters
US Sugar
Laboratory


TPRR 17
S5A
Reported Value (ug/L)
100
80
60
40
20
0
AEL-Gaines
CAS-Jax
DB Env.
ELAB
Everglades Lab.
FDEP
FIU-SERC
Harbor Branch
IRAS-ERSC
IFAS-TREC
IFAS-WEL
Jupiter Labs
Lee County
Miami Dade DERM
OCEPD
SFWMD
STL Denver
STL-Tal
Short Env.
TestAmerica
US Biologicals
US Sugar
Laboratory


TPRR 17
S10C
Reported Value (ug/L)
0
20
40
60
80
AEL-Gaines
CAS-Jax
DB Env.
ELAB
Everglades Lab
FDEP
FIU-SERC
Hall of Branch
IFAS-SREC
IFAS-TREC
IFAS-Wat.
Jupiter Labs
Lee County
Metro Dade-DERM
OCEPD
SFWMD
STL Denver
STL-Tal
Short Env.
TestAmerica
US Ecosystems
US Sugar
Laboratory


TPRR 17
U2
Reported Value (ug/L)
60
40
20
0
AEL-Gaines
CAS-Jax
DB Env.
ELAB
Everglades Lab.
FDEP
FIU-SERC
Harbor Branch
IRAS-ERSC
IFAS-TREC
IFAS-WEL
Jupiter Labs
Lee County
Metro Dade-DERM
OCEPD
SFWMD
STL Denver
STL-Tal
Short Env.
Test America
US Ecosystems
US Sugar
Laboratory

Appendix 6. Correspondence between FDEP and CWF providing laboratory experiments performed by DBE comparing two SRP preservation methods.

COMMUNITY
WATERSHED FUND

1 June 2005

Ms. Amy Wheeler
Environmental Assessment Section
Florida Department of Environmental Protection
Bureau of Laboratories

Re: Reflux QAPP, DEP Agreement No. G0097

Dear Ms. Wheeler:

Thank you for your recent comments (25 May 2005) regarding the QAPP for the above referenced project. For the sake of efficiency, I would like to address your comments herein before we revise the QAPP. I will be happy to telephone you at your convenience to discuss these issues.

Comment #1: Section B2: All surface water and sediment sample collection and handling must follow the procedures outlined in the FDEP SOPs (DEP-SOP-001/01, Revision Date: February 1, 2004).

Response: All water and sediment sample collection and handling will follow the procedures outlined in the FDEP SOPs (although, we hope to convince you that an exception can be made for ortho-phosphorus).

Comment #2: Section B2: The ortho-phosphorus holding time is 48 hours; there is no current approval for extension of that holding time for this project. All project management personnel would need to agree that ortho-phosphorus is not a key component of this project to allow freezing of the samples.

Response: Ortho-phosphorus is a key component of this project, as it was and is of other projects we conduct in the Everglades. We learned years ago the difficulty of collecting, transporting, and analyzing ortho-phosphorus within 48 hours. Consequently, we investigated an alternative

"Clean Water, Healthy Watersheds"
telephone: 321.639.4982 • fax: 321.631.3169 • www.cwfund.org • 365 Gus Hipp Road, Rockledge, FL 32955

1

to the 48-hour holding time on three separate occasions – June 1998, September 1998, and December 1999 – using samples from Everglades's projects. The data sets and analyses are attached for your information. We concluded from our investigations that ortho-phosphorus results are not affected by holding samples frozen for two weeks.

DB Environmental has employed the alternative method for ortho-phosphorus handling, including the two week holding time described in the QAPP, on many Everglades projects (Table 1). The Environmental Assessment Section of the FDEP reviewed QAPPs for the first three projects listed in Table 1.

Table 1. Projects in which ortho-phosphorus samples were frozen and held for up to two weeks before analysis.

Client	Date	Review	Project
SFWMD/FDEP	1998	QAPP	Submerged Aquatic Vegetation/Limerock Demonstration Project
SFWMD/FDEP	2000	QAPP	Submerged Aquatic Vegetation/Limerock Demonstration Project: Follow-on Study
FDEP	2001	QAPP	Assessment of Hydraulic and Ecological Factors Influencing Phosphorus Removal in STA-1W
SFWMD	2004	SAP	Baseline Tracer Study: STA-2 Cell 3
SFWMD	2004	SAP	C-139 Basin Particulate P and Sediments

Given the results of our independent investigations, and the repeated acceptance of protocol, we ask that you re-consider your directive to adhere to the 48 hour holding time for ortho-phosphorus.

Comment #3: Section B4: The laboratory must follow NELAC standards for analysis of total nitrogen (TN).

Response: We proposed to analyze total nitrogen in sediments and plant tissue. To the best of our knowledge, NELAC does not have a standard for these media.

Comment #4: Section B5, Table 3: Any additional quality control protocols stipulated in the DEP Agreement (No. G0097) must be followed.

Response: We will follow any additional quality control protocols stipulated in the DEP Agreement (G0097).

Comment #5: Section B5, Table 3: The stated MDLs for nitrate + nitrite (NOx) and total Kjeldahl nitrogen (TKN) are not acceptable for ambient waters. The target PQL for NOx in ambient waters is 0.04 mg/L. The stated MDL for NOx for this project is 0.05 mg/L, giving a PQL of 0.2 mg/L. The target PQL for TKN in ambient waters is 0.4 mg/L. The stated MDL for TKN for this project is 0.4 mg/L, giving a PQL of 1.6 mg/L.

Response: Thanks for pointing out the inadequacies of our MDLs for nitrogen. Our revised MDLs are 0.007 mg/L for NO_x and 0.05 mg/L for TKN.

Again, thanks for your help with our project. I look forward to speaking with you.

Sincerely,

Donald M. Kent, Ph.D.
Executive Director

Attachment: Ortho-phosphorus investigations

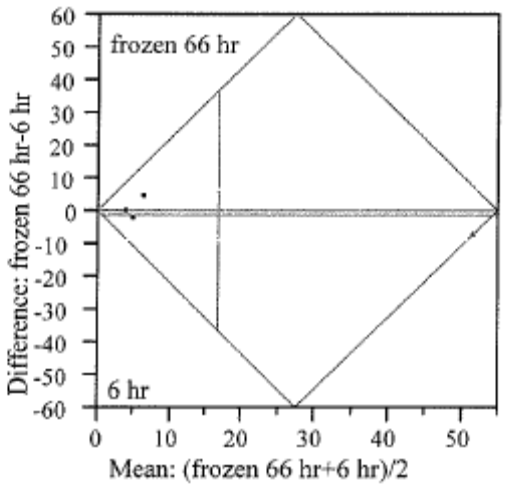
Cc: Aziz, Taufiqul
Kharbanda, Michelle

ORTHO-PHOSPHORUS HANDLING INVESTIGATIONS[1]

Investigation #1 – June 1998
One sample from each test apparatus analyzed within 48 h holding period; remainder of each sample frozen, thawed 66 hours later, and analyzed.

Station	6 hr ortho-P	Frozen 66 hr ortho-P
HRT M-2-Inf	55	48
HRT M-2 Eff	6	4
HRT M-2 LR Mid	4	9
HRT M-2 LR Eff	4	4

Matched Pairs Test (JMP Statistics, 2005)



Frozen 66 hr	16.25	t-Ratio	-0.40269
6 hr	17.25	DF	3
Mean Difference	-1	Prob > |t|	0.7142
Std Error	2.48328	Prob > t	0.6429
Upper95%	6.9029	Prob < t	0.3571
Lower95%	-8.9029		
N	4		
Correlation	0.99109		

[1] All values ppb

Investigation #2 – September 1998
One sample from each test apparatus analyzed within 48 h holding period; remainder of each sample frozen, thawed and analyzed 15 days later; frozen, thawed, and analyzed after another 7 days.

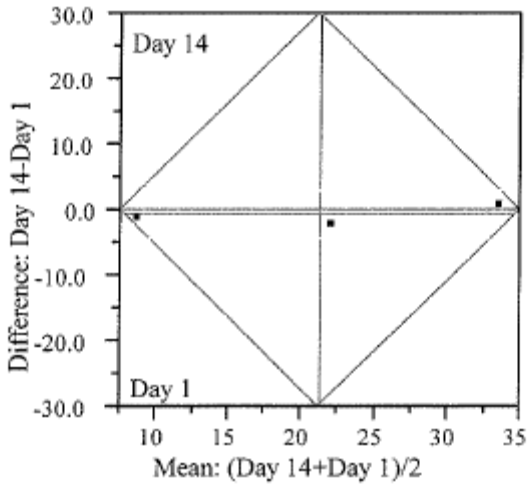
Station	< 48 hrs	Frozen 15 days	Frozen 22 days
L-1 Eff	5	5	4
L-1 LR Eff	7	8	10

Insufficient treatments to examine statistically; qualitatively, ortho-phosphorus similar through 15 days.

Investigation #3 – December 1999
One sample from each test apparatus analyzed within 48 h holding period; remainder of each sample frozen, thawed, and analyzed 14 days later.

Station	Day 1	Day 14
S-2 Inf	33	34
S-2 Eff	9	8
S-2 LR Eff	23	21

Matched Pairs Test (JMP Statistics, 2005)



		t-Ratio	-0.75593
Day 14	21	DF	2
Day 1	21.6667	Prob > |t|	0.5286
Mean Difference	-0.6667	Prob > t	0.7357
Std Error	0.88192	Prob < t	0.2643
Upper95%	3.12792		
Lower95%	-4.4612		
N	3		
Correlation	0.9954		

-----Original Message-----
From: Wheeler, Amy
Sent: Monday, June 20, 2005 10:27 AM
To: Aziz, Taufiqul
Cc: Tintle, Andrew
Subject: RE: FW: Review of QAPP for G0097

Aziz~
Here are my suggestions for the five comment responses found in the 6/1/2005 letter from Donald Kent. The responses to comments #1, #4, and #5 are acceptable. For the response to comment #2, the proposed holding time for ortho-phosphorus is longer than the standard of 48 hours. It is up to you as the Grant Manager to review the data provided in comparison to the needs of your project in order to decide what holding times and preservation method you will allow. For the response to comment #3, the comment was intended to emphasize that the method that they are using for TN must follow all applicable quality control standards outlined by NELAC.

I hope that my comments and recommendations have been helpful for this project. It is the policy of the Environmental Assessment Section to assist in the review of QAPPs for no more than two rounds per QAPP. This email constitutes the third round of review for this project and concludes our recommendations. Again, I hope that I have been helpful to you in your process of reviewing and overseeing this project.

Amy Wheeler
Environmental Assessment Section
FDEP Bureau of Laboratories