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Effects of sulfate amendments on mineralization and phosphorus release from South Florida (USA) wetland soils under anaerobic conditions

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ABSTRACT

We investigated the potential effects of elevated water-column sulfate (SO_4) levels on heterotrophic microbial respiration and net phosphorus (P) release for soils collected from impacted and unimpacted Everglades wetlands in south Florida. Soils from three sites, ranging from low P and low SO_4 to high P and high SO_4 environments, were examined under controlled laboratory conditions. The soils were subjected to anaerobic incubations to evaluate net P release and organic matter decomposition in response to SO_4 amendments of 32 or 96 mg l^{-1} (0.33 and 1.0 mM).

Three processes have been described in the literature to explain why SO_4 enrichment may lead to P release from soils under anaerobic conditions. First, alkalinization can lead to a more favorable pH environment for decomposition. For the soils examined here, alkalinization due to the hydrogen ion-consuming reaction of SO_4 reduction was not a prominent mechanism. We found that pH decreased in the incubation vessels, and that increases in alkalinity were more likely attributable to calcium carbonate dissolution than SO_4 reduction. Moreover, all the soils exhibited near circum-neutral pH levels, with moderate to high concentrations of native alkalinity.

Second, formation of iron sulfide (FeS_x) compounds has been shown to mobilize iron (Fe)-associated P. Soils from only one of the study sites had Fe concentrations that would be expected to be high enough to influence P mobility. Relatively high porewater Fe:soluble reactive P (SRP) ratios (>83:1) were observed at this site, which suggests that Fe could theoretically exert control over the release of P from the soil. However, soil P levels at this site were too low to measure any substantial influence of Fe on net P mobilization.

Finally, availability of electron acceptors such as SO_4 is a major determinant of decomposition rate, and thus rate of organic P release. Amending the soils with SO_4 did not result in either more heterotrophic microbial respiration as measured by carbon dioxide (CO_2) and methane (CH_4) production, or increased net P mobilization. In two of the SO_4-amended soils where post-incubation total sulfide concentrations were as high as 23.4 mg l^{-1}, SO_4 addition reduced production of respiratory carbon end products, suggesting hydrogen sulfide inhibition. Moreover, limitations imposed by substrate quality and low P contributed to the lack of meaningful enhanced decomposition of organic matter with the addition of 32 or 96 mg SO_4 l^{-1} to the oligotrophic wetland soils. Even though P release did occur under anaerobic conditions for the more enriched site, addition of SO_4 did not enhance P release.

1. Introduction

The Everglades historically represented a continuum of wetland ecosystems stretching from Lake Okeechobee southward to Florida Bay. Essentially all of the modern-day Everglades is now contained within Everglades National Park (ENP) to the south and the Water Conservation Areas (WCA) to the north. Much of the remainder of the northern Everglades was drained via a network of canals during the early to mid 1900s to develop the area into highly productive farmland (Everglades Agricultural Area [EAA]). The EAA lies to the northwest of the WCAs and immediately south of Lake Okeechobee, which places it upstream from both the WCAs and ENP (Fig. 1).

Decades of Lake Okeechobee water releases and stormwater runoff from the EAA have resulted in nutrient enrichment of

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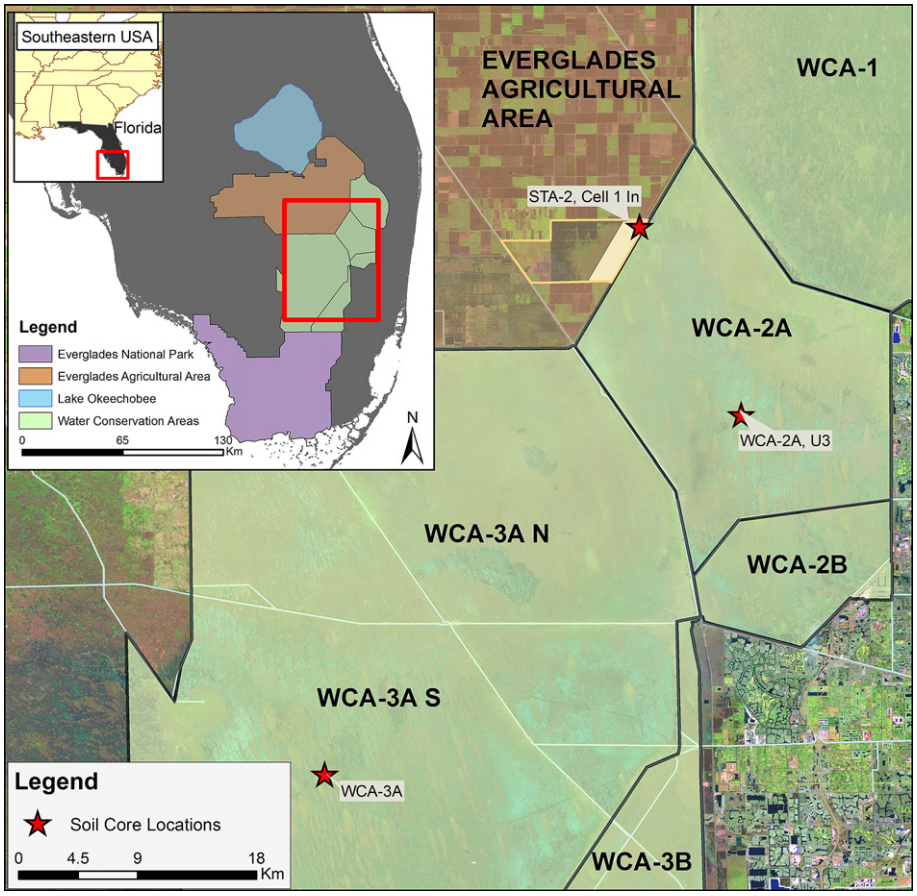

Fig. 1. Locations of soil cores retrieved for the laboratory incubations with and without SO_4 enrichment.

downstream Everglades wetlands, most notably in regions of the WCAs. Widespread ecological impacts associated with accelerated nutrient loading, primarily phosphorus (P), have included transformation of native sawgrass (*Cladium jamaicense* Crantz) marsh and open water sloughs to dense stands of cattail (*Typha domingensis* Pers. and *T. latifolia* L.) (Davis, 1994) and replacement of endemic periphyton communities by algal species typically associated with more eutrophic waters (McCormick and O'Dell, 1996; McCormick et al., 1996). Concerns over long-term impacts to Everglades ecosystems has prompted large-scale restoration efforts, including the construction of large treatment wetlands (referred to as Stormwater Treatment Areas [STA]) within the EAA, totaling more than 16,000 ha (40,000 acres).

Recently, concerns have been raised over the effects that elevated concentrations of sulfate (SO_4) in the STAs and northern regions of the WCAs may have on stimulating soil decomposition and subsequent P mobilization (Axelrad et al., 2008). Bates et al. (2002) reported SO_4 concentrations of 24 mg l^{-1} for Lake Okeechobee, and even higher values in canals that drain the EAA (mean of 68 mg l^{-1}). These SO_4 levels contrast with the low ambient surface water SO_4 levels (<1 mg l^{-1}) in the ENP (Scheidt and Kalla, 2007) and the low concentrations (<1–5 mg l^{-1}) reported for rainfall in south Florida (Bates et al., 2002; Scheidt and Kalla, 2007). Elevated water-column SO_4 levels can lead to sulfide formation, and porewater total sulfide (TS) levels over 5 mg l^{-1} have been measured in the Everglades WCAs (Scheidt and Kalla, 2007).

Understanding the biogeochemistry of sulfur (S) in the Everglades is important because elevated SO_4 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 flooded soils, and therefore may accelerate the decomposition of organic matter when other electron acceptors (e.g., oxygen, nitrate) are unavailable (Wright and Reddy, 2001). Sulfate reduction to TS also produces bicarbonate (HCO_3^-), which can buffer (neutralize) soil 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, TS produced because of microbial SO_4 reduction can reduce the availability of iron (Fe) to sequester phosphate (Roden and Edmonds, 1997; 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 SO_4 levels (Lamers et al., 1998).

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 influence of S on P cycling. This paper presents the results of laboratory incubations designed to test the potential effects of elevated water column SO_4 levels on anaerobic heterotrophic microbial respiration and net P release for soils from unimpacted and impacted wetlands (with respect to both P and S) in south Florida. Comparisons among soils exposed to incremental concentrations of SO_4 were made in terms of i) SRP release and ii) carbon dioxide (CO_2) and methane (CH_4) evolution, two of the common terminal carbon (C) compounds in heterotrophic microbial respiration of organic matter (Hines et al.,

2008). Our hypothesis was that SO_4 enrichment would result in higher net SRP mobilization and more mineralization of soil organic matter.

2. Methods

2.1. Experimental field sites

Soils from three separate sites, ranging from low P and low SO_4 to high P and high SO_4 water-column environments, were examined (Table 1; Fig. 1). The WCA-3A site was considered a low P and low SO_4 environment, while the STA-2 Cell 1 site is relatively high in both P and SO_4. The U3 site in WCA-2A was a very low P environment, but levels of SO_4 were relatively high.

The WCA-3A field site was located in the interior region of southern WCA-3A, and is well-removed form the direct influence of nutrient-laden waters in the major drainage canals (Fig. 1). 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-3A at several points around its northern perimeter, giving rise to downstream concentration gradients for P and other minerals (FDEP, 2001; Scheidt and Kalla, 2007). The landscape in WCA-3A consists of ridges of wet prairie and sloughs at slightly lower elevations. 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 dominated). For this study, soils were sampled in the slough community on January 19, 2009.

Site U3 in WCA-2A (Fig. 1) lies approximately 10 km downstream of the Hillsboro Canal S10 control structures, through which agricultural drainage water (ADW) was routed from the EAA prior to the construction of the STAs. It represents an environment that has been exposed to high SO_4, 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 SO_4 was not (Vaithiyanathan and Richardson, 1997). Site U3 provides an ideal environment for examining the effects of SO_4 enrichment in an environment that was SO_4-enriched, but P impoverished. The vegetation community at this site is similar to that in WCA-3A. On January 15, 2009, soils were sampled in the slough community.

The Stormwater Treatment Area STA-2 is located adjacent to the northwestern boundary of WCA-2A (Fig. 1), and receives relatively high P and high SO_4 runoff from the EAA via the S-6 structure on the Hillsboro Canal. Initially flooded in 1999, flow-through operations in STA-2 began in August 2002. Although it was historically part of the EAA, Cell 1 was never farmed. Cell 1 of STA-2 is dominated by emergent vegetation (mixed cattail and sawgrass), and has a treatment area of 728 ha (1798 acres) out of the total STA-2 treatment area of 3335 ha (8240 acres). STA-2 Cell 1 was sampled at the inflow region on June 2, 2009, two weeks after it had been rehydrated. Standing water previously had been absent in the cell for 76 days due to a prolonged drought.

2.2. Field methods

Three or four replicate soil cores were collected to a depth of 30 cm at each location with a 10-cm i.d. aluminum corer. After removing the benthic algal layer and any periphyton coating emergent plant stems (so-called "sleeves") present at WCA-2A U3 and WCA-3A, and prior to subsequent soil extrusion in the field, soil oxidation-reduction potential (ORP) was measured at a depth of 3–7 cm below the soil surface using a Pt electrode (referenced to a Ag/AgCl electrode in saturated KCl). 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 and 4–10 cm depth intervals, with each depth interval composited among the three or four replicate cores in zip-lock bags. The composited samples were transported on ice to the lab, where they were purged with N_2 gas and stored at 4 °C in the dark, pending chemical analysis and laboratory incubations.

2.3. Laboratory incubations

Laboratory incubations were conducted using soils collected from the 0–4 and 4–10 cm soil horizons at each field site. Soil samples were stored in the dark at 4 °C under N_2 until the start of the incubations, which ranged from one day (STA-2 Cell 1) to 25 days (WCA-3A) since the day of core retrieval in the field. For all experimental treatments, 120-ml serum bottles received 20 g of wet soil each, followed by 50 ml of low P and low SO_4-water (purged with N_2 gas beforehand) collected from WCA-3A. Soil-free controls with 70 ml of water were included with every set of soil incubations. Each serum bottle was capped with a butyl serum stopper aluminum-crimped top. All soils and depths (as well as water-only controls) received three levels of SO_4 amendment: (1) final water concentration of 0.33 mM (32 mg l^{-1}), (2) final water concentration of 1.0 mM (96 mg l^{-1}), or (3) no amendment. Sulfate solution (as Na_2SO_4) was added to each serum bottle as a 100 mM concentrate prior to rendering the bottle anaerobic by alternately evacuating (5 min) and purging (5 min) with ultra-high purity (UHP) N_2 or He gas 3 times (total 30 min). These SO_4 additions equaled 1.4–1.9 (0–4 cm soil horizon) and 0.9–1.2 (4–10 cm soil horizon) mg SO_4 g dry wt^{-1} of soil for the 0.33 mM, and 1.8–5.6 (0–4 cm soil horizon) and 2.7–3.7 (4–10 cm soil horizon) mg SO_4 g dry wt^{-1} of soil for the 1.0 mM SO_4 amendments, respectively. The added volumes were ≤1% of the total soil slurry volume.

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 three sites where soils were retrieved for the SO_4 amendment experiments performed as laboratory incubations.

Site	Sampling date	GPS coordinates (decimal degrees)		Historical water-column concentrations[a]		
		Latitude	Longitude	POR[b]	SO_4 (mg l^{-1})	TP (μg l^{-1})
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	February 1978–May 2008	39	7
STA-2 Cell 1 In	6/2/09	26.4166	−80.48783	January 2003–December 2007	66	78

[a] Data from https://my.sfwmd.gov/portal/page/portal/pg_grp_sfwmd_era/pg_sfwmd_era_dbhydrobrowser (WCA-2A U3; STA-2 Cell 1 In) and S. Newman (pers. comm.) (WCA-3A) of the SFWMD.
[b] POR = Period of Record.

We chose 1.0 mM as the highest SO_4 concentration since it represents an upper value for annual inflow SO_4 concentration to the STAs (Pietro et al., 2008). Each treatment and control was replicated in five (with soil) or four (without soil) serum bottles. Either one (without soil) or two (with soil) of the replicates for each treatment or control group was sacrificed for determination of initial concentrations of CO_2, CH_4, and solutes (pH, SRP, total soluble P [TSP], alkalinity, SO_4, TS, total ammoniacal nitrogen [TAN], and dissolved calcium [Ca] and iron [Fe]). The remaining three replicate bottles were incubated in the dark at 25.5 °C (range of 25–27 °C). Each serum bottle was vigorously shaken once each day for ~10 s.

At 1, 3, 5, 7, 10, and 14 days after initiating the incubations, 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 (sampling of headspace gases did not occur on day 1), respectively, within one hour of vigorously shaking the bottles to equilibrate dissolved CO_2 and CH_4 with the headspace. The water and gas volumes withdrawn at each time step were replaced with equal volumes of low P and low SO_4 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 the 14-day incubation. We also analyzed the aqueous phase within each control and treatment group at the end of the incubation for pH, TSP, alkalinity, SO_4, TS, TAN, and dissolved Ca and Fe concentrations. The ORP was measured at the end of the incubation only.

2.4. Analytical methods

Methods for chemical analysis of water and soil are listed in Table 2. A partial sequential inorganic P fractionation procedure was also performed (Hieltjes and Lijklema, 1980), grouping soil P into: (i) labile SRP (porewater, water soluble, and NH_4Cl extractable); (ii) Fe- and Al-bound P (NaOH-extractable SRP); and (iii) alkali-extractable organic P (NaOH-extractable organic P). Microbial biomass P (MBP) was analyzed according to Ivanoff et al. (1998), except the difference between the SRP (and not TP) concentrations in the 0.5 mol l^{-1} $NaHCO_3$ extracts of chloroform-fumigated and unfumigated samples was used to calculate MBP (Brookes et al., 1982). Additional soil analytes, along with a characterization of surface waters and porewaters (not described in this document), can be found in the corresponding South Florida Water Management District technical report for this study (DBE, 2009).

We measured the amount of each gas emitted over time in the unamended and SO_4-amended soils during the anaerobic incubation period to determine the effect of SO_4 on decomposition of organic matter. Carbon dioxide and CH_4 concentrations were measured simultaneously by injecting 0.5 ml of headspace gas directly into a Varian 450 gas chromatograph equipped with flame ionization (FID) and thermal conductivity (TCD) detectors. A detailed description of instrument settings, calibrations and quality assurance/control protocols are provided in DBE (2009).

Atmospheric pressure in the serum bottles was determined before sub-sampling the headspace on days 0, 3, 5, 7, 10 and 14 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):

$$\log\left[HCO_3^-\right] = -7.82 + \mathrm{pH} + \log CO_{2(g)} \quad (1)$$

The total mass of the CO_2–C and CH_4–C produced through day 10 of the incubation (only soils from WCAs 2A and 3A were measured through day 14) was divided by the initial dry weight of the soil to convert the terminal C flow to a basis of the soil mass incubated, e.g.,

$$(\mathrm{mg\ CO_2 - C})_{t=10\ \mathrm{days}}/(\mathrm{g\ soil\ dry\ wt})_{t=0} \quad (2)$$

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 total organic C (TOC) concentration originally contained in the serum bottle, e.g.,

$$\left[(\mathrm{g\ CO_2 - C + g\ CH_4 - C})_{t=10\ \mathrm{days}}/(\mathrm{g\ TOC})_{t=0}\right]100 \quad (3)$$

Table 2
Analytical method references for chemical analyses of water and soil.

Analyte	Matrix	Method(s)
Porewater + 1 M NH_4Cl-SRP	Soil	EPA 365.2[d]
0.1 M NaOH-SRP	Soil	EPA 365.2[d]
0.1 M NaOH-TP	Soil	3-227 in Plumb (1981)/EPA 365.2[d]
0.5 M $NaHCO_3$-SRP	Soil	EPA 365.2[d]
Alkalinity	Surface water, porewater	EPA 310.1[d]
TAN	Surface water, porewater	EPA 350.1[d]
Ca (dissolved)	Surface water, porewater	EPA 215.1[d]
DOP	Surface water	Calculation (TSP-SRP)
Fe (dissolved)	Surface water, porewater	Bathophenanthroline[a]
Lignin and Cellulose	Soil	AOAC 973.18[e]
MBP	Soil	Fumigation[b]
pH	Surface water, porewater	EPA 150.1[d]
PP	Surface water	Calculation (TP-TSP)
SRP	Surface water, porewater	4500P-F in APHA (1992)/EPA 365.2[d]
SO_4	Surface water, porewater	4110B in APHA (1992)
TS	Surface water, porewater	Modified 4500-S G in APHA (1998)
TC	Soil	CNS Elemental Analyzer
TCa	Soil	EPA SW 7140[f]
Total Recoverable Fe (TFe)	Soil	EPA SW 7380[f]
TIC	Soil	Calculation (TC-TOC)
TN	Soil	CNS Elemental Analyzer
TOC	Soil	CNS Elemental Analyzer
TOS	Soil	4110B in APHA (1992) [c]
TP	Soil	3-227 in Plumb (1981)/EPA 365.2[d]
TP	Surface water	4500P-F in APHA (1992)/EPA 365.2[d]
Total S	Soil	ASTM D-4239 and ASTM E-1915-05
TSP	Surface water, porewater	4500P-F in APHA (1992)

[a] Modification of Nürnberg (1984).
[b] Modification of Ivanoff et al. (1998).
[c] Modification of Smolders et al. (2006b).
[d] EPA (1979).
[e] AOAC (1990).
[f] EPA (1990).

2.5. Saturation index (SI)

Saturation indexes were calculated at the end of the 14-day incubation period for the 0–4 cm soil depth from WCA-3A to determine whether iron monosulfide (FeS) could be controlling the dissolved Fe and TS concentrations in the soil slurries:

$$\mathrm{SI} = \mathrm{IAP}/K_{sp} \quad (4)$$

where IAP is the ion activity product and K_{sp} is the solubility product constant for amorphous FeS (Chen and Liu, 2005):

$$H^+ + FeS_{(S)} = Fe^{2+} + HS^- \qquad K_{sp} = 1 \times 10^{-3} \tag{5}$$

2.6. Statistical analyses

For normally distributed data sets, we used a paired *t*-test to compare two means and a one-way analysis of variance (ANOVA) followed by a *post hoc* multiple comparison test (Tukey HSD) when the overall ANOVA was significant for testing three or more means. A two-way ANOVA model was used to determine the effects of sulfate treatment (no amendment, 0.33 mM and 1.0 mM SO_4-amendments) and soil depth (0–4 and 4–10 cm). Treatment comparisons were based on Tukey HSD. For non-normal data sets, we used nonparametric procedures for testing significant difference(s) between distributions of two (Mann–Whitney) or three (Kruskal–Wallis) independent samples. The level of significance (α) was either 0.01 or 0.05 for all comparisons resulting from either parametric or nonparametric procedures. Relationships between microbial activities (CO_2 and CH_4 generation) and soil properties, or between soil properties and P release, were derived from linear regression analysis, except for soil TFe:TP with respect to P release, which was expressed as a power function. All correlation coefficients are the result of the Pearson product-moment correlation analysis.

3. Results

3.1. Soil characteristics

Soils at the three sites were organic, with varying TOC content between 17.9–40.5% and 32.1–39.2% (oven dry wt) for the 0–4 and 4–10 cm soil horizons (Table 3), respectively. The total N (TN) content in surficial (0–4 cm) wetland soils ranged from 1.83% to 3.66%. Nitrogen concentrations decreased slightly with depth at the WCA-3A site, and increased with depth at the remaining two sites. Total recoverable Fe (TFe) concentration in the 0–4 cm soil horizon ranged from 0.08% at WCA-2A U3 to 1.00% at WCA-3A. As with TOC and TN, increases in TFe in the 4–10 cm horizon were observed at the WCA-2A U3 and STA-2 Cell 1 sites, but not at the WCA-3A site. The uppermost 0–4 cm of soil 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.

Total S content in the upper 0–4 cm of soil ranged between 0.44% and 0.76% (oven dry wt) for the three sites (Table 3). Soil total S concentrations at the 4–10 cm soil horizon decreased at the WCA-3A site, but increased at the other sites. Total oxidizable S (TOS), which is a measure of the reduced S in organic and inorganic matrices that can be oxidized within 29–30 days of continuous aeration, comprised only a small percentage (1–4%) of the total S. Concentrations of TOS, and TOS as percentage of total S, were higher in the 0–4 cm soil horizon and decreased with soil depth at the two slough sites (WCA-3A and WCA-2A U3). WCA-2A U3 soil contained the highest TOS concentration at 0–4 cm soil horizon (0.17 mg g dry wt^{-1}), while the highest TOS concentration at 4–10 cm soil horizon was observed at the STA-2 Cell 1 site (0.27 mg g dry wt^{-1}).

Total P concentrations in the soils varied widely; the slough soils in the WCAs contained substantially less P in the 0–4 and 4–10 cm soil horizons than in STA-2 Cell 1 (Table 3). The distribution of P among the different P pools also varied among sites. Concentrations of the porewater + NH_4-Cl extractable P pool (the labile pool) were low in the two WCA soils at both soil horizons (<0.1–0.3 μg g dry wt^{-1}; less than 0.2% of the TP), compared to STA-2 Cell 1 (27–52 μg g dry wt^{-1}; ca. 3.0% of TP). A larger proportion of the TP in the soil, but nevertheless still a relatively minor component, consisted of Fe- and Al-bound P. Concentrations in this pool increased with soil depth in the WCAs, and decreased with soil depth in STA-2 Cell 1 (Table 3). 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 wt^{-1}) compared to the same horizon in either WCA-3A (5 μg P g dry wt^{-1}) or WCA-2A U3 (3 μg P g dry wt^{-1}).

Biogenic P, which is the difference between the NaOH-extracted SRP and TP and represents organic P and polyphosphates, constituted a slightly higher proportion of the TP than the Fe-and Al-bound P in the WCA soils. The biogenic P concentration was approximately the same as the Fe- and Al-bound P concentration in STA-2 Cell 1 (Table 3). Biogenic P concentrations increased with soil depth (0–4 to 4–10 cm) in the WCA-3A soil, slightly increased with depth in the WCA-2A U3 soil, and no change with depth at STA-2

Table 3
Chemical characteristics of the 0–4 and 4–10 cm soil horizons from locations within WCA-3A, WCA-2A U3, and STA-2 Cell 1 In, where soils were collected for anaerobic laboratory incubations. Each value represents a single measurement from a composite of 3–4 soil cores collected at that location. LCI = [lignin (lignin + cellulose)$^{-1}$].

	WCA-3A Slough		WCA-2A U3 Slough		STA-2 Cell 1 In	
	0–4 cm	4–10 cm	0–4 cm	4–10 cm	0–4 cm	4–10 cm
TP (μg g^{-1})	400	311	190	234	1570	883
TN (%)	3.66	3.34	1.83	2.62	2.13	2.35
TC (%)	42.4	38.9	25.7	36.3	34.2	40.1
TOC (%)	40.5	39.2	17.9	32.1	29.2	36.5
TIC (%)	1.9	<0.3	7.8	4.2	5.0	3.6
Total S (%)	0.76	0.63	0.44	0.91	0.50	0.73
TOS (mg g^{-1})	0.11	0.06	0.17	0.14	0.13	0.27
Total Recoverable Fe (%)	1.00	0.71	0.077	0.16	0.14	0.22
Total Ca (%)	3.9	2.7	16.4	9.9	10.7	7.8
Lignocellulose Index (LCI)	0.71	0.76	0.68	0.74	0.65	0.69
MBP (μg g^{-1})[a]	53	58	41	31	116	57
Porewater +1 M NH_4Cl SRP (Readily Available) (μg g^{-1})[a]	<0.1	0.2	<0.1	0.3	52	27
0.1 M NaOH SRP (Fe- and Al-bound P) (μg g^{-1})[a]	5	12	3	7	59	48
0.1 M NaOH Organic P (Biogenic) (μg g^{-1})	10	18	12	13	51	51
TOC/TN	11.1	11.7	9.8	12.2	13.7	15.5
TOC/TP	1012	1260	942	1372	186	413
TN/TP	92	107	96	112	14	27
TFe/TP	25	23	4.1	6.8	0.9	2.5
Dry Bulk Density (g cc^{-1})	0.054	0.116	0.064	0.092	0.081	0.111

[a] P fractions that are considered labile.

Cell 1. The MBP concentration in the surface depth increment (0–4 cm) of the soil of STA-2 Cell 1 of 116 μg g dry wt^{-1} was two-to three-times higher than the MBP concentrations in the surficial soils from the WCAs; less of a MBP concentration difference occurred between the P-enriched STA-2 and P-limited WCAs in the 4–10 cm soil layer (Table 3).

3.2. Changes in aqueous concentrations of nutrients, sulfate, sulfide, metals, alkalinity and pH during anaerobic incubations

3.2.1. Low SO_4 and low P Environment: WCA-3A

Releases of SRP were negligible ($\leq$5 μg l^{-1}) for the SO_4 unamended and amended water-only controls (not shown) and the 0–4 cm soil horizon during the incubation period using slough soil from WCA-3A (Fig. 2). An initial SRP concentration of 17 μg l^{-1} was observed for the 1.0 mM SO_4-amended treatment of the 4–10 cm soil horizon, but the SRP levels decreased to the MDL (2 μg l^{-1}) after 5 days; the high initial concentration was likely due to carry-over of SRP originally contained in the soil.

Although the soil collected in the 4–10 cm soil horizon had higher initial DOP concentrations than the 0–4 cm soil horizon, there was no SO_4 treatment effect for either horizon ($P > 0.05$) (Table 4). Overall, DOP concentrations before and after the incubations were low ($\leq$15 μg l^{-1}), indicating DOP was not mobilized by microbial processes such as was observed for TAN.

Due to the reducing conditions during the incubation (Table 4), SO_4 amendments to the 0–4 cm soil horizon all but disappeared during the incubation. Sulfate also was depleted in the 0.33 mM, but not in the 1.0 mM, amended 4–10 cm soil horizon. In contrast, the water-only controls retained most ($\geq$78%) of the SO_4 added after two weeks of incubation. The highest TS concentration at the end of the incubation period was 0.27 mg l^{-1} in the 1.0 mM amended 0–4 cm deep soils. The controls without soil did not respond to SO_4 amendments (TS concentrations $\leq$ 0.006 mg l^{-1}).

The unamended soil cored from the 0–4 cm soil horizon released the most dissolved Fe during the incubation, attaining a water concentration of 3.0 mg l^{-1}. Dissolved Fe concentrations for the 0.33 mM amended treatments to the two soil horizons were higher than for the 1.0 mM amendment (Table 4) because of the greater FeS_x binding at the higher sulfide concentrations produced by the 1.0 mM SO_4 amendment. The Saturation Index (SI) for FeS was twice as high for the 1.0 mM SO_4-amended than unamended 0–4 cm soil horizon (SI = 16 vs. 32 under unit-activity conditions), suggesting that the higher sulfide concentrations in the 1.0 mM amended soil were more oversaturated with respect to FeS than the unamended soil. The final dissolved Fe concentrations in the 4–10 cm soil horizon were considerably lower at the end of the incubation than their respective treatment levels in the 0–4 cm soil horizon ($P < 0.05$). This indicates that relative to the 0–4 cm soil horizon, the Fe-reducible pool was smaller in the 4–10 cm soil horizon. Dissolved Fe concentrations were below the MDL of

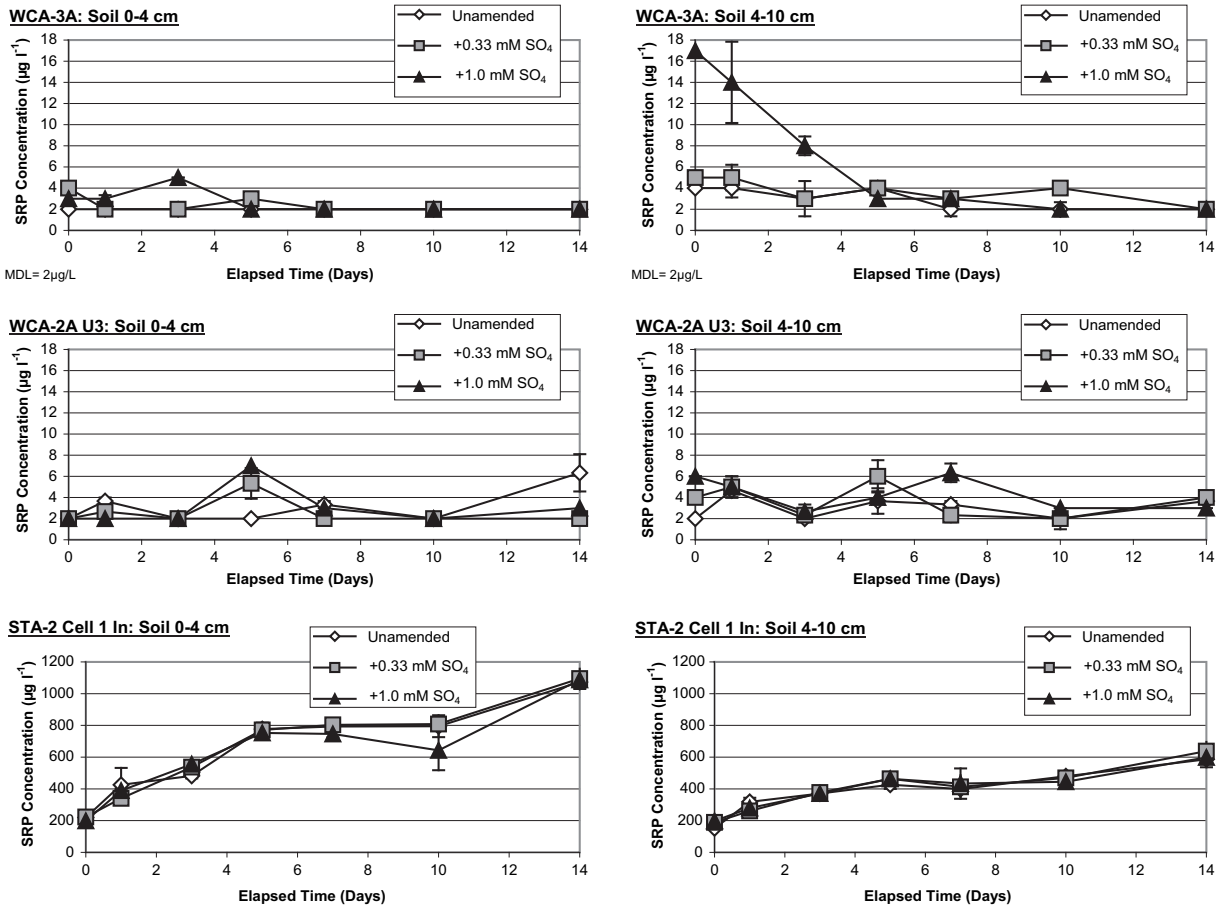

Fig. 2. Release (mean ± 1 S.E.) of soluble reactive phosphorus (SRP) during a 14-day anaerobic incubation of 0–4 cm (top) and 4–10 cm (bottom) soil horizons retrieved from slough communities in WCA-3A and WCA-2A U3 on January 19 and 15, 2009, respectively, and from the inflow region of Cell 1 in STA-2 on June 2, 2009. The soils were exposed to unamended and SO_4-amended (with 0.33 mM (32 mg l^{-1}) or 1.0 mM (96 mg l^{-1})) low P and low SO_4 surface water from WCA-3A. Each data point represents the mean of three replicates. Note the differences in the *Y*-axis scales.

Table 4
Concentrations of key chemical parameters before and after a 14-day anaerobic incubation of soils from WCA-3A with SO_4-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water.

		0–4 cm horizon			4–10 cm horizon		
		Unamended	+0.33 mM SO_4	+1.0 mM SO_4	Unamended	+0.33 mM SO_4	+1.0 mM SO_4
TAN (mg l^{-1})	Initial[a]	1.45	1.68	1.39	0.85	1.24	1.34
	Final[b]	3.74 ± 0.11	3.22 ± 0.12	2.76 ± 0.15	3.51 ± 0.1	3.29 ± 0.12	3.14 ± 0.24
	Δ	2.29	1.54	1.37	2.66	2.05	1.80
SRP (μg l^{-1})	Initial	<2	4	3	4	5	17
	Final	<2	<2	<2	<2	<2	<2
	Δ	0	−3	−2	−3	−4	−16
DOP (μg l^{-1})	Initial	8	5	6	13	11	18
	Final	7 ± 0.9	6 ± 0.3	6 ± 0	15 ± 1	12 ± 0	11 ± 0.9
	Δ	−1	1	0	2	1	−7
SO_4 (mg l^{-1})	Initial	6.5	39	106	5	46	121
	Final	<0.5	<0.5	0.7 ± 0.2	<0.5	<0.5	47 ± 1.7
	Δ	−6.3	−39	−105	−5	−46	−74
TS (mg l^{-1})	Initial	0.03	0.04	0.04	0.12	0.14	0.15
	Final	0.05 ± 0.01	0.07 ± 0.02	0.27 ± 0.04	0.08 ± 0	0.12 ± 0	0.16 ± 0.01
	Δ	0.02	0.03	0.23	−0.04	−0.02	0.01
Dissolved Fe (mg l^{-1})	Initial	0.072	0.057	0.13	0.080	0.088	0.072
	Final	2.97 ± 0.161	2.14 ± 0.367	1.19 ± 0.055	0.126 ± 0.012	0.461 ± 0.087	0.420 ± 0.082
	Δ	2.90	2.08	1.06	0.046	0.373	0.348
pH (units)	Initial	8.14	8.00	8.03	8.02	7.79	7.83
	Final	7.31 ± 0.04	7.31 ± 0.02	7.35 ± 0.04	7.59 ± 0.02	7.54 ± 0.01	7.53 ± 0.02
	Δ	−0.83	−0.69	−0.68	−0.43	−0.25	−0.30
Alkalinity (mg $CaCO_3$ l^{-1})	Initial	175	174	169	225	193	199
	Final	248 ± 2	259 ± 1	290 ± 2	249 ± 5	259 ± 6	268 ± 5
	Δ	73.0	85.0	121	24.0	66.0	69.0
Dissolved Ca (mg l^{-1})	Initial	56	56	57	50	53	55
	Final	79 ± 1.3	76 ± 0.9	68 ± 1.2	74 ± 0.9	69 ± 0.7	71 ± 0.7
	Δ	23	20	11	24	16	16
Redox Potential (mV)	Final	39.8 ± 3.67	27.5 ± 2.65	15.2 ± 0.67	21.5 ± 7.51	1.8 ± 2.33	−14.5 ± 1.73

[a] Initial values represent a single measurement of two composited samples.
[b] Final values represent the mean ± 1 standard error for 3 replicate incubation bottles.

0.025 mg l^{-1} at the beginning and end of the incubation for the unamended and SO_4-amended water-only controls.

The pH, alkalinity, and dissolved Ca concentrations remained essentially unchanged (mean initial vs. final (14-day) values for pH: 8.48 vs. 8.38; alkalinity: 158 vs. 156 mg $CaCO_3$ l^{-1}; dissolved Ca: 51 vs. 50 mg l^{-1}) in the unamended and amended water-only controls during the incubation. The presence of soil increased 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 (Table 4). There appeared to be a slight increase in alkalinity (likely due to the titration of soil particles contained in the unfiltered sample), coincident with a slight decrease in dissolved Ca concentrations, with increasing SO_4 amendments in the 0–4 cm soil horizon ($P < 0.05$). This was not observed for the 4–10 cm soil horizon.

3.2.2. High SO_4 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 (data not shown), SRP concentrations remained below 7 μg l^{-1} during the incubation period for all treatments and controls. More importantly, there was no evidence ($P > 0.05$) that amending with the two SO_4 levels (0.33 and 1.0 mM) enhanced the mobilization of SRP from the soils (Fig. 2)). Soluble reactive P concentrations were frequently near or at the MDL in both soil horizons at the end of the incubation (Table 5).

The final concentrations of DOP were ≤12 μg l^{-1} for all soil treatments (Table 5). The initial DOP concentrations in the unamended soils from both soil horizons were reduced after the 14-day incubation, as was also observed in the water-only controls (data not shown). Although the final DOP concentration for both soil horizons were positively correlated with the initial SO_4 concentration ($r = 0.79$ and 0.95 for the 0–4 cm and 4–10 cm soil horizons, respectively), both relationships were nevertheless insignificant ($P > 0.05$).

Although all SO_4-amended vessels had measurable SO_4 concentrations remaining, most of the added SO_4 decreased during the incubation period under the reducing conditions present in the incubation vessels containing soil (Table 5). The exception was the 1.0 mM SO_4 amendment to the 4–10 cm soil horizon where approximately one-half of the added SO_4 remained after 14 days. The unamended soils, which initially had 7.5 mg SO_4 l^{-1} (0–4 cm soil horizon) and 5.4 mg SO_4 l^{-1} (4–10 cm soil horizon), completely reduced their background SO_4 levels to below the MDL (<0.5 mg l^{-1}).

Total sulfide concentrations were directly proportional to the amount of SO_4 reduced ($r = 0.97$; $P < 0.05$) (Table 5). The highest TS concentration (23.4 mg l^{-1}) measured in any of the incubations during this study was in the 0–4 cm WCA-2A U3 soils that were amended with 1.0 mM SO_4. For the 4–10 cm soil horizon, TS concentrations were nearly the same in the 0.33 mM and 1.0 mM SO_4 treatments. The initial and final TS concentrations in the water-only controls were below the MDL (<0.006 mg l^{-1}). The high TS concentrations measured in the SO_4-amended soils at the end of

Table 5
Concentrations of key chemical parameters before and after a 14-day anaerobic incubation of soils from WCA-2A U3 with SO_4-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water.

		0–4 cm horizon			4–10 cm horizon		
		Unamended	+0.33 mM SO_4	+1.0 mM SO_4	Unamended	+0.33 mM SO_4	+1.0 mM SO_4
TAN (mg l^{-1})	Initial	1.26	1.17	2.10	1.17	1.31	1.11
	Final	4.36 ± 0.07	4.53 ± 0.30	4.45 ± 0.14	2.55 ± 0.88	2.22 ± 0.03	2.23 ± 0.07
	Δ	3.10	3.36	2.35	1.38	0.91	1.12
SRP (μg l^{-1})	Initial	<2	<2	2	2	4	6
	Final	6 ± 1.8	2 ± 0.6	<3 ± 0	4 ± 0.7	<4 ± 0	<3 ± 0
	Δ	5	1	−0.5	2	−2	−4.5
DOP (μg l^{-1})	Initial	5	5	5	10	9	10
	Final	2 ± 1.2	6 ± 0.9	7 ± 0.3	5 ± 0.7	9 ± 1	12 ± 0.7
	Δ	−3	1	2	−5	0	2
SO_4 (mg l^{-1})	Initial	7.5	42	114	5.4	40	108
	Final	<0.5 ± 0	2.2 ± 0.1	18 ± 1.2	<0.5 ± 0	2.5 ± 0.3	54 ± 5.3
	Δ	−7.3	−40	−96	−5.2	−38	−54
TS (mg l^{-1})	Initial	0.04	0.04	0.04	0.11	0.18	0.27
	Final	1.05 ± 0.41	5.86 ± 0	23.4 ± 0.6	0.88 ± 0.1	9.24 ± 0.63	9.08 ± 2.73
	Δ	1.01	5.82	23.4	0.77	9.06	8.81
Dissolved Fe (mg l^{-1})	Initial	0.049	0.042	0.026	0.026	0.034	0.034
	Final	0.033 ± 0.004	<0.025	<0.025	<0.025	<0.025	<0.025
	Δ	−0.016	−0.030	−0.014	−0.014	−0.022	−0.022
pH (units)	Initial	7.93	8.02	7.87	7.90	7.84	7.86
	Final	7.78 ± 0.03	7.66 ± 0.01	7.56 ± 0.02	7.86 ± 0.01	7.73 ± 0.04	7.62 ± 0.09
	Δ	−0.15	−0.36	−0.31	−0.04	−0.11	−0.24
Alkalinity (mg $CaCO_3$ l^{-1})	Initial	272	305	345	570	770	1070
	Final	385 ± 2	409 ± 7	451 ± 17	825 ± 23	929 ± 50	958 ± 42
	Δ	113	104	106	155	159	−112
Dissolved Ca (mg l^{-1})	Initial	50	51	51	48	49	49
	Final	62 ± 1.8	62 ± 1.9	55 ± 0.3	49 ± 0.9	48 ± 0.6	53 ± 2.7
	Δ	12	11	4	1	−1	4
Redox Potential (mV)	Final	−109 ± 5.2	−148 ± 5.2	−180 ± 2.6	−122 ± 1.7	−170 ± 3.8	−170 ± 11.5

* Initial values represent a single measurement of two composited samples.
** Final values represent the mean ± 1 standard error for 3 replicate incubation bottles.

the incubation was due to the low concentrations (≤0.033 mg l^{-1}) of dissolved Fe found in the post-incubated amended soils. Dissolved Fe reacts with sulfide under low ORP to form insoluble FeS_x (Smolders et al., 2006a). The dissolved Fe concentrations were low initially, and most treatment and control vessels ended with dissolved Fe concentrations below the MDL of 0.025 mg l^{-1}. There were no significant differences ($P > 0.05$) in the final dissolved Fe concentrations among sulfate treatments for either the 0–4 or 4–10 cm soil horizon.

Compared to the pH (8.45) in the water-only controls, the pH decreased to 7.56–7.86 after the 14-day incubation in the soil-containing vessels (Table 5). There was a SO_4-treatment effect for the 0–4 and 4–10 cm soil horizons, with significantly ($P < 0.05$) lower pH values corresponding to higher SO_4 amendments for the 1.0 mM (0–4 and 4–10 cm soil horizons) and 0.33 mM (4–10 cm soil horizon) SO_4-amended than unamended soils. There were no significant pH differences ($P > 0.05$) between the 0–4 and 4–10 cm soil horizons. Alkalinity levels during the incubation increased by nearly the same amount in the amended 0–4 cm soil horizon. Higher alkalinity increases occurred in the unamended and 0.33 mM SO_4 amended soil, but not in the 1.0 mM SO_4 treated soil, from the 4–10 cm soil horizon. Alkalinity levels remained unchanged in the water-only controls (means of 162 mg $CaCO_3$ l^{-1}) for initial and final concentrations.

Dissolved Ca concentrations increased during the incubation period in the unamended and amended 0–4 cm soil horizon, with the 1.0 mM SO_4-amended soil exhibiting the smallest response (Table 5). Dissolved Ca concentration increases were not as obvious in the 4–10 cm soil horizon incubation as they were for the 0–4 cm soil horizon. Ca levels decreased slightly (means of 53 and 49 mg l^{-1} for initial and final concentrations, respectively) during the incubation in the water-only controls.

3.2.3. High SO_4 and high P environment: STA-2 cell 1

The initial SRP concentration of 200 μg l^{-1} at the onset of the incubation was unexpectedly high given the low-SRP (2 μg l^{-1}) water from WCA-3A that was added to the soil (Fig. 2). The higher labile soil P concentrations in STA-2 Cell 1 compared to the soils in the WCAs (Table 3) resulted in elevated SRP concentrations immediately after flooding the soil. As a result of the labile P pools, five and three times the mass of the initial SRP in the water was released from the 0–4 and 4–10 cm soil horizons, respectively, after 14 days of incubation (Fig. 2). This resulted in SRP concentrations reaching between 1000 and 1100 μg l^{-1} on day 14 in the soil horizon incubations and between 586 and 638 μg l^{-1} in the 4–10 cm soil horizon incubations. As observed in the incubations on soils collected from WCAs, there was no effect ($P > 0.05$) of adding SO_4 on the release of SRP from the soil. Concentrations of SRP were at or less than the MDL (2 μg l^{-1}) in the water-only controls.

Along with SRP, DOP concentrations also increased during the incubation (Table 6). The extent of DOP release was not as great as for SRP release, but the same pattern of lower DOP release associated with the 4–10 cm compared to the 0–4 cm soil horizons was observed. Dissolved organic P releases were independent of the SO_4 treatment ($P > 0.05$).

Table 6
Concentrations of key chemical parameters before and after a 14-day anaerobic incubation of soils from STA-2 Cell 1 with SO_4-amended (0.33 mM or 1.0 mM) and unamended WCA-3A surface water.

		0-4 cm horizon			4-10 cm horizon		
		Unamended	+0.33 mM SO_4	+1.0 mM SO_4	Unamended	+0.33 mM SO_4	+1.0 mM SO_4
TAN (mg l^{-1})	Initial[a]	1.38	1.45	1.42	1.05	1.14	1.1
	Final[b]	16.0 ± 0.32	16.7 ± 0.81	14.8 ± 0.41	5.8 ± 0.11	5.3 ± 0.42	4.8 ± 0.07
	Δ	14.6	15.3	13.4	4.8	4.2	3.7
SRP (μg l^{-1})	Initial	220	223	199	152	189	198
	Final	1073 ± 44	1095 ± 63	1087 ± 47	586 ± 19	638 ± 59	598 ± 62
	Δ	853	872	888	434	449	400
DOP (μg l^{-1})	Initial	96	97	129	66	67	70
	Final	283 ± 52	249 ± 65	240 ± 74	126 ± 18	84 ± 17	109 ± 30
	Δ	187	152	111	60	17	39
SO_4 (mg l^{-1})	Initial	18	52	125	14	50	118
	Final	<0.5 ± 0	1.7 ± 0	5.6 ± 0.2	<0.5	0.8 ± 0.1	21 ± 11
	Δ	−18	−50	−119	−14	−49	−97
TS (mg l^{-1})	Initial	0.15	0.17	0.21	0.27	0.31	0.28
	Final	4.6 ± 0.13	8.7 ± 0.24	21.0 ± 0.60	2.5 ± 0.12	5.7 ± 0.16	12.7 ± 2.6
	Δ	4.5	8.5	21	2.0	3.4	12.4
Dissolved Fe (mg l^{-1})	Initial	0.047	0.063	0.055	0.051	0.055	0.070
	Final	0.114 ± 0.015	0.065 ± 0	0.060 ± 0.002	0.101 ± 0.003	0.084 ± 0.003	0.073 ± 0.005
	Δ	0.067	0.002	0.005	0.050	0.029	0.003
pH (units)	Initial	7.69	7.68	7.73	7.61	7.66	7.57
	Final	7.68 ± 0.08	7.51 ± 0.05	7.28 ± 0.01	7.70 ± 0.12	7.46 ± 0.02	7.39 ± 0.02
	Δ	−0.01	−0.17	−0.45	0.09	−0.20	−0.18
Alkalinity (mg $CaCO_3$ l^{-1})	Initial	370	410	420	400	630	620
	Final	393 ± 3	398 ± 7	410 ± 0	306 ± 7	330 ± 6	350 ± 0
	Δ	23	−12	−10	−94	−300	−270
Dissolved Ca (mg l^{-1})	Initial	58	56	61	54	54	56
	Final	106 ± 1.2	104 ± 0.6	93 ± 1.2	77 ± 0.3	75 ± 0.3	79 ± 0.9
	Δ	48	48	32	23	21	23
Redox Potential (mV)	Final	−117 ± 7.0	−138 ± 2.5	−156 ± 1.5	−129 ± 2.0	−144 ± 1.5	−156 ± 0.5

[a] Initial values represent a single measurement of two composited samples.
[b] Final values represent the mean ± 1 standard error for 3 replicate incubation bottles.

Sulfate concentrations decreased significantly in the SO_4-amended soils (Table 6); smaller reductions (5–18 mg l^{-1}) were observed for the water-only controls. Total sulfide concentrations varied directly with the SO_4 treatment for both the 0–4 cm and 4–10 cm soil horizons ($P < 0.05$). For a given initial SO_4 concentration, more TS production occurred in the 0–4 cm than 4–10 cm horizons.

Because of the low ORP (−117 mV to −156 mV) during the incubations, dissolved Fe concentrations increased in the soil slurries, but concentrations never exceeded 0.12 mg l^{-1} by day 14 (Table 6). For both horizons, there was a significant SO_4-treatment effect ($P < 0.05$), with the highest final dissolved Fe concentrations occurring within the unamended soils.

With the exception of the unamended 4–10 cm soil layer, the pH decreased during the incubation in the remaining water-only controls (data not shown) and soil treatments (Table 6). However, the magnitude of the decrease was minor, never more than 0.5 units lower than the initial pH. Alkalinity levels decreased in the post-incubated SO_4-amended 4–10 cm soil horizons. Such decreases were not observed during the incubation of the 0–4 cm soil horizon.

Dissolved Ca concentrations increased in all the vessels containing soil at the end of the incubation (Table 6), whereas Ca levels remained nearly the same (55–56 mg l^{-1}) in the water-only controls. More Ca was released in the 0–4 cm than 4–10 cm soil horizons. The release was dependent on the SO_4 treatment ($P < 0.05$) to the 0–4 cm soil horizon, except for the 0.33 mM SO_4 amendment. There was no SO_4-treatment effect ($P > 0.05$) on the final Ca concentrations measured in the 4–10 cm soil horizon.

3.3. Heterotrophic microbial respiration and methanogenesis during anaerobic incubations

3.3.1. Methane production

Increasing SO_4 additions resulted in less CH_4 production in two (WCA-2A U3 and WCA-3A) of the three incubated 0–4 cm soil horizons ($P < 0.05$) (Fig. 3). Total mass of CH_4-C released from the unamended 0–4 cm horizon soils during the 10-day incubation was comparable (0.46–0.52 mg C g dry wt^{-1}; $P > 0.05$) at all three wetland locations. In comparison, CH_4 released from the 1.0 mM SO_4-amended 0–4 cm deep soils was considerably lower than the unamended control soils from the same depth ($P < 0.05$), ranging from 0.11 (WCA-3A and WCA-2A U3) to 0.36 mg C g dry wt^{-1} (STA-2 Cell 1). Methane concentration in the 0.33 mM SO_4-amended incubations was intermediate between the unamended and 1.0 mM amended soils from the WCA sites, but was slightly higher than the unamended soil from the STA-2 Cell 1 site. The STA-2 Cell 1 soil exhibited the least CH_4 response to different SO_4 amendments for the 0–4 cm soil horizon compared to the soils from the WCAs, likely due to the high ambient SO_4 concentration (18 mg l^{-1}). The 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.

3.3.2. Carbon dioxide production

The amounts of C produced as CO_2 per initial dry weight of soil after 10 days of incubation varied among wetland locations, soil horizons, and SO_4 treatment (Fig. 3). As observed for CH_4, production of CO_2 was generally lower in the 4–10 cm than at

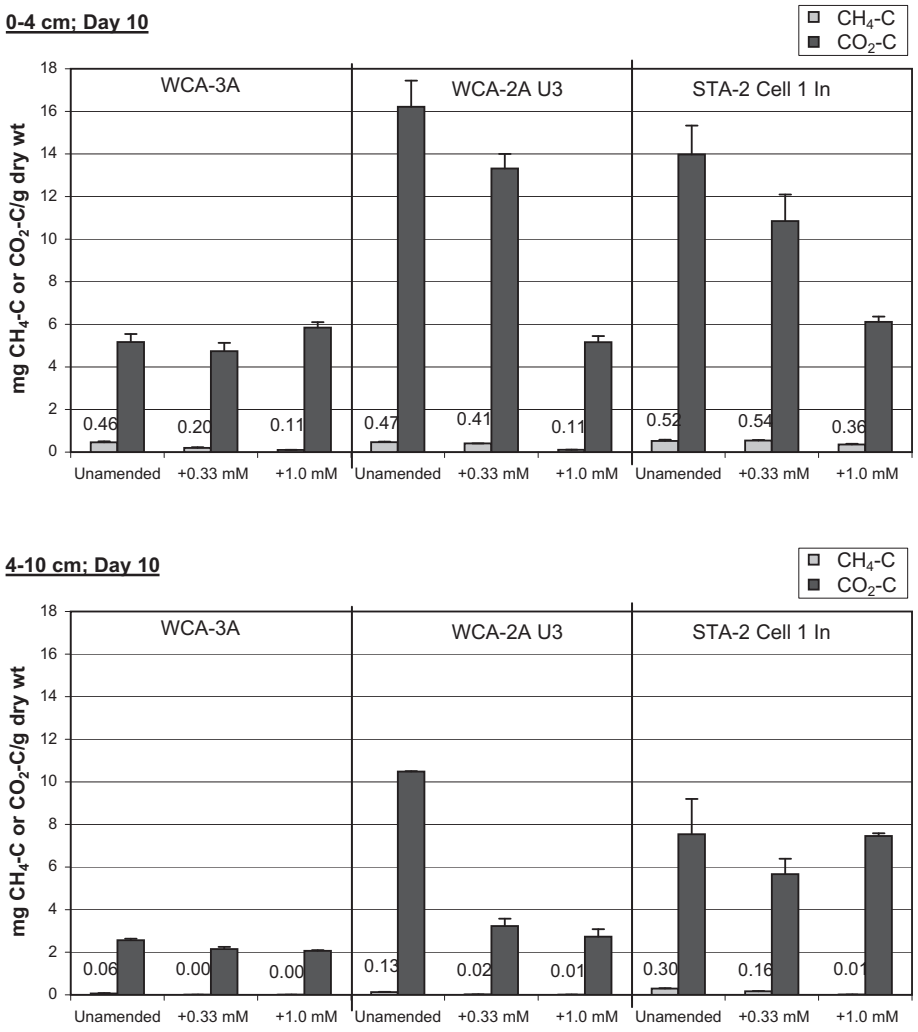

Fig. 3. The mass of methane (CH_4) and carbon dioxide (CO_2) released per initial dry weight of soil in the 0–4 cm (top panel) and 4–10 cm (bottom panel) soil horizons of WCA-3A, WCA-2A U3, and STA-2 Cell 1 In after 10 days of anaerobic incubation. The soils were exposed to unamended and SO_4-amended (with 0.33 mM (32 mg l^{-1}) or 1.0 mM (96 mg l^{-1})) low P and low SO_4 surface water from WCA-3A. Each column represents the mean of three replicates.

0–4 cm soil horizon ($P < 0.05$) (Fig. 3). Both the 0.33 and 1.0 mM SO_4 additions inhibited heterotrophic microbial activity relative to the unamended soil in the 0–4 cm soil horizons of WCA-2A and STA-2 Cell 1 ($P < 0.05$); such treatment inhibition was not observed for the 4–10 cm soil horizons collected from WCA-3A and STA-2 Cell 1 ($P > 0.05$).

3.3.3. Organic carbon mineralization

Carbon dioxide accounted for up to 9.1% of the soil C mineralized in 10 days for the unamended 0–4 cm soil horizon from WCA-2A U3 . The lowest amount (1.3%) of initial organic soil C mineralized in 10 days for the same soil horizon under unamended conditions was at WCA-3A. For the 4–10 cm soil depth horizon, the unamended WCA-2A U3 was again the highest evolving CO_2-C soil with 3.3% of the soil organic C mineralized after 10 days. The lowest mineralized soil at the 4–10 cm soil horizon was from 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 production, which represents the sum of the respiratory and methanogenic microbial processes, clearly demonstrated higher mineralization rates in the 0–4 cm than the 4–10 cm soil horizons for each of the three wetland locations ($P < 0.05$). The inhibition in CO_2 production rates by SO_4 for the WCA-2A U3 and STA-2 Cell 1 soils, particularly in the 0–4 cm horizon (Fig. 3), is contrary to the original hypothesis that mineralization of organic matter is enhanced with increasing SO_4 concentrations. Also, the lack of a significant correlation between the percentage of the original soil organic C mineralized to CO_2–C + CH_4–C and the amount of SRP released also violated the hypothesis that the soil mineralization rate under continuously flooded, anaerobic conditions is linked to P release.

4. Discussion

4.1. Anaerobic organic matter mineralization and methanogenesis

4.1.1. Mineralization

Sulfate reduction rates among the three wetland locations varied within narrow ranges for the 0–4 cm (3.75–4.58 μmol SO_4 g dry wt^{-1} d^{-1}) and 4–10 cm (1.56–2.29 μmol SO_4 g dry wt^{-1} d^{-1}) soil horizons. These rates are comparable to the rates reported by D'Angelo and Reddy (1999) for P-enriched and unenriched soils (upper 15 cm) in WCA-2A (2.56 and 2.59 μmol SO_4 g dry wt^{-1} d^{-1}), and the 4.53 μmol SO_4 g dry wt^{-1} d^{-1} rate measured by McLatchey and Reddy (1998) for the upper 15 cm of soil in a restored central Florida wetland. We found no evidence that increasing

concentrations of SO_4 (an electron acceptor) resulted in enhanced microbial respiration at any of the wetland sites. Several independent lines of evidence demonstrate that adding SO_4 to the soils did not enhance mineralization or P release, as discussed below.

The SO_4-reduction process associated with organic matter decomposition consumes H^+ ions, thereby increasing the pH and alkalinity (Smolders et al., 2006a). However, the pH decreased during the incubations of the wetland soils with only one exception (Tables 4–6), suggesting that microbial processes other than organic matter oxidation – with SO_4 serving as the terminal electron acceptor – were dominant. The alkalinity increased in most of the experiments, with higher alkalinities measured for the SO_4-amended than unamended soils at the end of the incubation (Tables 4–6). These increases in alkalinity were more likely due to the dissolution of $CaCO_3$ (Ca concentrations increased during the soil incubations with one exception (Tables 4–6)) caused by the lowered pH values as a result of acidogenic fermenters, rather than by only the action of sulfate-reducing bacteria (SRB). Furthermore, if SO_4 reduction dominated the terminal step in organic matter decomposition, the SO_4-reduction-to-CO_2-production molar ratio should equal 0.5 if complete oxidizers dominate the dissimilatory SRB (Reddy and DeLaune, 2008). However, the ratio in our incubations ranged from 0.04 to 0.1 in the 1.0 mM SO_4-amended soils, indicative of metabolic pathways other than sulfate-reduction by complete oxidizers as a source of CO_2.

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 release rates of those gases, we found no evidence of enhanced organic matter mineralization with 0.33 mM or 1.0 mM SO_4 amendments to soils from WCA-3A, WCA-2A U3, or STA-2 Cell 1 (Fig. 3). D'Angelo and Reddy (1999) found no significant difference in soil organic C mineralization ($CO_2 + CH_4$ production) under denitrifying, SO_4-reducing, and methanogenic conditions within each of 10 different wetland soils. If the bioavailability of organic C limits soil mineralization among different groups of electron acceptors in anaerobic respiration as these investigators report, then increasing concentrations of SO_4 would not be expected to result in higher decomposition rates. Indeed, D'Angelo and Reddy (1999) observed no enhanced soil mineralization (as measured by CO_2 production rates) with SO_4 enrichment between some of the unamended (control) and SO_4-amended (320 mg SO_4 l^{-1}) soils.

In our study we observed that SO_4 amendments had an apparent inhibitory effect on the production of CO_2 and CH_4 in the WCA-2A U3 and STA-2 Cell 1 soils, but not in the WCA-3A soils (Fig. 3). Concentrations of total recoverable Fe in WCA-3A soils were high (Table 3), which we surmise resulted in low TS concentrations at the end of the anaerobic incubation (Table 4) due to the precipitation of FeS (SI for FeS $\geq$ 16 for unit-activity conditions). On the other hand, total recoverable Fe concentrations in the soils from WCA-2A U3 and STA-2 Cell 1 were lower than from WCA-3A, and therefore TS concentrations were free to increase at the end of the incubations in proportion to the initial SO_4 concentrations ($r = 0.84$ and 0.93, $P < 0.05$), reaching as high as 23.4 mg l^{-1} (Tables 5 and 6).

Besides sulfide inhibition (McCartney and Oleszkiewicz, 1991; Okabe et al., 1995; Icgen and Harrison, 2006), a variety of environmental factors may affect the composition and activities of syntrophic consortia in the Everglades, including the availability of C (Amador and Jones, 1995, 1997; DeBusk and Reddy, 1998; D'Angelo and Reddy, 1999; Chauhan and Ogram, 2006; Wright and Reddy, 2007; Wright et al., 2009) and nutrients (Amador and Jones, 1993, 1995, 1997; DeBusk and Reddy, 1998, 2005; Wright and Reddy, 2001, 2007). We attribute the lack of a response by SRB in WCA-3A to SO_4 enrichment to substrate and/or P limitation, which caused the early decline in CO_2 emissions in the 0–4 cm and 4–10 cm soil horizons. The CO_2 production ceased after five days in the 0–4 cm and three days in the 4–10 cm soil horizon incubations regardless of the SO_4 treatment (DBE, 2009). Whether this was due to the recalcitrant nature of the C bonds in the substrate and/or to P limitation inherent in the C compounds is uncertain, as both labile organic C and inorganic P concentrations have been found to limit heterotrophic microbial activity at oligotrophic sites in WCA-2A (DeBusk and Reddy, 1998; Wright and Reddy, 2007). Both reasons are plausible given the high lignocellulose index (LCI) values of 0.71 and 0.76 and soil TOC:TP ratios of 1012 and 1260 (wt wt^{-1}) for the 0–4 cm and 4–10 cm soil horizons from that location. The LCI values for both horizons lie within the 0.7 and 0.8 range where Melillo et al. (1989) found the major limitation to decomposition was lignin degradation, which is slow in anaerobic environments and a function of environmental conditions alone such as exogenous labile C and N compounds. Stevenson (1986) reported a TOC:TP ratio > 300 (wt wt^{-1}) can limit decomposition under aerobic conditions, but this ratio is likely to be higher under anaerobic conditions since P requirements are lower for anaerobic than aerobic bacteria (Reddy and DeLaune, 2008). Qualls and Richardson (2000) and Newman et al. (2001) found SRP loadings to mesocosms in the oligotrophic regions of WCA-2A and WCA-1, respectively, resulted in increased decomposition, indicating that an exogenous source of labile P, per se, can induce faster decomposition of organic matter in these P-limited wetlands.

The substrates in the soils from the more enriched STA-2 Cell 1 were not as limiting for decomposition, since the organic matter was more labile (lower LCI) and contained higher labile P pools and total P concentrations (Table 3). This was the only wetland whose soils released SRP during the incubations, but still without an effect of added SO_4 (Fig. 2). However, even though the organic matter was more labile at this location, organic C limitation may still have occurred. For example, Wright and Reddy (2007) reported labile organic C limitation for a P-enriched site in WCA-2A. In our study, SO_4 amendments did not increase mineralization rates in the STA-2 Cell 1 soil (Fig. 3).

Carbon mineralization, as indicated by CO_2 and CH_4 release in the WCA-2A U3 soils, was the highest for any of the three wetland locations (Fig. 3). This was unexpected given that DeBusk and Reddy (1998) and Wright and Reddy (2001, 2007) reported both organic C and P limitation at oligotrophic sites in WCA-2A. Carbon and P limitation notwithstanding, Wright and Reddy (2001) reported unamended (basal) anaerobic soil mineralization rates (1.1 to 1.8 mg CO_2–C g dry wt^{-1} d^{-1} for the detritus and 0–10 cm soil horizon) that were similar to the sulfate-unamended rates we measured in the 0–4 cm (1.6 mg CO_2–C g dry wt^{-1} d^{-1}) and 4–10 cm (1.04 mg CO_2–C g dry wt^{-1} d^{-1}) soil horizons retrieved from WCA-2A U3. In a later study, Wright and Reddy (2007) found lower basal CO_2 production rates in P-unimpacted areas of WCA-2A: 0.19 mg CO_2–C g dry wt^{-1} d^{-1} for detritus and 0.05 mg CO_2–C g dry wt^{-1} d^{-1} for the uppermost 10 cm soil horizon, which are close to the 0.20 (detritus) and 0.12 (0–10 cm upper soil horizon) mg CO_2–C g dry wt^{-1} d^{-1} reported by DeBusk and Reddy (1998).

Under anaerobic SO_4-amended conditions during a 10-day incubation in the unimpacted area of WCA-2A, Wright and Reddy (2001) measured CO_2 production rates of 0.29 and 0.15 mg CO_2–C g dry wt^{-1} d^{-1} for the detrital and 0–10 cm soil horizons, respectively, compared to 0.52 and 0.27 mg CO_2–C g dry wt^{-1} d^{-1} for the 0–4 cm (included the detritus layer) and 4–10 cm soil horizons that we amended with 1.0 mM SO_4. Their initial SO_4 amendment was 6.97 mg SO_4 g dry wt^{-1}, 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 wt^{-1} we added as a 1.0 mM amendment. Since incubation conditions in the laboratory (sulfate concentrations, temperature, agitation, duration, source of added water) and environmental conditions (hydrology,

site location, soil horizon, season, P loading history) at the time of field coring varied among studies, direct comparisons of these laboratory incubation studies should be made with caution. Changes in soil properties within WCA-2A also may have also affected rates of heterotrophic microbial activity since more than a decade separated the previous studies (DeBusk and Reddy, 1998; Wright and Reddy, 2001, 2007) from our investigation. In spite of these temporal, incubation, and potential site differences, rates of heterotrophic microbial activity were in general agreement among some of the investigations.

The inverse relationship between mineralization (i.e., CO_2–C + CH_4–C production as a percentage of soil TOC) and soil TOC ($r = -0.83$; $P < 0.01$) or soil TN ($r = -0.64$; $P < 0.01$) across the wetland locations and both soil depths suggests that it was likely the quality, rather than quantity, of TOC or TN that determined the amount of organic matter mineralized in our incubations. Hence, even though WCA-3A had the highest soil TOC and TN concentrations, the substrate quality was likely to have been the most recalcitrant among the wetlands. On the other hand, we found a positive relationship ($P < 0.01$) between soil P pools and soil organic C mineralization in WCA-3A and STA-2 Cell 1, but not in WCA-2A ($P > 0.05$). Other investigators have reported inconsistent results between soil P pools and organic C mineralization: DeBusk and Reddy (1998), Wright and Reddy (2001), and Wright et al. (2009) found a positive relationship between soil TP and CO_2 production rate, whereas D'Angelo and Reddy (1999) and Wright et al. (2009) found no significant relationships between heterotrophic respiration and available P pools such as MBP and readily labile (water and exchangeable) P.

Higher CO_2 production occurred within the 0–4 cm than in the 4–10 cm soil horizon at all three wetland locations (Fig. 3), indicating substrate quality was less desirable as an electron donor during decomposition of the deeper soils. For all three soils, the LCI was higher in the 4–10 cm than 0–4 cm soil horizons (Table 3). A higher LCI indicates that more lignin is present than the more easily degradable cellulose component of organic matter (Melillo et al., 1989). The TOC:TP ratio also increased with soil depth at all wetland locations (Table 3), indicating that decomposition of organic matter for both soil horizons was likely P limited since all of the soil TOC:TP ratios except for the 0–4 cm STA-2 Cell 1 soil horizon were >300 (wt wt^{-1}), a cutoff point where decomposition of organic matter can be P limited in aerobic environments (Stevenson, 1986). The MBP, a P pool that can readily contribute labile P, decreased in the 4–10 cm compared to the 0–4 cm soil horizon in two of the three soils (Table 3), consistent with the reduced MBP with soil depth reported by Qualls and Richardson (2000) for P-enriched soils in WCA-2A. Other investigators (Schipper and Reddy, 1994; DeBusk and Reddy, 1998; Wright and Reddy, 2001, 2007) also have reported reduced organic mineralization with soil depth, which corresponded with lower substrate quality.

4.1.2. Methanogenesis

Sulfate-reducing bacteria 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 (Winfrey and Zeikus, 1977; Lovley and Klug, 1983; Holmer and Storkholm, 2001), although sulfide toxicity may also be present (Winfrey and Zeikus, 1977). This likely occurred in our anaerobic soil slurries where CH_4 production in the 0–4 cm and 4–10 cm horizons for all three wetland locations was inhibited by SO_4 addition (Fig. 3). Other investigators (Drake et al., 1996; D'Angelo and Reddy, 1999) have found SO_4-induced inhibition of CH_4 production in P-enriched and unenriched soils from the WCAs.

Methanogenesis as the terminal C flow in our incubations (Fig. 3) was of minor importance compared to other fermentative processes and respiration from SO_4-reduction. This was likely due in large measure to the added SO_4; CH_4 emission rates clearly decreased with increased SO_4 levels. The short incubation times chosen for this study of 10 and 14 days also may have contributed to the low CH_4 emission rates, as the SO_4 amendments would have remained inhibitory to methanogenesis during most, if not all, of the incubation period. The CH_4 generation profiles typically showed higher rates towards the end of the incubations when the amended SO_4 concentrations would have been reduced to very low levels (DBE, 2009).

Compared to CO_2 production, CH_4 generation was relatively low in the unamended soils from all three wetland locations. Some sulfate was carried over with the soils transferred to the serum bottles, resulting in initial SO_4 concentrations of 6.5, 7.5, and 18 mg l^{-1}, respectively, in WCA-3A, WCA-2A U3 and STA-2 Cell 1unamended soils. Based on the rates of sulfate reduction (3.9–8.5 mg l^{-1} d^{-1} [40–90 μM d^{-1}]) that occurred in the 1.0 mM sulfate-amended soils, only 1–2 days would have been required to eliminate the initial sulfate amounts in the unamended soils. Although this may have resulted in a slight delay in the onset of methanogenic conditions, headspace analysis collected on day 3 during the incubation indicated CH_4 production did occur at all three sites for the 0–4 cm soil horizon (DBE, 2009). A more likely reason for low CH_4 production, particularly in the oligotrophic WCA sites, was the dominance of incomplete acetate oxidizing SRB (Castro et al., 2002; Chauhan and Ogram, 2006).

Notwithstanding the lower rates of CH_4 production in our incubations, CH_4 production in the WCA-2A U3 0–4 cm soil horizon was nearly 400 times higher than the production rates measured by Schipper and Reddy (1994) at an unimpacted site in WCA-2A. Other investigators (D'Angelo and Reddy, 1999; Wright and Reddy, 2001, 2007) working in the unimpacted areas of WCA-2A have reported CH_4 production rates that were comparable to our values. Schipper and Reddy (1994) also found CH_4 production accounted for 70% of the C loss (CO_2–C + CH_4–C) at their WCA-2A site, a finding that has not been repeated in this or any other investigations in the umimpacted regions of WCA-2A (D'Angelo and Reddy, 1999; Wright and Reddy, 2001, 2007).

As was found for CO_2 production, methanogenesis also decreased with soil depth. This again points to the likelihood of soil C being more available in surface soils to fermenters responsible for producing the substrates (acetate, H_2) required by methanogens (Bachoon and Jones, 1992; Schipper and Reddy, 1994; Wright and Reddy, 2001, 2007).

4.2. Soluble reactive P release

After 7 years of operation, the soil P concentrations at the STA-2 Cell 1 inflow region are much higher than those at the WCA sites that are remotely located from canal discharges (Table 3; DBE, 2009). Thus, the high SRP release during the incubation of the STA-2 Cell 1 soils was predictable. However, for all three wetland locations, we did not observe a sustained net increase in SRP due to SO_4 enrichment compared to unamended soils (Fig. 2). We also conducted SO_4-amendment investigations on soils collected from STA-5 (operational for 8 years), and again from WCA-3A, using the same experimental design (DBE, 2009). The results were the same: there was no effect of SO_4 enrichment on net SRP release. Although there is ample evidence from U.S. (Roden and Edmonds, 1997) and European (Smolders et al., 2006a; Zak et al., 2006; Guerts et al., 2008) studies that SO_4 enrichment leads to enhanced soil P release, there are exceptions reported in the literature (e.g., Lamers et al., 2002).

While the European studies point to the importance of SO_4 as a precursor for TS buildup and net P release, there are several

important differences between the European wetlands and the Everglades. Many of the European wetlands had significant groundwater intrusion that altered the water chemistry in surface and porewaters. Soil and water in those wetlands frequently contained high Fe and low Ca concentrations, which contrasts to the low Fe and high Ca concentrations in the Everglades. Finally, the climate and plant communities are vastly different in the Everglades and wetlands in Europe.

Contrary to the negative correlations with soil TOC and TN contents, soil P pools were strongly directly correlated ($P < 0.01$) with final SRP concentrations in the incubation waters for all wetland locations and soil horizons. The implication is that the sizes of the total and readily available soil P pools affected the release of SRP into initially low P and low SO_4 incubation water during anaerobic incubations. Lamers et al. (2002) found that the level of P eutrophication was mainly determined by the P concentration of the sediment, and not by dissolved Fe concentrations in the porewater.

The size of available and total P pools is primarily related to the nutrient enrichment history at a site (Koch and Reddy, 1992; Reddy et al., 1998; Qualls and Richardson, 2000; Wright et al., 2009). Wright et al. (2009) found that mineralizable P (i.e., extracted in 10^{-3} M HCl), MBP, and TP were more responsive indicators than N and C pools to nutrient enrichment in the Everglades, a finding consistent with the higher TP and labile P pools found in the soils of the more eutrophic inflow region of Cell 1 of STA-2 than at the oligotrophic sites in the WCAs. Moreover, another aspect of substrate quality (the composition of easily degraded cellulose vs. more recalcitrant lignin) as measured by the LCI was also correlated ($P < 0.01$) with P release.

The TFe content by itself in the soils was not significantly correlated to net SRP release ($r = -0.42$; $P > 0.05$), but Fe-associated P was correlated with the release of SRP (r −0.97; $P < 0.01$). Other investigators (Zak et al., 2006; Guerts et al., 2008; Loeb et al., 2008) have reported a correlation of net P release with the soil Fe-P fractions, amorphous Fe:Fe-P fractions, or TFe:TP concentrations. Guerts et al. (2008) suggested that porewater Fe:SRP and soil Fe:TP ratios could be a valuable prognostic tool for the restoration of water quality and biodiversity in fen waters. High surface water SRP and TP concentrations appeared to be SO_4 induced below threshold porewater Fe:SRP ratios of 6.3:1 and total soil Fe:P ratios of 18.1:1 by weight.

The higher soil and porewater Fe concentrations at WCA-3A (Table 3; DBE, 2009) point to the possibility that Fe minerals may be important in regulating surface water P concentrations at this site (Roden and Edmonds, 1997). Porewater Fe:SRP and soil TFe:TP ratios (wt wt^{-1}) were 83:1 and 23:1 to 25:1, respectively, for the slough soils in WCA-3A (DBE, 2009), well above the ratios where Fe could control the P release in a SO_4-rich environment (Guerts et al., 2008). Yet there was no net release of SRP from WCA-3A soils under the reducing conditions present during the anaerobic incubations (Fig. 2), even though the TFe concentration was the highest in WCA-3A soils among the three sites (Table 3). We believe the reason was that the Fe-P component comprised a very small fraction of TFe: only 0.05 and 0.17% of the TFe was associated with P in the 0–4 cm and 4–10 cm soil horizons in P-limited WCA-3A, respectively. Loeb et al. (2008) found that whereas soil Fe-P:amorphous Fe ratio correlated well with porewater P concentration in iron-rich soils, soil Fe-P by itself did not. This indicates that the release of P due to the reduction of Fe depends on both the reduction rate and the extent of saturation of Fe with P.

In contrast to WCA-3A, the porewater Fe:SRP and soil TFe:TP ratios (wt wt^{-1}) were <6.3:1 and <18.1:1, respectively, in WCA-2A U3 and STA-2 Cell 1 (DBE, 2009), suggesting that Fe would not control P solubility and release to overlying waters in sulfidic environments (Guerts et al., 2008). For the WCA-2A U3 soils, the low levels of TP and labile P pools limited P release, as was found in the WCA-3A soils. We propose that for both WCA soils, a low P pool in the soil was the controlling factor regulating net P release, and not the Fe content or mineralization of organic matter.

Although organic P mineralization may have contributed to some of the observed SRP increases in the overlying water of soils from STA-2 Cell 1 during the incubation, we believe the preponderance of the SRP originated from labile inorganic P pools associated with $CaCO_3$ and Ca-P precipitates. We base this on two reasons: 1) Ca concentrations increased during the incubation (Table 6), indicating $CaCO_3$ dissolution and subsequent release of any SRP loosely adsorbed to the mineral; 2) a mass balance calculated for SRP released in the unamended 0–4 cm soil horizon incubation vs. the inorganic P initially present as labile P (i.e., porewater + NH_4Cl-extractable P) in the soil indicated that the labile inorganic P pool was high enough (52 μg g dry^{-1}) to supply more than the observed amount of net SRP released (38 μg g dry^{-1}) during the 14-day incubation. Other investigators (Koch and Reddy, 1992; Qualls and Richardson, 1995; Reddy et al., 1998) have invoked Ca solubility, as well as organic P mobilization, as controlling P mobility, especially in soils near points where high nutrient ADW enters the Everglades.

Across all three wetland locations, two soil horizons (0–4 and 4–10 cm), and SO_4 treatments (unamended, +0.33 mM, and +1.0 mM), we observed no relationship between the amount of soil organic C mineralized and net SRP released during the incubations. The wetland soil that deviated the greatest was WCA-2A U3, where the highest mineralization rates were measured (Fig. 3), albeit with very low P release (Fig. 2). These data suggest other factors, such as sulfide inhibition, total and labile P and C pool sizes, and inorganic P substrates (i.e., $CaCO_3$) played an important role in the mobilization of soil P.

It should be noted that our batch laboratory incubations may have introduced artifacts related to environmental conditions (e.g., no water exchange; no sunlight; small enclosure “wall” effects), which along with the short-term nature of the observations, might have influenced our findings. We reviewed available data for the one field site (WCA-2A site U3) that has been exposed to SO_4 enrichment for over four decades to assess potential SO_4 effects on P release. The mean TP concentration at this SO_4-enriched (mean of 39 mg SO_4 l^{-1}) site and others within a radius of 1000 ft was 7 μg l^{-1}, which is based on 370 surface water observations dating back to 1974 (South Florida Water Management District, unpublished). Annual porewater SRP concentrations (quarterly samples collected at the 10–20 cm soil horizon interval) from 1995–2001 ranged from 5 to 24 μg l^{-1} (Fink, 2003) at site U3. Data from these sites substantiate our laboratory incubation results: even though high prevailing water column SO_4 levels were observed in this wetland for several decades, surface water P concentrations have remained low.

5. Conclusions

The lack of a soil P mobilization response with SO_4 enrichment during anaerobic incubations is due to a combination of biogeochemical factors present in the northern Everglades marsh and STA soils. These include high alkalinity, P-limited and C-limited substrates, low Fe-associated P pools, sulfide inhibition, and dissolution of an inorganic substrate ($CaCO_3$) associated with P. Results from the laboratory incubations are consistent with the historical record collected at SO_4-enriched WCA-2A U3. While further investigations on SO_4 impacts using controlled field mesocosms are underway, our laboratory findings demonstrate that

SO_4 enrichment neither enhances the mobilization of soil P in northern Everglades soils, nor impairs soil P retention of the STA.

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