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Elevated sulfate reduction in metal-contaminated freshwater lake sediments

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[1] Although sulfate-reducing prokaryotes have long been studied as agents of metals bioremediation, impacts of long-term metals exposure on biologically mediated sulfur cycling in natural systems remains poorly understood. The effects of long-term exposure to metal stress on the freshwater sulfur cycle were studied, with a focus on biologic sulfate reduction using a combination of microbial and chemical methods. To examine the effects after decades of adaptation time, a field-based experiment was conducted using multiple study sites in a natural system historically impacted by a nearby zinc smelter (Lake DePue, Illinois). Rates were highest at the most metals-contaminated sites ($\sim$35 μmol/cm^3/day) and decreased with decreased pore water zinc and arsenic contamination levels, while other environmental characteristics (i.e., pH, nutrient concentrations and physical properties) showed little between-site variation. Correlations were established using an artificial neural network to evaluate potentially non-linear relationships between sulfate reduction rates (SRR) and measured environmental variables. SRR in Lake DePue were up to 50 times higher than rates previously reported for lake sediments and the chemical speciation of Zn was dominated by the presence of ZnS as shown by X-ray Absorption Spectroscopy (XAS). These results suggest that long-term metal stress of natural systems might alter the biogeochemical cycling of sulfur by contributing to higher rates of sulfate reduction.

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1. Introduction

[2] It is well established that metal contamination significantly alters the microbiology of impacted environments [e.g., *Bååth*, 1989; *Giller et al.*, 1998; *Konstantinidis et al.*, 2003; *Moffett et al.*, 2003]. Many studies have examined aerobic processes in soils [e.g., *Doelman et al.*, 1994; *Sverdrup et al.*, 2006; *Vaasquez-Murrieta et al.*, 2006], but much less is known about the effects of metals on microbial communities in anaerobic environments. Yet, in aquatic habitats, anoxic sediments are a major repository for metal contaminants [*Sprenke et al.*, 2000; *Webster et al.*, 2000], in part by formation of highly insoluble metal sulfides [*Morse et al.*, 1987; *Peltier et al.*, 2003]. A main source of sulfides in sediments is by biologic production by the sulfate-reducing prokaryotes (SRP). In addition to producing sulfides, SRP contribute to both carbon and sulfur nutrient cycling in freshwater systems [*Smith and Klug*, 1981a] Formation of metal-sulfides is also thought to reduce the metal's bioavailability [*Gadd and White*, 1993; *Mori et al.*, 2000].

[3] Although SRP have been studied for possible application to metals bioremediation [e.g., *Barnes et al.*, 1994; *Gadd*, 2000], the response of sulfate reduction activity following chronic metal exposure remains unresolved for natural freshwater systems. Many bench-scale studies of pure cultures and enrichments of SRP have shown sensitivity to metals [e.g., *Booth and Mercer*, 1963; *Capone et al.*, 1983; *Chardin et al.*, 2002; *Nordgren et al.*, 1988; *Radha and Seenayya*, 1992; *Ueki et al.*, 1991; *Utgikar et al.*, 2001, 2003], suggesting that metal contamination might repress sulfate reduction. However in apparent contradiction, other studies have reported stimulation of sulfide production following metals stress [*Harithsa et al.*, 2002; *Loka Bharathi et al.*, 1990]. Recent microcosm studies have established that SRP populations adapted to metal contaminated sediments may greater metal tolerance [*Jin et al.*, 2007]. As an additional complicating factor, trophic interactions that sustain SRP in natural environments [*Hamilton*, 1998] can be modified as a result of metals exposure and consequently that long-term adaptation in open systems might differ from responses seen in shorter-term laboratory studies. To examine the impact of metal stress on natural

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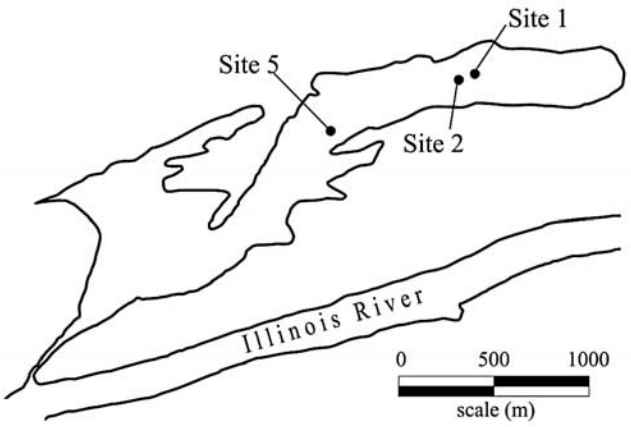

Figure 1. Location of sampling sites in Lake DePue, Illinois, USA (latitude 41°19′N, longitude 89°18′W).

freshwater SRP activities, the present study examined the in situ biological sulfate reduction rates (SRR) in relation to metal contamination levels in the sediments of Lake DePue (Illinois), a lake chronically exposed to metals contamination over multiple decades. A field-based approach designed around multiple study sites within the contaminated lake was used to specifically compare in situ conditions in sediments with long-term adaptation to different levels of contamination. Comparison of SRR and other environmental conditions monitored at the study sites demonstrated a positive trend between increased rates of sulfate reduction and higher levels of metals contamination, suggesting a significant impact of chronic metals contamination on the biogeochemistry of this lake.

2. Methods

2.1. Site Description

[4] Lake DePue, Illinois, USA (latitude 41°19′N, longitude 89°18′W) is a shallow, eutrophic, backwater lake connected by a narrow channel to the Illinois River (Figure 1). The lake is located in the floodplain of the Illinois River and is surrounded by wetlands and marshlands. Similar to other backwater lakes, the lake depth is highly influenced by rainstorm events with a bottom current that drains the lake in the direction of the Illinois River (on-site observation). The lake averages about 2 m in depth at the center during the summer months. Sedimentation rates in the lake have been estimated at between 1.5 and 3 cm per year [*Cahill and Steele*, 1986]. Sediments within this lake become anoxic within the first 1–2 millimeters below the sediment-water interface (data not shown). Metal contamination migrated to the lake sediments from a zinc smelter that operated near the north lakeshore for approximately 80 years [*Cahill and Bogner*, 2002]. This adjacent facility is currently listed on the United States Environmental Protection Agency (USEPA) National Priority List (NPL, aka Superfund, Site ID# ILD062340641). In addition to metal smelting tailings, gypsum deposits are also reported at the facility, potentially explaining the elevated sulfate concentrations monitored in Lake DePue sediments. A man-made creek draining into the northeast corner of the lake is a likely migration path. Past chemical analysis of the lake sediments showed the presence of large concentrations of metal-sulfides [*Webb et al.*, 2000], suggesting the presence of potentially active SRP. Previous work at the study sites in Lake DePue demonstrated that microbial biomass decreased in association with increased pore water Zn and As concentrations [*Gough et al.*, 2008].

2.2. Field Sampling Strategy and Methods

[5] The locations of the study sites were previously identified, and precisely monitored using a hand-held GPS-unit. Past monitoring at the study sites has shown total organic carbon concentrations as high as 50,000 mg/kg. These concentrations were equivalent among the sampling sites, but can vary with time [*Gough et al.*, 2008]. The sediments at the study sites consist of unconsolidated dark grey clays [*Gough et al.*, 2008], and have historically shown zinc contamination levels ranging between 21,400 mg/kg (Site 1) to 3100 mg/kg (Site 5) [*Gough et al.*, 2008].

2.2.1. Preliminary Sampling to Evaluate Alternative SRR Sample Quenching Methods

[6] The initial sampling event (3 May 2001) was primarily to test an altered quenching protocol and was also used to evaluate appropriate incubations times in accordance with the suggestions of *Jørgensen* [1978a]. In earlier site visits, the method conventionally used to quench the on-site incubations for SRR measurements did not quench sulfate reduction in the Lake DePue sediments (data not shown). As the conventional quench relies on the toxicity of zinc acetate [*Fossing et al.*, 2000], a potentially high zinc tolerance of the SRP in these zinc-contaminated sediments could account for this observation. An alternative quench solution was tested that was composed of 50 mM $Na_2MoO_4.2H_2O$ and 6.4% formaldehyde, for a final concentration of approximately 3% after sediment addition. To prevent loss of sulfide during transport, samples were stored in dry-ice.

[7] Sediments were collected from Site 1 (Figure 1) by direct pushing of incubation cores. Incubation cores were constructed from 61-cm-long PVC schedule 40 piping with a 3.8 cm inner diameter, which were modified by beveling the ends and drilling sampling ports down one side in 1 cm intervals. Sampling ports were filled with aquarium grade silicone. In eight cores, 6 μL carrier free $Na^{35}SO_4$ (approx. 10 μCi) were injected across the inner diameter of the top three 1-cm intervals, a method previously shown to facilitate even diffusion of the tracer throughout each 1 cm sediment core interval [*Jørgensen*, 1978a]. Intact cores were incubated approximately 30, 60 and 90 minutes (duplicates for 30 minutes and triplicates for 60 and 90 minute time points) in a large container of lake water that maintained temperatures comparable to the shallow lake water throughout the incubation period. To terminate incubation, 1-cm thick sediment slices were extruded into 10 mL of quenching solution, and frozen in dry ice for transport. Controls were established by quenching the top three 1-cm increments of two replicate cores prior to addition of $Na^{35}SO_4$. Sediments from a final (eleventh) replicate core were extruded in 1-cm intervals into 50-ml screw top disposable centrifuge tubes, and transported to the laboratory on ice under N_2 gas for sulfate analysis. Extrusion and all on-site

sediment manipulations were done under a stream of N_2 gas.

2.2.2. Main Sampling Event for Between Site Comparisons of SRR and Metal Concentrations

[8] In the main study (18 June 2001), sediments were collected from three sites (Figure 1, site names correspond with our earlier studies in the lake [*Gough et al.*, 2008]). Four replicate cores from each site were used for SRR (triplicate incubations and one quench control), samples from one core were dedicated for zinc speciation using X-ray absorption fine structure (XAFS) and samples from the other two cores were used for pore water chemical analysis and bulk sediment density determination. On the day of sampling, the incubation cores (as described for the preliminary sampling event) were too short to be directly pushed from the side of the boat because the lake water level was deeper than normal (1.5 m instead of 0.5 m) as a result of a storm event 1 wk earlier. Instead, incubation cores were sub-cored from larger-diameter piston cores. The piston cores were made of thin-walled PVC sleeves (183-cm-long and 6-cm inner diameter) and equipped with metal extension rods. An expandable O-ring in the piston core preserved the sediment–water interface and minimized compression of the sediment layers. At each site, the top four 1-cm increments were studied in two cores, and the top ten 1-cm increments in the third core, with incubations of approximately 60 minutes; actual times were recorded. A fourth incubation core from each site was used as a quenched control as described for the preliminary study. Extrusion and all on-site sediment manipulations were done under a stream of N_2 gas.

[9] The three additional cores collected in June 2001 used 244-cm-long, schedule 40 PVC tubing with a beveled end. Sediments extruded for XAFS analysis were immediately mounted into sample holders, secured with Kapton tape, and placed into a portable liquid nitrogen Dewar vessel to minimize chemical transformation prior to analysis. The two remaining cores were processed to provide duplicate samples for chemical analysis at each site. After extruding a targeted interval, sediments were briefly homogenized and divided between 50 mL disposable centrifuge tubes for pore water analysis (DOC, sulfate and dissolved metal) and large-mouth sterile disposable sampling cups for bulk analysis (wet density, moisture content, and total metal concentration). Both containers were transported on ice in airtight boxes under N_2 gas.

[10] One replicate incubation control core from Site 5 was discarded as radio-labeled injections were not properly delivered as evidenced by low total activity counts. Coordinate mapping indicated that one incubation core and one of the parallel cores collected for bulk sediment and pore water analysis from Site 2 were collected from outside the predetermined Site 2 area. Results from these two cores were excluded from further analysis and the samples reported as ''lost'' in the subsequent sections of this manuscript.

2.2.3. Follow-Up Sampling Event for Evaluations of Cultivable SRB Concentrations

[11] During a follow-up sampling event (October 2002), sediment was collected for most-probable-number (MPN) culture analysis from Sites 1 and 5 using an Eckman dredge grab sampler. Large-mouth sterile disposable sampling cups were filled with sediment, leaving no headspace, and transported on ice.

2.3. Sulfate Reduction Rates

[12] Sulfate reduction rate (SRR) samples were processed as previously described [*Fossing and Jørgensen*, 1989; *Jørgensen*, 1978a] using the modified quenching protocol described above. Prior to thawing, 37 μL of 30% zinc acetate was added to ensure that sulfide was immobilized. Note that zinc acetate addition at earlier processing steps caused a precipitant to form in the presence of molybdate (data not shown). 100 μL supernatant of centrifuged samples (4,500 g, 10 minutes) were added to 3 mL scintillation fluid (Ultima Gold High-Flash-Point Universal LSC cocktail, Packard) and the activity was measured using a liquid scintillation counter (Tricarb 1900 TR from Packard) to determine the amount of ^{35}S-sulfate remaining in the samples. To release sulfides from the sediments, approximately 1 g of sediment pellet was distilled by heating with 12 M HCl, and 1 M Cr^{+2} in 0.5 M HCl. Liberated H_2S (including $H_2^{35}S$) was trapped in 7 ml of 5% zinc acetate. This solution was added to 14 ml of scintillation fluid and the activity associated with the $H_2^{35}S$ was measured using a scintillation counter.

[13] SRRs were calculated using previously published equations [*Fossing and Jørgensen*, 1989; *Jørgensen*, 1978a] as follows:

$$SRR\left(\frac{nmol}{cm^3 \cdot d}\right) = \frac{[\text{fraction of } {}^{35}SO_4 \text{ converted}]\left[\text{sulfate concentration}\left(\frac{nmol}{cm^3 \text{sediment}}\right)\right]}{[\text{incubation time(days)}]} \times [\text{isotope fractionation factor}]$$

Select calculations used to determine the fraction of sulfate conversion are presented in Table 1. Procedural controls were established by adding the sediments to the quenching solutions prior to inoculation with the radio-labeled sulfate to demonstrate that the quenching solution was effective in prohibiting further sulfate conversion. An isotope fractionation factor of 1.06 was used as a correction factor for the biologic preference for lighter sulfate, a value typically used with these methods, though a range of fractionation factors has been suggested [*Detmers et al.*, 2001]. Samples found to have substantially lower total activity (either controls or incubations) were considered to have not received proper $Na^{35}SO_4$ inoculations. Specifically, the total ^{35}S activity was significantly lower for Site 1 Core A 8–9 cm and Site 3 Core C 3–4 cm (Table 1, ''total disintegrations in sample'' shown at the bottom) than for the other samples processed that day, and were removed from further data analysis, recording these as ''lost''.

2.4. Most Probable Number Determination

[14] Dilution series were established in triplicate using defined media for the cultivation of SRP from freshwater lake sediments [*Widdel and Bak*, 1992] and electron donors and carbon sources common for eutrophic lake sediments (acetate/H_2/CO_2, propionate, or lactate) [*Smith and Klug*, 1981b]. Neither carbon source nor electron donor was added to a fourth control series. Sediment was first diluted in an anaerobic glove box by adding approximately 1 g sediment from the center of the sampling cups to 45 ml of media (approximate initial dilution of 1:46, actual dilution

Table 1. Calculations to Determine the Fractions of Radiolabeled Sulfide and Sulfate Following Incubation for Select Samples and Process Controls

			Calculations for Sulfide Fraction					Calculations for Sulfate Fraction							
Core ID	Sample Depth	Total Sediment Sample Mass (g)	Mass of Sediment After Centrifuge (g)	Mass of Ccentrifuged Sediment Distilled (g)	Wet[b] Sediment Density (g/cc)	Instrument Count (dpm)	Disintegrations[c] per Sediment Volume (dpm/cc)	Water[b] Content (mass %)	Porosity[b] (Pore vol: Total vol)	Total Sample Pore Water (ml)	Total Quench Volume (ml)	Instrument Count (for 0.1 ml Liquid) (dpm)	Disintegrations[d] per Sediment Volume (dpm/cc)	Total[e] Disintegrations in Sample (dpm/cc)	Fraction of Sufate Converted
							Controls[a]								
							May 2001 sampling event to evaluate alternative quenching methods								
Site 1, Control A	0–1	13.47	10.96	1.23	1.16	41.7	32.1	57%	0.66	7.69	10.35	158,890	2,468,056	2,468,089	0.001%
	1–2	12.72	10.64	1.91	1.17	50.2	25.7	58%	0.68	7.40	10.41	114,468	1,871,064	1,871,090	0.001%
	2–3	16.10	13.08	1.31	1.25	31.2	24.2	52%	0.65	8.38	10.42	130,166	1,904,563	1,904,588	0.001%
Site 1, Control B	0–1	15.72	12.46	1.56	1.16	57.8	34.1	57%	0.66	8.98	10.38	157,867	2,254,497	2,254,531	0.002%
	1–2	14.18	11.59	2.30	1.17	97.6	40.5	58%	0.68	8.24	10.38	112,054	1,718,609	1,718,650	0.002%
	2–3	16.64	13.92	2.28	1.25	40.8	18.8	52%	0.65	8.67	10.33	114,847	1,641,856	1,641,874	0.001%
							July 2001 sampling event for comparative site study								
Site 1, Control	0–1	11.92	8.25	2.13	1.16	892	336	68%	0.79	8.16	10.43	61,339	1,108,693	1,109,029	0.030%
	1–2	13.85	10.02	2.37	1.17	461	164	62%	0.73	8.63	10.40	55,976	898,082	898,246	0.018%
	2–3	17.20	13.68	2.45	1.25	71.7	29.2	61%	0.77	10.53	10.40	51,513	785,153	785,182	0.004%
	3–4	15.47	12.49	2.19	1.18	1226	532	61%	0.72	9.42	10.36	58,931	887,762	888,294	0.060%
Site 2, Control	0–1	17.20	13.51	3.44	1.05	1386	331	71%	0.75	12.27	10.34	45,915	631,036	631,367	0.052%
	1–2	16.68	13.80	2.28	1.02	146	54	65%	0.67	10.88	10.33	55,922	728,360	728,414	0.007%
	2–3	14.31	12.16	2.12	1.12	2359	1055	65%	0.72	9.26	10.32	58,525	893,014	894,068	0.118%
	3–4	16.15	14.01	1.92	1.17	937	494	63%	0.74	10.18	10.35	62,655	931,039	931,533	0.053%
							Replicate cores for select intervals								
							May 2001 sampling event to evaluate alternative preservative methods								
Site 1, Core A	0–1	18.66	13.83	2.31	1.16	21642	8045	57%	0.66	10.66	10.40	96,194	1,259,034	1,267,079	0.63%
Site 1, Core B	0–1	13.29	10.91	1.84	1.16	20729	10746	57%	0.66	7.59	10.44	102,620	1,614,747	1,625,493	0.66%
Site 1, Core A	2–3	19.82	16.37	1.83	1.25	7903	4471	52%	0.65	10.32	10.30	53,339	695,098	699,569	0.64%
Site 1, Core B	2–3	17.31	14.00	1.57	1.25	21503	13899	52%	0.65	9.01	10.45	114,483	1,612,253	1,626,152	0.85%
							July 2001 sampling event for comparative site study								
Site 1, Core A	1–2	16.20	12.16	2.53	1.17	860,198	297,955	62%	0.73	10.09	10.40	11,748	173,504	471,459	63%
Site 1, Core B	1–2	16.15	12.98	2.42	1.17	989,020	382,938	62%	0.73	10.06	10.37	11,910	175,958	558,896	69%
Site 1, Core C	1–2	12.05	9.50	1.96	1.17	471,069	221,064	62%	0.73	7.51	10.38	12,486	216,456	437,519	51%
Site 1, Core A	2–3	14.76	11.91	2.51	1.25	893,861	360,315	61%	0.77	9.03	10.39	7,973	131,397	491,712	73%
Site 1, Core B	2–3	15.52	12.40	2.64	1.25	1,003,862	380,701	61%	0.77	9.50	10.39	6,426	103,185	483,886	79%
Site 1, Core C	2–3	14.21	11.80	2.75	1.25	1,199,181	453,391	61%	0.77	8.70	10.40	8,044	135,435	588,826	77%
Site 2, Core A	1–2	16.55	12.76	2.81	1.02	317,125	89,118	65%	0.67	10.79	10.36	36,886	483,078	572,196	16%

Table 1. (continued)

			Calculations for Sulfide Fraction					Calculations for Sulfate Fraction							
Core ID	Sample Depth	Total Sediment Sample Mass (g)	Mass of Sediment After Centrifuge (g)	Mass of Ccentrifuged Sediment Distilled (g)	Wet[b] Sediment Density (g/cc)	Instrument Count (dpm)	Disintegrations[c] per Sediment Volume (dpm/cc)	Water[b] Content (mass %)	Porosity[b] (Pore vol: Total vol)	Total Sample Pore Water (ml)	Total Quench Volume (ml)	Instrument Count (for 0.1 ml Liquid) (dpm)	Disintegrations[d] per Sediment Volume (dpm/cc)	Total[e] Disintegrations in Sample (dpm/cc)	Fraction of Sufate Converted
Site 2, Core C	1–2	13.34	10.72	2.63	1.02	1,120,661	350,775	65%	0.67	8.70	10.38	9,303	136,361	487,136	72%
Site 2, Core A	2–3	15.23	12.24	2.26	1.12	1,117,342	442,510	65%	0.72	9.85	10.44	25,046	372,251	814,761	54%
Site 2, Core C	2–3	16.65	12.35	2.79	1.12	924,204	274,111	65%	0.72	10.77	10.36	8,100	114,646	388,758	71%
Site 5, Core A	1–2	14.88	12.30	2.67	1.18	415,436	152,140	66%	0.78	9.78	10.42	21,550	345,952	498,092	31%
Site 5, Core B	1–2	12.17	8.64	2.17	1.18	545,918	211,533	66%	0.78	7.99	10.36	23,752	423,772	635,305	33%
Site 5, Core C	1–2	24.76	17.23	2.25	1.18	818,322	298,645	66%	0.78	16.27	10.36	30,145	383,347	681,992	44%
Site 5, Core A	2–3	15.15	12.82	2.62	1.17	895,872	337,990	64%	0.75	9.73	10.40	23,921	371,333	709,323	48%
Site 5, Core B	2–3	14.77	10.76	2.04	1.17	406,911	169,834	64%	0.75	9.48	10.38	41,952	659,376	829,210	20%
Site 5, Core C	2–3	19.94	15.18	2.97	1.17	327,127	97,916	64%	0.75	12.81	10.36	8,917	121,005	218,922	45%
July 2001 samples that were excluded due to poor labeled-sulfate injections															
Site 2, Core A	8–9	17.87	12.72	2.63	1.26	32,483	11,063	58%	0.73	10.43	10.34	2,469	36,117	47,180	23%
Site 3, Core C	3–4	11.52	9.34	2.86	1.16	29,253	9,648	63%	0.73	7.22	10.36	852	15,106	24,753	39%

[a]Controls were established by mixing the sediments with the quenching solution prior to addition of the radiolabeled sulfate to demonstrate that the preservation technique adequately stopped sulfate reduction.
[b]Calculated in parallel cores collected for each site in July 2001.
[c]Calculated as: (instrument count x wet density/mass of sediment distilled) (mass of sediment after centrifuge/total sediment sample mass).
[d]Calculated as: [Instrument count/(0.1 ml processed/porosity)] x [(quench volume + total pore volume)/total pore volume].
[e]Samples (including controls) with significantly lower total sample dpm concentrations were assumed to have jeopardized during ^{35}S-sulfate inoculation, and were not included in data analysis.

determined by mass). Subsequent dilutions were prepared using syringe transfers. Dilution series tubes were incubated in the dark at room temperature and sulfide production was determined colorimetrically in comparison to incubation controls [*Widdel and Bak*, 1992] at 1 wk, 2 wks, 4 wks, 2 mos, and 10 mos. The MPN of cultivable SRP and mean error of estimates were calculated using the methods originally developed by *Thomas* [1942].

2.5. Basic Sediment Characterization

[15] Bulk sediment density was calculated by simultaneously recording water displacement (volume) and mass of a sediment aliquot. Water content was determined by recording the mass lost during overnight drying at 100°C. Pore water was recovered by centrifugation (4500 g, 10 minutes). Dissolved organic carbon (DOC) was measured in filtered pore water (0.45-μm glass fiber, Millipore) using a Tekmar-Dohrmann Apollo 9000 TOC Combustion Analyzer operated in general accordance with USEPA Method 415.1 [*USEPA*, 1983]. Sulfate concentrations were measured using filtered porewater (0.22 μm polycarbonate, Fisherbrand) with a Waters Capillary Ion Analyzer (CIA) Quanta 2000.

2.6. Metal Analysis

2.6.1. Pore Water Metal Analysis

[16] Filtered pore water (0.22 μm cellulose acetate, Millipore) was preserved using trace metal grade HNO_3 (2% final concentration). Samples were diluted with de-ionized water (Milli-Q RG) as necessary to match the instrumental analytical window, and maintained at the same final pH by addition of HNO_3. Dissolved pore water metals (As, Cd, Cr, Cu, Mn, Pb, and Zn) were analyzed in general accordance with EPA Method 200.8 [*USEPA*, 1991] using a VG elemental inductively coupled plasma-mass spectrometer (ICP-MS) Model PQ ExCell.

2.6.2. Total Metal Analysis

[17] Total acid leachable (''total'') metal concentrations were determined using a concentrated (70%) HNO_3 extraction of oven dried sediments. The supernatant was filtered (0.22 μm cellulose acetate, Millipore) and analyzed by flame atomic absorption spectroscopy (FAAS) using a GBC model 920.

2.6.3. X-ray Absorption Fine Structure (XAFS)

[18] Metal speciation analysis was performed on the bending magnet beam line of the DND-CAT at the Advanced Photon Source, Argonne National Laboratory. Briefly, a Si(111) monochromator was used to vary the X-ray energy from approximately 200 eV below to approximately 1000 eV above the absorption K edge of Zn (9659 eV) [*Gaillard et al.*, 2001]. The incident and transmitted intensities were measured with IC Spec ionization detectors (Oxford Instruments—now Oxford-Danfysik Instruments). Fluorescence was measured with a Stern-Heald ''Lytle'' detector. XAFS measurements were acquired in continuous scanning mode (CS-XAS). The contribution of various Zn species to the spectra was determined using quadratic linear programming [*Vandenberghe and Boyd*, 1996] to fit the sample spectra to a linear combination of standard reference spectra.

2.7. Data Analysis

[19] Differences in environmental parameters and metal concentrations recorded between the three sites were monitored using analysis of variance (ANOVA) with a 5% level of significance. When statistical differences were found, Tukey's method (pair-wise differences) was used to identify the source of variation [*Larsen and Marx*, 1986].

[20] Neuroet, an artificial neural network (NN) package [*Noble and Tribou*, 2006], was used to investigate nonlinear relations among independent variables (inputs: sample depth, sulfate concentration, and pore water concentrations of Zn, As, Cr, and Cd) and dependent variables (output: sulfate reduction rate). As a control, two sets of random data (Microsoft EXCEL® random number generator) were also included as input variables. All combinations of up to four independent variables were analyzed. The following settings were used to train the NNs: input data scaling (−1 and 1); output data scaling (mean of 0 and a standard deviation of 1); the hyperbolic tangent transfer function was used for two hidden neurons and pure linear transfer function was used for one output neuron; and during training, error was minimized using a Levenberg–Marquardt algorithm. Training was terminated when the sum of squares of the residuals (SS) ceased to decrease by 0.001 over a minimum of 10 billion clock cycles on a Motorola G4 processor or 10 training iterations (whichever was longer). For each combination of input variables, 15 NN runs were generated and compared using the corrected Akaike's Information Criterion (AIC_c) [*Motulsky and Christopoulos*, 2003]. The lower 25th percentile of replicate model runs were considered as potentially fixed in local error minima and removed. This procedure was then repeated 10 times for each input variable combination, and the averaged AIC_c were used for ranking the models. Relative differences between the ranked models were considered by calculating the probability (p_{model}) that one model was more predictive than another and the evidence ratio (E) as previously described [*Motulsky and Christopoulos*, 2003].

3. Results

3.1. Optimization of Sulfate-Reduction Protocol

[21] The altered quenching protocol was shown to work for the Lake DePue sediments, as demonstrated by four key observations. The control samples had substantially lower sediment-associated ^{35}S than soluble ^{35}S (Table 1), demonstrating that (1) the formaldehyde and molybdate solution adequately stopped sulfate reduction and (2) sulfate had not been liberated during the acid reduction of the sediments. Further the incubated samples had much higher levels of sediment-associated activity than soluble activity in comparison to the controls (Table 1), demonstrating that (3) the zinc acetate added just prior to centrifugation had adequately trapped the sulfides during sample processing, and (4) that molybdate-complexes had not prevented liberation of the sulfides during the acid-reduction of the sediments.

[22] Comparison of time series incubations revealed that, for the 1–2 cm sample depth, the amount of sulfate uptake potentially decreased at the last time point (Figure 2), indicating that nutrient limitations may have impacted the activities measured in sediments incubated for 90 minutes. As a 30-minute was found to be inadequate time for on-site sample processing, a 60-minute incubation time was selected for subsequent studies. Using the data from the 60-minute incubations, SRR was calculated to range from

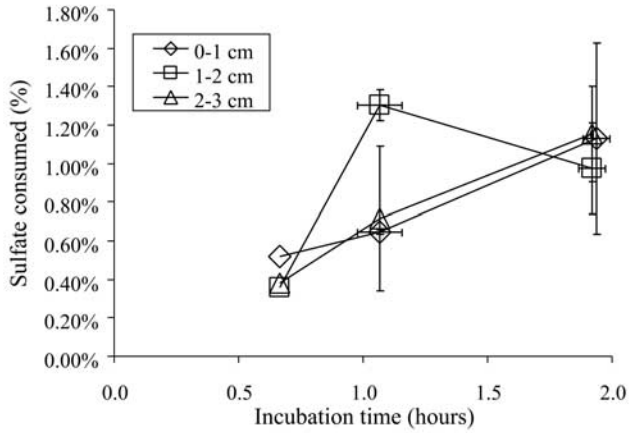

Figure 2. Sulfate consumption during intact core incubations as a function of time for three sample depths (Site 1, May 2001). Error bars represent average deviation of duplicate cores. Replicate data for the (approximately) 0.5 hr incubations were lost during processing.

200 to 1000 nmol/cc/d among the Site 1 depth layers in May 2001.

3.2. Sulfate Reduction Rates and Sulfate Concentrations

[23] Sulfate concentrations in the pore waters ranged from 0.2 to 4 mM. SRR in June 2001 ranged from 500 to 37,000 nmol/cm^3/day among the Sites and depths (Figure 3), and declined with increased distance from the contamination source (statistically significant difference between sites when one compares the top 6 cm intervals, F = 8.81, $p(2,15) = 0.0029$). When integrated over the sampling depth, the resulting flux rates for sulfate were 1.62 mol/m^2/day at Sites 1, 1.16 mol/m^2/day at Sites 2, and 0.71 mol/m^2/d at Sites 5. Similarly, sulfate concentrations were lower at Site 5 than at Sites 1 and 2 (Figure 3); however differences among the sampling sites could not be discerned statistically (F = 0.294, $p(2,27) = 0.748$) because of the high level of variability along the depth profiles. Sulfate concentrations at Sites 1 and 2 generally decreased with sampling depth, whereas at Site 5 no specific trends were observed with depth in part because of the higher variation between the duplicate cores. SRR at Site 1 were substantially higher in June while sulfate concentrations were five times lower. Similar relative SRR increases between early May and mid-June have previously been documented in other lake systems in association with natural seasonal variation [*Li et al.*, 1999].

3.3. MPN of SRP

[24] Evaluation of three substrates previously identified as potential carbon/energy sources for SRB in eutrophic freshwater sediments[*Smith and Klug*, 1981b] revealed that the most probable number (MPN) concentrations for cultivable SRP grown on acetate/H_2/CO_2 were slightly higher at Site 1 than at Site 5 (Figure 4), while the opposite was seen for growth on lactate. Sums of the two MPN concentrations of the SRB capable of growing under laboratory conditions using either acetate/H_2/CO_2 or lactate were equivalent between the two sites, within the error of the MPN technique. Little or no growth was observed with propionate.

3.4. Metal Concentrations

[25] Pore water metal concentrations varied both between sites and with sample depth (select data, Figure 3). Duplicate cores showed variable results, as reflected by the large error bars in Figure 3. Pore water Mn concentration were more consistent with depth, and averaged 37 μM ± 3, 28 μM ± 7, and 17 μM ± 7 along the depth profiles of Sites 1, 2 and 5 respectively. Averaged site concentrations of pore water Zn, As, and Mn were statistically different between the sites (Zn, F = 6.73, $p(2,27) = 0.00425$; As, F = 11.352, $p(2,27) = 0.00026$; Mn, F = 22.24, $p(2,37) = 0.000002$). Statistical differences were not detected among the sampling sites for pore water concentrations of Cd or Cr (Cd, Site 1: 2.4 nM ± 1.5, Site 2: 3.5 nM ± 3.4, and Site 5: 1.0 nM ± 0.7; and Cr, Site 1: 65 nM ± 17, Site 2: 71 nM ± 31, and Site 5: 63 nM ± 11).

[26] Total metal concentrations generally decreased with distance from the contamination source (Figure 5), and remained consistent along the depth profile (variation presented as error bars in Figure 5). Of those monitored, Fe and Mn did not vary statistically between the sites. Total Pb concentrations were highest at Site 2, an exception to the general distribution pattern.

[27] The chemical speciation of Zn in sediments determined by XAFS (Figure 6) revealed that a significantly larger fraction of Zn was associated with sulfides at Site 5 than at either Sites 1 or 2 (F = 9.04, $p(2,15) = 0.0027$). Differences in the carbonate associated fraction of zinc between the sampling sites were not statistically significant (F = 2.26, $p(2,15) = 0.138$) and no statistical change in speciation was observed as a function of sediment depth (data not shown).

3.5. Sediment Characteristics

[28] Wet bulk sediment density ranged from 1.02 to 1.29 g/cc, and moisture content ranged from 65% to 82% (select data, Table 1). Because all samples were saturated, porosity could be determined from moisture content and bulk density. Neither wet sample density nor moisture content varied significantly among the sampling sites (respectively, F = 2.627, $p(2, 27) = 0.091$; and F = 2.698, $p(2, 27) = 0.085$). Wet sample density generally increased and moisture content decreased with sampling depth (data not shown), reflecting decreasing void space with depth. Conversely, DOC, which ranged from 0.70 to 2.41 mM C, was higher at Site 1 than at Site 5 (F = 7.633, $p(2,27) = 0.0002$). Within each site, increased SRR was associated with decreased DOC, however DOC did not predict between-site SRR differences (Figure 7).

3.6. Relating SRR to Metal Concentrations

[29] Data used in the neural network analysis were collected during a single day (June 2001) to focus the results on identifying the influential metal concentrations rather than on variation associated with temporal seasonal changes, which have previously been documented to potentially influence microbial sediment activities [*Bosshard et al.*, 2000; *Newton et al.*, 2006; *RooneyVarga et al.*, 1997] including up to two order-of-magnitude increases in sulfate reduction during a similar seasonal time frame [*Li et al.*,

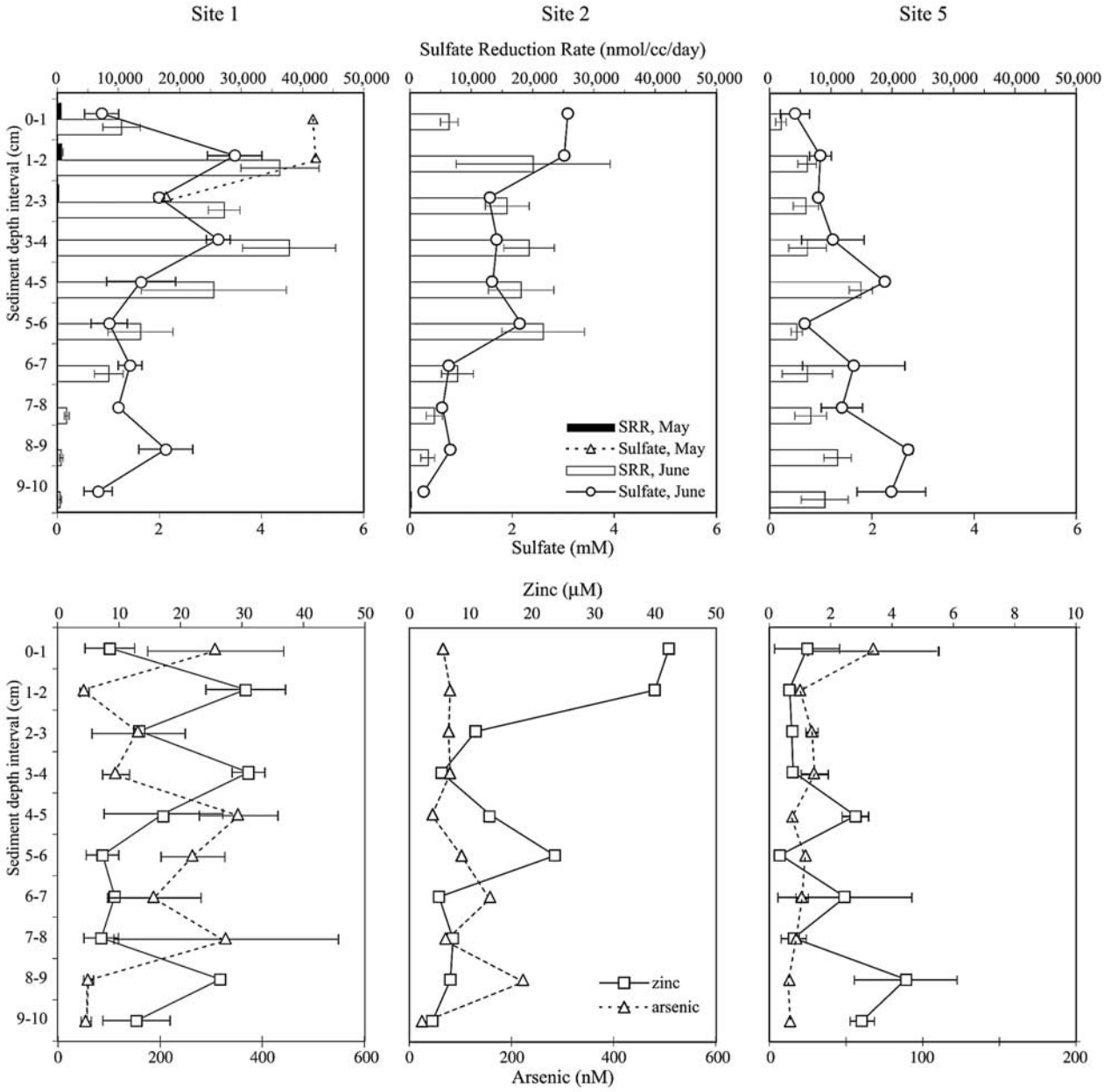

Figure 3. Sulfate reduction rates (SRR) and sulfate concentrations (top panels, May 2001 for Site 1; May and June 2001 for Sites 2 and 5), and pore water concentrations of Zn and As (bottom panels, June 2001) along a sediment depth profile for three sites in Lake DePue. Error bars for SRR at and above 4 cm indicate the average deviation of triplicate cores. Below 4 cm sample depth error bars indicate the combined errors of parameters used to calculate SRR (sulfate concentrations, wet densities and water content in duplicate cores, and averaged scintillation count variation in experimental replicates). Error bars for sulfate and metals analysis indicate the average deviation of duplicate cores. For Site 2, sulfate and metal concentrations represent results from a single core and SRR incubation cores were in duplicate.

1999]. Using this approach, pore water Zn concentrations appeared in the highest 3 ranked NN models, and pore water As appeared in the second highest ranked model (Table 2). In comparison to models using only sulfate concentration and depth, addition of pore water Zn or pore water As improved prediction of SRR ($p = 99.9\%$ and $p = 98.5\%$ respectively; $E = 1200$ and $E = 65$, respectively). The addition of random input data decreased predictability (data not shown), further demonstrating that the variability in the measured SRR followed a predictable pattern and was not simply associated with random variation.

4. Discussion

[30] Biologic sulfate reduction has been long been studied in freshwater and marine sediments to understand the

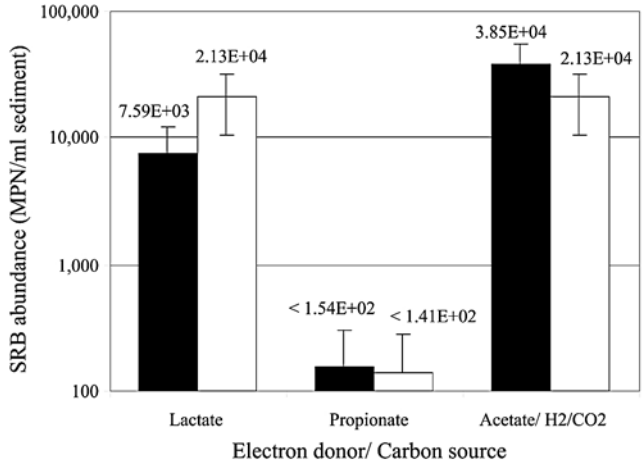

Figure 4. Most probable number of SRB abundance in sediments from Sites 1 and 5 using specified electron donors and carbon sources. Black bars show data from Site 1, and white bars show data from Site 5. Error bars indicate the calculated mean error of estimates [*Thomas*, 1942].

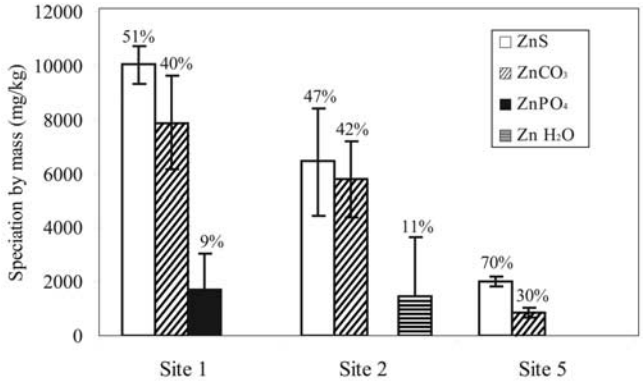

Figure 6. Zinc speciation averaged over the top 10 cm of Lake DePue sediments, as determined by XAFS. Speciation is shown by mass (bars) and by percent (number at the top of each bar). Error bars indicate the average deviation of speciation in ten 1-cm depth intervals (n = 10).

influences of this process on both the carbon and the sulfur cycles [e.g., *Ingvorsen and Brock*, 1982; *Smith and Klug*, 1981a; *Thamdrup et al.*, 2000]. It has been established that the biologic actions of the SRP community work to continually recycle the relatively low concentrations of sulfur found in freshwater systems back to the water column, preventing burial of this essential nutrient [*Holmer and Storkholm*, 2001]. Metal contaminants have been implicated in altering many biologic activities [*Lee et al.*, 2002; *Renella et al.*, 2004], and indeed, this study has shown a significant disruption in normal biogeochemical processing of sulfate associated with long-term metal contamination. However, unlike the inhibition often expected with metals contamination, sulfate reduction rates were higher in more contaminated sediments within the study (Figure 3) in association with pore water As and Zn concentrations (Table 2). This result was in contrast to many bench-scale studies that have reported decreased activities associated with metal stress both for sulfate reduction [*Capone et al.*, 1983; *Jin et al.*, 2007] and for other anaerobic activities [*Kong et al.*, 1994; *Togna et al.*, 2001]. Still, our field-based experimental results are not without laboratory precedent, as metals have also been reported to stimulate community respiration [*Fliessbach et al.*, 1994; *Khan and Scullion*, 2002; *Renella et al.*, 2004], including sulfide production in enrichment studies [*Harithsa et al.*, 2002; *Loka Bharathi et al.*, 1990]. While it is interesting to note that the MPN concentrations of cultivable SRP were similar between Sites 1 and 5, it is important to consider both that MPN testing

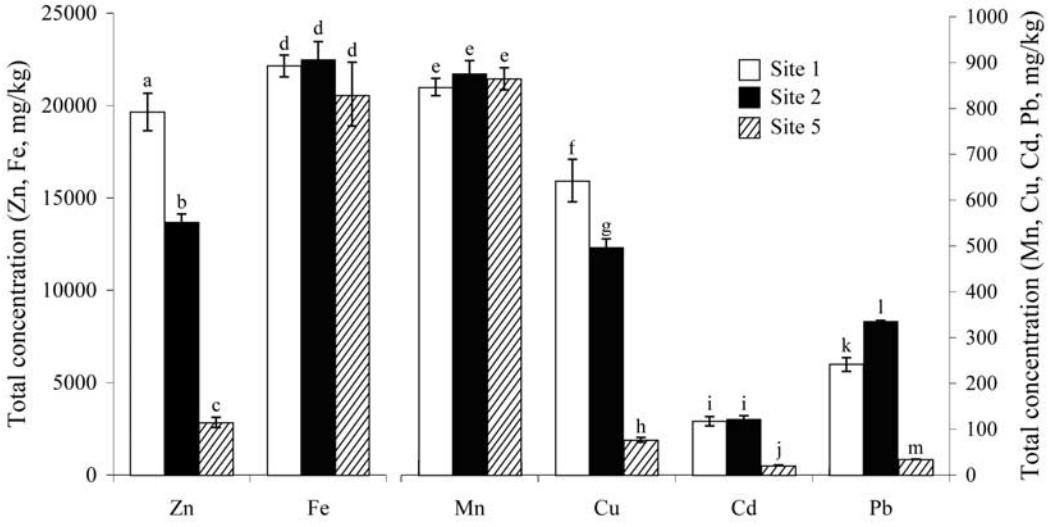

Figure 5. Total metal concentrations (Zn, Fe, Mn, Cu, Cd, and Pb) in Lake DePue sediments. Error bars indicate the average deviation of two depth intervals (0–1 and 5–6 cm depths) from duplicate cores (n = 4). The duplicate core for Site 2 was lost in processing (n = 2). Concentrations within each metal grouping with different lower case letters were significantly different as determined by Tukey's method (α = 0.01).

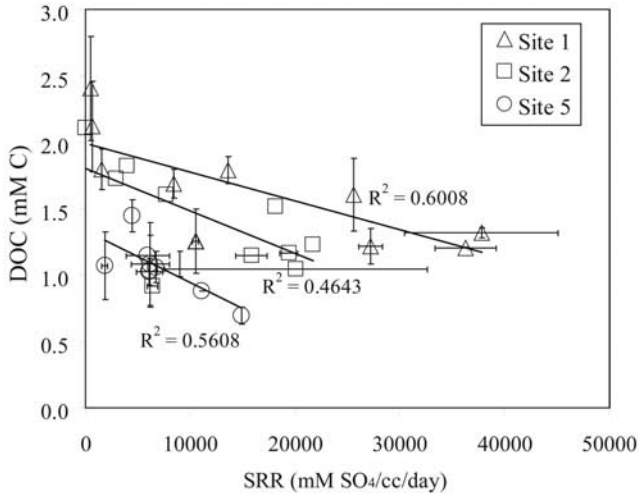

Figure 7. Dissolved organic carbon (DOC) as a function of sulfate reduction rates (SRR). Correlation trends (solid-lines) and correlation index (R^2) are indicated for each site.

indicates the concentrations of viable microbes capable of growth under the specified conditions, and that population concentrations do not necessarily predict population activities [*Roling*, 2007]. Reports of cellular SRP respiration vary by as much as three-orders-of-magnitude [*Jørgensen*, 1978b; *Ravenschlag et al.*, 2000; *Sahm et al.*, 1999]; a range that is broad enough to encompass SRR detected among the sites in this study at Lakes DePue, as well as those rates reported for uncontaminated sediments in nearby Lake Mendota and Lake Wintergreen (Table 2).

[31] Because of the high levels of natural heterogeneity, comparisons among lakes can be complicated and selection of appropriate controls is inherently difficult for field-based studies. When studying the impacts of an environmental contaminant, a seemingly reasonable approach might be to compare results to samples collected from an uncontaminated control site. However the extent of natural variation expected in sediment ecology between two lakes is not well established. Indeed, while both sulfate and SRR were higher than typically reported for freshwater sediments (Table 3) without detailed study of each system it is difficult to identify the factors most contributing to the observed differences. Microbial activity in sediments is influenced by many factors such as nutrient availability (including sulfate) [*Schallenberg and Kalff*, 1993; *Sobczak et al.*, 1998], sediment texture (i.e., sand versus clay content) [*Albrechtsen and Winding*, 1992; *Girvan et al.*, 2003], light availability, wet/dry cycles [*Fierer et al.*, 2003], benthic plant growth [*England et al.*, 1993] as well as many other factors. Therefore, differentiating between variation associated with a contaminant, and natural variation may not be possible when comparing two different lake systems. As an alternative approach, we selected three sites within the same lake system that had differing levels of metal contamination. We were able to reduce the influence of potentially confounding variables on the results by selecting sampling sites with similar environmental characteristics. Still, site monitoring revealed two potentially important parameters that varied significantly among the samples: DOC and sulfate concentrations. Examination of relations between DOC and SRR within a single site revealed that increased SRR was associated with DOC depletion (Figure 7), as might be expected if the SRP were responsible for DOC consumption. However trends between sites were more ambiguous and did not seem to predict the observed high SRR at Site 1 (Figure 7). Similarly, several lines of evidence suggest that sulfate concentration alone did not control the SRR in this study. Firstly, since sulfate concentrations were consistently above the established limiting concentration of 0.2 mM [*Ingvorsen et al.*, 1981; *Smith and Klug*, 1981a; *Spear et al.*, 2000]. Further study is needed to determine if this limiting concentration is applicable to metal-contaminated sediments, though, higher sulfate concentrations have been measured in freshwater sediment without correspondingly higher sulfate reduction rates (e.g., *Meier et al.* [2000], Table 3). Secondly, neural network analysis supported metal contaminants as likely influences in the SRR rates. Thirdly, the fraction of sulfate converted during the incubation was also higher in the metals contaminated sediments (data for select intervals are shown in Table 1), and calculation of this parameter is independent of the measured pore water sulfate concentrations. Of additional interest, sulfate depletion could not account for the observed reduced activity with increased depth in Lake DePue, and the parameter responsible for decreased activity with depth was not identified in this study.

[32] In addition to high sulfate reduction rates, the Lake DePue sediments had high levels of zinc-bound sulfides (Figure 6). Using the calculated sulfate consumption (sulfide production) rates observed in Lake DePue, it would be estimated to take 4 years to generate the amount of sulfide bound to zinc along the 10 cm depth profile monitored at Site 1. When the sulfide bound to iron and other metals are also considered this time frame would be substantially longer. Thus the sulfide concentrations observed in the sediments are more than adequate to account for the high levels of sulfide generated by the SRP in these sediments.

[33] Further investigation is needed to identify the mechanisms that cause SRR stimulation associated with heavy metal contamination. However, based on previously published work, several mechanisms might be postulated. One potential mechanism is stimulation of sulfate reduction by metals sequestration of sulfide. Metal concentrations in Lake DePue are significantly higher than in other lake sediments where SRR has previously been studied. In fact, sediment zinc concentrations in some areas of Lake DePue

Table 2. Top Ten Neural Network Models Ranked by Corrected Akaike's Information Criterion (AICc)

Model Parameters	AICc Score[a]
Depth, sulfate, Zn	−3.52 ± 1.80
Depth, sulfate, Zn, As	2.37 ± 3.10
Sulfate, Zn	5.27 ± 1.99
Depth, sulfate, As	6.08 ± <0.01
Sulfate, Zn, Cd	6.29 ± <0.01
Depth, sulfate, Zn, Cr	7.11 ± 1.31
Depth, sulfate	10.71 ± <0.01
Depth, Zn	10.86 ± 1.30
Depth, sulfate, Cd	10.95 ± 3.22
Sulfate, Zn, As	12.07 ± 2.09

[a]Average of ten sets of 15 analyses ±SD.

Table 3. Summary of Reported Sulfate Reduction Rates From Various Sediments Environments

Sample Source	Sulfate (mM)	Maximum SRR (nmol/cc/d)	Reference
Freshwater Sediments			
DePue study	3.5	37,800	This study
Freshwater lake (Lake Mendota)	0.07	600	*Ingvorsen et al.* [1981]
Coastal lake	0.4 to 1.2	300	*Holmer et al.* [1999]
Minning lake	5.2	171	*Meier et al.* [2000]
Freshwater lake (Wintergreen Lake)	0.071	171	*Smith and Klug* [1981a]
Freshwater lake	0.32	134	*Meier et al.* [2000]
Stream	0.09	61	*Fossing and Jørgensen* [1989]
Mesotrophic freshwater lake	0.023	10.1	*Li et al.* [1996]
Marine Sediments			
Root zone of marine macrophytes	nr[a]	1,052	*Nielsen et al.* [2001]
Organic-rich coastal sediment	25	750	*Arnosti and Holmer* [1999]
Brackish water	6.3	178	*Meier et al.* [2000]
Black Sea shelf sediment	14 to 17	100	*Thamdrup et al.* [2000]
Fjord	8	84	*Fossing and Jørgensen* [1989]
Marine Bay	15	74	*Fossing and Jørgensen* [1989]
Coastal marine sediment	nr	40	*Sahm et al.* [1999]
Marine artic sediment	nr	20	*Ravenschlag et al.* [2000]
Continental margin sediments	30	20	*Fossing et al.* [2000]
High artic sound (Greenland)	nr	20	*Glud et al.* [2000]
Other Systems			
Sewer biofilm	0.5	69,680	*Ito et al.* [2002]
Moderately-saline sediment (Great Salt Lake)	14	6,000	*Brandt et al.* [2001]
Rice paddy root	0.15	500	*Liesack et al.* [2000]
Culture	nr	90	*Jørgensen* [1978a]
Hyper-saline sediment (Great Salt Lake)	208	32	*Brandt et al.* [2001]

[a]nr, not reported.

are similar to iron concentrations (Figure 5). The solubility product of ZnS is much lower than the solubility product of FeS and zinc interacts with iron sulfide resulting in a kinetic exchange of the sulfide following the reaction: $Zn^{+2} + FeS \rightarrow ZnS + Fe^{+2}$ [*Hao et al.*, 1996], thus potentially increasing the hydrogen sulfide sorbing capacity in the Lake DePue sediments. This is of potential importance because many SRP are sensitive to sulfide toxicity [*Icgen and Harrison*, 2006; *Maillacheruvu and Parkin*, 1996], and stimulation of biologic sulfate reduction by binding of hydrogen sulfide to zinc and ferrous iron has previously been demonstrated for cultured SRP [*Miller*, 1950]. Further investigation is required to determine if the stimulatory effects of hydrogen sulfide sequestration is plausible under the conditions observed in the Lake DePue sediments.

[34] An alternative mechanism may be associated with the high energy needs associated with common metal resistance mechanisms [e.g., *Hantke*, 2001; *Naz et al.*, 2005; *Tsai et al.*, 1997]. Increased maintenance energy needs might contribute to the elevated respiration rates observed in Lake DePue as in other metal stressed systems [*Fliessbach et al.*, 1994; *Khan and Scullion*, 2002; *Renella et al.*, 2004]. The phenomena of increased respiration rates has been more thoroughly explored for macro-organisms in stressed ecosystems [*Odum*, 1985], while less is known about the response of microbial communities in metal stressed systems.

[35] Surprisingly, the rate of sulfate depletion reported here for Lake DePue sediments is highly similar to the rates discernable from reported studies of other freshwater systems. The estimated time to deplete sulfate in Lake Depue sediments at Site 1 is 1.67 hours, at Site 2 is 2.7 hours, and at Site 3 is 3 hours. Applying the same calculations to previously reported SRR, the study by *Ingvorsen et al.* [1981] predicts sulfate depletion in Lake Mendota sediments at ~2.1 hours, the study by *Smith and Klug* [1981a] predicts sulfate depletion in Wintergreen Lake sediments at ~7.5 hours, and the study by *Liesack et al.* [2000] predicts sulfate depletion in rice paddy soils at 5.4 hours. Advective delivery of sulfate might contribute to these apparently diffusion limiting systems, as has been documented in other subsurface systems [for examples see: *Bussmann et al.*, 1999; *Krest and Harvey*, 2003]. Additionally, advective flow from oxygenated surface waters might contribute to rapid biological reoxidation of sulfide [*Roden and Tuttle*, 1993]; however this has not been studied in Lake DePue or (to our knowledge) in the other systems mentioned above.

5. Conclusions and Implications

[36] The most significant finding of this study was the differences of activity observed among the sampling locations. These differences were best explained when pore water concentrations of both Zn and As were included in predictive NN models, suggesting that metal contamination was associated with increased activity of the SRP community. This is an intriguing finding, as metal toxicity is more often associated with decreased biologic activity. Although additional research is needed to determine the relationship between the precise mechanism of elevated sulfate reduction rates and the biogeochemistry of this impacted lake system, our results have documented an unprecedented increase in SRP activity associated with chronic metals contamination.

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