Biogeochemistry **9**: 99–116, 1990

Establishment of anaerobic, reducing conditions in lake sediment after deposition of acidic, aerobic sediment by a major storm

PAMELA E. BELL, ALAN T. HERLIHY[1] & AARON L. MILLS
Department of Environmental Sciences, University of Virginia, Clark Hall, Charlottesville, Va 22903, USA ([1]Present address: United States Environmental Protection Agency, Corvallis, Oregon)

Key words: Sediment, disturbance, sulfate reduction, bacterial communities

Abstract. Hurricane Danny resulted in the rapid deposition of 10 cm of oxidized, acidic sediment in the Contrary Creek arm of Lake Anna, Virginia. Several biological and geochemical parameters were monitored with time to ascertain how long it took the newly-deposited lake sediments to attain the anaerobic, circumneutral, actively sulfate-reducing state normally observed in this portion of the lake. The sediment platinum-electrode potential dropped from 350 mV to 100 mV within the first week after the storm. The pH of the pore water increased from 4.5 to 5.8 within three weeks, and titratable alkalinity was detected within two weeks and three weeks at 3 cm and 1 cm depths, respectively. Accumulation of reduced products of sulfate reduction (acid volatile sulfide) began by three to four weeks after the storm event. Both methanogens and sulfate reducers were present in high and approximately equal numbers in the freshly deposited material. The rapid neutralization of the acidity in the fresh sediment prior to the onset of sulfate reduction suggests that reactions other than sulfate reduction caused the initial increase in pH and alkalinity in this system.

Introduction

Homeostasis, the ability to recover from disturbance, is a fundamental property of communities and ecosystems. Much of microbial ecology is concerned with the recovery of the functions carried out by microbial communities after slight or severe disturbance, either by adaptation of the organisms present or by selection of replacement strains that carry out the same function. The latter is a process termed congeneric homotaxis (Hill & Wiegert 1980).

The activities and abundance of heterotrophic microbial communities in the water column of the Contrary Creek arm of Lake Anna, Virginia, recover from the effects of acid mine drainage entering the lake from Contrary Creek within the first kilometer from the mouth of the stream (Wassel & Mills 1983; Carpenter et al. 1983; Mills 1985). The increase in

heterotrophic activity is coincident with a decrease in abundance and activity of *Thiobacillus ferrooxidans* (Baker & Mills 1982). Other observations have shown that the community of sediment anaerobes is abundant and highly active, even in close proximity to the source of the acid pollution (Wassel & Mills 1983; Herlihy & Mills 1985; P.E. Bell, Ph.D. thesis, University of Virginia, 1988; Mills et al. 1989).

As opposed to the acidic habitat (pH 3–4) of the water column community in the Contrary Creek arm of the lake, the sediment community habitat is circumneutral (pH 6.5; Herlihy & Mills 1985, 1986). The genesis of circumneutral conditions in the sediment and the rapid neutralization of acidity in the water column have been ascribed to the activity of the sulfate reducing bacteria (SRB) that generate alkalinity (Mills 1985; Mills & Herlihy 1985; Tuttle et al. 1969). Generation of buffering capacity in other freshwater systems has also been ascribed to the activity of sulfate reducing bacteria (Schindler et al. 1980; Kelly et al. 1982; Cook et al. 1986; Rudd et al. 1986).

Hurricane Danny (August, 1985) was a highly erosive storm (30.5 cm of rain fell in the area around Lake Anna in a 24 hour period) that resulted in the deposition of 10–11 cm of oxidized, acidic sediment in the Contrary Creek arm of Lake Anna. This event provided the opportunity to evaluate the recovery of trophic structure of the anaerobic microbial community using several indirect measures of anaerobic microbial community metabolism.

Because studies of the response of microbial community metabolism to this type of disturbance have not been reported, it was not clear which parameters should be measured. We had approximately 24 hours in which to plan the study, and because episodic events of this magnitude are unpredictable and infrequent, we chose to sacrifice a desirable level of sample replication in favor of collecting a greater diversity of information with more frequent sampling. As a result, we cannot comment on the statistical significance of observed individual differences in the data. Several trends are obvious, and those trends reveal much about the efficiency of homeostatic mechanisms in disturbed lake sediments. We have a long observational record for this particular site both before and after the event; given our experience with variability and patchiness we are confident that the results presented here are representative of overall trends in this portion of Lake Anna after the storm.

The objective of this work was to examine if, and how rapidly, pre-storm conditions were established after a major depositional event. Results showed that reducing conditions and anaerobic community metabolism recovered rapidly (within one week after deposition of the new sediments). Sulfate-reducing bacteria (SRB) and methanogens (MB) were found in the

freshly deposited sediment and were assumed to be carried in with the sediment from the surrounding catchment. Increases in pH and alkalinity were observed in the sediment before products of sulfate reduction (SR) accumulated, suggesting that reactions other than SR may be important in the initial rapid buffering of acidity and the recovery of the anaerobic community in these sediments.

Materials and methods

Sample collection and processing

Sediment samples were collected at a station approximately 0.8 km from the mouth of Contrary Creek (station C3 [Herlihy & Mills 1985]) on the day after the storm and several times over the next six weeks. During each sampling trip, three sediment cores were collected by hand by a SCUBA diver. After collection, the cores were returned to the laboratory where the stratigraphy of each core was noted. Processing began within 2 hours of collection.

Redox potential (Eh; Pt-electrode potential) was determined at 1-cm intervals over the top 14 cm of sediment on all of the cores by inserting a platinum wire electrode through a port in the side of the core sealed with silicone (Dow-Corning). In order to form a complete circuit, a silver-silver chloride electrode was placed in the water overlying the core. Voltage was read using a digital millivolt meter. The system was standardized using ZoBell's solution (ZoBell 1946).

After Eh was measured, the top 14 cm of two cores were extruded at 2-cm depth intervals. The pore water was squeezed out of each sediment layer through a #3 Whatman filter paper using N_2 gas at a pressure of 30–40 lb in^{-2} in a Reeburgh-type squeezer (Reeburgh 1967). The pH and alkalinity of the interstitial water was determined within one hour after squeezing. pH was measured using a Sensorex pH probe calibrated with pH 4.00 and pH 7.00 buffers. Alkalinity was measured by titration against 0.1 *N* HCl using a Radiometer autotitrator, and the endpoint was determined by plotting the first derivative of the titration against 0.1 *N* HCl using a Radiometer autotitrator, and the endpoint was determined by plotting the first derivative of the titration curve (American Public Health Association 1971). Chloroform was added to the pore water samples collected from the second core, and the samples were refrigerated until analysis for concentrations of SO_4^{2-}, fatty acids, and alcohols.

The third core was subcored for sediment sulfide analysis at depths of 1, 3, 5, 7, 9, 11, and 13 cm. At weeks 4 and 6, an additional core was collected

in the field so that duplicate samples for this analysis were available at each depth. Subcores were obtained by inserting a detipped 10-ml syringe through the sealed ports in the side of the core (Herlihy & Mills 1985). The subcores were sealed with a serum-vial stopper and frozen until analysis. After removing the subcore, sediment for bacterial counts was obtained by placing a detipped 1-mL syringe into the hole and removing 1 mL of sediment material. The sample was placed in 2% formaldehyde and kept at 5 °C until analysis.

Bacterial cultures for fluorescent antibody counts

Organisms were isolated from the sediments of Contrary Creek using a serum bottle modification of the Hungate technique with butyl rubber stoppers (Miller & Wolin 1974; Fulghum & Worthington 1977). Oxygen was scrubbed from gases by passing them over heated (350 °C) copper turnings.

A sulfate reducer (strain SM) was isolated using Postgate's medium B and E modified with the addition of 0.1% acetate (Postgate 1984). This organism was identified as *Desulfovibrio* sp. by the presence of the pigment desulfoviridin. The optimal growth temperature was 30°C.

A methanogen (strain CA) was isolated using the following modification of Balch's medium (Balch et al. 1979): *vitamins* (mg 100 mL^{-1} stock) biotin, 0.2; folic acid, 0.2; pyridoxine·HCl; 1.0; thiamine·HCl, 0.5; riboflavin, 0.5; nicotinic acid, 0.5; D-L Ca-pantothenate, 0.5; Vitamine B_{12}, 0.01; p-aminobenzoic acid, 0.5; lipoic acid, 0.5: *minerals* (mg 100 mL^{-1} stock) nitrilotriacetic acid, 150; $MgSO_4 \cdot 7H_2O$, 300; $MnSO_4 \cdot H_2O$, 30; NaCl, 100; $FeSO_4 \cdot 7H_2O$, 10; $CoSO_4$, 10; $CaCl_2 \cdot 2H_2O$, 10; $ZnSO_4$, 10; $CuSO_4 \cdot 5H_2O$, 1; $AlKSO_4$, 1; H_3BO_3, 1; $Na_2MoO_4 \cdot 2H_2O$, 1; $NiCl_2 \cdot 6H_2O$, 2; NaSe, 2.

The methanogen contains the pigment F_{420} and produced methane from CO_2 and H_2 but not from formate, acetate, or lactate. It had an optimal growth temperature of 40°C. This organism forms short straight rods when grown in organic media and forms long "corkscrew" shapes when grown in inorganic media.

Fluorescent antibody assay

The sulfate reducer and methanogen were used to generate polyclonal antibody in New Zealand white rabbits. Pure cultures were suspended for three days in 1% formaldehyde, then washed in phosphate-buffered saline (PBS) and resuspended in 0.1% formaldehyde to a concentration of about 10^9 cells mL^{-1}.

One rabbit was used for each organism. Increasing doses of antigen were

injected into the marginal ear vein every four days for a total of four injections (0.5 mL, 1.0 mL, 2.0 mL, 3.0 mL) (Garvy 1977). After the last injection, the rabbits were rested for one week, then a sample of blood was drawn to determine the antibody titer. If the titer was too low (< 640) a booster injection was given (1 mL, subcutaneous in the neck). The final titers were 1280 and > 5120 for the methanogen and the sulfate reducer, respectively.

The antibody was purified by precipitation with $(NH_4)_2SO_4$ and dialysis against PBS (Kawamura 1977). Electrophoresis demonstrated the successful removal of the albumin fraction from the serum and the gamma fraction as the major protein peak.

Specificity of each preparation was determined by slide agglutination using 30 aerobic and anaerobic isolates from Contrary Creek and local water sources. All questionable reactions were checked using indirect fluorescent antibody. No cross reactions were observed. The antibody to the methanogen did not stain *Methanobacterium formicicum* (donated by J.G. Ferry).

Cell counts

To count bacteria in the sediments, 1-mL subcores were blended for 1 min in alkaline yeast extract (0.25% Yeast Extract, pH = 9.7). After blending, 10 drops (0.5 mL) of iso-amyl alcohol were added to break the foam. The sediment was allowed to settle for 3 min, and 0.01 mL of the liquid was removed and placed in the circle of a fluorescent antibody slide (Clay Adams). Four such subsamples were taken from each homogenate.

The smears were allowed to air dry and were then heat fixed. Rhodamine-conjugated acid-hydrolyzed gelatin (Bohlool 1968) was placed on the smears and allowed to dry in order to decrease nonspecific fluorescence. Dilutions (1/20) of the antibody were applied to the smears and the slides were incubated in a moist chamber for 45 min before gentle rinsing for 10 min in PBS. FITC-Goat-anti-rabbit IgG (heavy and light chain; Jackson Immunoresearch) was applied, and the slides were incubated in the moist chamber for 45 min. Again, the slides were rinsed for 10 min in PBS and allowed to air dry. After drying, the smears were mounted in buffered glycerol. Cells were counted using the oil immersion (100×) objective and epifluorescence illumination.

Organic product concentrations

Alcohols were determined by injecting 2 μL of pore water into a Varian model 3700 gas chromatograph equipped with a FID. The column (2 m

stainless steel) was packed with Porapak Q (100/120 mesh). Operating conditions were: injector, 200 °C; column, 180 °C; detector, 250 °C; N_2 and H_2 flow, 30 mL min^{-1}; and air flow, 300 mL min^{-1}. Supelco alcohols were used as standards for determination of retention time and quantitative detector response.

Organic acids were analyzed by Ion Exclusion Chromatography with a Dionex 030888 anion separator column and a Dionex 030891 suppressor column (Keene et al. 1983). The eluent was 0.001 *N* HCl, and the flow rate was 1 mL min^{-1}. Supelco fatty acids were used as standards for determination of retention times and detector response.

Sediment sulfur analysis

Each sediment subcore was analyzed for acid-volatile sulfide (AVS), acetone extractable sulfur (AES), and chromium-reducible sulfide (CRS) using the methodology of Herlihy et al. (1988). Sediment subcores were frozen in a dry ice-ethanol bath after sampling and kept at −5 °C until analysis. Each frozen subcore was placed in a modified Johnson-Nishita distillation apparatus and acidified with 2 mL of concentrated HCl. The liberated sulfide was distilled with N_2 for one hour and trapped in two test tubes linked in series, each containing 20 mL of 0.2 *N* NaOH. This technique measures all aqueous sulfides, amorphous iron monosulfides (Fe_xS), and poorly crystallized iron monosulfides.

The acidified sediment was then filtered and rinsed, air dried overnight, and extracted with 60 mL of acetone for four hours in a Soxhlet extractor. The acetone from the extraction was placed in the distillation apparatus and sulfide volatilized from the extracted sulfur by adding 10 mL of 1 *M* $CrCl_2$ in 0.5 *N* HCl (Zhabina & Volkov 1978). The liberated sulfide was trapped as described above. The chromium reduction assay is specific for inorganic sulfur; thus, the acetone extractable fraction represents elemental sulfur (Canfield 1986; Howarth & Jorgensen 1984). After acetone extraction, the sediment was air dried and placed in a distillation apparatus. Eight milliliters of ethanol, 5 mL of concentrated HCl, and 16 mL of reduced chromium were added and the mixture was boiled for one hour. The liberated sulfide was recovered using the procedures described earlier. The sulfur in this fraction is a measure of the crystalline iron sulfide minerals, mainly pyrite (Wieder et al. 1985; Zhabina & Volkov 1978).

Sulfide in each trap fraction was quantified using the spectrophotometric method of Cline (1969). Sulfide standard curves were determined using $Na_2S \cdot 9H_2O$ solutions standardized by iodometric titration (American Public Health Association 1971).

Sulfate concentration

SO_4^{2-} was determined by ion chromatography (Moses et al. 1984) with a Dionex ion chromatograph equipped with an HPLC AS-3 analytical column, an HPLC-AG3 guard column and an anion fiber suppressor. A 3.5 m*M* $NaCO_3$ eluent at a flow rate of 3 mL min^{-1} was used along with a 0.025 *N* H_2SO_4 regenerant solution for the fiber suppressor. All samples were filtered through preleached 0.22 μm pore diameter filters (Gelman Metricel) before analysis.

Results

Eh, pH, alkalinity

The depth of sediment deposited by Hurricane Danny was determined by measurements made on several stakes in the area that had been implanted a year before the storm as references for the evaluation of general sedimentation in this arm of Lake Anna. The stake nearest the coring area was about 2 m away. Measurements at that stake indicated that 10 cm of fresh material were deposited in that area as a result of Hurricane Danny. The depth of deposition varied from 1 to 2 cm throughout the area. For cases in which the records existed, the values obtained from the post-depositional sediment at depths greater than 10 cm closely matched values typical of the pre-storm conditions in the sediment.

The measured Eh in the newly deposited sediment decreased by 200 mV between weeks 0 and 1, and remained fairly stable throughout the remainder of the study period except for an increase at the 1-cm depth interval during week 2 (Fig. 1). The region below 10 cm (old sediment) was usually more reduced than the overlying sediment. Such measurements in pre-storm sediments often ranged from negative values to about + 150 mV; the latter value was rarely observed in sediments at depths greater than 1 cm (McIntire et al. 1988). Platinum-electrode potential is a relatively subjective measurement for many reasons (Stumm 1966; Whitfield 1974), and while the values should not be translated as absolute Nernstian electrode potentials, the trends are clear and reproducible in many laboratories. Because Lake Anna sediments contain high concentrations of dissolved Fe(II) (up to 7 m*M*, Herlihy et al. 1988), these measurements probably represent the electroactive ferric-ferrous couple.

During the first six weeks, the bottom water pH increased from 3.5 to 4.2 (Fig. 2). The pH in the deeper sediment remained stable with small fluctuations around 6.4 (the "typical" value for pre-storm sediments, Herlihy &

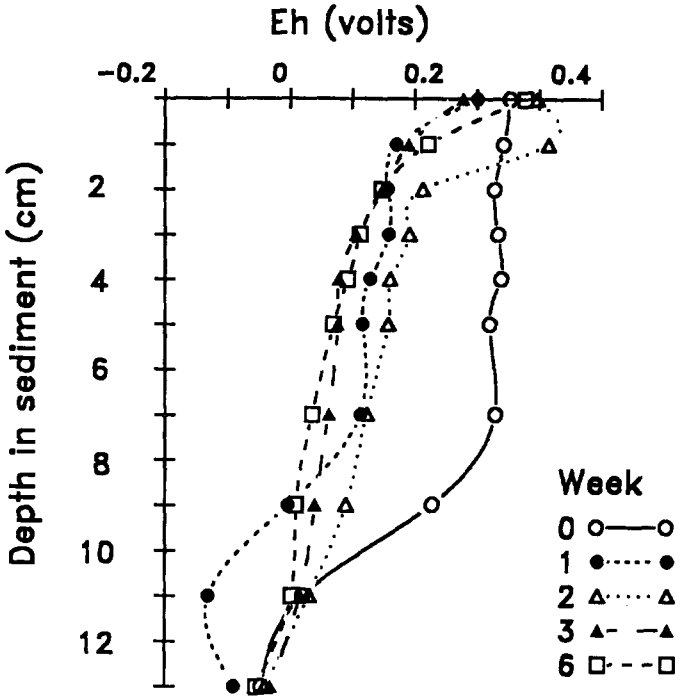

Fig. 1. Platinum electrode potential (Eh) profiles in Lake Anna sediments after Hurricane Danny. Values in undisturbed sediments in this part of Lake Anna are usually in the range of -0.1 to $+0.1$ v at depths greater than 1 cm in the sediment.

Mills 1986). At 3, 5, and 7 cm depths, the largest change in pH (ca. 0.6 units) occurred during the first week after the storm. At a depth of 1 cm, the largest change in pH (4.75–5.72) occurred between the second and third week after the storm. The pH increase started just above the old sediment layer and proceeded toward the sediment surface during the observation period.

Initially and at week 1, alkalinity was measurable only in the old sediment (11 cm, Fig. 3), but by the second week, the alkalinity in the new material had increased, reaching a peak of 5.5 meq liter^{-1} at 9 cm. By the third week, even the 1-cm layer contained measurable alkalinity (0.9 meq liter^{-1}). While the largest change in alkalinity occurred between weeks 1 and 2 in the 3–9 cm layer, the highest alkalinity at all depths occurred during weeks 3 and 4. The increases in alkalinity usually lagged behind increases in pH by about one week.

Sulfate and solid iron sulfides

Initially there was 1–4 mmol SO_4^{2-} liter^{-1} present in the new sediment. This

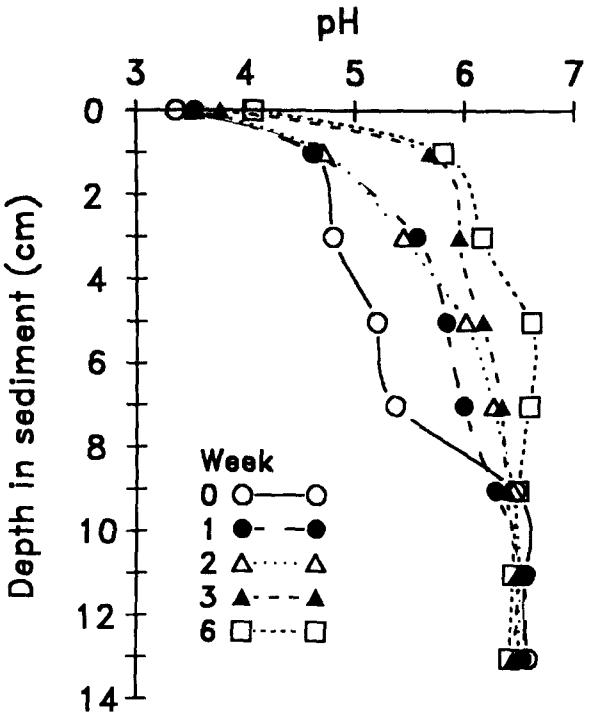

Fig. 2. pH profiles in sediment pore water at Lake Anna after Hurricane Danny. Below the top 1 cm of sediment, the pH is nearly always 6.4–6.6 in all parts of the Contrary Creek arm.

value is, of course, high compared to most freshwater systems, and was higher than the pre-storm conditions in the Lake Anna sediments. By the first week, however, the SO_4^{2-} concentration in the pore water had increased. At that time, depth profiles of SO_4^{2-} concentration took on the typical "exponentially decreasing" shape usually observed in these sediments (Herlihy & Mills 1985). In the third week after the storm, there was a large pulse of SO_4^{2-} in the pore water (11–16 mmols liter^{-1}) (Fig. 4).

Depth-integrated values of AVS, AES, and CRS were calculated for the top 8 cm of sediment (Fig. 5) and therefore represent only sulfide partitioning after the storm. Because the coefficient of variation for CRS was approximately 30% of the mean in replicated samples, it could not be demonstrated that the slight increase in pyrite was or was not be due to the formation of new mineral material. AVS increased with time after the storm; the largest increase occurred in the 5–9 cm layer, which is the region immediately overlying the old sediment. Profiles for the reduced sulfur compounds (not shown) indicated that the CRS concentrations were uniform with depth while AVS and AES both increased with depth.

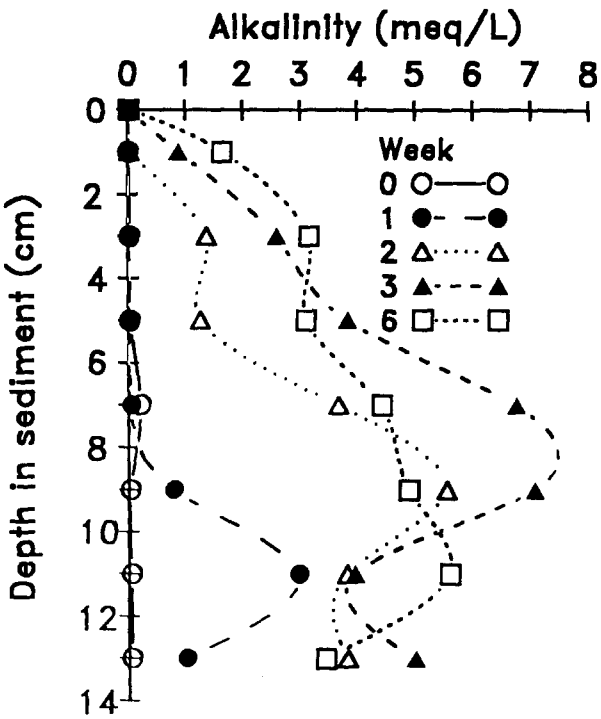

Fig. 3. Profiles of titratable alkalinity in the sediment pore water at Lake Anna after Hurricane Danny. Typical pore water alkalinities are in the range of 0.1 to 0.5 meq liter^{-1}.

Bacteria and products of anaerobic metabolism

The counts of the methanogen and the sulfate reducer were highly variable (Table 1). Both organisms were present in the freshly deposited sediment in numbers of $4 \cdot 10^6$ to $6 \cdot 10^6$ cells mL^{-2} sediment, and their numbers tended to fluctuate about $4 \cdot 10^6$ cells mL^{-1}. A greater separation in the numbers of the sulfate reducer and the methanogen was usually seen at this station with the sulfate reducer outnumbering the methanogen. Most-probable-number counts for each of these organisms produced 1–2 orders of magnitude lower numbers than did the corresponding fluorescent-antibody counts (P.E. Bell, Ph.D. thesis, University of Virginia, Charlottesville, 1988).

Acetate concentrations in the 1-cm depth interval were initially high (190 μM), but later, approximated those at depth (50–100 μM) (Fig. 6). Butyrate and propionate (not shown) appeared sporadically throughout the study period. Butyrate was present throughout the profile at concentrations between 130 and 360 μM by week 6. Ethanol concentrations were initially low (ca. 297 μM), but increased with time to around 1500–2000 μM (Fig. 7).

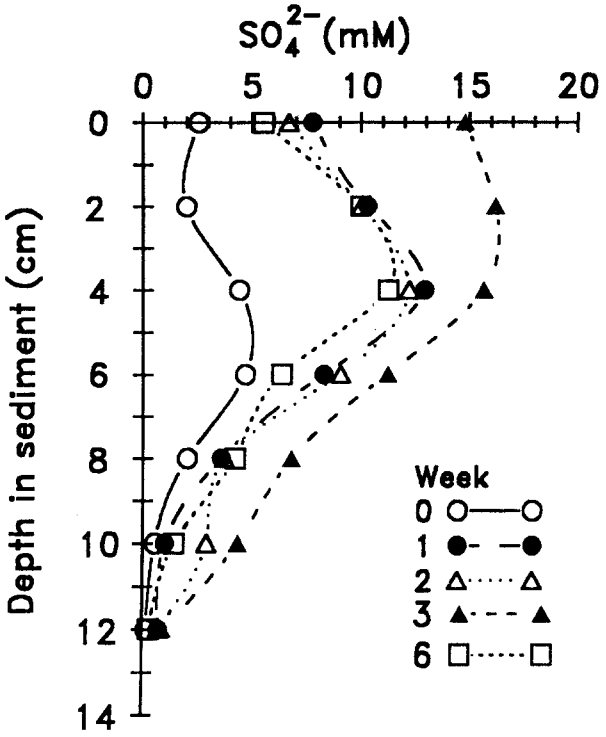

Fig. 4. Concentrations of SO_4^{2-} in pore water at Lake Anna during the period after Hurricane Danny. In undisturbed sediments, the SO_4^{2-} concentrations are as high as 1 mM at the surface (depending on the SO_4^{2-} concentration in the overlying water), but always fall to undetectable levels within the first 4 cm in the sediments.

Discussion

Conditions in the storm-deposited sediments approached those in the older layers within 3 weeks of the storm. While we have implied that the sequence of events constitutes a "re-establishment" of prior conditions, the sequence is actually the establishment of those conditions in new material. Given that consideration, the rapidity of the sequence of events is even more striking.

The day after the storm, visibility in the water column was essentially zero (the diver's hand could not be seen in front of the mask). All data indicated that the top 8–10 cm of the sediment was freshly mixed. Both SRB and methanogens were present at around 10^6 cells mL^{-1} of sediment. In depth profiles taken at the study site before hurricane Danny, the SRB significantly outnumbered the methanogens at nearly all depths sampled (Mills et al. 1989). The fact that SRB and methanogens occurred in the post storm profiles in approximately equal numbers suggests that their source was likely

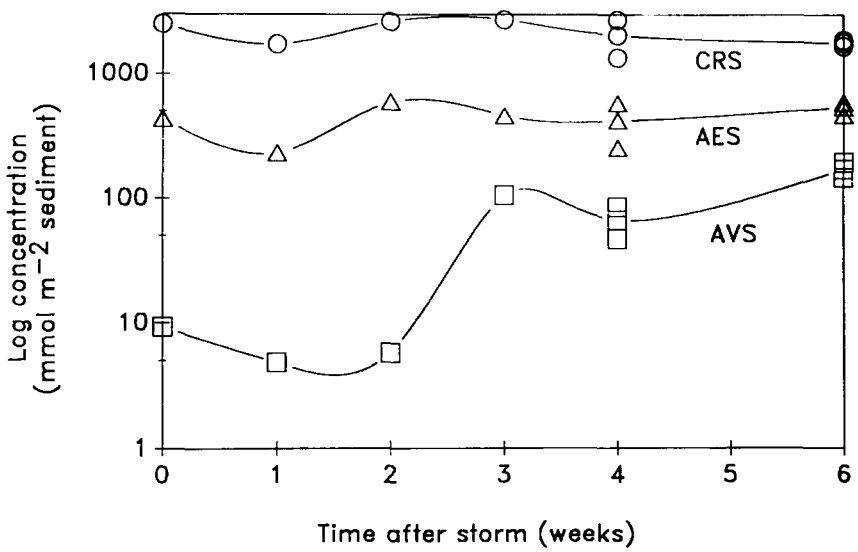

Fig. 5. Chromium-reducible sulfide (CRS), acetone-extractable sulfur (AES), and acid-volatile sulfide (AVS) in Lake Anna sediment after Hurricane Danny. The values represent integrations of depth profiles for the top 8 cm to ensure that only storm-deposited sediment was considered. Points at weeks 4 and 6 represent the mean and range of duplicate cores.

not from resuspension of near surface sediments (although such an occurrence cannot be entirely ruled out). The only other probable source for the organisms is the sedimentary material (soil, etc.) from the surrounding catchment. It is not known whether the anaerobes were functional in the soil in reduced microniches or whether they are dormant.

In many acidified systems, ion exchange reactions are an important means of rapidly neutralising incoming acidity (Cosby et al. 1985). Ion exchange reactions are rapid and would probably occur within the first day after the storm while the particulates settled out of suspension. In Lake Anna, ion exchange reactions are likely to be less important in proton consumption due to the low cation exchange capacity and base saturation of the soil material in the surrounding watershed, which is derived from the Ca-deficient sericitic, granitic schists of the Chopawamsic formation (Pavlides 1981).

Metal oxides and hydroxides control surface chemistry in most freshwater systems (Stumm & Morgan 1981). Because iron is the dominant metal in the Contrary Creek arm of Lake Anna, iron oxides and hydroxides should dominate surface interactions in this system, and it is likely that much of the transport of SO_4^{2-} to the sediment is due to co-precipitation and adsorption of SO_4^{2-} with and to the oxidized iron compounds (Sigg & Stumm 1980;

Table 1. Numbers of the methanogen (strain CA) and sulfate reducer (strain SM) in sediments of the Contrary Creek arm of Lake Anna, Virginia after Hurricane Danny. Values are the mean ± 1 SD (n = 4).

Depth (cm)	Methanogen (10^6 cells mL^{-1})				
	Week 0	Week 1	Week 2	Week 3	Week 6
1	4.07 ± 1.12	9.03 ± 4.50	10.61 ± 2.34	2.74 ± 1.11	3.42 ± 1.37
3	3.08 ± 6.84	7.53 ± 3.26	3.08 ± 3.21	3.08 ± 1.72	4.11 ± 1.94
5	2.71 ± 1.61	6.16 ± 1.77	6.16 ± 5.06	4.79 ± 2.62	3.42 ± 2.37
7	3.76 ± 2.82	7.55 ± 1.75	4.79 ± 2.62	2.39 ± 1.31	3.08 ± 0.68
9	2.39 ± 1.31	3.42 ± 0.79	3.08 ± 3.42	1.65 ± 0.73	2.74 ± 0.00
11	1.04 ± 0.22	1.66 ± 0.73	4.45 ± 1.31	3.42 ± 0.79	3.02 ± 1.41
13	1.32 ± 0.48	3.42 ± 3.26	1.68 ± 0.71	3.42 ± 0.79	2.74 ± 1.94
Depth (cm)	Sulfate reducer (10^6 cells mL^{-1})				
	Week 0	Week 1	Week 2	Week 3	Week 6
1	6.16 ± 2.62	3.65 ± 0.79	2.39 ± 1.31	5.47 ± 1.94	6.50 ± 3.42
3	2.74 ± 1.58	4.45 ± 0.68	2.74 ± 1.94	5.81 ± 1.31	4.13 ± 1.72
5	3.76 ± 1.72	4.11 ± 1.58	4.79 ± 0.78	4.45 ± 1.72	3.42 ± 1.77
7	5.47 ± 1.11	3.76 ± 2.05	5.13 ± 2.82	2.05 ± 1.37	5.47 ± 1.94
9	1.71 ± 0.68	8.26 ± 4.40	5.13 ± 2.05	3.76 ± 1.31	1.65 ± 0.73
11	2.05 ± 0.79	3.36 ± 1.86	5.13 ± 3.60	1.71 ± 0.68	2.74 ± 0.00
13	1.30 ± 0.13	2.01 ± 0.81	3.42 ± 1.77	1.71 ± 0.68	1.65 ± 0.73

Herlihy & Mills 1989). The zero point of charge (ZPC) of most iron oxides and hydroxides ranges from pH 5 to pH 9. This implies that when the pH of the solution is below the ZPC, as in the immediate post-storm situation, colloid surfaces become protonated and are positively charged. The protonation will consume H^+ and will result in an undefined level of immediate buffering capacity. As the pH rises above the ZPC of the dominant colloids, biogenic processes such as SO_4^{2-} reduction, iron reduction, ammonification, etc. generate alkalinity to maintain circumneutral conditions.

The low initial concentrations of sulfate in the sediment are probably the result of sulfate adsorption onto iron-rich soil particles (Chao et al. 1962; Hingston et al. 1972). This bound sulfate could then be released either by solid phase dissolution during iron reduction, or by desorption when the pH increased and anion exchange potential decreased (Hingston et al. 1972). These processes would account for the increased concentrations of SO_4^{2-} in the sediment pore water after week 1. Microbial reduction of released SO_4^{2-} would result in the subsequent observed increase in AVS in the sediments.

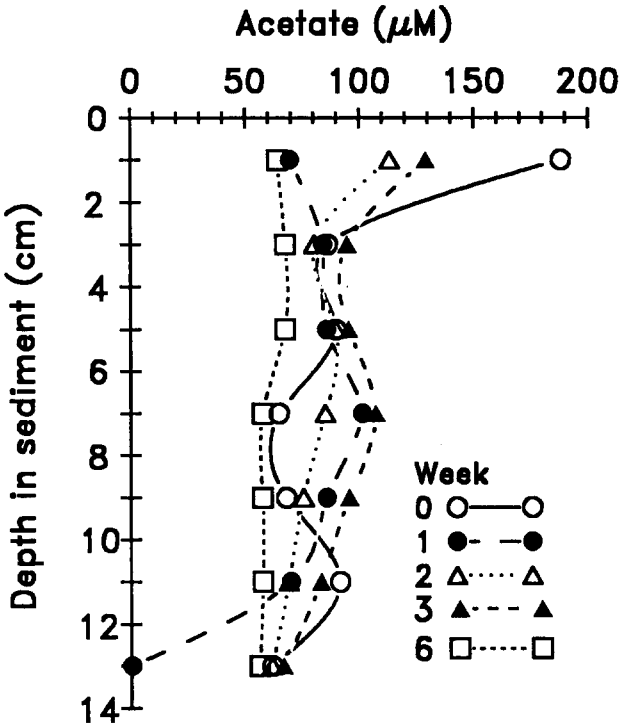

Fig. 6. Profiles of acetate in the sediment pore water at Lake Anna after Hurricane Danny.

Alcohol and fatty acid concentrations are indicative of bacterial fermentation and illustrate the dynamics of the anaerobic community. Alcohols are end products of anaerobic metabolism (though they are sources of carbon and electrons for many members of the anaerobic community) and are not usually present under oxidizing conditions. Ethanol, butanol, and propanol concentrations were initially low and increased with time after the storm event. Acetate was present at concentrations from 50 to 200 μM during the study; pre-storm acetate concentrations fluctuated between 50 and 100 μM (R.L. Snyder, M.S. thesis, University of Virginia, 1985). Propionate and butyrate did not appear until after the sediments had become reducing. These parameters may typify initial stages of the recovery process in any disturbed sediment that is becoming anaerobic. While acetate concentrations were similar to those reported in the literature (ca. 100 μM, Lovley & Klug 1982), ethanol concentrations were always higher in Contrary Creek than in other systems (viz. in the mM range as opposed to the 35 μM reported by Lovley & Klug 1982; Phelps & Zeikus 1985). When other fatty acids were present (propionate, and butyrate) they appeared in higher

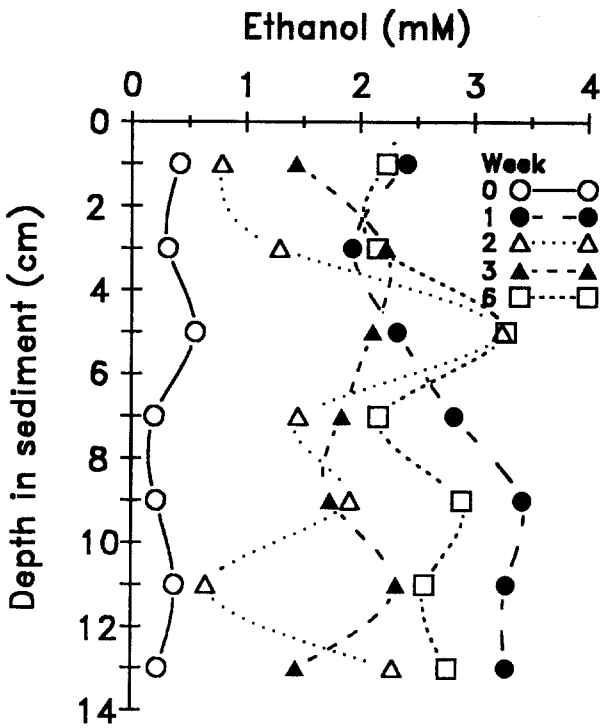

Fig. 7. Profiles of ethanol in the sediment pore water at Lake Anna after Hurricane Danny.

concentrations than reported in Lake Mendota (Phelps & Zeikus 1985). This could result from a rebound effect where the anaerobic community is not yet operating efficiently.

The chief product of sulfate reduction in Contrary Creek is AVS (Herlihy et al. 1988), and it did not increase until after the pH and alkalinity had already increased. This suggests that processes other than sulfate reduction were initially buffering the system. While SO_4^{2-} reduction may have been occurring during that early period, it is possible that rapid re-oxidation of the biogenic sulfides may have prevented measurable buildup of AVS in Lake Anna sediments near Contrary Creek (Herlihy et al. 1987).

The experience with Hurricane Danny has not only demonstrated how rapidly the anaerobic sediment community can become re-established in oxidized, acidic sediments, it has also provided more insight into how a SR community can become established in unfavorable conditions. The initial pH of the sediment was < 5.4, a value generally thought too low for active sulfate reduction (Postgate 1984). While sulfate-reducing bacteria active at low pH values (pH = 3.0) have been isolated from strip-mine lakes (Konopka et al. 1985; Gyure RA Brooks A Doemel W Konopka A & Miner

J Abstr. Ann. Meet. Am. Soc. Microbiol., 1986. I42.), it appears that SRB may not generally be the guild that catalyzes initial buffering of acidic sediments. The data from the current study suggest that a succession of events, including proton adsorption, iron-reduction, and fermentative activity, occurs that can account for the rise in pH from 3.4 to greater than 5.5 before the onset of measurable SR. The data further suggest that those events are a combination of physical, chemical, and biological processes, and implicate other bacterial guilds in the neutralization process.

The data support the conclusion that both physical and biological processes were important in the rapid neutralization of acidity brought into the Contrary Creek arm of Lake Anna with this storm. Reducing conditions and anaerobic community metabolism were established rapidly (within one week after deposition of the oxidized, acidic sediments). pH and alkalinity increased before products of sulfate reduction were produced. The generation of permanent alkalinity in these sediments is necessarily a coupled physical and biological process involving several anaerobic microbial guilds.

Acknowledgements

This work was supported by Grants-In-Aid to ATH and PEB from The Society of the Sigma Xi.

References

American Public Health Association (1971) Standard methods for the examination of water and wastewater. 13th ed. APHA, Washington, D.C.

Baker KH & Mills AL (1982) Determination of the number of respiring *Thiobacillus ferrooxidans* cells in water samples by using combined fluorescent antibody -2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride staining. Appl. Environ. Microbiol. 43: 338–344

Balch WE, Fox GE, Magrum LG, Woese CR & Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43: 260–296

Bohlool BB & Schmidt EL (1968) Nonspecific staining; its control in immunofluorescence examination of soil. Science 162: 1012–1014

Canfield DE, Raiswell R, Westrich JT, Reaves CM & Berner RA (1986) The use of chromium reduction in the analysis of reduced inorganic sulphur in sediments and shales. Chem. Geol. 54: 149–155

Carpenter JM, Odum WE & Mills AL (1983) Decomposition of leaf litter in a lake receiving acid mine drainage. Oikos. 41: 165–172

Chao TT, Harward ME & Fang SC (1962) Adsorption and desorption phenomena of sulfate ions in soils. Soil Sci. Soc. Am. Proc. 26: 234–237

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454–458

Cook RB, Kelly CA, Schindler DW & Turner MA (1986) Mechanisms of hydrogen ion neutralization in an experimentally acidified lake. Limnol. Oceanogr. 31: 134–148

Cosby BJ, Hornberger GM, Galloway JN & Wright RF (1985) Time scales of catchment acidification. Environ. Sci. Technol. 19: 1144–1149

Fulghum RS & Worthington JM (1977) Butyl rubber stoppers increase the shelf life of prereduced, anaerobically sterilized media. Appl. Environ. Microbiol. 33: 1220–1221

Garvey JS, Cremer NE & Sussdorf DH (1977) Methods in Immunology. WA Benjamin, Inc., Reading M.A.

Herlihy AT & Mills AL (1985) Sulfate reduction in freshwater sediments receiving acid mine drainage. Appl. Environ. Microbiol. 49: 179–186

Herlihy AT & Mills AL (1986) The pH regime of sediments underlying acidified waters. Biogeochemistry 2: 377–381

Herlihy AT, Mills AL, Hornberger GM & Bruckner AE (1987) The importance of sediment sulfate reduction to the sulfate budget of an impoundment receiving acid mine drainage. Water Resour. Res. 23: 287–292

Herlihy AT, Mills AL & Herman JS (1988) Distribution of sulfide minerals resulting from sulfate reduction in sediments of an acidified lake. Appl. Geochem. 3: 333–344

Herlihy AT & Mills AL (1989) Factors controlling the removal of sulfate and acidity from the waters of an acidified lake. Water Air Soil Pollut. 45: 135–155

Hill J & Wiegert RG (1980) Microcosms in ecological modelling. In: Geisy JP (Ed) Microcosms in Ecological Research (pp 138–163) US Dept. of Energy Symp. 52. NTIS Springfield, Va.

Hingston FJ, Posner AM & Quirk JP (1972) Anion adsorption by geothite and gibbsite. I. The role of the proton in determining adsorption envelopes. J. Soil Sci. 23: 177–192

Howarth RW & Jorgensen BB (1984) Formation of ^{35}S-labelled elemental sulfur and pyrite in coastal marine sediments Limfjorden and Kysing Fjord, Denmark. during short-term $^{35}SO_4^{2-}$ reduction measurements. Geochim. Cosmochim. Acta. 48: 1807–1818

Kawamura A (1977) Fluorescent antibody techniques and their applications. Univ. of Tokyo Press, Tokyo

Keene WC, Galloway JN & Holden JD Jr. (1983) Measurement of weak organic acidity in precipitation from remote areas of the world. J. Geophys. Res. 88: 5122–5130

Kelly CA, Rudd JWM, Cook RB & Schindler DW (1982) The potential importance of bacterial processes in regulating the rate of lake acidification. Limnol. Oceanogr. 27: 868–882

Konopka A, Gyure RA, Doemel W & Brooks A (1985) Microbial sulfate reduction in extremely acid lakes. Technical Report 173. Purdue Univ. Water Resour. Res. Center, W. Lafayette, Indiana, 50 pp

Lovley DR & Klug MJ (1982) Intermediary metabolism of organic matter in the sediments of a eutrophic lake. Appl. Environ. Microbiol. 43: 552–560

McIntire PE, Mills AL & Hornberger GM (1988) Groundwater lake interactions and the occurrence of high sulfate concentrations at depth in the sediment of Lake Anna, Virginia. Hydrolog. Process 2: 207–217

Miller TL & Wolin MJ (1974) A serum bottle modification of the Hungate technique for culturing obligate anaerobes. Appl. Microbiol. 27: 983–987

Mills AL (1985) Acid mine waste drainage: Microbial impact on the recovery of soil and water ecosystems. In: Tate R.L. & Klein D (Eds) Soil Reclamation Processes (pp 35–81) Marcel Dekker, Inc., New York

Mills AL, Bell PE & Herlihy AT (1989) Microbes, sediments, and acidified water: the importance of biological buffering. In: Rao SS (Ed) Acid Stress and Aquatic Microbial Interactions (pp 1–45) CRC Press, Inc, Boca Raton

Mills AL & Herlihy AT (1985) Microbial ecology and acidic pollution of impoundments. In: Gunnison D (Ed) Microbial Processes in Reservoirs (pp 169–189) Dr W. Junk Publishers, Dordrecht, The Netherlands

Moses CO, Nordstrom DK & Mills AL (1984) Sample and analyzing mixtures of sulfate, sulfite, thiosulfate, and polythionate. Talanta 31: 331–339

Pavlides L (1981) The central Virginia volcanic-plutonic belt: an island arc of Cambrian (?) age. U.S. Geol. Surv. Prof. Paper 1231-A

Phelps TJ & Zeikus JG (1985) Effect of fall turnover on terminal carbon metabolism in Lake Mendota sediments. Appl. Environ. Microbiol. 50: 1285–1291

Postgate JR (1984) The sulphate-reducing bacteria. Cambridge University Press. Cambridge, UK

Reeburgh WS (1967) An improved interstitial water sampler. Limnol. Oceanogr. 12; 163–165

Rudd JWM, Kelly CA, St. Louis V. Hesslein RH, Furutani A & Holoka MH (1986) Microbial consumption of nitric and sulfuric acids in acidified north temperate lakes. Limnol. Oceanogr. 31: 1281–1291

Schindler DW, Wagemann R, Cook RB, Ruszczynski T & Prokopowich J (1980) Experimental acidification of lake 223, Experimental Lakes area: background data and the first three years of acidification. Can. J. Fish. Aquat. Sci. 37: 342–354

Sigg L & Stumm W (1980) The interactions of anions and weak acids with the hydrous goethite (α-FeOOH) surface. Colloids and Surfaces. 2: 101–117

Stumm W (1966) Redox potential as an environmental parameter; conceptual significance and operational limitation. Adv. Water Pollut. Res. 3: 283–306

Stumm W & Morgan JJ (1981) Aquatic Chemistry. J. Wiley and Sons, Inc., NY

Tuttle JH, Dugan PR & Randles CI (1969) Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. Appl. Microbiol. 17: 297–302

Wassel RA & Mills AL (1983) Changes in water and sediment bacterial community structure in lake receiving acid mine drainage. Microb. Ecol. 9: 155–169

Whitfield M (1974) Thermodynamic limitations on the use of the platinum electrode in Eh measurements. Limnol. Oceanogr. 19: 857–865

Wieder RK, Lang GE & Granus VA (1985) An evaluation of wet chemical methods for quantifying sulfur fractions in freshwater wetland peat. Limnol. Oceanogr. 30: 1109–1115

Zhabina NN & Volkov II (1978) A method of determination of various sulfur compounds in sea sediments and rocks. In: Krumbien WE (Ed) Environmental Biogeochemistry and Geomicrobiology, Vol 3 (pp 735–746) Ann Arbor Sci.

Zobell CA (1946) Studies on redox potential of marine sediments. Bull. Am. Assoc. Petrol. Geologists 30: 477–509