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# Impact of Water Column Acidification on Protozoan Bacterivory at the Lake Sediment-Water Interface

SARAH C. TREMAINE AND AARON L. MILLS*

*Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903*

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**Although the impact of acidification on planktonic grazer food webs has been extensively studied, little is known about microbial food webs either in the water column or in the sediments. Protozoon-bacterium interactions were investigated in a chronically acidified (acid mine drainage) portion of a lake in Virginia. We determined the distribution, abundance, apparent specific grazing rate, and growth rate of protozoa over a pH range of 3.6 to 6.5. Protozoan abundance was lower at the most acidified site, while abundance, in general, was high compared with other systems. Specific grazing rates were uncorrelated with pH and ranged between 0.02 and 0.23 $h^{-1}$, values similar to those in unacidified systems. The protozoan community from an acidified station was not better adapted ($P = 0.95$) to low-pH conditions than a community from an unacidified site (multivariate analysis of variance on growth rates for each community incubated at pHs 4, 5, and 6). Both communities had significantly lower ($P < 0.05$) growth rates at pHs 4 and 5 than at pH 6. Reduced protozoan growth rates coupled with high grazing rates and relatively higher bacterial yields (ratio of bacterial-protozoan standing stock) at low pH indicate reduced net protozoan growth efficiency and a metabolic cost of acidification to the protozoan community. However, the presence of an abundant, neutrophilic protozoan community and high bacterial grazing rates indicates that acidification of Lake Anna has not inhibited the bacterium-protozoon link of the sediment microbial food web.**

The consequences of lake acidification for planktonic grazer food chains have been well documented (30, 62, 70). Trophic reorganization occurs as fish species die out with concomitant shifts in the structure and function of insect (32, 48, 70), zooplankton (17, 44), and phytoplankton (18, 46, 62) communities. In contrast to the macrograzer food chain, there have been no studies on the impact of acidification on microbial food webs either in the water column or the sediments. Microbial food chains are a source of recycled detrital carbon and nutrients in aquatic ecosystems (5, 54, 55, 63, 80). Bacterivorous protozoa play a principal role in nonacidified, pelagic microbial food webs (65, 71), grazing 80 to 100% (or more) of the daily bacterial production (22, 56, 59). Associated with these substantial biomass fluxes, bacterivorous protozoa have been shown to be efficient mineralizers of nitrogen and phosphorus (2, 16). Recycling of carbon and nutrients via microbial food chains may be especially important in nutrient-poor ecosystems, such as acidified and/or oligotrophic lakes, as well as oligotrophic marine systems (e.g., coral reefs), where trophic interactions are tightly coupled (53, 58, 72).

We chose to study the microbial food web at the sediment-water interface of an acid mine drainage (AMD)-acidified portion of a lake in central Virginia. The sediment-water interface is a dynamic and complex lake subsystem in Lake Anna; it is the site of steep gradients of oxygen, nutrients, organic matter, and high bacterial abundance and activity (34, 45, 67). Additionally, the sediment environment offers a refuge from water column low-pH conditions because of biological buffering (4, 35, 37, 38, 60).

This study focused on bacterium-protozoon interactions of the microbial food web. Reports in the literature indicate that in the water column ciliate abundance and biomass decrease with increasing acidity, while cell size increases and species composition shifts (6, 10). Other studies report observations of acid-tolerant (10) and acidophilic protozoa in AMD streams (24, 42, 49) and bogs (49). There is a well-described group of protozoa known as acetate flagellates which comprises some species previously observed in AMD streams and is capable of tolerating extremely low (1.2) pH values (3, 49).

The effects of acidity on the protozoa and on protozoan bacterivory were evaluated by using three approaches: determination of the abundance and distribution of microorganisms, dilution grazing experiments, and experimental determination of protozoan community growth rates. These methods address ecosystem, community, and population effects of acidification (61, 62, 66). Of particular interest was whether chronic acidification of the water column had resulted in selection for an acidophilic protozoan community in the interface adjacent to biologically buffered sediments. The results indicate that sediment buffering was sufficient to maintain a neutrophilic protozoan community. In this system, acidification does decrease the protozoan growth rate but not the grazing rate, implying that there are increased physiological costs, which can be translated into reduced efficiency for the microbial food web at the sediment-water interface of an acid lake.

## MATERIALS AND METHODS

**Site.** The research was conducted in the Contrary Creek and Freshwater Creek arms of Lake Anna, Va. (Fig. 1). The Contrary Creek arm of the lake is acidified by AMD from abandoned mines and tailing piles in the creek watershed. Pyrite mining operated in this area from 1850 to 1920, and acid drainage with a pH of 2.5 to 3.5 (34) has continued to enter the creek and the impounded lake unabated. Water column pH values increase with increasing distance from the creek mouth: at site 2, pH values range between 3.5 and 4.5; at site 5, pH values range between 4.2 and 6.5 (34).

* Corresponding author.

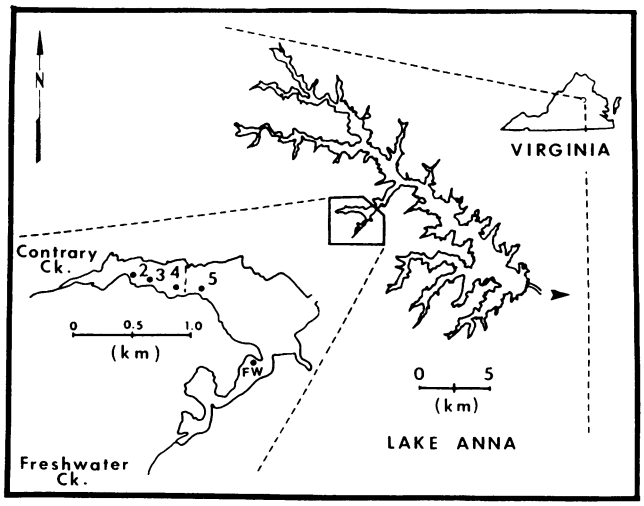


FIG. 1. Map of Lake Anna, Va., showing the acidified stations in the Contrary Creek (Ck.) arm of the lake (stations 2 through 5) and the nonacidified (control) Freshwater Creek arm of the lake (station FW). The dashed line between stations 4 and 5 shows the underwater transect.

In contrast to the acid water column, sediment porewater pH values are generally circumneutral (6.5 to 7.0) at a depth of 3 cm into the sediments (35). Acidification of the water column has been shown to be diminished by anaerobic microbial processes (iron and sulfate reduction) in the sediments of Contrary Creek (9, 45).

The Freshwater Creek arm of the lake (location of the FW site [Fig. 1]) has a watershed of similar size but without AMD; pH values range from 5.9 to 7.0 (33). Earlier studies reported that total sediment bacterial abundance at the FW site was similar to that in the sediments of acidified site 2 (78).

**Protozoan abundance.** To examine whether the abundance of sediment-water interface bacteria and protozoa varied spatially, temporally, and/or on a pH gradient, samples were collected at sites along the axis of the lake (stations 3 and 5) and along a transect perpendicular to the lake axis. Samples were collected in 1986 and 1987 by a scuba diver drawing undisturbed watery floc from the sediment-water interface into 10-ml syringes. All microbiological samples were preserved with 10% glutaraldehyde buffered at pH 7.0 with 0.1 M sodium cacodylate; the final preservative concentration was 1% (vol/vol) (15). Bacteria were enumerated by using the acridine orange direct count method (36) within 2 weeks, and protozoa were enumerated by using the primulin method (15) within 2 days.

The pH was measured to the nearest 0.1 pH unit in one of three ways: in situ (waterproofed Nester pH pen), in a sample immediately after collection (Nester pH pen or Corning pH stick), or in a sample immediately upon return to the laboratory (Corning 130 pH meter with a Sensorex combination electrode). The pH probes were calibrated with standard pH 4.00 and 7.00 buffers and checked for intercalibration to within 0.1 pH unit.

**Protozoan grazing.** To determine the impact of acidification on bacterivory, rates of protozoan grazing on bacteria were quantified by using the dilution method (21, 43) in samples from different sites (and therefore different pH values). The dilution method estimates bacterivory on the basis of the difference in apparent bacterial growth in diluted versus undiluted (whole) water samples. The dilution technique was extensively tested for violations of three critical assumptions (76) and was selected as the best method for the sediment-water interface subsystem (75, 77). In this method, triplicate samples were collected from the sediment-water interface by using a peristaltic pump fitted with a device to draw watery floc from the interface. Samples were pumped via autoclaved tubing into ethanol-washed, insulated containers and returned to the laboratory, where the experiments were initiated within 3 h after sample collection.

The dilution treatment and undiluted control flasks were set up in triplicate 2-liter Erlenmeyer flasks with a final volume of 500 ml per flask. The undiluted control flasks contained 500 ml of unaltered water collected from the sediment-water interface and contained both bacteria and protozoa. The dilution treatment flasks were a 1:3 dilution in which 1 part of whole water was diluted with 2 parts of filter-sterilized (Whatman no. 5, followed by 0.2-μm-pore-diameter Nuclepore filters) sample water. The flasks were incubated under subdued light at constant temperature, within 5°C of the in situ temperature. Subsamples (10 ml) were collected from each flask every 4 to 6 h for at least 24 h and preserved with buffered glutaraldehyde, and bacteria and protozoa were enumerated as described earlier.

Least-squares regressions (40) were conducted on logarithmically transformed bacterial concentration-versus-time plots (69) to estimate bacterial apparent specific growth rates in the presence ($r_n$) and relative absence ($r_d$) of grazing (76). Protozoan apparent specific grazing rates ($g$; units are per hour [hereafter, specific grazing rates]) were calculated from the difference between the bacterial apparent specific growth rates in the two treatments and corrected by the dilution factor ($X = N_{DIL}/N_{CTL}$ at time zero; where $N$ is the number of bacteria per milliliter; i.e., $g = [r_d - r_n] \cdot [1 - X]^{-1}$) as described previously (21, 43, 76). Bacterial specific growth rates ($\mu$) were calculated from the relationship $\mu = r_n + g$.

**Protozoan community growth.** An experiment was conducted to determine whether the protozoan community from the acidified arm of the lake (ACID; station 3) was better adapted to acid conditions than a protozoan community not exposed to AMD. The latter protozoan community was collected from a station (FW) in the adjacent and unpolluted arm of the lake which served as an in-lake control (34, 78). The experiment compared apparent specific growth rates of mixed protozoan communities from these two sites exposed in the laboratory to different pH regimens (independent variables were the two sites and four pH treatments, giving eight treatment combinations). To obtain sufficient inoculum, protozoa were collected from each station by using polyfoam blocks (13) which were deployed at the sediment-water interface of each station 2 weeks prior to the beginning of the experiment. The blocks were collected and placed into ethanol-washed, insulated containers and returned to the laboratory. When the blocks were collected, water was also pumped from the sediment-water interfaces of the two stations.

In the laboratory, the polyfoam blocks obtained from the ACID (a) and FW (f) sites were squeezed into separate beakers to obtain inocula $I_a$ and $I_f$. The interface water samples used as the diluent ($D_a$ and $D_f$) were filtered (Whatman GF-C; 1.2-μm nominal pore size) to remove the grazers without removing the bacteria (77). The incubations were carried out in 250-ml polystyrene tissue culture flasks. Each flask received 30 ml of one of the protozoan inocula and 60 ml of diluent, resulting in a 1:3 dilution (Tables 1 and 2). Initial pH values were established by adding different

TABLE 1. Samples used to test the effect of pH on the acid sensitivities of protozoan communities

| Station | Source | pH |
|---|---|---|
| ACID | Protozoan inoculum | 4.11 |
| ACID | Diluent water | 3.57 |
| FW | Protozoan inoculum | 6.28 |
| FW | Diluent water | 6.35 |

TABLE 2. Design of the experiment done to test the effect of pH on the acid sensitivities of two protozoan communities[a]

| Target pH | Actual pH | Inoculum[b] size (ml) | Diluent vol (ml) and source |
|---|---|---|---|
| 6 | 6.4 | 30 | 60 FW |
| 5 | 5.1 | 30 | 30 FW, 30 ACID |
| 4 | 4.0 | 30 | 60 ACID |
| Control | 3.5 (ACID), 6.3 (FW) | 0 | 90 FW or ACID |

[a] From an acidified station (ACID) versus a nonacidified control station (FW).
[b] From ACID or FW.

proportions of diluent to the inocula (i.e., for pH 6, 100% $D_f$ was added; for pH 5, 50% $D_a$ and 50% $D_f$ were added; for pH 4, 100% $D_a$ was added). In an unbuffered, preliminary experiment, the initial pH in the low-pH treatments (5.0 and 4.0) increased to between 5.5 and 6.0 within 24 h in all of the flasks. Citrate-phosphate buffer (0.01 M) was added to the treatment flasks in the subsequent experiment, which had an incubation time of 7 days, to counteract these pH shifts. The 60 ml of diluent with the appropriate buffers was added to the flasks at a rate of 10 ml/h to minimize the pH shock to the protozoa in the experimental manipulation. Two sterile wheat grains were added to each flask to provide an organic substrate for bacterial growth. Diluent control flasks were also run to account for protozoan regrowth in the diluent; these flasks contained 90 ml of the diluent with wheat grains and no buffer (two replicates per diluent).

Subsamples (5.5 ml) were taken at 0, 1, 2, 4, and 7 days. The pH of each subsample was measured immediately, and the sample was then preserved with buffered glutaraldehyde (final concentration, 1% [vol/vol]). Protozoans (flagellates and ciliates, not amoebae) were enumerated within 3 days, and bacteria were enumerated within 1 week.

Protozoan specific growth rate was determined as the slope of the least-squares regression of protozoan abundance versus time. Heteroscedastic variance was observed in residual plots, and therefore, protozoan abundance data were logarithmically transformed and regressions were rerun; the resulting residual plots were observed to be homoscedastic (40). An analysis of variance (ANOVA; two protozoan sources by four treatments by three replicates) was performed by using SPSSx with three a priori contrasts to test hypotheses concerning the source of variance in the calculated protozoan specific growth rates (69).

## RESULTS AND DISCUSSION

**Protozoan abundance.** Bacterial concentrations at the sediment-water interface ranged between $10^6$ and $10^7$ cells $ml^{-1}$ and were intermediate between concentrations in the water column ($10^6$ cells $ml^{-1}$) and the sediments ($10^8$ to $10^9$ cells $ml^{-1}$). Sediment and water column bacterial abundances at Lake Anna were similar to values published in the literature for lakes and marine systems (12, 39, 41, 57, 79).

Total protozoan abundance at the sediment-water interface of Lake Anna ranged from $10^3$ to $10^4$ cells $ml^{-1}$ (greater than 90% flagellates) and was 2 to 4 orders of magnitude greater than the number of ciliates in the water column (flagellates were not enumerated in these samples; 9a) (Table 3). There were more protozoa in the sediments ($10^4$ to $10^5$ cells $ml^{-1}$) than at the interface; Lake Anna sediment concentrations were higher than other published values in freshwater and marine systems (1, 28, 29). Water column ciliate abundance was similar to values reported for other mesotrophic lakes (7, 41, 57).

The spatial and temporal distributions of bacteria and protozoa were evaluated to see whether acidification had an effect on microorganism abundance. The data were screened to check for normality of distribution and the presence of outliers (73). Bacterial and protozoan concentration data were not normally distributed and were therefore logarithmically transformed for the following analyses. Data analyses were divided into samples collected parallel and perpendicular to the axis of the lake.

Analysis of samples taken parallel to the axis of the lake was problematic, as sites were strongly correlated with pHs: pH was always lower at site 3 (2.9 to 4.2) than at site 5 (4.6 to 5.4). An ANOVA was conducted with date and site as the main effects to examine how these factors influenced protozoan and bacterial abundances. Date and site were both significant contributors to the variance in protozoan abundance ($P = 0.008$ and $P < 0.001$, respectively); the two main effects accounted for 66% of the variance. This was not the case for bacterial abundance, in which site alone was evaluated, and variance between sites was no greater than within-site variance ($F = 0.43$, $P > 0.05$).

Samples collected along the perpendicular transect were pooled into three groups corresponding to the southern, midlake and northern regions of the transect. An ANOVA was conducted for pH with date and site as factors to examine how acidity at the interface varied with time and

TABLE 3. Abundances of protozoa in the water column, sediment-water interface, and sediment of Lake Anna compared with values reported in the literature

| No. of protozoa[a] $ml^{-1}$ (reference[s]) | | | Source(s) |
|---|---|---|---|
| Water column | Sediment-water interface | Sediment | |
| $10^0$–$10^1$ C (9a) | $10^3$–$10^4$ C, F (this study) | $10^4$–$10^5$ C, F (this study) | Lake Anna |
| $10^{-1}$–$10^1$ C (7, 14, 50, 57), $10^3$ F (57) | | $10^3$–$10^4$ C (28, 29) | Other lakes |
| $10^0$–$10^1$ C (41), $10^3$ F (41) | $10^2$–$10^3$ C (39) | $10^0$–$10^2$ C (1), $10^1$–$10^3$ F (1) | Marine systems |

[a] C, Ciliates; F, flagellates.

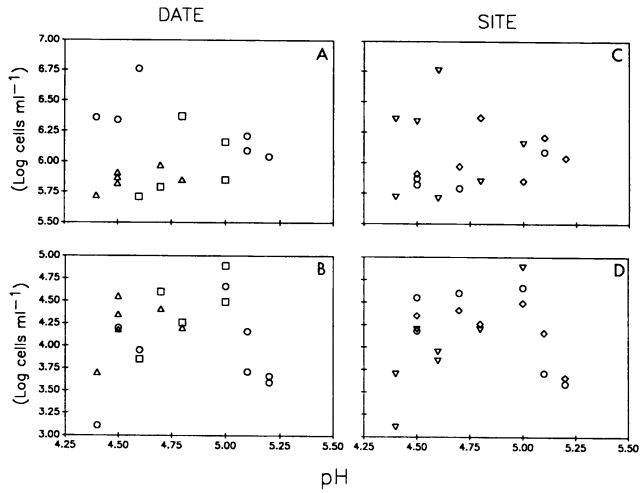


FIG. 2. Bacterial (A and C) and protozoan (B and D) abundances along a transect perpendicular to the axis of the acid arm of Lake Anna as a function of pH. In graphs A and B, the data were plotted by using the symbols ○, △, and □ for 20 May, 30 May, and 2 June 1986, respectively, while in graphs C and D, the symbols ▽, ◇, and ○ indicate south, midlake, and north sites across the lake, respectively.

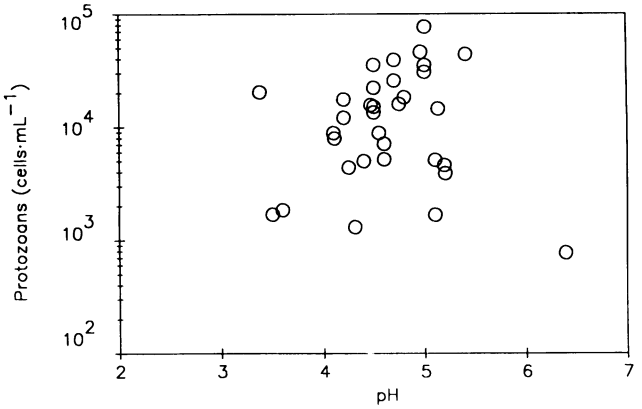


FIG. 3. Effect of pH on heterotrophic protozoan concentration. Each datum is the concentration of an individual sample. The counting error was about 10%.

site across the lake. Significant contributions to the variance were made by date ($P = 0.020$) and site ($P = 0.027$) but not the date-by-site interaction term ($P > 0.05$).

Plots of bacterial and protozoan abundances versus pH indicate that there was no overall linear relationship with pH for either microbial group; therefore, pH could not be added to the ANOVA as a covariate (Fig. 2) (73). The overall pattern of bacterial abundance had the greatest variance at low pH, with decreasing variance at higher pH, and implies a greater dependence of bacteria on microsites at the low-pH sites. The plots (Fig. 2) suggest that bacterial abundance was inversely related to pH on one date and positively related on another date. This was borne out in the ANOVA, in which date accounted for 42% of the variance in bacterial concentration (significant at the 0.05 level) while site and the date-by-site interaction term did not.

The overall protozoan abundance pattern showed the lowest numbers at low and high pH values and maximum abundance at intermediate pH values. Neither date nor site contributed significantly to the variance in protozoan abundance.

The most interesting analyses of these data were based on bacterial yield (the log of the ratio of bacterial-to-protozoan abundance). Bacterial yield was evaluated for pH effects blocked separately for date and site via least-squares regression of microbial abundance by pH. Date had all negative regression coefficients, indicating that as pH increased, bacterial yield decreased. This suggests that the low-pH protozoan community required a larger standing stock of bacteria than the high-pH community.

Regressions of bacterial yield blocked by site were quite different. Site 1 had a negative regression coefficient and was significantly different from the coefficients for sites 2 and 3, which were positive. Sites 2 and 3 had smaller bacterial standing stocks per protozoan when the sites had low pH than when the pH was high, whereas site 1 was the opposite. Therefore, pH had different effects on the microbial community, depending on the site.

Patchy microbial distribution in sediments, which has been widely reported for both bacteria (68) and protozoa (74), hampers hypothesis testing on sediment and interface systems. A regression of all protozoan abundance data from all sites and dates versus pH was not significant (Fig. 3; slope = 0.31, $r^2 = 0.2$). This is not surprising, as date and site confounded the analysis of pH effects in a subset of the data as described above. Date is likely to reflect factors such as the amount of acid loading from runoff events, temperature, incident radiation, and carbon loading in this system, while site is a composite of water depth, percent organic matter, aquatic vegetation density, macrophyte species composition, mineralogy [abundance of $Fe(OH)_x$] (8), and water velocity.

Given these limitations of patchy distribution and multiply confounded variables, several conclusions can be drawn concerning microbial abundance and distribution at Lake Anna. (i) Protozoan abundance in the sediments and the interface was greater than the values reported for other systems, although abundance was significantly lower at the site closest to the AMD source. (ii) The distribution of protozoa across the lake was complex, with peak abundance between pHs 5.0 and 5.3. (iii) Bacterial yield generally showed greater numbers of bacteria per protozoan at low pH regardless of date, but this was not consistently observed at all sites across the lake.

**Grazing.** Twelve dilution grazing experiments were conducted over the research period and over a pH range of 3.60 to 6.39 to examine the effect of acidification on protozoan bacterivory (e.g., Fig. 4). Bacterial growth in the undiluted controls was near zero because of continued grazing pressure, while bacterial growth was greater than zero in the dilution treatments. Two experiments directly compared protozoan grazing rates on the same dates at sites with contrasting pHs (Fig. 4 and 5). In both experiments, grazing rates were higher at the more acid site; these differences were significant ($t$ test) in one experiment but not for the other because of unusually high variance in the acid treatment (Table 4). Regression of protozoan specific grazing rate versus pH revealed no significant trend (slope = 0.017, $r^2 = 0.11$). This result concurs with the lack of correlation between community zooplankton grazing rates and pH (11). Protozoan grazing rates varied seasonally, with the maximum occurring in late summer and into the autumn and are likely to be a function of temperature (Fig. 6).

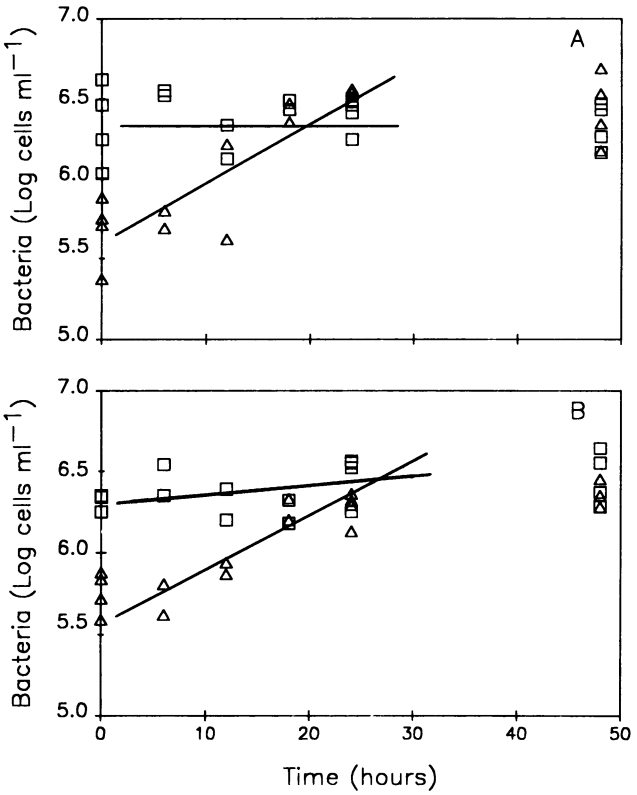


FIG. 4. Bacterial concentration as a function of time in dilution grazing experiments conducted in September 1984 at sites with pH values of 4.39 (A) and 5.24 (B). The lines are least-squares regression slopes indicative of bacterial growth rates for the control (□) and the 1:3 dilution treatment (△). Regression coefficients (± the standard errors) for the control and the dilution treatment, respectively, are as follows: graph A, 0.0016 ± 0.0049 and 0.0380 ± 0.0053; graph B, 0.0037 ± 0.0036 and 0.0244 ± 0.0037. The grazing rates calculated from these data, contrasting the two sites, were statistically significantly different.

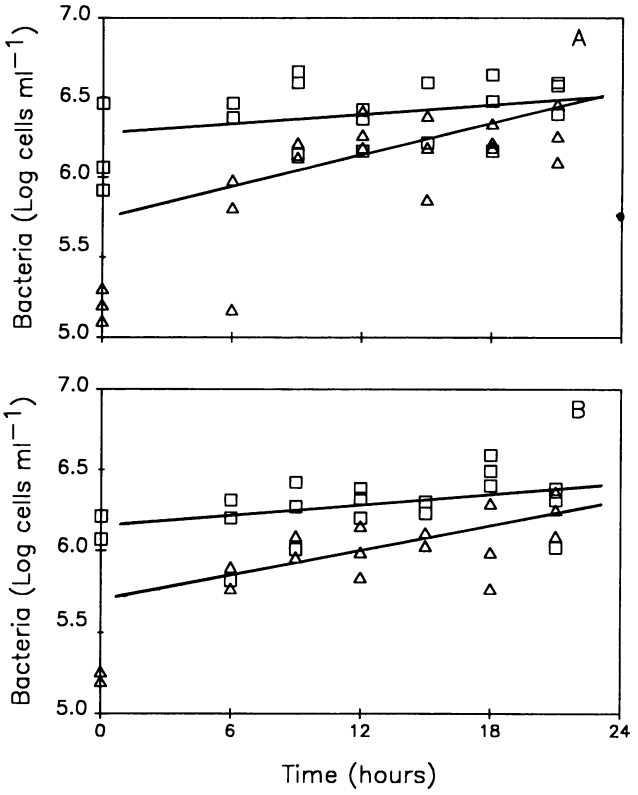


FIG. 5. Bacterial concentration as a function of time in dilution grazing experiments conducted in July 1986 at sites with pH values of 4.24 (A) and 6.39 (B). Lines indicate least-squares regression slopes indicative of bacterial growth rates for the control (□) and the 1:3 dilution treatment (△). Regression coefficients (± the standard errors) for the control and the dilution treatment, respectively, are as follows: graph A, 0.0048 ± 0.0090 and 0.0320 ± 0.122; graph B, 0.0108 ± 0.0054 and 0.0246 ± 0.0071. The grazing rates calculated from these data, contrasting the two sites, were not statistically significantly different.

Specific grazing rates ranged over an order of magnitude (0.02 and 0.23 $h^{-1}$, median = 0.075; Table 3); half of the grazing rates were higher than any other published value (Table 5). Clearance rates (Table 4) ranged from 18 to 65 nl $grazer^{-1}$ $h^{-1}$ with a mean of 45 nl ± 8 (standard error of the mean) nl $grazer^{-1}$ $h^{-1}$ and were greater than all other published values, except for marine ciliates in a Georgia salt marsh tidal creek and the adjacent estuary (Table 5) (64). Feeding rates ranged from 7 to 111 bacteria $grazer^{-1}$ $h^{-1}$, with a mean of 62.9 ± 15. The rates are higher than those published for water column studies in both freshwater and marine systems and are surpassed only by published feeding rates for marine sediment ciliates (Table 5) (39).

Previously, we have described an analysis of substrate kinetics for the Lake Anna sediment bacterial community in which the natural dissolved organic carbon concentration was 5.7 mg of C $liter^{-1}$ and the calculated half-saturation constant was 5.4 mg of C $liter^{-1}$ (76, 77). These data suggest that the bacterial community was not substrate limited and was growing at maximal rates. Such a situation would allow for high rates of protozoan bacterivory. The protozoan community consumed 0.53 × $10^6$ to 11 × $10^6$ bacteria $ml^{-1}$ $day^{-1}$ or 47 to 150% of the daily bacterial production. The ranges of specific grazing rates and bacterial specific growth rates were nearly equal (0.02 to 0.23 $h^{-1}$ versus 0.03 to 0.24 $h^{-1}$). These results are consistent with reports in which protozoan grazing rates were determined independently of bacterial production (uptake of tritiated thymidine) (22). Calculated bacterial production rates at Lake Anna (specific growth rates; range = 0.03 to 0.24 $h^{-1}$; Table 4) are in the range of the most productive bacterial marine systems, such as the Chesapeake Bay, the Great Barrier Reef, and the Baltic Sea (47).

**Protozoan growth.** Reports in the literature document species of acidophilic protozoa and their association with AMD. Given the results described above, which show abundant populations and active grazing, we wondered whether the AMD in this system had acted as a selective force on the protozoan community to produce such an acidophilic community. Alternatively, maybe the acid stress was insufficient (compensated for by sediment microbial alkalinity generation) to select an acidophilic protozoan community. If the protozoan community from the acidified arm of the lake had adapted to the acid stress, then higher protozoan growth rates at low pH and reduced growth rates at high pH should result. A community not adapted to low pH should have growth rates with the opposite pattern. To test this, we measured the specific growth rates of protozoan

TABLE 4. Protozoan apparent specific grazing rates and other population parameters calculated from dilution grazing experiments

| Date (mo-day-yr) | pH | Bacterial abundance ($10^6$ cells $ml^{-1}$) | Specific grazing rate ($h^{-1}$) (SD) | Bacterial specific growth rate ($h^{-1}$) | % of bacteria grazed | Protozoan concn ($10^3$ cells $ml^{-1}$) | Clearance rate (nl $grazer^{-1}$ $h^{-1}$) |
|---|---|---|---|---|---|---|---|
| 9-21-84 | 4.39 | 2.47 | 0.106 (0.017) | 0.110 | 96 | | |
| 9-21-84 | 5.24 | 2.29 | 0.065 (0.012) | 0.074 | 88 | | |
| 5-8-84 | 4.28 | 1.43 | 0.016 (0.014) | 0.033 | 47 | | |
| 5-12-84 | 3.60 | 2.58 | 0.052 (0.008) | 0.080 | 66 | | |
| 7-13-85 | 3.60 | 1.87 | 0.064 (0.012) | 0.044 | 150 | 1.84 | 35.0 |
| 8-1-85 | | 2.14 | 0.212 (0.012) | 0.194 | 110 | 6.84 | 31.0 |
| 6-2-86 | 4.20 | 1.28 | 0.097 (0.031) | 0.075 | 142 | 17.6 | 55.1 |
| 7-15-86 | 5.10 | 1.89 | 0.109 (0.027) | 0.104 | 106 | 1.67 | 65.2 |
| 7-31-86 | 4.25 | 2.24 | 0.080 (0.035) | 0.091 | 88 | 4.40 | 18.2 |
| 7-31-86 | 6.39 | 1.34 | 0.050 (0.021) | 0.074 | 68 | 0.77 | 64.9 |
| 10-16-86 | | 2.33 | 0.032 (0.008) | 0.055 | 59 | | |
| 11-6-86 | | 0.75 | 0.228 (0.087) | 0.243 | 93 | | |
| Minimum | 3.60 | 0.75 | 0.016 | 0.033 | 47 | 0.77 | 18.2 |
| Maximum | 6.39 | 2.58 | 0.228 | 0.243 | 150 | 17 | 65.2 |

communities from the acid and nonacid sites incubated at pHs 6, 5, and 4. The pH of each flask was measured as it was subsampled over time; pH increased less than 0.6 pH unit over the 7-day experimental period in all flasks. Heterotrophic protozoa were enumerated from subsamples taken over a 28-day period (Fig. 7). Generally, heterotrophic protozoa increased in number over the course of the experiment. Least-squares regressions were performed on logarithmically transformed protozoan concentrations versus time to obtain protozoan specific growth rates from the slope (Table 6).

The three-way ANOVA (three replicates by two protozoan sources by four treatments [pHs 4, 5, and 6 and the diluent control]) conducted on the heterotrophic protozoan specific growth rates included specific (special) contrasts to examine more closely the sources of error variance within the treatments (multivariate ANOVA subroutine) (69). The ANOVA assumption of homogeneity of variance was tested (Cochran's $C$ test with 2 and 6 degrees of freedom = 0.364, $P$ = 0.623); the populations were found to have equal variances. There were two ANOVA results. (i) Heterotrophic protozoan growth at pH 6 was significantly greater than that at pH 5 or 4 ($P$ = 0.001 and 0.002, respectively; Fig. 8). (ii) The source of the protozoan inoculum (acid versus nonacid) was not a significant contributor to error variance associated with the estimate of protozoan specific growth rate ($P$ = 0.95). Therefore, the protozoan community from the acidified site was not acidophilic relative to the community derived from the nonacidified site.

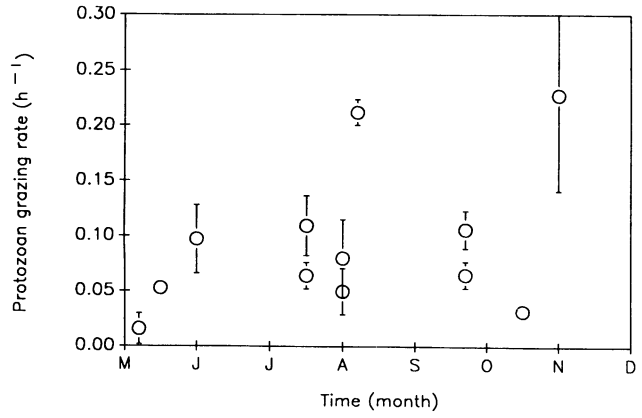


FIG. 6. Temporal distribution of protozoan apparent specific grazing rates calculated from dilution grazing experiments.

While protozoan abundance was high and specific grazing rates were not limited by low pH, site-specific protozoan abundance and community growth rate were depressed under acidic conditions. This suggests that low pH stresses the protozoa, resulting in reduced fecundity. Energy budget studies have shown that the major energy sink for protozoa is production of macromolecules, which is directly coupled to growth and reproduction (25, 27). Only a small fraction of protozoan energy is spent on mechanical, electrical, and osmotic work. Under pH stress, energy is diverted to mechanical and osmotic work. The large ciliate *Paramecium caudatum* exhibits decreased swimming velocity and increased ciliary reversal (avoidance reactions) under low-pH conditions; both are expected to impair feeding efficiency (20). Freshwater protozoan cytoplasmic osmolalities are 110 to 117 mOsm $liter^{-1}$, whereas their environment is usually 0 to 6 mOsm $liter^{-1}$ (51). As a result, protozoans continually pump water out via contractile vacuoles to compensate for osmotic water gain (25); low pH increases protozoan cell membrane permeability (19, 23, 31) and, therefore, raises the energetic cost of iono-osmoregulation for protozoa in acidified systems. Given all of the data, we can hypothesize that under acid stress in disturbed lake sediments (pH $\leq$ 5), higher maintenance costs probably cause reduced community growth rates (despite equal or greater grazing), resulting in the observed lower abundance at the most acid site.

Despite reduced community growth rates at low pH, there was no environmental selection for an acid-adapted protozoan community of acidophiles. This most likely occurred because the sediments were temporally (and spatially) variable with respect to pH. The Lake Anna sediment-water interface environment is highly dynamic. Periodic scouring and new sediment deposition at the interface by storm events expose protozoa to low-pH conditions (9) and population washout. After storm events, the protozoan community likely suffers from reduced fecundity as the cellular water-ion balance is disrupted by increased proton concentrations; however, these conditions are short lived. Within 3 weeks of a major storm event, the bulk porewater pH had increased from 3.5 to 6.5; moderate-pH conditions were probably reestablished even more quickly in sediment microenvironments (9). Thus, high rates of bacterial alkalinity

TABLE 5. Comparison of protozoan grazing, clearance, and feeding rates for Lake Anna, other lakes, and marine systems

| Source and organisms[a] | Grazing rate ($h^{-1}$) | Clearance rate(s) (nl $grazer^{-1}$ $h^{-1}$) | Feeding rate (cells $grazer^{-1}$ $h^{-1}$) | Site (reference) |
|---|---|---|---|---|
| Water column | | | | |
| F | 0.0003–0.0017[b] | 0.27–1.7[b] | 2.7–17 | Lake Vechten (12) |
| C, F | 0.0014–0.014[b] | 0.1–20 (F), 2–220 (C) | NA[c] | Lake Oglethorp (57) |
| F | 0.021–0.045 | 20 | 26.9 | Marine coast (43) |
| C, F | 0.017 ± 0.01 | NA | NA | Warm Core Rings (21) |
| F | 0.003–0.009[b] | 0.6–5.3 | 2.0–36.8 | Baltic Sea (41) |
| C, F | 0.0007[b] | 1.65 (F), 138 (C) | 19[b] | Open sound (64) |
| C, F | 0.019[b] | 2.7 (F), 168 (C) | 81[b] | Tidal creek (64) |
| Sediment | | | | |
| C | 0.0001–0.001[d] | 0.01–0.16[b] | 37–525 | Salt marsh-pond (39) |
| C, F | 0.02–0.23 | 18–65 | 7–111 | Lake Anna (this study) |

[a] C, Ciliates; F, flagellates.
[b] Calculated from available data.
[c] NA, Not available.
[d] The first number is for a salt marsh, and the second number is for sediment from a saline pond.

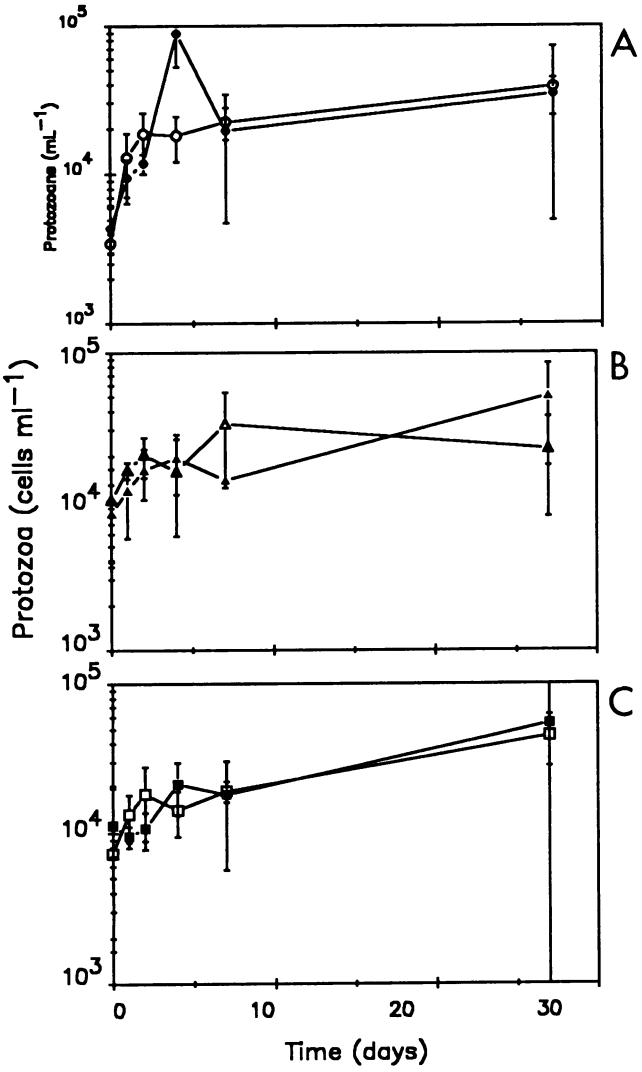


FIG. 7. Change in protozoan concentration with time for mixed protozoan communities collected at the acidified (ACID; station 3) station (open symbols) and the nonacidified (FW) station (solid symbols) exposed to pH 6 (A), 5 (B), or 4 (C).

generation and iron geochemistry (8) stabilize porewater pH while the gradient across the interface is highly dynamic.

**Conclusions.** Protozoan bacterivory is a major component of the microbial food web and has been widely documented in many pelagic ecosystems (12, 22, 57, 64). In Lake Anna, low pH did not reduce bacterivory, which consumed a substantial portion (often 100%) of the bacterial production. Researchers have attempted to determine whether the microbial food web is significantly linked to higher trophic levels; accumulating evidence suggests that carbon flux from bacteria to the metazoa via protozoa is small (22, 55, 71). This, however, does not preclude significant nutrient fluxes to higher trophic levels.

This study suggests that acidification decreases the net

TABLE 6. Heterotrophic protozoan apparent specific growth rates of mixed protozoan communities collected from ACID and FW sites and exposed to pHs 6, 5, and 4

| Treatment pH | Specific growth rate (SE) ($h^{-1}$) | |
|---|---|---|
| | ACID inoculum | FW inoculum |
| 6 | 0.582 (0.288) | 0.532 (0.180) |
| | 0.893 (0.265) | 0.244 (0.113) |
| | 0.922 (0.202) | 0.420 (0.801) |
| Mean | 0.799 (0.115) | 0.525 (0.161) |
| 5 | 0.081 (0.370) | 0.147 (0.088) |
| | 0.156 (0.071) | 0.450 (0.219) |
| | 0.039 (0.021) | 0.083 (0.092) |
| Mean | 0.092 (0.120) | 0.227 (0.094) |
| 4 | 0.272 (0.028) | 0.196 (0.157) |
| | 0.044 (0.159) | 0.117 (0.196) |
| | 0.106 (0.214) | 0.463 (0.290) |
| Mean | 0.140 (0.081) | 0.258 (0.134) |
| Control | 0.774 (0.325) | 0.569 (0.223) |
| | 0.474 (0.246) | 0.617 (0.454) |
| Mean | 0.620 (0.166) | 0.689 (0.269) |

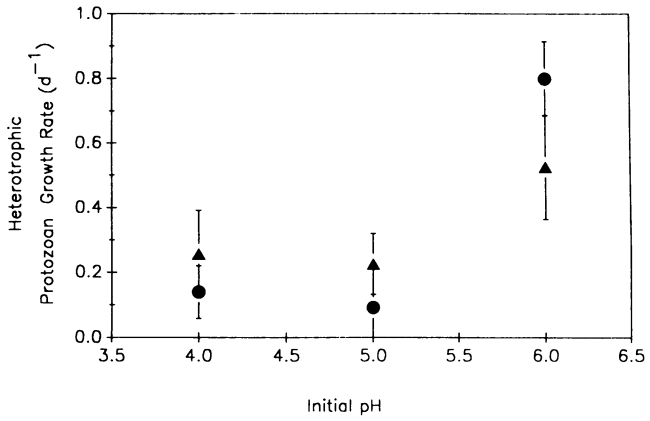


FIG. 8. Effect of initial pH on heterotrophic protozoan apparent specific growth rate for protozoans inoculated from the acidified (●) or nonacidified station (▲). Error bars, 1 standard deviation.

growth efficiency of protozoa, as evidenced by higher bacterial yields at lower-pH sites, equal (or perhaps greater) grazing rates, and decreased protozoan growth rates at low pH. The trophic efficiency of the conversion of detritus to bacterial biomass is related to the nutrient quality of the detrital material (16, 26). Protozoan bacterivory, in turn, repackages small, high-quality, bacterial biomass into larger, high-quality protozoa with efficiencies in excess of 60% (25). Lakes acidified by acid rain are frequently oligotrophic and nutrient poor; phosphorus availability often limits pelagic phytoplankton and bacterial production (52, 62). It is likely that oligotrophic lakes rely on nutrient recycling by the microbial food web to support metazoan, pelagic food webs (56). The effect of acid stress-induced decreased protozoan growth efficiency remains to be studied, but it is likely to be increased nutrient excretion (recycling).

Acidification did not dramatically inhibit the protozoan community at the sediment-water interface of a lake receiving AMD. This conclusion was reached because grazing rates were high and not inhibited by low pH and protozoan abundances were higher than any other value reported in the literature. Additionally, there was no evidence of an acidophilic protozoan community. These data suggest that protozoan populations are not limited by acidification in Lake Anna, and we hypothesize that this is due to the mitigating effects of a refuge from acid stress provided by sediment bacterial alkalinity generation. Because moderate sediment porewater pH has been widely reported in temperate acid lakes (4, 35, 38, 62), the results of our experiments can be generalized to other systems.

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