# Direct and indirect effects of mine drainage on bacterial processes in mountain streams

DEV K. NIYOGI[1] AND WILLIAM M. LEWIS, JR.

*Center for Limnology, Cooperative Institute for Research in Environmental Sciences,
University of Colorado, Boulder, Colorado 80309-0216 USA*

DIANE M. MCKNIGHT

*Institute for Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309-0450 USA*

*Abstract.* The effects of mine drainage on 2 bacterial processes were examined in Rocky Mountain streams affected by mine drainage. Thymidine incorporation into bacterial DNA was measured as an index of bacterial production, and nitrification was examined because it is sensitive to a variety of stressors. These processes, as well as pH, concentration of dissolved Zn, and deposition rate of metal oxides, all of which are influenced by mine drainage, were measured at 37 sites. The concentrations of inorganic nutrients and dissolved organic carbon (DOC), as well as algal biomass (as chlorophyll *a*), also were measured at each site. The rate of incorporation of thymidine into DNA was negatively related to deposition of metal oxides and positively related to pH, algal biomass, and concentration of DOC. pH, DOC, and algal biomass together explained 59% of the variation in thymidine incorporation among sites in a multiple regression. Metal oxide deposition probably influenced thymidine incorporation through suppression of algal biomass and through sorption of a fraction of the DOC. The nitrification potential for microbes on gravel substratum removed from streams was estimated as the rate of $NO_3^-$ production following addition of $NH_4^+$. Nitrification was undetectable at pH < 5.3 or Zn > 2 mg/L. Low pH (<6, as a categorical variable), concentration of Zn (which had a negative effect), and algal biomass (which had a positive effect) explained 77% of the variation in nitrification potential. The results suggest that bacterial processes vary in their sensitivity to the stressors from mine drainage, and that they can be disrupted both directly and indirectly. Nitrification was sensitive to direct influences of acid mine drainage, namely acidity and dissolved Zn. Low pH also affected thymidine incorporation. In contrast, acid mine drainage indirectly affected both thymidine incorporation and nitrification potential through its influence on algal biomass, which declined in response to deposition of metal oxides.

*Key words:* bacterial processes, thymidine incorporation, bacterial production, nitrification, mine drainage, streams.

Ecosystem processes are an important indicator of ecosystem health (Meyer 1997, Hill et al. 2002), but often are overlooked in considering the effects of anthropogenic stress on ecosystems (Cairns 1983). Bacteria perform many important processes in stream ecosystems. They break down both dissolved and particulate organic matter (Suberkropp 1998), and are mediators in many biogeochemical pathways, such as the oxidation and reduction of N, S, and Fe. Bacteria can be consumed by higher trophic levels and enter aquatic food webs through the microbial loop (Meyer 1994). Thus, bacterial processes warrant measurement and understanding in

streams under anthropogenic stress. The purpose of our research was to examine the effects of mine drainage on 2 bacterial processes, thymidine incorporation and nitrification, in mountain streams.

The substratum in mountain streams typically is composed of gravels, cobbles, and boulders that support epilithic bacteria in close association with other biota, especially algae, as a biofilm. Heterotrophic bacteria in the epilithon rely on organic C for energy, which can come from several sources. Bacteria may obtain C from living or dead algae in the biofilm, and from particulate and dissolved organic C (DOC) in the stream. Bacterial productivity covaries with algal abundance and productivity in some streams (Kaplan and Bott 1989, Romaní and Sabater 1999), but not in others (Findlay et al.

[1] Present address: Department of Biological Sciences, University of Missouri-Rolla, 105 Schrenk Hall, 1870 Miner Circle, Rolla, Missouri 65409-1120 USA. E-mail: niyogid@umr.edu

1993, Sobczak and Burton 1996). Addition of labile C to stream water usually increases bacterial productivity (Maltby 1992, Sobczak 1996).

Microbial metabolism is responsible for most processes in the N cycle, including nitrification, which is the microbial oxidation of $NH_4^+$ to $NO_3^-$ by chemoautotrophic bacteria. The nitrification rate of a stream can influence the export of N because the product, $NO_3^-$, is more mobile than $NH_4^+$. At the same time, $NO_3^-$ can be denitrified to gaseous end products, which can be an important sink for N in polluted streams (e.g., Sjodin et al. 1997). The rate of nitrification in streams is often related positively to concentrations of its reactants, $NH_4^+$ and $O_2$ (Strauss and Dodds 1997, Kemp and Dodds 2001). Nitrification rate can be related negatively to concentrations of DOC because heterotrophic bacteria outcompete nitrifying bacteria for limiting $NH_4^+$ (Strauss and Lamberti 2000, 2002).

Mine drainage may affect the microbial processes of C and N cycling in streams through 3 major stressors: 1) acidity, 2) high concentrations of dissolved metals, and 3) deposition of hydrous metal oxides (Sode 1983, McKnight and Feder 1984, Kelly 1988). Few studies have examined the effects of these stressors on bacterial processes. Wassel and Mills (1983) noted altered community structure of sediment bacteria in a lake receiving mine drainage. Palumbo et al. (1987) and Osgood and Boylen (1990) found low production of epilithic bacteria in streams with low pH. Nitrification can be disrupted by low pH (Rudd et al. 1988) or metal toxicity (Singha et al. 1998). However, the various stressors from mine drainage may differ in their effects on stream biota and ecosystem processes (McKnight and Feder 1984, Niyogi et al. 1999, 2001). A comprehensive study examining these effects on stream microbes is lacking.

We chose 2 bacterial processes to monitor in streams under stress from mine drainage: thymidine incorporation because it is an index of bacterial growth rate, and nitrification because it is sensitive to stress (Rudd et al. 1988). We expected nitrification, which is conducted by specific taxa of bacteria, would be more sensitive than thymidine incorporation, which measures growth of many bacterial taxa that could have varying sensitivities to the effects of mine drainage. We examined both the direct and indirect effects of the individual stressors from mine drainage on these 2 processes. Bacteria may be directly affected by low pH and metal toxicity, but also may be indirectly affected by mine drainage because of its effects on algal biomass and DOC.

## Methods

### Site descriptions

Study sites were located on low-order streams at high elevation (2700–3400 m asl) in the Rocky Mountains of Colorado in regions with a history of mining (Fig. 1). Thymidine incorporation and nitrification potential were measured at 37 sites. The study sites varied greatly in amount of mine drainage entering streams. Some sites were unaffected by mine drainage and were considered pristine, whereas others were severely polluted. Most of the streams had slopes of 2 to 6%, and stream substrata consisted of cobble, gravel, and boulder. Study sites were situated in well-lit riffles, which was the dominant habitat type of most of the streams. The hydrographs of the streams are dominated by snowmelt, which occurs predominantly during May and June and is accompanied by peak concentrations of DOC and low algal biomass caused by scour and turbidity. Measurements for this study were conducted during baseflow, in late August to early September of 1998, when algal biomass had become established in pristine streams.

### Abiotic and biotic characteristics of streams

The measurement of abiotic variables (pH, concentrations of metals and nutrients, rate of metal oxide deposition) is described in detail by Niyogi et al. (1999, 2001). pH was measured with an ion-specific electrode. Water samples for analysis of dissolved metals were filtered through 0.45-μm filters, acidified with ultrapure nitric acid, and analyzed by ICP-AES or AA spectroscopy (Kimball et al. 1994). Additional samples were analyzed as duplicates and spiked samples (every 10 samples). Among dissolved metals, concentrations of Zn were commonly in excess of US Environmental Protection Agency aquatic life criteria at most sites, whereas concentrations of other metals usually were not. Consequently, Zn was the only metal to be included in data analysis as a measure of stress. The detection limit for Zn in our analyses was 5 μg/L.
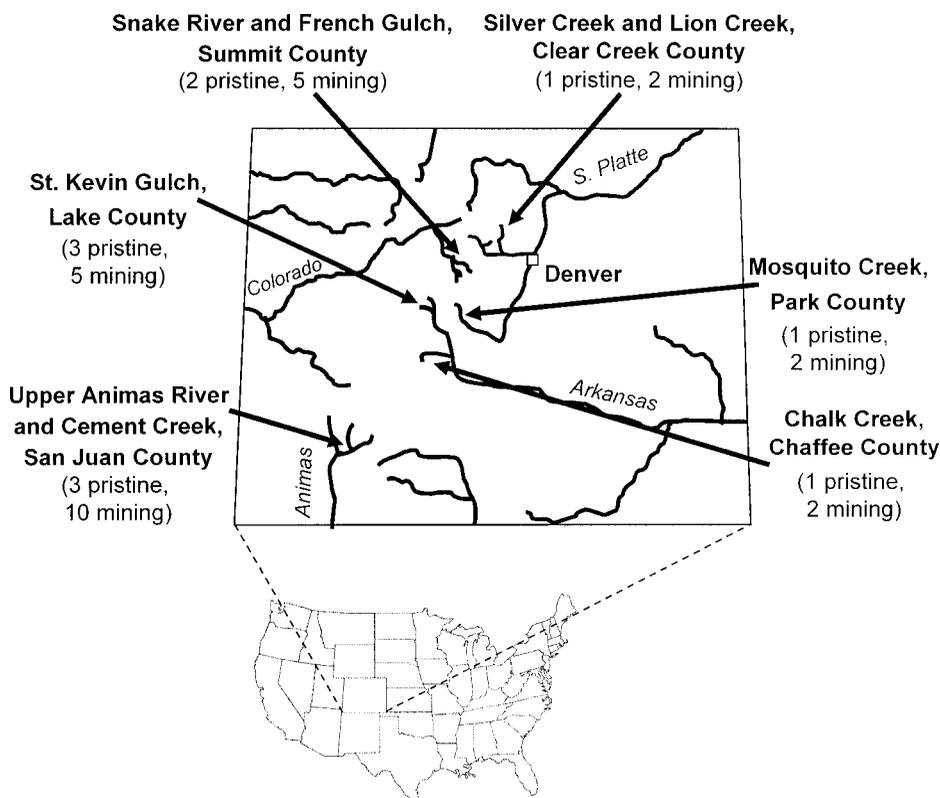
FIG. 1.   Study areas in Colorado, USA. Numbers of pristine sites and sites affected by mine drainage in each study area are listed.

The rate of metal oxide deposition was estimated as the rate of accumulation of deposits on cobbles placed in streams for known periods of time. Cobbles used for this purpose were removed from the stream, brushed clean, and placed in areas of moderate stream velocity (surface velocity 10–30 cm/s). The cobbles were left in the stream for 4 to 8 wk, at which time they were removed, placed in plastic bags, and transported to the laboratory. Deposits from the upper surface of the cobbles were brushed into a tared weighing boat for determination of dry and ash mass. The upper surfaces of the cobbles were covered with aluminum foil, which was then weighed, and converted to area by use of standardized (100-cm²) squares (Steinman and Lamberti 1996). Deposition rates were calculated as the mass of ash material accumulated on cobbles per unit of surface area per unit time. Deposited material from most sites was acid-digested and analyzed (Kimball et al. 1994) to confirm that the deposits were composed of oxides of Fe and Al.

Water samples for analyses of nutrients and DOC were filtered through glass-fiber filters (Whatman GF/F, nominal pore size = 0.7 μm) and analyzed by standard protocols (Lewis et al. 1984). $NH_4^+$-N was measured by a phenol hypochlorite colorimetric test, $NO_3^-$-N was measured by ion chromatography, and soluble reactive P (SRP) was measured by an acid molybdate colorimetric procedure. DOC was analyzed by high-temperature combustion using a Dohrman DC-190 analyzer. Detection limits were determined as the lowest standard that could be detected reliably above background, and were as follows: $NH_4^+$-N = 2 μg/L, $NO_3^-$-N = 2 μg/L, SRP = 1 μg/L, and DOC = 0.2 mg/L.

Biomass of primary producers in the epilithon was estimated as the amount of chlorophyll a per unit area of substrate. Algae were scraped

from cobbles and filtered onto glass-fiber filters, which then were ground with a glass homogenizer, extracted with hot ethanol, and analyzed spectrophotometrically for mass of chlorophyll *a* after correction for phaeophytin (Lewis et al. 1984).

*Thymidine incorporation*

Thymidine incorporation into DNA was measured on unglazed, ceramic tiles that had been in the stream for at least 6 wk to allow epilithic communities to become established. Tiles were placed in containers with stream water and returned to the laboratory, where thymidine incorporation by epilithic bacteria was measured within 12 h as described by Findlay (1993). Tritiated thymine (0.6 nmol thymidine, $^3$H-methyl, specific activity = 35 $\mu$Ci/nm) was added to a tube containing 20 mL of filtered stream water and one tile (2.5 × 2.5 cm). Incubations were conducted in the dark at 10°C and lasted 2 h, after which time formalin was added. Preliminary trials indicated that thymidine incorporation was linear over a 4-h period. Formalin was added at the beginning of incubation for some samples, which served as killed controls to correct for abiotic processes that lead to labeled thymidine being present in DNA extracts. Samples were filtered after incubation, and the tile and filter were shaken with alkaline extract (0.3 N NaOH, 0.1% SDS, 0.025 M EDTA) overnight. DNA was precipitated by addition of cold trichloroacetic acid (TCA) and washed several times with 5% cold TCA. The DNA was then hydrolyzed with heat and counted by liquid scintillation, with quench correction. Rates of thymidine incorporation are reported as pmol of tritiated thymidine incorporated into DNA per cm$^2$ substrate (tile) per hour, corrected for killed controls.

*Nitrification potential*

Nitrification potential was measured for microbes on gravel (8–32 mm) collected from the stream and incubated in the laboratory (150 g of gravel in 100 mL of stream water). Gravel sizes did not vary visually among sites. Excess $NH_4^+$ (final concentration of ~1 mg N/L) was added to ensure that free $NH_4^+$ was available to nitrifying bacteria after sorption and uptake by algae and heterotrophic bacteria. Incubations were conducted in the dark at 10°C and lasted 1 to 2 d; containers were gently shaken during incubation. Water from the containers was then filtered (Whatman GF/F filter, nominal pore size = 0.7 $\mu$m) and analyzed for $NO_3^-$ and $NO_2^-$ by ion chromatography. Concentrations of $NH_4^+$ also were measured (as described above) in test containers at the end of incubations to ensure that $NH_4^+$ was not limiting nitrification. Blanks (stream water only) and killed controls always showed undetectable changes in concentration of $NO_3^-$. Rates of nitrification potential are reported as the increase in $NO_3^-$-N per gram of gravel per day.

The effects of nitrogenous and non-nitrogenous organic matter on nitrification rates were tested for gravel taken from one pristine site (Deer Creek, Summit County; see McKnight and Feder 1984 for site description). We hypothesized that algal organic matter (biomass or exudates) may lead to high rates of nitrification because its low C:N ratio allows $NH_4^+$ to be available to both heterotrophic and nitrifying bacteria. Labile organic C sources without any N inhibit nitrification because heterotrophic bacteria outcompete nitrifiers for $NH_4^+$ (Strauss and Lamberti 2000, 2002). Glucose was chosen as a non-nitrogenous, labile C source, and glutamate was used as a surrogate for algal organic matter (labile, low C:N). The control had no additions of $NH_4^+$ or C, and thus was a measure of net nitrification (net increase in $NO_3^-$ over time). One treatment had $NH_4^+$ added (to 1 mg N/L), which was the same treatment as in the nitrification potential measurements described above. The 2 other treatments had glucose or glutamate (Na salt) added to achieve a final concentration of 20 mg C/L in the test containers.

*Data analysis*

The effects of stressors from mine drainage and other variables on bacterial processes were analyzed by simple and multiple linear regression using SAS (release 8.00, SAS Institute, Cary, North Carolina). In addition, logistic regression was used to examine the effects of pH on nitrification, which exhibited a threshold response to low pH. Given the effect of low pH on nitrification (see Results), low pH (<6) was then included as a categorical variable in further analyses using multiple regression. Data were transformed as necessary to improve consistency

with assumptions of parametric statistics. A full stepwise regression procedure was used to determine best predictive models. The stepwise procedure added and retained independent variables to the model if they were significant at $p < 0.10$. Results from the stepwise procedure were compared to a best subsets procedure (given the reduced number of variables) to ensure consistency between methods. Collinearity among independent variables was checked by calculating variance inflation factors (VIF) in the regressions. All VIF values from our regressions were <2, indicating collinearity did not have large effects on the results of the regressions.

A path analysis (Schumacker and Lomax 1996) was also done for each bacterial process to test an overall model of direct and indirect effects of mine drainage as predicted by previous studies (e.g., Rudd et al. 1988, Kaplan and Bott 1989, Sobczak 1996). Specifically, we expected thymidine incorporation to be affected directly by low pH, and indirectly by effects on algal biomass and DOC. Metal oxide deposition was negatively related to both algal biomass and DOC in previous studies (McKnight et al. 1992, Niyogi et al. 2002). Our model of nitrification potential predicted that low pH and elevated Zn would affect nitrification, as would indirect effects through algal biomass. Path analysis allows one to examine the statistical validity of a given model of relations among variables. The overall fit of our models was evaluated by the Normed Fitted Index (NFI) of Bentler and Bonnet (1980). An NFI value ≥0.90 is considered acceptable. Path analysis also allows one to compare pathways of effects on a response variable. Standardized regression coefficients are calculated for causal relationships in the proposed model, and correlation coefficients are determined for relationships between independent variables. The magnitude of an indirect effect can be estimated by multiplying path coefficients (either standardized regression or correlation coefficients) in a series of paths. Path analysis was used in this manner to compare direct and indirect effects of mine drainage on bacterial processes.

## Results

### Water chemistry

The pH of the study sites ranged from 2.7 to 7.8, concentrations of dissolved Zn ranged from <0.01 to 80 mg/L, and the deposition rate of metal oxides ranged from <0.01 to 1.2 g m$^{-2}$ d$^{-1}$ (Figs 2–4). All of the pristine sites had circumneutral pH (7–8), low concentrations of Zn (<0.05 mg/L), and low rates of deposition (<0.01 g m$^{-2}$ d$^{-1}$) of metal oxides or other inorganic material. Concentrations of $NH_4^+$ usually were low (<5 µg N/L); however, one site had very high $NH_4^+$ (460 µg N/L), and 2 sites with low pH had high concentrations of $NH_4^+$ (25 and 82 µg/L). Concentrations of $NO_3^-$ varied from undetectable (<2 µg N/L) to 210 µg N/L. SRP concentrations usually were low (<5 µg/L), but 2 sites with very low pH (<3) had high SRP (56 and 81 µg/L). DOC ranged from 0.2 to 3.8 mg/L, and algal biomass ranged from <1 to 144 mg chlorophyll $a$/m$^2$ (Figs 2, 3).

### Thymidine incorporation

Thymidine incorporation ranged from 0.055 to 0.205 pmol thymidine cm$^{-2}$ h$^{-1}$ at pristine sites and from 0.005 to 0.161 pmol thymidine cm$^{-2}$ h$^{-1}$ at sites affected by mine drainage (Fig. 2). Of the stressors from mine drainage (pH, Zn, metal oxide deposition), metal oxide deposition was most closely related to the rate of thymidine incorporation ($R^2 = 0.39$). Deposition of metal oxides was significantly negatively related to rates of thymidine incorporation. Thymidine incorporation was also significantly related to pH. Most sites with low pH had low rates of thymidine incorporation, but 3 sites with pH <5 had rates comparable to those of pristine sites (>0.050 pmol thymidine cm$^{-2}$ h$^{-1}$). Thymidine incorporation was not significantly related to concentration of Zn. Several sites with high concentrations of Zn had high rates of thymidine incorporation.

Thymidine incorporation was significantly positively related to algal biomass and DOC concentration (Fig. 2). A stepwise regression procedure indicated that pH, algal biomass, and DOC together explained 59% of the variation in thymidine incorporation (Table 1). Although metal oxide deposition entered first into the stepwise model, it was removed once algal biomass and DOC were included in the regression. Metal oxide deposition was negatively related to both algal biomass and DOC (Fig. 4). Low pH had a negative effect on thymidine incorporation after accounting for the effects of algal biomass and DOC. No other variables, including

concentrations of inorganic nutrients (N, P), stream width, and location (areas shown in Fig. 1), were significantly related to thymidine incorporation in the final model.

The path analysis for thymidine incorporation (Fig. 5) illustrates how mine drainage affected thymidine incorporation either directly by low pH or indirectly through effects of metal oxide deposition on algal biomass and DOC. Algal biomass was the strongest predictor for thymidine incorporation, followed closely by DOC. The indirect effects of metal oxide deposition on thymidine incorporation were 0.54 ($0.68 \times 0.44 = 0.30$ via algal biomass and $0.66 \times 0.36 = 0.24$ via DOC), whereas the direct effect of pH was 0.32. Thus, indirect effects of mine drainage were greater than direct effects in predicting thymidine incorporation as revealed by path analysis. pH also was related to metal oxide deposition (correlation coefficient $= -0.53$) because it influences the solubility of metal oxides, so pH indirectly affects biological responses (algal biomass, thymidine incorporation) because of its relation to metal oxide deposition. The calculated NFI value for this model was 0.96, indicating a very good fit to the data.

*Nitrification potential*

Nitrification potential ranged from 0.04 to 0.37 $\mu$g N g$^{-1}$ d$^{-1}$ at our 11 pristine sites and from undetectable ($<0.002$ $\mu$g N g$^{-1}$ d$^{-1}$) to 0.24 $\mu$g N g$^{-1}$ d$^{-1}$ at 26 sites that were affected by mine drainage. Nitrification potential was undetectable at all 8 sites with pH $<5.3$ (Fig. 3). Nitrification potential also was undetectable at 2 sites that had neutral pH and concentrations of dissolved Zn $>2$ mg/L. Nitrification was detectable at all other sites.

Low pH had a significant effect on whether nitrification was detectable or not in a logistic regression (Wald $\chi^2 = 6.74$, df $= 1$, $p = 0.009$). Low pH ($<6$) was included as a categorical variable in a multiple regression to see what other factors influenced nitrification potential at our sites. In addition to low pH, Zn and algal biomass also were related ($p < 0.001$ and $p = 0.056$, respectively) to nitrification potential (Table 1). These 3 variables explained 77% of the variation in nitrification potential across all sites.

For sites with detectable nitrification, nitrification potential was significantly negatively related to concentration of dissolved Zn ($R^2 =$

0.39), but not to metal oxide deposition or DOC concentration (Fig. 3). Nitrification potential was also positively related to algal biomass, but one site with a high concentration of NH$_4^+$ (460 $\mu$g N/L) had low algal biomass and a high rate of nitrification. When this site was excluded from analysis, algal biomass explained 43% of the variation in nitrification potential. Algal biomass and Zn concentration together accounted for 61% of the variation in nitrification potential at sites with detectable nitrification (Table 1).

The addition of C and N to gravel from Deer Creek, a pristine stream, led to significantly different rates of nitrification. Net nitrification was detectable at this site (0.035 $\mu$g N g$^{-1}$ d$^{-1}$). When NH$_4^+$ was added at the concentration used for measures of nitrification potential, the rate of nitrification (0.11 $\mu$g N g$^{-1}$ d$^{-1}$) was $\sim$3 times higher. However, with the addition of glucose (no NH$_4^+$ added), the net rate of nitrification ($-0.025$ $\mu$g N g$^{-1}$ d$^{-1}$) was $<0$; the initial concentration of NO$_3^-$ in the incubation water was 80 $\mu$g N/L and the final concentration was undetectable ($<2$ $\mu$g N/L). The rate of nitrification (0.10 $\mu$g N g$^{-1}$ d$^{-1}$) after the addition of glutamate (no NH$_4^+$ added) was significantly higher (ANOVA, $p < 0.01$) than for the glucose treatment or the control and similar to the rate when NH$_4^+$ was added.

The path analysis for nitrification potential (Fig. 5) illustrates how mine drainage affected nitrification either directly by low pH and Zn or indirectly through effects on algal biomass. Low pH (as a categorical variable) and Zn were strong predictors for nitrification, and algal biomass was also important. The indirect pathway of metal oxide deposition affecting nitrification (via algal biomass) was only 0.10 ($0.61 \times 0.17$), whereas the direct effects of pH and Zn were much higher (path coefficients of $\sim$0.50). The calculated NFI value for this model was 0.95, indicating a good fit to the data.

**Discussion**

*Thymidine incorporation*

The rate of thymidine incorporation measured in pristine streams in our study is comparable to rates measured in other streams (Osgood and Boylen 1990, Sobczak 1996). Bacterial biomass and production in streams often are related to algal biomass (Goulder 1988, Kaplan
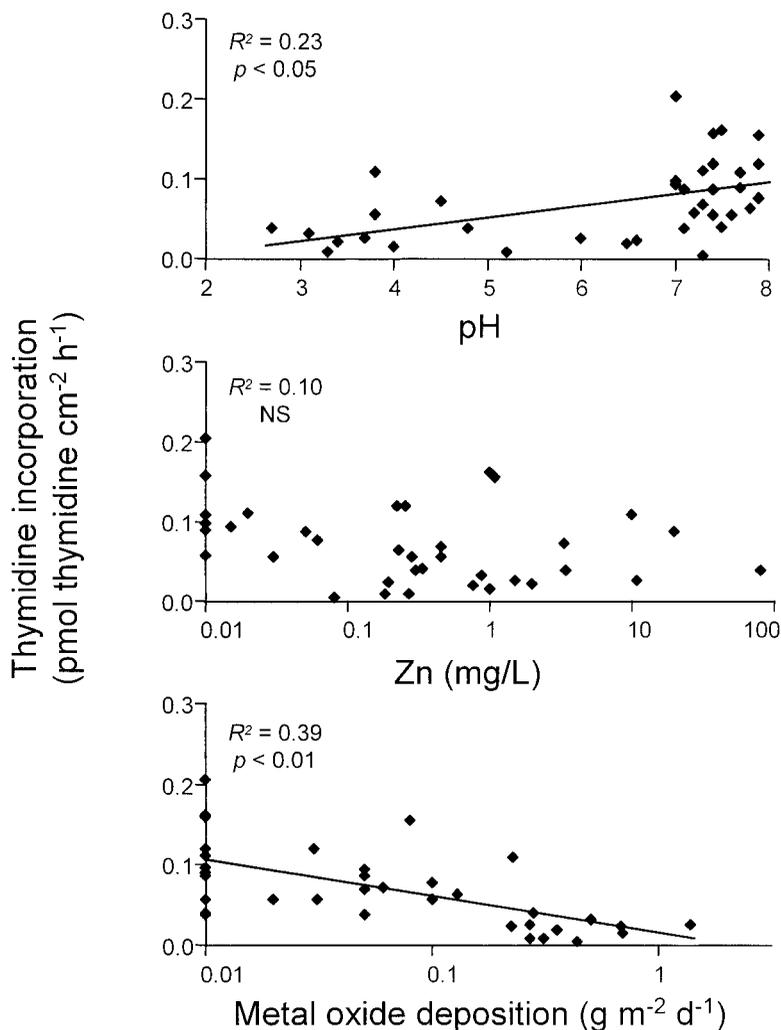
FIG. 2. Rate of thymidine incorporation versus pH, Zn, metal oxide deposition, algal biomass (chlorophyll *a*), and dissolved organic C (DOC). NS = not significant ($p > 0.05$).

and Bott 1989, Sobczak 1996, Ledger and Hildrew 1998, Romaní and Sabater 1999, Rier and Stevenson 2001) or DOC concentration (Bott et al. 1984, Sobczak 1996), as in our streams. DOC serves as a substrate for bacteria, and algal biomass may stimulate growth of epilithic bacteria through exudation of labile DOC (Haack and McFeters 1982, Neely and Wetzel 1995). Algae may also provide additional surface area for attachment of bacteria. Addition of glucose or nutrients (N, P) during 2-h incubations of tiles from several of our sites did not stimulate thymidine incorporation (DKN, unpublished data), but longer incubations may be necessary to in-

crease bacterial biomass and production (e.g., Sobczak 1996).

The negative correlation between metal oxide deposition and thymidine incorporation most likely was related to low algal biomass (chlorophyll *a* concentration) at sites with high rates of metal oxide deposition (Fig. 4, Niyogi et al. 2002). Also, metal oxides can adsorb DOC (McKnight et al. 1992), especially compounds with aromatic moieties and carboxylic acid functional groups. The sorption process could affect bacterial production, depending on the bioavailability of this fraction of DOC. If the sorbed DOC was biologically labile (Meyer et al.
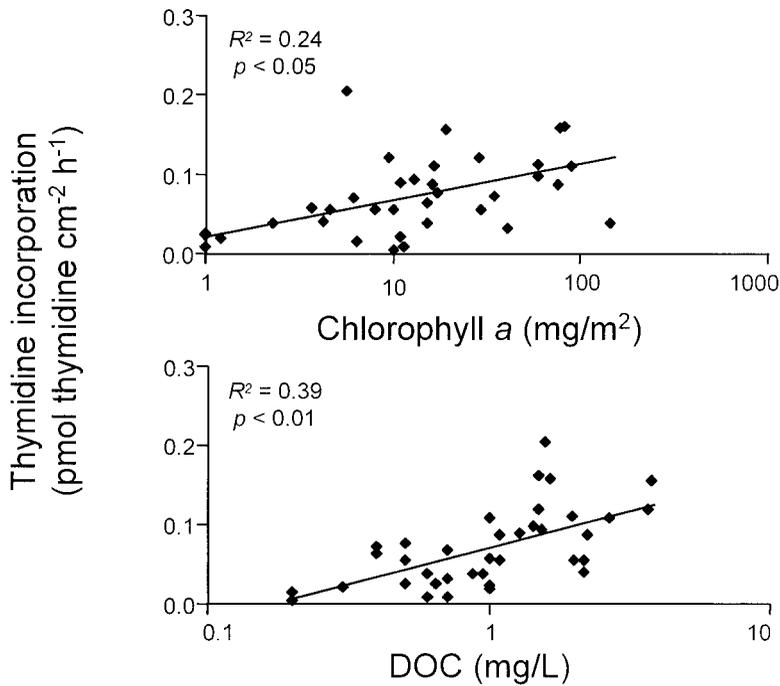
FIG. 2.　Continued.

1987, Volk et al. 1997), metal oxides could negatively affect bacterial production.

Low pH generally had a negative effect on thymidine incorporation after the effects of DOC and algal biomass were taken into account (Table 1). Low pH has been associated with low rates of epilithic thymidine incorporation in other studies (Palumbo et al. 1987, Osgood and Boylen 1990). Low pH also reduced bacterial production on leaf litter undergoing decomposition in acidic streams (Mulholland et al. 1987, Osgood and Boylen 1992). In another study, however, uptake of glucose by epilithic bacteria was higher in a stream of low pH (2.9) than a stream of neutral pH (Mills and Mallory 1987). Three of the sites in our study had low pH ($<5$) but high rates of thymidine incorporation. Thus, acidity often has a negative association with bacterial production, but there are exceptions where epilithic bacteria appear able to be productive despite low pH. High amounts of algal biomass or DOC at such sites may compensate for the stress from low pH. In addition, the loss of grazing protists and animals may allow greater bacterial biomass to develop than would otherwise be present in pristine conditions. A reduction in grazing pressure contributes to increased algal biomass and function in streams stressed by low pH or metals (Elwood and Mulholland 1989, Niyogi et al. 2002).

Concentrations of dissolved Zn were not significantly related to thymidine incorporation when pH, DOC, and algal biomass were taken into account. In fact, thymidine incorporation was higher at 2 sites with elevated Zn ($>1$ mg/L) than in pristine streams nearby. These sites also had higher algal biomass than the pristine streams. In another study, thymidine incorporation was not affected by high concentrations of Zn at 2 sites downstream from a Zn factory (Admiraal et al. 1999). A community of Zn-tolerant bacteria may develop and be productive at sites where Zn concentrations are high (Horner and Hilt 1985, Dean-Ross 1991, Admiraal et al. 1999). As in streams with low pH, a reduction in grazing and an increase in algal biomass in Zn-contaminated streams may compensate for stress from the high Zn in such environments.

*Nitrification*

We measured rates of potential nitrification because rates of net nitrification were 0 (or $<0$)
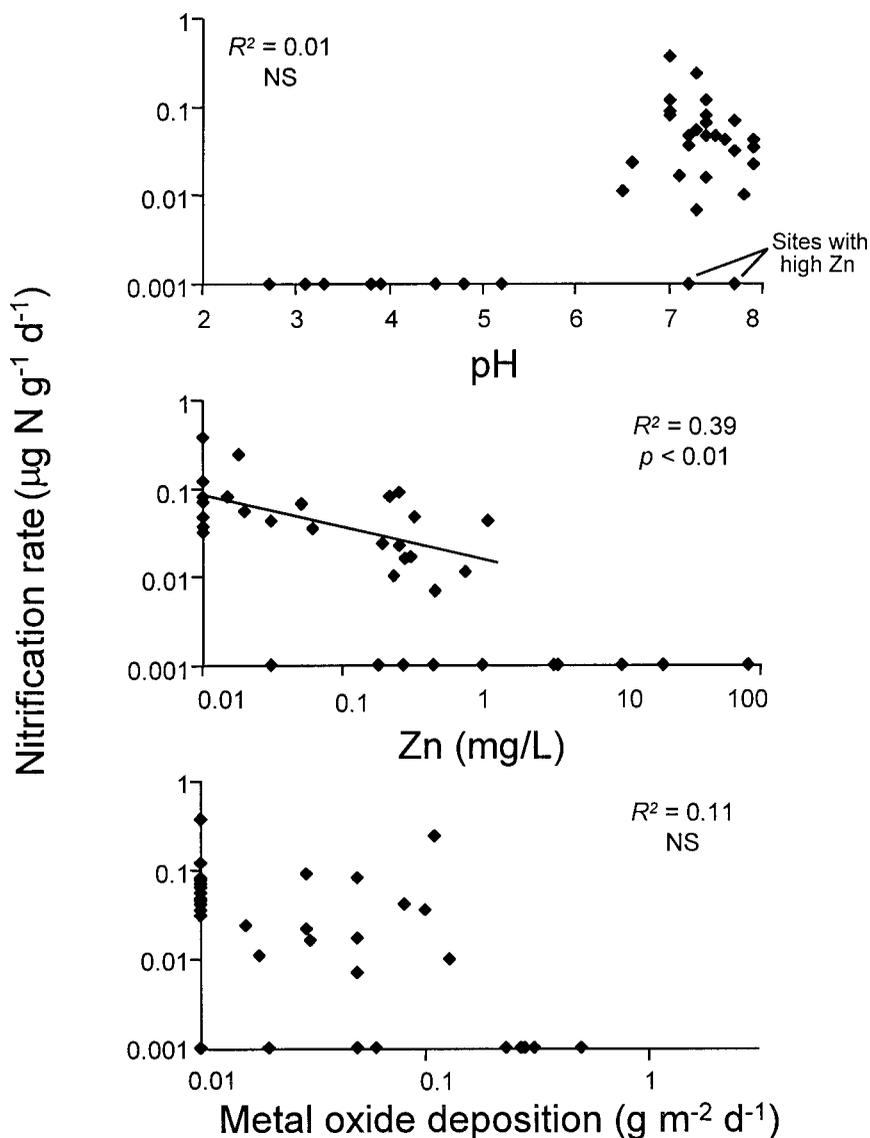
FIG. 3.   Rate of nitrification potential versus pH, Zn, metal oxide deposition, algal biomass (chlorophyll *a*), and dissolved organic C (DOC). Regression lines and statistics do not include sites where nitrification was undetectable (see text). Regression line for algal biomass does not include outlier with high $NH_4^+$ concentration. NS = not significant ($p > 0.05$).

at several of our study sites; uptake of $NO_3^-$ by N-limited biota led to negative or undetectable rates of net nitrification (data not shown). Nitrification potential was measurable even at pristine sites with undetectable inorganic N (<2 $\mu$g/L $NO_3^-$ and $NH_4^+$). Thus, populations of nitrifying bacteria at such sites probably compete with algae and heterotrophic bacteria for $NH_4^+$ that is transiently available from mineralization.

Recent studies using stable isotopes of N supported similar conclusions (e.g., Dodds et al. 2000, Peterson et al. 2001).

The nitrification potentials reported here generally are lower than nitrification rates measured in aquatic sediments (0.8–49 $\mu$g N $g^{-1}$ $d^{-1}$, reviewed in Strauss and Dodds 1997). These results were expected because gravel has much less surface area per unit mass than the fine sed-
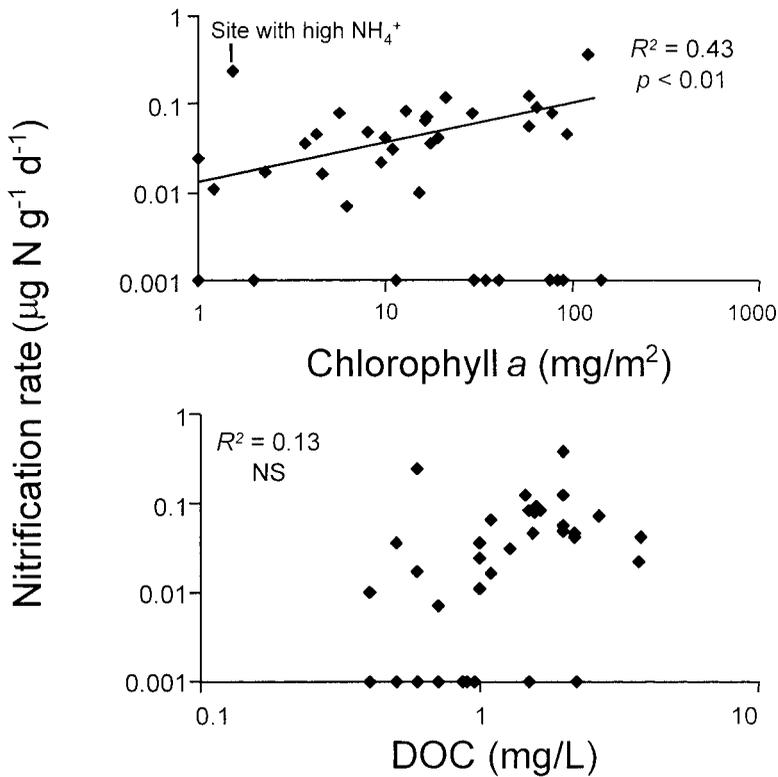
FIG. 3.  Continued.

iments in lakes and springs where most measurements have been made. Rates of nitrification per unit mass for coarse sand from streams in this study were 2 to 5 times higher than for gravel (DKN, unpublished data).

The concentration of $NH_4^+$ often controls the rate of nitrification in aquatic systems (Triska et al. 1990, Jones et al. 1995, Strauss and Dodds 1997, Strauss and Lamberti 2000). In our study, one site with a very high concentration of $NH_4^+$ also had a high rate of nitrification. Concentrations of $NH_4^+$ were usually very low at other sites, and were not related to nitrification potential. Nitrification rates also can be controlled by dissolved $O_2$ concentrations in some streams (Cooper 1984, Kemp and Dodds 2001), but $O_2$ probably did not limit nitrification on gravels in the well-aerated mountain streams of our study. Although additions of DOC can limit the rate of nitrification in stream sediments (Strauss and Lamberti 2000, and see below), we did not see a significant relationship between nitrification potential and DOC across our sites, which had a fairly narrow range of DOC.

Nitrification in aquatic systems appears to be inhibited below a threshold of pH ~6 and was undetectable at all sites of pH <5.3 in this study. Similarly, Rudd et al. (1988) found that nitrification became inhibited in 2 lakes as pH was lowered experimentally to <6. Nitrification also was undetectable in 2 mountain streams with pH <5 (Kopáček and Blažka 1994). These results indicate that nitrifying bacteria in streams cannot sustain nitrification at low pH, although some nitrifiers can tolerate transient episodes of low pH (Kopáček and Blažka 1994, DKN, unpublished data). Nitrification can occur in acidic soils (De Boer and Kowalchuk 2001), although the rate is often reduced compared to neutral pH. Nitrifying bacteria oxidize unionized $NH_3$, which decreases in concentration (in an acid–base relation with $NH_4^+$) as pH decreases. Thus, substrate limitation at low pH may affect the growth of nitrifiers (Prosser 1989). However, other factors, including toxicity, may also account for reduced (or totally inhibited) nitrification at low pH.

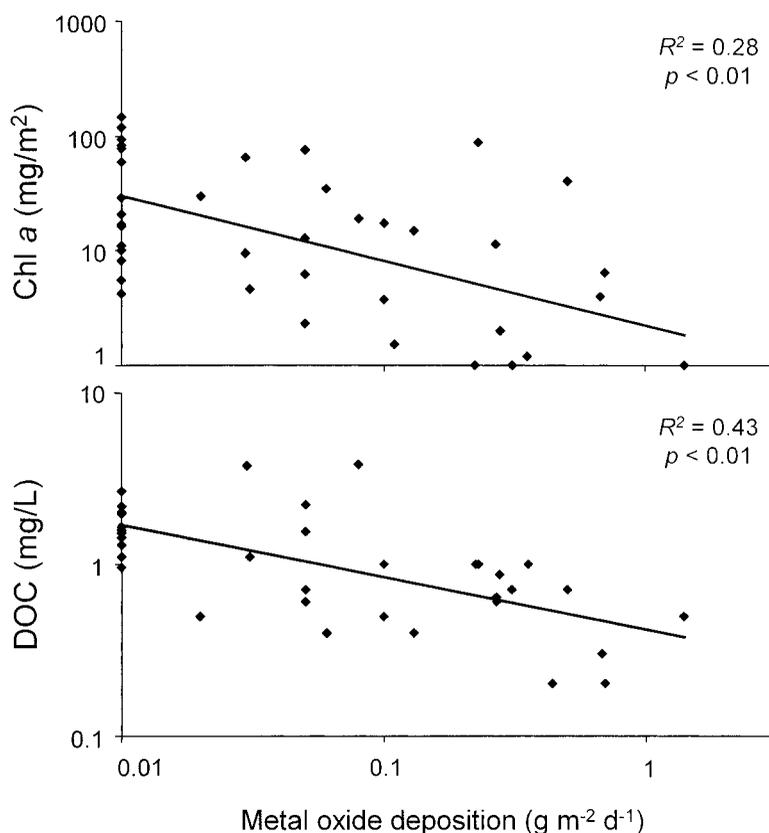Nitrification also was undetectable at 2 sites

FIG. 4. Algal biomass (chlorophyll [chl] *a* concentration) and dissolved organic C (DOC) concentration versus rate of metal oxide deposition for sites used in analysis of thymidine incorporation.

in our study that had neutral pH but high concentrations of dissolved Zn (>2 mg/L). Where it was detectable, nitrification potential was negatively related to Zn (Fig. 3). Nitrifying bacteria are sensitive to elevated concentrations of metals in soils (Dusek 1995, Singha et al. 1998). Because nitrifying bacteria are sensitive to low pH, metals, and other toxic chemicals (Smith et al.

TABLE 1. Multiple regression analysis of thymidine incorporation and nitrification potential. DOC = dissolved organic C.

| Dependent variable | df | Overall $R^2$ | Overall *p*-value | Independent variable | Standardized regression coefficient | *p*-value |
|---|---|---|---|---|---|---|
| Rate of thymidine incorporation | 3,33 | 0.59 | 0.001 | pH | 0.32 | 0.022 |
| | | | | Algal biomass | 0.44 | 0.001 |
| | | | | DOC | 0.36 | 0.014 |
| Nitrification potential | 3,33 | 0.77 | 0.001 | Low pH | −0.52 | 0.001 |
| | | | | Zn concentration | −0.50 | 0.001 |
| | | | | Algal biomass | 0.17 | 0.056 |
| Nitrification potential[a] | 2,23 | 0.61 | 0.001 | Algal biomass | 0.51 | 0.001 |
| | | | | Zn concentration | −0.46 | 0.003 |

[a] Sites with undetectable nitrification and one outlier (see Fig. 3) not included
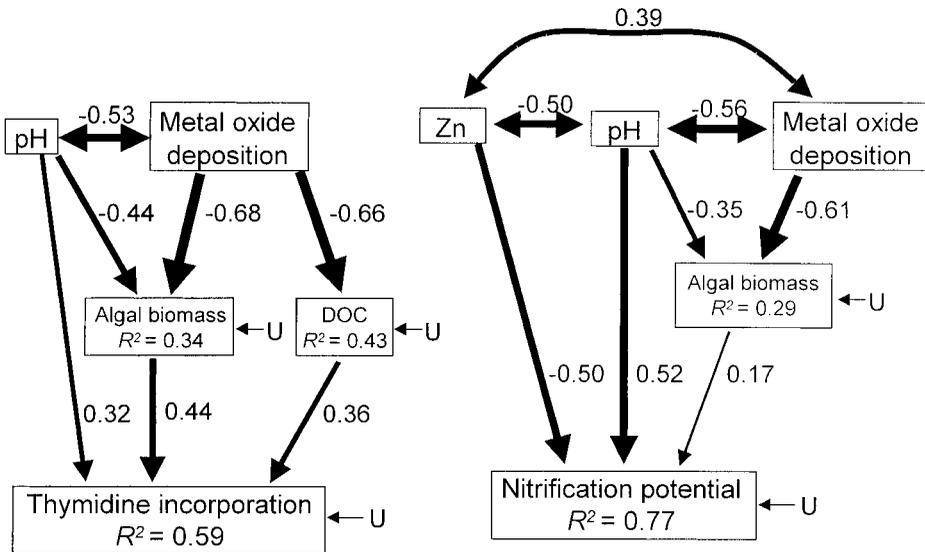
FIG. 5. Path analyses of the effects of mine drainage on thymidine incorporation (left) and nitrification potential (right). Double-headed arrows indicate correlations (numbers are correlation coefficients, $r$). Single-headed arrows indicate suggested causal relationships (numbers are standardized regression coefficients). The thickness of an arrow indicates the magnitude of the correlation or standardized regression coefficient. $R^2$ is the coefficient of determination, and U indicates residual variation. pH was included as a categorical variable in path analysis for nitrification potential depending on whether it was above or below 6 (see text). The 37 sites for the two processes were not identical, so correlation coefficients (between stressors) differ between the 2 data sets. DOC = dissolved organic C.

1997, Laursen and Carlton 1999), nitrification is often one of the first ecosystem processes to be disrupted under chemical stress (Schindler 1990, Niyogi 1999).

We observed a close linkage between algal biomass and nitrification in our streams (Fig. 3). Ammonium was usually low and was not significantly related to either algal biomass or nitrification. Although bacterial production often is related to algal biomass, it is not obvious why nitrification was related to algal biomass. High concentrations of C often depress nitrification in sediments (Strauss and Dodds 1997, Strauss and Lamberti 2000) because heterotrophic bacteria may outcompete nitrifying bacteria for $NH_4^+$ that becomes available through mineralization. Algal biomass in streams, however, has a lower C:N ratio than detrital organic matter in most sediments. Consequently, upon mineralization of algal biomass or exudates, heterotrophic bacteria may release $NH_4^+$, which would then be available to nitrifiers. By this mechanism, nitrifiers may occur in greatest abundance at sites with highest algal biomass because nitrifying bacteria indirectly rely on algae for $NH_4^+$ (Jones

et al. 1995). This mechanism was tested in the present study by the addition of glutamate, a surrogate for algal-derived C (C:N = 5), to stream gravels. The addition of glutamate stimulated nitrification, whereas the addition of glucose led to a negative rate of net nitrification (net uptake of $NO_3^-$). Glucose probably stimulated growth of heterotrophic bacteria, which then took up all available N ($NH_4^+$ and $NO_3^-$). Thus, the N content of dissolved organic matter is important in determining its effect on nitrification rates (Jones et al. 1995, Strauss 1999).

*Implications*

We measured 2 bacterial processes in mountain streams. Much of the variation in thymidine incorporation (59%) and nitrification potential (77%) across sites was accounted for by stressors related to mine drainage (pH, Zn, metal oxide deposition) or other stream characteristics (algal biomass, DOC). The 2 processes, however, responded differently to the stressors from mine drainage. Nitrification was undetectable at all sites with low pH and 2 circumneutral sites

with high Zn, whereas thymidine incorporation was affected somewhat by low pH but was not related to Zn (at concentrations measured at our study sites). Just as different taxa of invertebrates have varying sensitivities to stressors from mine drainage (e.g., Clements 1994), bacteria that control different processes also have different sensitivities. Thymidine incorporation measures growth of the heterotrophic bacterial community, which includes a variety of taxa with varying sensitivities to stressors from mine drainage. Nitrification, on the other hand, is conducted by a more restrictive group of chemoautotrophic bacteria, which are sensitive to a variety of stressors.

Because our results are based mainly on statistical relations, we cannot confirm the exact mechanisms by which acid mine drainage affects bacterial processes. The data indicate, however, that mine drainage affected bacterial processes both directly and indirectly (Fig. 5). In addition to direct effects of low pH and Zn, both thymidine incorporation and nitrification potential were indirectly affected by mine drainage through its effects on algal biomass (Fig. 4, Niyogi et al. 2002), which probably acts as a source of DOC and mineralized $NH_4^+$ to bacteria.

The disruption of bacterial production and nitrification affects C and N cycling in streams. Heterotrophic bacteria contribute to the turnover of organic matter and can support higher trophic levels (Meyer 1994, Hall and Meyer 1998). Thymidine incorporation in our streams was less sensitive to stressors from mine drainage than some invertebrates (Clements 1994, Niyogi et al. 2001) or fish (Kelly 1988), so reduced bacterial production in streams affected by mine drainage probably would not affect higher trophic levels as much as direct effects (e.g., toxicity) to animals. Because thymidine incorporation was closely related to algal biomass, we would expect this bacterial process to have similar responses and thresholds to mine drainage as algae (Niyogi et al. 2002).

In contrast to thymidine incorporation, nitrification was more sensitive than some invertebrate taxa (e.g., shredders, Niyogi et al. 2001). Thus, nitrification may be disrupted at levels of stress similar to those that affect structural indicators of stream health. Given the established sensitivity of nitrifying bacteria, nitrification is used as an indicator of metal toxicity in soils

(Cela and Sumner 2002), and we suggest that it can serve a similar purpose in streams affected by low pH or various toxins. Our protocol for nitrification potential in streams was simple and inexpensive compared to the measures of thymidine incorporation.

The reduction of nitrification in streams may increase concentrations of $NH_4^+$ (as seen in some of our streams with low pH), which generally is taken up faster than $NO_3^-$ in streams (Peterson et al. 2001). Alternatively, $NO_3^-$ can serve as a terminal electron acceptor in denitrification, and the loss of nitrification in stressed streams may decrease rates of denitrification, which can control the amount of N transported downstream. Consequently, the overall effects of reduced nitrification in stressed streams can be complex and warrant further study.

## Acknowledgements

## Literature Cited

Admiraal, W., H. Blanck, M. Buckert-de Jong, H. Guasch, N. Ivorra, V. Lehmann, B. A. H. Nystrom, M. Paulsson, and S. Sabater. 1999. Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. Water Research 33:1989–1999.

Bentler, P. M., and D. G. Bonnet. 1980. Significance tests and goodness of fit in the analysis of covariance structures. Psychological Bulletin 88: 588–606.

Bott, T. L., L. A. Kaplan, and F. T. Kuserk. 1984. Benthic bacterial biomass supported by streamwater dissolved organic matter. Microbial Ecology 10:335–344.

Cairns, J. 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100:47–57.

CELA, S., AND M. E. SUMNER. 2002. Critical concentrations of copper, nickel, lead, and cadmium in soils based on nitrification. Communications in Soil Science and Plant Analysis 33:19–30.

CLEMENTS, W. H. 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado. Journal of the North American Benthological Society 13:30–44.

COOPER, A. B. 1984. Activities of benthic nitrifiers in streams and their role in oxygen consumption. Microbial Ecology 10:317–334.

DEAN-ROSS, D. 1991. Effects of combined treatments of zinc and pH on attached bacterial abundance and activity. Canadian Journal of Microbiology 37:491–496.

DE BOER, W., AND G. A. KOWALCHUK. 2001. Nitrification in acid soils: micro-organisms and mechanisms. Soil Biology and Biochemistry 33:853–866.

DODDS, W. K., M. A. EVANS-WHITE, N. M. GERLANC, L. GRAY, D. A. GUDDER, M. J. KEMP, A. L. LÓPEZ, D. STAGLIANO, E. A. STRAUSS, J. L. TANK, M. R. WHILES, AND W. M. WOLLHEIM. 2000. Quantification of the nitrogen cycle in a prairie stream. Ecosystems 3:574–589.

DUSEK, L. 1995. The effect of cadmium on the activity of nitrifying populations in two different grassland soils. Plant and Soil 177:43–53.

ELWOOD, J. W., AND P. J. MULHOLLAND. 1989. Effects of acidic precipitation on stream ecosystems. Pages 88–135 in D. C. Adriano and M. Havas (editors). Acid precipitation. Volume 2: Biological and ecological effects. Springer-Verlag, New York.

FINDLAY, S. 1993. Thymidine incorporation into DNA as an estimate of sediment bacterial production. Pages 479–586 in P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (editors). Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, Florida.

FINDLAY, S., K. HOWE, AND D. FONTVIELLE. 1993. Bacterial-algal relationships in streams of the Hubbard Brook Experimental Forest. Ecology 74:2326–2336.

GOULDER, R. 1988. Epilithic bacteria in an acid and calcareous headstream. Freshwater Biology 19:405–416.

HAACK, T. K., AND G. A. MCFETERS. 1982. Nutritional relationships among microorganisms in an epilithic biofilm community. Microbial Ecology 8:115–126.

HALL, R. O., AND J. L. MEYER. 1998. The trophic significance of bacteria in a detritus-based stream food web. Ecology 79:1995–2012.

HILL, B. H., A. T. HERLILY, AND P. R. KAUFMANN. 2002. Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern U.S.A. Freshwater Biology 47:185–194.

HORNOR, S. G., AND B. A. HILT. 1985. Distribution of zinc-tolerant bacteria in stream sediments. Hydrobiologia 128:155–160.

JONES, J. B., S. G. FISHER, AND N. B. GRIMM. 1995. Nitrification in the hyporheic zone of a desert stream ecosystem. Journal of the North American Benthological Society 14:249–258.

KAPLAN, L. A., AND T. L. BOTT. 1989. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: effects of temperature, water chemistry, and habitat. Limnology and Oceanography 34:718–733.

KELLY, M. 1988. Mining and the freshwater environment. Elsevier, New York.

KEMP, M. J., AND W. K. DODDS. 2001. Centimeter-scale patterns in dissolved oxygen and nitrification rates in a prairie stream. Journal of the North American Benthological Society 20:347–357.

KIMBALL, B. A., R. E. BROSHEARS, K. E. BENCALA, AND D. M. MCKNIGHT. 1994. Coupling of hydrologic transport and chemical reactions in a stream affected by acid mine drainage. Environmental Science and Technology 28:2065–2073.

KOPÁČEK, J., AND P. BLAŽKA. 1994. Ammonium uptake in alpine streams in the High Tatra Mountains (Slovakia). Hydrobiologia 294:157–165.

LAURSEN, A. E., AND R. C. CARLTON. 1999. Responses to atrazine of respiration, nitrification, and denitrification in stream sediments measured with oxygen and nitrate microelectrodes. Federation of European Microbiological Societies Microbiology Ecology 29:229–240.

LEDGER, M. E., AND A. G. HILDREW. 1998. Temporal and spatial variation in the epilithic biofilm of an acid stream. Freshwater Biology 40:655–670.

LEWIS, W. M., JR., J. F. SAUNDERS, D. W. CRUMPACKER, AND C. M. BRENDECKE. 1984. Eutrophication and land use. Springer-Verlag, New York.

MALTBY, L. 1992. Heterotrophic microbes. Pages 165–194 in P. Calow and G. E. Petts (editors). The rivers handbook. Volume 1: Hydrological and ecological principles. Blackwell Scientific Publications, Cambridge, Massachusetts.

MCKNIGHT, D. M., K. E. BENCALA, G. W. ZELLWEGER, G. R. AIKEN, G. L. FEDER, AND K. A. THORN. 1992. Sorption of dissolved organic carbon by hydrous aluminum and iron oxides occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado. Environmental Science and Technology 26:1388–1396.

MCKNIGHT, D. M., AND G. L. FEDER. 1984. The ecological effect of acid conditions and precipitation of hydrous metal oxides in a Rocky Mountain stream. Hydrobiologia 119:129–138.

MEYER, J. L. 1994. The microbial loop in flowing waters. Microbial Ecology 28:195–199.

MEYER, J. L. 1997. Stream health: incorporating the human dimension to advance stream ecology. Jour-

nal of the North American Benthological Society 16:439–447.

Meyer, J. L., R. T. Edwards, and R. Risley. 1987. Bacterial growth on dissolved organic carbon from a blackwater stream. Microbial Ecology 13:13–29.

Mills, A. L., and L. M. Mallory. 1987. The community structure of sessile heterotrophic bacteria stressed by acid mine drainage. Microbial Ecology 14:219–232.

Mulholland, P. J., A. V. Palumbo, J. W. Elwood, and A. D. Rosemond. 1987. Effects of acidification on leaf decomposition in streams. Journal of the North American Benthological Society 6:147–158.

Neely, R. K., and R. G. Wetzel. 1995. Simultaneous use of $^{14}$C and $^{3}$H to determine autotrophic production and bacterial protein production in periphyton. Microbial Ecology 30:227–237.

Niyogi, D. K. 1999. Effects of stress from mine drainage on ecosystem functions in Rocky Mountain streams. PhD Dissertation, University of Colorado, Boulder, Colorado.

Niyogi, D. K., W. M. Lewis, Jr., and D. M. McKnight. 2001. Litter breakdown in mountain streams affected by mine drainage: biotic mediation of abiotic controls. Ecological Applications 11:506–516.

Niyogi, D. K., W. M. Lewis, Jr., and D. M. McKnight. 2002. Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. Ecosystems 5:554–567.

Niyogi, D. K., D. M. McKnight, and W. M. Lewis, Jr. 1999. Influences of water and substrate quality for periphyton in a montane stream affected by acid mine drainage. Limnology and Oceanography 44:804–809.

Osgood, M. P., and C. W. Boylen. 1990. Seasonal variations in bacterial communities in Adirondack streams exhibiting pH gradients. Microbial Ecology 20:211–230.

Osgood, M. P., and C. W. Boylen. 1992. Microbial leaf decomposition in Adirondack streams exhibiting pH gradients. Canadian Journal of Fisheries and Aquatic Sciences 49:1916–1923.

Palumbo, A. V., M. A. Bogle, R. R. Turner, J. W. Elwood, and P. J. Mulholland. 1987. Bacterial communities in acidic and circumneutral streams. Applied and Environmental Microbiology 53:337–344.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Martí, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86–90.

Prosser, J. I. 1989. Autotrophic nitrification in bacteria. Advances in Microbial Physiology 30:125–181.

Rier, S. T., and R. J. Stevenson. 2001. Relation of environmental factors to density of epilithic lotic bacteria in 2 ecoregions. Journal of the North American Benthological Society 20:520–532.

Romani, A. M., and S. Sabater. 1999. Effect of primary producers on the heterotrophic metabolism of a stream biofilm. Freshwater Biology 41:729–736.

Rudd, J. W. M., C. A. Kelly, D. W. Schindler, and M. A. Turner. 1988. Disruption of the nitrogen cycle in acidified lakes. Science 240:1515–1517.

Schindler, D. W. 1990. Experimental perturbations of whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos 57:25–41.

Schumacker, R. E., and R. G. Lomax. 1996. A beginner's guide to structural equation modeling. Lawrence Erlbaum Associates, Mahwah, New Jersey.

Singha, D. D., A. Singh, and A. P. Gupta. 1998. Nitrogen transformation in sewage-sludge amended soil as influenced by addition of zinc, cadmium and nickel. Indian Journal of Agricultural Sciences 68:96–100.

Sjodin, A. L., W. M. Lewis, Jr., and J. F. Saunders. 1997. Denitrification as a component of the nitrogen cycle for a large plains river. Biogeochemistry 39:327–342.

Smith, R. V., L. C. Burns, R. M. Doyle, S. D. Lennox, B. H. L. Kelso, R. H. Foy, and R. J. Stevens. 1997. Free ammonia inhibition of nitrification in river sediments leading to nitrite accumulation. Journal of Environmental Quality 26:1049–1055.

Sobczak, W. V. 1996. Epilithic bacterial responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization. Journal of the North American Benthological Society 15:143–154.

Sobczak, W. V., and T. M. Burton. 1996. Epilithic bacterial and algal colonization in a stream run, riffle, and pool: a test of biomass covariation. Hydrobiologia 332:159–166.

Sode, A. 1983. Effect of ferric hydroxide on algae and oxygen consumption by sediment in a Danish stream. Archiv für Hydrobiologie Supplement 65:134–162.

Steinman, A. D., and G. A. Lamberti. 1996. Biomass and pigments of benthic algae. Pages 295–313 in F. R. Hauer and G. A. Lamberti (editors). Methods in stream ecology. Academic Press, New York.

Strauss, E. A. 1999. The effects of organic carbon and nitrogen availability on nitrification rates in stream sediments. PhD Dissertation, University of Notre Dame, Notre Dame, Indiana.

Strauss, E. A., and W. K. Dodds. 1997. Influence of protozoa and nutrient availability on nitrification rates in subsurface sediments. Microbial Ecology 34:155–165.

STRAUSS, E. A., AND G. A. LAMBERTI. 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45:1854–1859.

STRAUSS, E. A., AND G. A. LAMBERTI. 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshwater Biology 47:65–74.

SUBERKROPP, K. F. 1998. Microorganisms and organic matter decomposition. Pages 120–143 in R. J. Naiman and R. E. Bilby (editors). River ecology and management: lessons from the Pacific Coastal Ecoregion. Springer, New York.

TRISKA, F. J., J. H. DUFF, AND R. J. AVARINO. 1990. Influence of exchange flow between the channel and hyporheic zone on nitrate production in a small mountain stream. Canadian Journal of Fisheries and Aquatic Sciences 47:2099–2111.

VOLK, C. J., C. B. VOLK, AND L. A. KAPLAN. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. Limnology and Oceanography 42:39–44.

WASSEL, R. A., AND A. L. MILLS. 1983. Changes in water and sediment bacterial community structure in a lake receiving acid mine drainage. Microbial Ecology 9:15–169.