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Microbial Mobilization of Major and Trace Elements from Catchment Rock Samples of a High Mountain Lake in the European Alps

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Abstract

At a high mountain lake in catchments of mica schist and gneiss rock in the European Alps, substantial increases in solute concentrations of sulfate, magnesium, calcium, silica, manganese, and nickel were observed over the past two decades. We hypothesized that microbial interactions with rock in the catchment of the lake might play an important role. We studied the chemolithotrophic activities resulting in the production of metal mobilizing metabolites (mineral acids). The potential of nitrifying and sulfur-oxidizing cultures derived from rock to mobilize elements from this rock when augmented with ammonium and thiosulfate was investigated in a 35 day laboratory study. Bacterial species prevailing in the indigenous nitrifying and sulfur-oxidizing mixed cultures were determined by 16S rRNA gene sequence based analysis. The average mineralogical composition of the rock sample was quartz (50%), feldspar (27%), muscovite (15%), chlorite (6%), and dolomite (2%). The increase of each soluble element in the presence of cultures relative to the conditions without microbes was related to the total element in the rock sample (leaching efficiency in percent). After 35 days, leaching efficiency was 7% (Ca), 2.4% (Mg), and 6.3% (Mn) in the presence of the nitrifying culture. In the presence of the sulfur-oxidizing culture, leaching efficiency was 13% (Ca), 5.7% (Mg), 5.4% (Mn), 1.3% (Zn), 0.2% (Fe), and 0.1% (Al). The results suggest that under conditions of abundant substrate availability, chemolithotrophic activity on catchment rock can contribute to the increase in soluble Ca, Mg, and Mn in lake water.

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Introduction

Over the past two decades, substantial rise in solute concentration at high mountain lakes in catchments of metamorphic rocks in the European Alps was observed (Thies et al., 2007). At Lake Rasass, the electrical conductivity increased 18-fold from 1986 to 2005 (24 to about $450 \mu\text{S cm}^{-1}$). Increase in solutes at Lake Rasass was 68-fold for magnesium, more than 26-fold for sulfate, and more than 13-fold for calcium; silica concentration increased by almost twofold. In 2005, the mean concentration found for Ca, Mg, Si, and sulfate in Lake Rasass was (in mg L^{-1}): 34.0; 27.4; 2.3, and 211, respectively. High concentrations for Mn and Ni in Lake Rasass waters were also found (559 and $243 \mu\text{g L}^{-1}$); the concentration found for Al, Fe, and Zn was 143, 1.0, and $181 \mu\text{g L}^{-1}$, respectively. Potential factors that may have contributed to the pronounced solute increase at Lake Rasass include atmospheric deposition, rising air temperature and weathering, and the impact of active rock glaciers (Thies et al., 2007). Based on the levels of calcium, magnesium, and sulfate in atmospheric deposition at long-term monitoring sites in the vicinity of the lake, the impact of atmospheric deposition on the recent strong rise of base cations and sulfate at Lake Rasass was considered to be negligible. In a study of 57 remote high mountain lakes in glaciated and non-glaciated catchments in the Alps (Tyrol, Austria), Sommaruga-Wögrath et al. (1997) found that between 1985 and 1995, the concentration of sulfate, base cations, and silica had increased, while atmospheric input of sulfate had decreased. Those authors proposed that the changes in lake chemistry were therefore likely to be caused by enhanced

weathering and increased biological activity possibly resulting from an increase in air temperature. An increase in mean annual air temperature of more than 1°C was reported for the Alps for the period of 1980–2003 with respect to the reference period 1901–2000 (Auer et al., 2007). As the air temperature increases, the average height of the freezing line moves upwards. The running average of the 0°C line for May reached the elevation of Lake Rasass during the mid of 1990s, thus favoring earlier and more intense melt processes in the lake catchment than had previously occurred (Thies et al., 2007). The strong increase in solutes at Lake Rasass was therefore attributed to an intensified melting and release of substances from the rock glacier in the lake catchment as a response to climate warming.

To date, the interactions between microorganisms and rocks in glaciated and non-glaciated/non-vegetated catchments have not been investigated in relation to the enrichment of sulfate and major and trace elements in the water of high mountain lakes in the Alps.

Microbial activities play an important role in the weathering of rocks and minerals. In cold climate environments, however, biological weathering has not yet received the level of attention it has received in other environments (Hall et al., 2002). It is likely that a warmer climate and the more favorable conditions it brings for microbial activity will probably lead in short- to mid-term to an increased loss of elements from rocks. Thus the effect of microorganisms on weathering processes in high altitudes (like high mountain lakes in the Alps) might have increased over the past two decades in response to climate warming.

In this study we focused on nitrifying, sulfur-, and iron-oxidizing bacteria because of their ability to use inorganic energy substrates and to produce mineral acids and ferric iron. These mineral acids and ferric iron represent potent metal-leaching agents. Many chemolithotrophic bacteria are also able to use carbon dioxide as the sole source of carbon.

We investigated the potential of indigenous nitrifying, sulfur-, and iron-oxidizing cultures to mobilize major and trace elements from the catchment rocks of Lake Rasass. Analysis of the microbial potential to mobilize elements from rock requires culturing of organisms. Under natural field conditions, biogenic leaching activity is difficult to quantify. Among the reasons for these difficulties are elution of solutes by runoff or percolating water and the confinement of activity to areas where abundant substrate is supplied and environmental conditions are favorable for biological activity. Therefore, we performed a laboratory experiment and simulated abundant substrate supply to test the potential of cultures to mobilize major and trace elements from rock samples collected in the catchment of the lake. We monitored changes in soluble elements and microbial activity by quantitative analysis of metabolic products.

Site Description and Methodology

DESCRIPTION OF SAMPLING AREA AND ROCK SAMPLE

A detailed site description of Lake Rasass is provided in Thies et al. (2007). Lake Rasass is a high alpine lake in headwater catchments of the Central Eastern Alps in Europe. This lake is located in the upper Vintschgau in South Tyrol (Italy), south of the main alpine divide near the Swiss and Austrian borders; 46°44'50"N, 10°27'23"E, watershed altitude: 2682–2870 m a.s.l., watershed area: 0.22 km², rock glacier area: 0.038 km², lake surface area: 0.015 km². The bedrock consists of bare rock and talus of the Oetztal metamorphic complex (i.e., paragneisses, micaschists, and orthogneisses). Soil coverage in the catchment was sparse (~10% of catchment area) and characterized by alpine grass vegetation. The mean annual precipitation was about 900 mm, and the mean annual air temperature was –2.7 °C. Rock samples from the catchment of Lake Rasass were collected in autumn 2005. Sample points were distributed randomly in the area under investigation (catchment talus exposed to the atmosphere, non-glaciated). About 6000 g of rock (lumps of varying size) were aseptically collected and transferred to the laboratory in a cooling box. Procedures to develop enrichment cultures were started immediately upon sample arrival. To obtain the suspension used as inoculum to setup culture enrichments, rock lumps were submerged in sterile distilled water, brushed, and washed off, using sterile equipment. Rock samples were stored dry at 4 °C until analysis and use in leaching experiments. For chemical and mineralogical analysis, and for use as leaching substrate in leaching experiments, rock samples were crushed, passed through a sample divider, and ground to ≤250 μm particle size. Table 1 presents chemical and mineralogical properties of the rock sample.

Mineralogical analysis was carried out by X-ray diffraction- and Rietveld-analysis. For analysis the rock sample was milled to <20 μm and analyzed with X-ray powder diffraction (Type Philips PW 1820 Goniometer with Cu X-ray tube, Philips, Netherlands). Quantitative analysis of the powder diffraction pattern was performed by Rietveld-method using SiroQuant software (Sietronics Pty Ltd, Australia). Elemental composition and total sulfur of the rock sample was determined by X-ray fluorescence analysis. For analysis the rock sample was milled to <63 μm; 10.0 g of the sample powder were mixed with 2.0 g of Hoechst

TABLE 1
Properties of the catchment rock sample.

Chemical composition (mg g ⁻¹ dry weight, ± SD)	Mineralogical composition (%)
Ca (16.4 ± 2.07)	Quartz (50)
Mg (15.9 ± 1.34)	Feldspar (27)
K (17.3 ± 2.54)	Muscovite (15)
Fe (49.8 ± 0.27)	Chlorite (6)
Al (102.2 ± 19.8)	Dolomite (2)
Mn (0.597 ± 0.002)	
Cu (< 0.02)	
Zn (0.09 ± 0.002)	
Ni (0.024 ± 0.006)	
Si (241.8 ± 20.8)	
P (0.7 ± 0.09)	
Ti (10.9 ± 1.44)	
S _(total) (0.59 ± 0.01)	
SO ₄ (water-soluble) (0.18 ± 0.002)	

Wax C, pressed into 40 mm pellets, and analyzed using a wavelength-dispersive X-ray fluorescence spectrometer (Type S8 Tiger, Bruker AXS, Karlsruhe, Germany). Water-soluble sulfate of the rock sample was extracted according to ISO 11048 (International Standardization Organization, 1995) and quantified by ion chromatography.

ENRICHMENT CULTURES

Enrichment cultures of nitrifying, sulfur-, and iron-oxidizing bacteria were developed in 100 mL Erlenmeyer flasks. For the enrichment of nitrifiers, a culture medium as described by Koops and Möller (1992) was used, with the modification that the addition of trace elements was omitted. The pH of the medium was adjusted to 7.7. Waksman medium was supplemented with thiosulfate as described in Karavaiko et al. (1988) to enrich sulfur-oxidizers; the pH of the medium was adjusted to 5.0. Enrichment of ferrous iron-oxidizing bacteria was set up in a nutrient solution as described by Mackintosh (1978) with the modification that the addition of trace elements was omitted. The pH of the medium was adjusted to 2.0. To accomplish enrichment, 15 mL of double-concentrated medium was inoculated with 15 mL of the suspension obtained by submerging, brushing, and washing rock lumps in sterile distilled water (described in the preceding paragraph). The flasks were incubated at 20 °C on a rotary shaker (150 rpm). Progress of enrichment was assessed by analyzing the growth medium for pH and the content of sulfate (sulfur-oxidizers), nitrite and nitrate (nitrifiers), and ferrous/ferric iron (ferrous iron oxidizers). The pH was determined with a glass electrode. The concentration of nitrate and nitrite was determined by ion pair chromatography; sulfate content was measured by ion chromatography. A photometric procedure using 2,2'-dipyridyl as a reagent producing complexes with ferrous iron was performed to quantify ferrous and ferric iron; absorption was measured at 515 nm (Lange and Vejdelek, 1980). For inoculation in leaching experiments, enrichment cultures with cell numbers approximating 10⁸ per mL of culture were used. The total number of cells in the cultures was determined by the direct counting method using a counting chamber and a phase contrast light microscope.

LEACHING EXPERIMENTS

Shake-flask incubation experiments using 100 mL Erlenmeyer flasks were performed. For each of the different setups, ground

rock (particle size $\leq 250 \mu\text{m}$) was weighed into flasks to give a substrate density of 2% and autoclaved separately from the medium. For leaching experiments, the same media were used as for enrichment. From the eight flasks prepared for each sampling date, four were amended with 27 mL of sterile culture medium and inoculated with 3 mL of enrichment culture (experimental flask). The remaining four flasks received 30 mL of the sterile culture medium only (uninoculated control flask). The flasks were incubated at 20 °C on a rotary shaker (150 rpm). After 0, 7, 14, 21, 28, and 35 days, eight flasks each (four experimental and four control flasks) were sampled for assay. For assays at the specified times, solutions and solids from each experimental and control flask were separated by centrifugation ($7000 \times g$) for 10 min. Solutions were assayed for pH, the concentration of K, Ca, Mg, Al, Fe, Zn, Mn, Ni, and Cu and for either the content of thiosulfate and sulfate (sulfur-oxidizing enrichment), or ammonium, nitrite, and nitrate (nitrifying enrichment). The pH was determined with a glass electrode. Soluble metals were quantified by inductively coupled plasma-atomic emission spectrometry, except potassium, which was determined by flame-atomic emission spectrometry. The concentration of ammonium was assayed colorimetrically by the Nessler method (Allen et al., 1974). Nitrate and nitrite determination was carried out by ion pair chromatography; thiosulfate and sulfate determination was performed by ion chromatography. The method described by Lange and Vejdelek (1980) was used to determine ferrous and ferric iron concentration.

GENOTYPING ANALYSIS

Genotyping analysis was performed with aliquots of culture from the experimental flasks in which leaching of rock was promoted. Aliquots were taken after three weeks of incubation. DNA was extracted using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, California) according to the manufacturer's protocol. Solids of the culture were harvested by centrifugation ($10,000 \times g$) for 10 min. Near full-length 16S rRNA gene clone libraries were constructed and sequenced. Universal bacterial 16S rDNA primers 8F: 5'- Universal bacterial 16S rDNA primers 8F: 5'- AGA GTT TGA TYM TGG CTCAGA GTT TGA AG and R1401: 5'- CGGTGTGTACAAGGCCCG-3' were used for PCR amplification of the region corresponding to positions 8 to 1400 of the *Escherichia coli* 16S rDNA gene (GenBank J01859) (Felske et al., 1996; Heuer et al., 1997). Primers used for amplification of archaeal rDNA were: Arch21F: 5'- TTCCGGTGATC-CYGCCGGA and 1492R: GGTTACCTTGTTACGACTT (De-Long, 1992). PCR amplification was performed under the following reaction conditions (final concentrations): 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.01% gelatin, 0.1% Triton X-100, 3 mM MgCl_2 , 0.2 mM dNTPs, 50 pmol of primers in a total volume of 50 μL . Each of the 30 (bacteria) or 40 (Archaea) amplification cycles entailed denaturation at 95 °C for 60 s, annealing at 52 °C (bacteria) or 50 °C (Archaea) for 60 s, and primer extension at 72 °C for 60 s (Gao et al., 2007). DNA from *Sulfolobus solfataricus* was used as a positive control for Archaea PCR. PCR products were analyzed on 1.5% agarose gels stained with ethidium bromide. The PCR fragments were cloned in pGEM-T (Promega, Madison, Wisconsin) and transformed into *E. coli* DH5 α competent cells. Sequencing of PCR products was performed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems/Hitachi) using cycle sequencing. The nucleotide sequences were identified by searches in NCBI-BLASTN and RDP II (Ribosomal Database Project) databases. All sequences were examined for chimerism using Greengenes (DeSantis et al., 2006).

STATISTICAL DATA ANALYSES

Leaching experiments were carried out using four replicates. Chemical properties of the rock material were determined using three replicates. Results are presented as mean values with standard deviation giving a measure of variability. In leaching experiments, normal distribution of the data was tested by the Kolmogorov-Smirnov test, and two-tailed *t*-test for independent samples was performed to locate significant differences between experimental setup and control setup; Pearson product-moment correlation technique was used to reveal relations between the concentration of nitrate, nitrite, or sulfate in solution and soluble elements at the specified sampling dates (Wardlaw, 1985; Hoshmand, 1998). Statistical tests were performed at $\alpha < 0.05$.

Results

MOBILIZATION OF ELEMENTS BY INDIGENOUS NITRIFYING CULTURE

In the experimental flask (inoculated with nitrifying culture enrichment), the value of pH significantly decreased in the first two weeks of incubation, which was parallel to an increase in the production of nitrite and nitrate compared to the uninoculated control (Fig. 1). After 35 days, in the inoculated flask, the concentration measured for soluble Ca and Mg increased up to twofold and the one measured for Mn was up to 16-fold greater, compared to the control ($P < 0.0001$; Fig. 2). Significant correlations were obtained between nitrite and soluble Ca, Mg, and Mn ($P < 0.001$) from day seven on; the value of correlation coefficients was 0.99 (Ca), 0.95 to 0.99 (Mg) and 0.96 to 0.99 (Mn).

Biogenic mobilization of K, Al, Cu, Ni, Fe, and Zn was not significant; soluble amounts of Cu, Ni, Fe, and Zn remained below the limit of determination ($< 0.04 \mu\text{g mL}^{-1}$, $< 0.03 \mu\text{g mL}^{-1}$, $< 0.01 \mu\text{g mL}^{-1}$, and $< 0.009 \mu\text{g mL}^{-1}$, respectively). The amount of soluble K remained at about $60 \mu\text{g mL}^{-1}$ during the experiment and did not differ significantly between the experimental setup and the control. Table 2 presents the leaching efficiency of the mixed culture after 14 and 35 days.

Sequence analysis of the bacterial clone library derived from the culture of the experimental flask in which leaching of rock was promoted indicated the presence of a mixed culture (Table 3, A). In total, 49 clones were analyzed. Genotyping did not detect members of the domain *Archaea* in the mixed culture.

MOBILIZATION OF ELEMENTS BY INDIGENOUS SULFUR-OXIDIZING CULTURE

Sulfate production, solution pH, and mobilization of elements is presented in Figures 3 and 4. In the experimental flask, the value of pH was significantly higher after 7 days, and significantly lower after 21, 28, and 35 days ($P < 0.0001$) than in the control flask. The contrary was observed for soluble Ca, which was significantly lower after seven days and significantly higher after 21, 28, and 35 days ($P < 0.0001$). Soluble Mg, Al, and Fe were significantly higher in the experimental flask after 21, 28, and 35 days of incubation (Mg, Al: $P < 0.0001$; Fe: $P = 0.001$ and < 0.001). Soluble Mn was significantly higher in the experimental flask after 28 and 35 days ($P = 0.002$, $P < 0.0001$). The difference in soluble Zn between experimental and control flask was significant ($P = 0.0017$) after 35 days. After 35 days, the amount of element entering the solution in the experimental flask was about twofold (Ca, Mg), threefold (Zn, Mn), and fourfold (Al, Fe) greater than in the control flask. Biogenic mobilization of K, Cu,

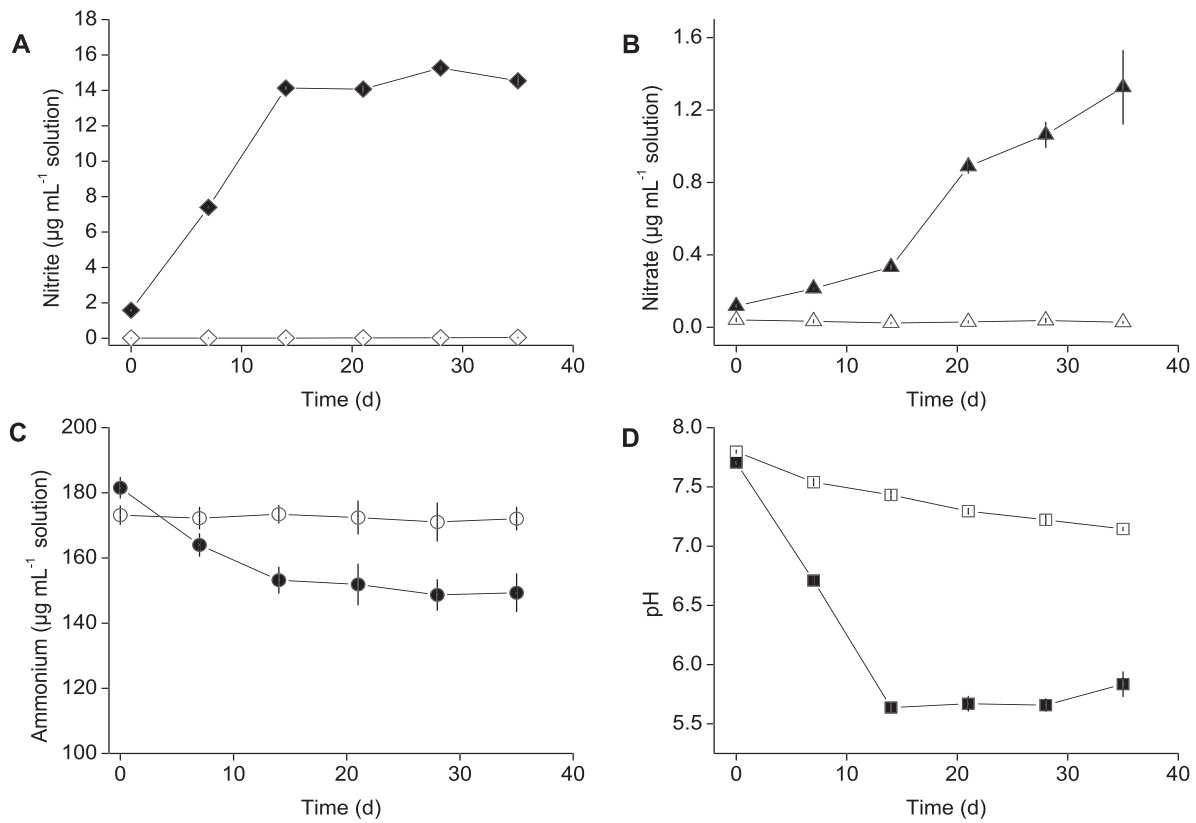


FIGURE 1. Nitrite and nitrate production, and acidification by an indigenous nitrifying mixed culture in a leaching experiment with catchment rock under conditions of ammonium supply. (A) Nitrite. (B) Nitrate. (C) Ammonium. (D) pH. Solid symbols: experimental flask. Open symbols: uninoculated control flask. Error bars show the SD of four replicates.

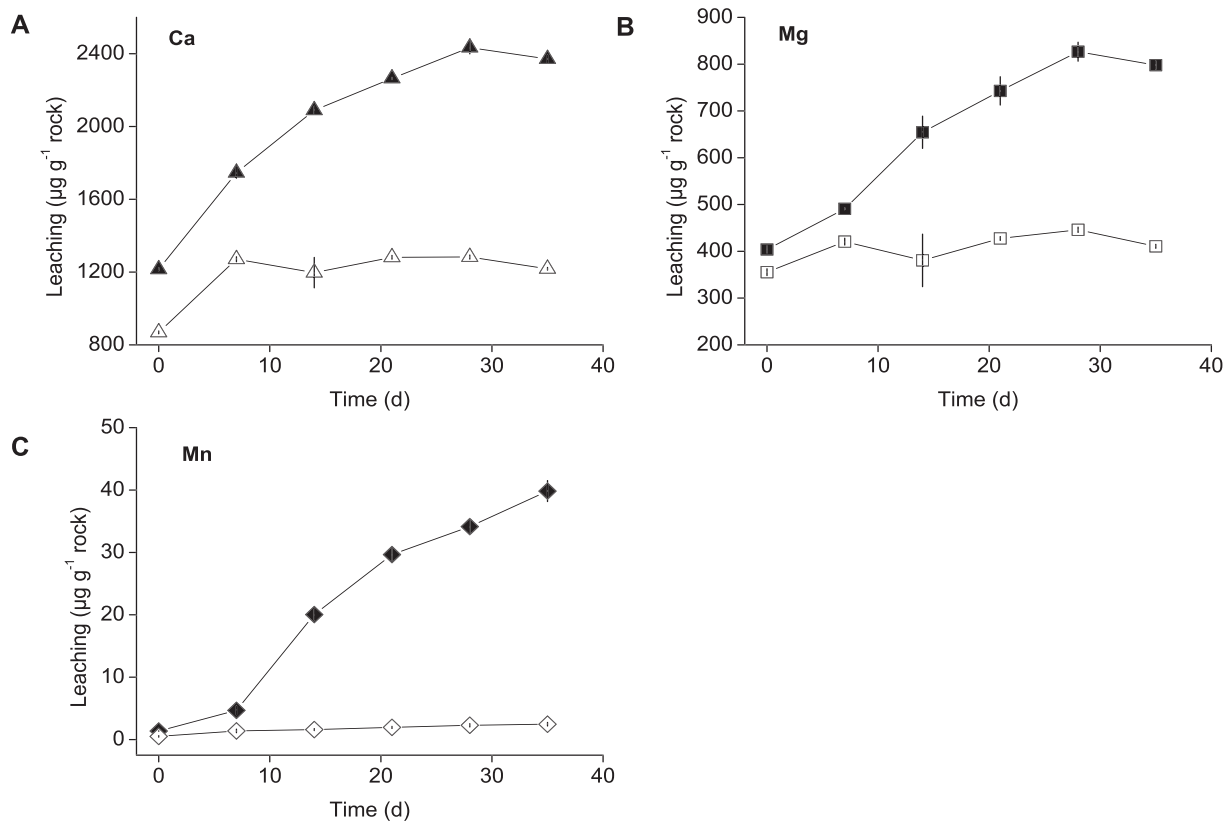


FIGURE 2. Mobilization of major and trace elements from catchment rock by an indigenous nitrifying mixed culture. (A) Calcium. (B) Magnesium. (C) Manganese. Solid symbols: experimental flask. Open symbols: uninoculated control flask. Error bars show the SD of four replicates.

TABLE 2

Leaching efficiency (%) of indigenous nitrifying and sulfur-oxidizing cultures after day 14 (first value) and day 35 (second value). Leaching efficiency: biologically mobilized amount of an element related to total element in the catchment rock sample.

Culture	%					
	Ca	Mg	Fe	Al	Zn	Mn
nitrifying	5.4/7.0	1.7/2.4	-/-	-/-	-/-	3.1/6.3
sulfur-oxidizing	1.5/13	0.08/5.7	-/0.16	-/0.08	0.06/1.3	0.04/5.4

and Ni was not significant; soluble Ni remained below the limit of determination ($<0.03 \mu\text{g mL}^{-1}$). Soluble K did not differ significantly between the experimental setup and the control during the experiment; the range of soluble K was between about $650 \mu\text{g mL}^{-1}$ (day 0, 14, 21) and $1200 \mu\text{g mL}^{-1}$ (day 28, 35). Table 2 presents the leaching efficiency of the mixed culture after 14 and 35 days of incubation.

The concentration of sulfate correlated negatively ($P < 0.001$) with soluble Ca ($r = -0.99$) and Al ($r = -0.96$) after seven days of incubation. After 14 days, no significant correlations between sulfate and soluble Ca, Mg, Al, Mn, Fe, and Zn were observed. From day 21 on, sulfate correlated significantly with soluble Ca ($P < 0.001$, $r = 0.98$ to 0.99), Mg ($P < 0.001$, $r = 0.96$ to 0.99), Al ($P < 0.001$, $r = 0.96$ to 0.98), and Fe ($P \leq 0.001$, $r = 0.92$ to 0.98). A significant correlation between sulfate and soluble Mn ($P \leq 0.002$, $r = 0.90$ to 0.96) was observed, from day 28 on. After 35 days, sulfate concentration and soluble Zn correlated significantly ($P = 0.003$, $r = 0.88$).

Sequence analysis of the bacterial clone library derived from the culture of the experimental flask in which leaching of rock was promoted revealed the presence of a mixed culture (Table 3, B). In total, 47 clones were analyzed. Genotyping did not detect members of the domain Archaea in the mixed culture.

TABLE 3

Prevalent bacterial species in (A) nitrifying and (B) sulfur-oxidizing cultures.

Bacterial species	GenBank	% nt	% of clones
	No of closest representative	sequence identity	
(A)			
<i>Pseudomonas</i> sp.	AM084028	99	25
<i>Herbaspirillum</i> sp.	DQ337592	99	23
<i>Massilia</i> sp.	GQ339887	99	18
<i>Acidobacterium</i> sp.	DQ351783	98	18
<i>Nitrosovibrio</i> sp.	AY123803	99	6
<i>Methylobacterium</i> sp.	EF165044	99	2
<i>Delftia</i> sp.	EU304256	99	2
<i>Burkholderia</i> sp.	DQ381722	99	2
<i>Alcaligenes</i> sp.	AF430122	98	2
<i>Variovorax</i> sp.	FJ527675	99	2
(B)			
<i>Pseudomonas</i> sp.	AM084028	99	36
<i>Rhodanobacter</i> sp.	EU196308	98	21
<i>Burkholderia</i> sp.	DQ381722	99	13
<i>Brevundimonas</i> sp.	AB526328	99	11
<i>Variovorax</i> sp.	FJ527675	99	7
<i>Arthrobacter</i> sp.	DQ094184	99	6
<i>Methylobacterium</i> sp.	EF165044	99	2
<i>Pedobacter</i> sp.	AM279216	98	2
<i>Sulfitobacter</i> sp.	FJ460049	98	2

Discussion

The present study aimed to investigate the potential of chemolithotrophic bacteria, enriched from rock sampled in the catchment of Lake Rasass, to mobilize major and trace elements from this rock. The microorganisms selected use ammonia, reduced inorganic sulfur compounds, or ferrous iron to obtain their basic energy needs.

Our results revealed the ability of indigenous nitrifying and sulfur-oxidizing cultures to release the elements Ca, Mg, and Mn or Ca, Mg, Mn, Al, Fe, and Zn, respectively, from the rock samples. Attempts to enrich iron-oxidizing bacteria were not successful.

In the presence of nitrifying culture after 35 days, we observed a twofold increase for Ca and Mg, and Mn increased to 16-fold greater in solution compared to the control. Investigations to the biodeterioration of building stone indicated that nitrifying bacteria occurred more frequently on calcareous stones and a pH between 7 and 9 (Bock and Sand, 1993; Mansch and Bock, 1998). In our experiment, about 10% of the added ammonium was converted to nitrite and nitrate by the mixed culture. In the inoculated flask, pH had decreased to 5.6 in the first two weeks of incubation. Since ammonia availability decreases with decreasing pH, ammonium oxidation leveled off in the later period of incubation.

We obtained evidence for the precipitation of calcium and magnesium as sulfates in the flask inoculated with sulfur-oxidizing enrichment after seven days. However, from day 21 on, significant increases in the concentration of sulfate and the amount of elements entering the solution were observed. After 35 days, the amount of elements entering the solution in the experimental flask was about twofold (Ca, Mg), fourfold (Al, Fe), and threefold (Zn, Mn) greater than in the control flask. After 21, 28, and 35 days, the sulfur-oxidizing enrichment mobilized calcium and magnesium with higher efficiency from the rock sample than the nitrifying enrichment.

Ammonium, thiosulfate, and ferrous iron were provided in abundant supply during the experiment. Hence the minerals composing the rock substrate were not major suppliers of energy to the microorganisms. Microbial activity affected mineral substrates by metabolites produced from provided energy substrates. Acidolysis is the dominant mechanism of metal mobilization from minerals under nitrifying and sulfur-oxidizing conditions. Acidolysis is affected by the inorganic acids (nitrous, nitric, sulfuric) produced under these conditions.

X-ray diffraction analysis identified quartz, feldspar, muscovite, chlorite, and dolomite in the rock sample. Our data indicate leaching of dolomite and of silicates in the rock. It appears that there are differences in the effects on the rock for certain elements. The elements Ca, Mg, and Mn were mobilized in greater quantities in both nitrifying and sulfur-oxidizing cultures. Ca and Mg can be found in the dolomite of the rock. Their instability in acid solution is a chemical basis for the decomposition of carbonates. At low pH values of solution as observed in our experiment Ca and Mg will

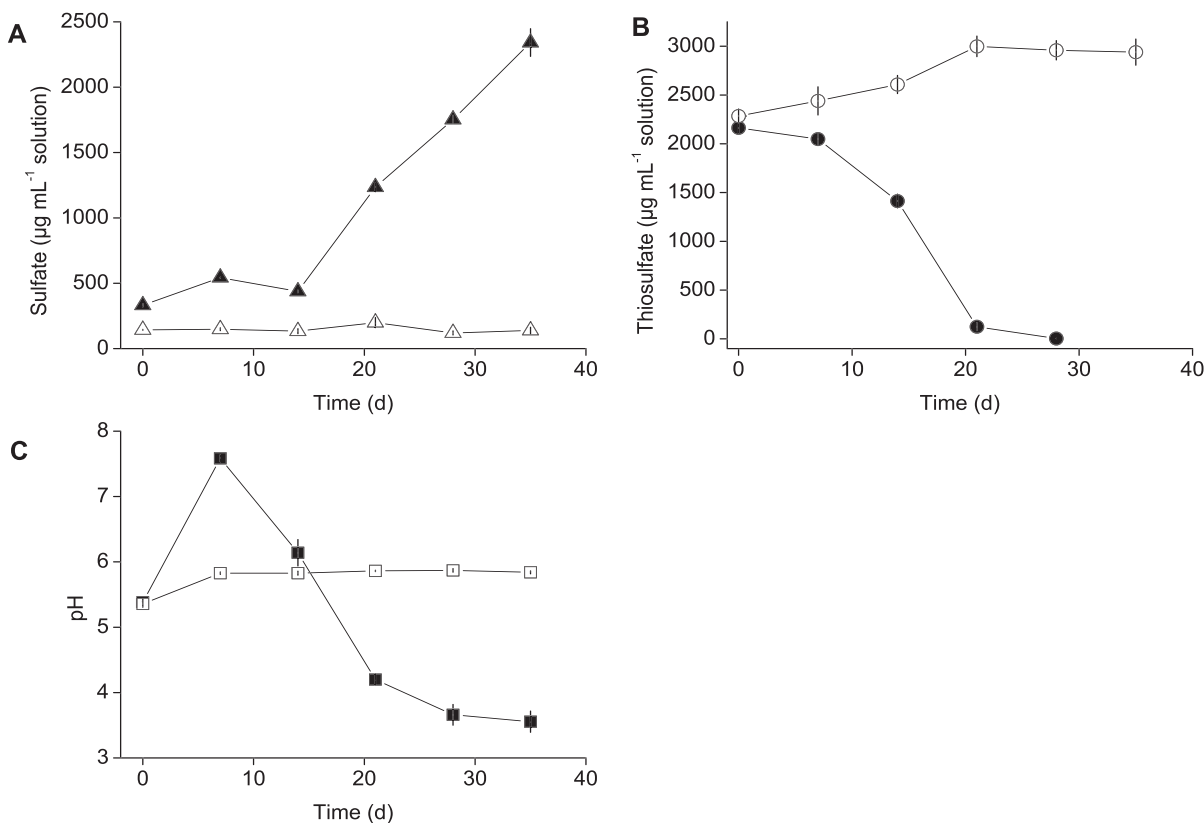


FIGURE 3. Sulfate production and acidification by an indigenous sulfur-oxidizing mixed culture in a leaching experiment with catchment rock under conditions of thiosulfate supply. (A) Sulfate. (B) Thiosulfate. (C) pH value. Solid symbols: experimental flask. Open symbols: uninoculated control flask. Error bars show the SD of four replicates.

be released from dolomite. The molecular ratio of Ca and Mg in solution, however, suggests that the stoichiometric ratio of Ca:Mg of dolomite (1:1) is exceeded as far as Ca is concerned. Therefore, there was another source of Ca in the rock mineralogy. A part of the Ca present in solution might have been released from silicates, most likely from feldspar. Potassium was present in the rock, but we did not observe leaching of K in the presence of the nitrifying and the sulfur-oxidizing enrichment. Quantitative analysis was made difficult by the relatively high background concentration of K in the culture medium used to prepare the experimental setup and the control. This was especially true for the sulfur-oxidizing experiment (initial K concentration in the culture medium was 860 $\mu\text{g mL}^{-1}$).

The set of elements released into solution in the presence of nitrifying enrichment was not the same observed in the presence of sulfur-oxidizing enrichment. Manganese was released in both nitrifying and sulfur-oxidizing experiments, but more Mn was released from rock in the presence of the nitrifying enrichment. Manganese occurs in form of oxides, carbonates, and silicates (e.g. chlorites). It is not clear whether Mn release was promoted by reductive microbial processes or by biogenic acid attack on manganous minerals. For Mn a different relation of time and element release between the two types of enrichment culture was observed. In the presence of nitrifying culture, Mn release into solution was observed early and increased in the course of the experiment (Fig. 2, C). In the sulfur-oxidizing experiment, release of Mn became significant towards the end of incubation (Fig. 4). The differences in the relation of time and Mn release might be related to the different biogenic acids produced by the experimental cultures and the way these acids affected the minerals. In the presence of nitrifying culture, nitrous and nitric acid were

produced (Fig. 1). Acidolysis is proposed as a mechanism of Mn release from manganous minerals under these conditions. Nitrous acid also might have effected Mn release from minerals by its capability to act as a reducing agent. In the presence of sulfur-oxidizing culture, sulfuric acid was produced and acidolysis is proposed as the dominant mechanism of metal release from the rock substrate. Under sulfur-oxidizing conditions, metal sulfates with reduced solubility in aqueous solution can be formed (e.g. calcium sulfates). Evidence for the formation of calcium sulfate was obtained in the sulfur-oxidizing experiment in the early period of incubation (Figs. 3, A and C; 4, A). With increasing sulfur-oxidizing activity, proton promoted release of Mn from minerals has apparently gained in importance (Figs. 3, A and C; 4, C). Sulfuric acid is an oxidizing acid, thus Mn release from minerals by a reductive process, as suggested to be of relevance in the presence of nitrous acid (nitrifying experiment) will not occur in the sulfur-oxidizing experiment.

Release of Al, Fe, and Zn was not observed in the nitrifying experiment. The loss of aluminum, iron, and zinc to solution from the rock was observed only in the experiment with sulfur-oxidizing culture. In the rock substrate, aluminum and iron were present in silicates, with iron essentially occurring in chlorites. The release of Zn to solution was low. It appeared that Zn was present in a mineral phase, most likely in a sulfide, that was only slightly affected by microbial activity. *Acidithiobacillus ferrooxidans*, which is very versatile in attacking metal sulfides, could not be detected in the culture enrichment. The higher amount of soluble elements in the setup with sulfur-oxidizing culture is attributable to the stronger acidification of the solution ($\text{pH} < 3.7$) as a result of sulfuric acid production. In addition, ferric iron might have gained importance as a leaching agent in the later period of

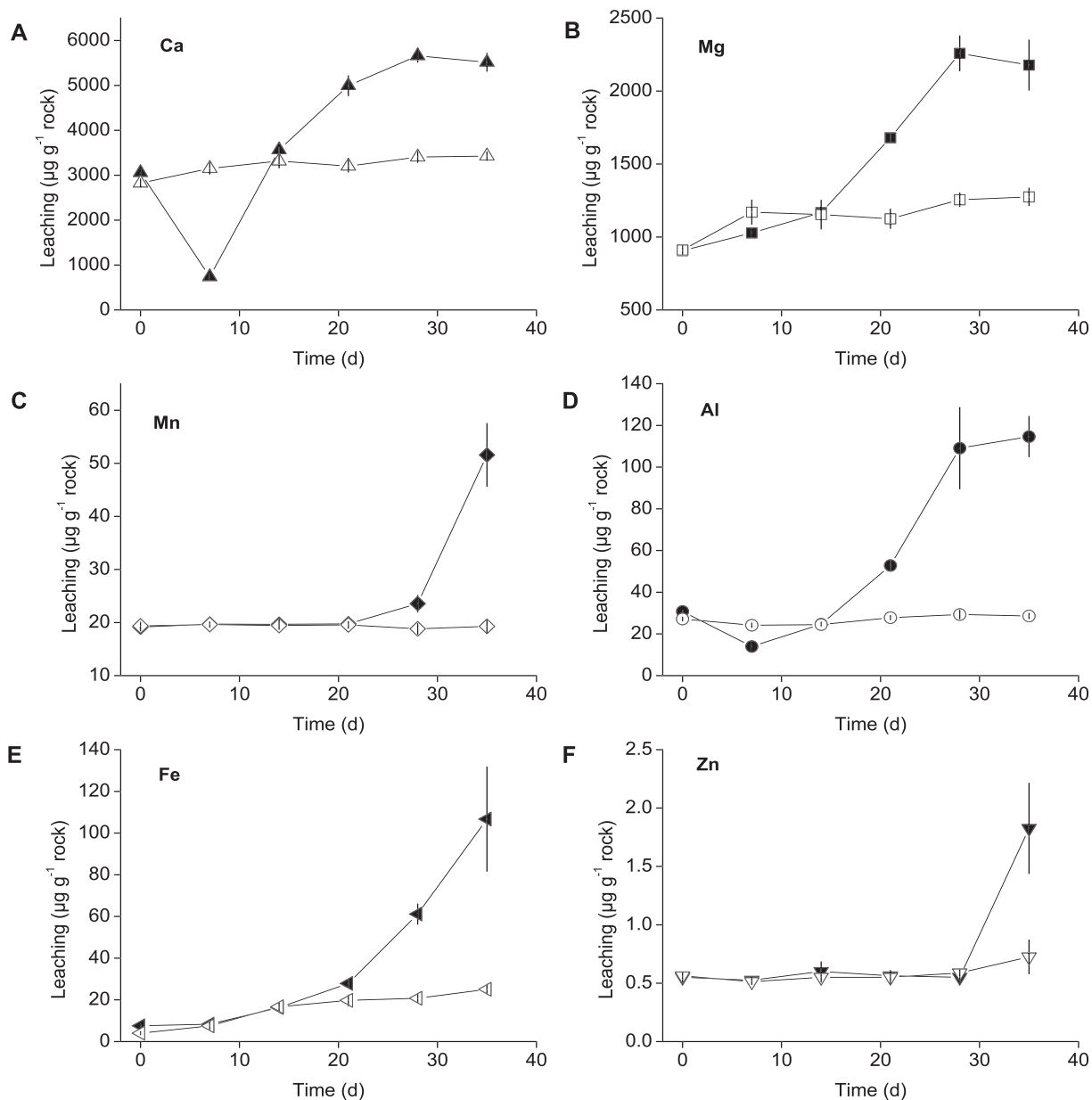


FIGURE 4. Mobilization of major and trace elements from catchment rock by an indigenous sulfur-oxidizing mixed culture. (A) Calcium. (B) Magnesium. (C) Manganese. (D) Aluminum. (E) Iron. (F) Zinc. Solid symbols: experimental flask. Open symbols: uninoculated control flask. Error bars show the SD of four replicates.

incubation. After 35 days, the concentration measured for iron was $2.1 (\pm 0.49) \mu\text{g mL}^{-1}$ of solution in the inoculated flask. Of this total soluble iron, the share of ferric iron was 40%. The high concentration of Ni in Lake Rasass waters could not be related to leaching of the rock substrate by nitrifiers or sulfur oxidizers because the rock was low in Ni.

In the nitrifying culture, chemolithoautotrophic ammonia oxidizing bacteria were associated with chemoorganoheterotrophic bacteria and with genera containing chemolithotrophic representatives (e.g. genus *Herbaspirillum*). Heterotrophic nitrification does not appear to contribute to the increased biogenic mobilization of our experiment. This process may be of relevance only when high concentrations of electron donors are available (van Loosdrecht and Jetten, 1998). Clones affiliated with *Acidobacterium* kingdom were found in our culture enrichment. Members of this kingdom are genetically and metabolically diverse and environmentally widespread (Barns et al., 1999).

Representatives of this group were also found in chemolithoautotrophically based cave systems (Schabereiter-Gurtner et al., 2004; Meisinger et al., 2007). Clones assigned to the genus *Massilia* were present in the culture enrichment. The genus *Massilia* belongs to the family Oxalobacteriaceae (Gallego et al., 2006); representative species were isolated from soil and drinking water.

In case of the sulfur-oxidizing culture enrichment, genotyping analysis did not reveal the presence of representatives of the genera *Acidithiobacillus* or *Thiobacillus*. We found clones assigned to genera *Pseudomonas*, *Rhodanobacter*, *Burkholderia*, and *Methylobacterium* in the culture enrichment. Representatives of these genera have been reported to be able to oxidize inorganic sulfur compounds. Representatives of the genus *Pseudomonas* oxidized thiosulfate to tetrathionate or sulfate (Trudinger, 1967; Vitolins and Swaby, 1969; Schook and Berk, 1978; Mason and Kelly, 1988). Chemolithoheterotrophic growth by oxidation of thiosulfate to sulfate was observed for *Burkholderia* spp. (Jung et al.,

2005), *Limnibacter thiooxidans* (Spring et al., 2001), *Rhodanobacter thiooxydans* (Lee et al., 2007), and *Methylobacterium oryzae* (Anandham et al., 2007). The culture medium used in our study was not supplemented with organic substrates. However, microbial metabolism and necromass represented potential sources of organic substrates for heterotrophic activity in our setup. Organic substrates originating from these sources and the inorganic substrate might have been used concomitantly by microorganisms.

Several organic metabolites such as carboxylic acids and amino acids are known as potential leaching agents. In a preliminary analysis we screened for the presence of such acids in experimental and control flasks. However, the concentration of acids was too low to allow reliable quantification.

In our study we provided abundant substrate supply to test the potential of indigenous nitrifying and sulfur-oxidizing cultures to mobilize elements from the catchment rock sample. Under field conditions, on bare rock or in pristine mineral soil like soil of glacier forefields, boulders, alluvial mineral soil, desert soil, and erosion sites, microorganisms may be supplied with inorganic energy sources in different ways. Substrates may be directly supplied by rocks as most rocks contain inclusions of sulfide minerals (e.g. pyrite), at least in small quantities (Ehrlich, 2002). Some rocks contain reduced inorganic nitrogen (Stevenson, 1959; Wlotzka, 1961). According to Wlotzka (1961), magmatic rocks contain an average of 0.02 mg NH₃-N g⁻¹ and the maximum is up to 0.15 mg g⁻¹. The NH₃-N content of sedimentary rocks is higher and clay and clay slates contain on average 0.58 mg g⁻¹. Atmospheric deposition is an important source for reduced nitrogen and sulfur compounds, which represent potential microbial substrates.

The amount of reduced nitrogen and sulfur supplied to nitrifiers and sulfur oxidizers in the field is small compared to that supplied in our study. However, in the field, micro-areas in the rock can be rich geochemically in potential microbial substrates (Wlotzka, 1961; Ehrlich, 2002) or get enriched with substrates by atmospheric deposition (Galloway et al., 2004; Rogora et al., 2006). Such areas represent hotspots for microbial activity and for interactions between microorganisms and the rock. Results of laboratory investigations cannot be transferred directly to conditions in the field but they do indicate the potential of microorganisms to enhance the weathering release of minerals into the surrounding waters.

Conclusions

Our laboratory study revealed that nitrifying and sulfur-oxidizing cultures derived from rock sampled in the catchment of Lake Rasass have the potential to mobilize significant amounts of major and trace elements from this rock. In addition to dolomite dissolution, we observed some dissolution of silicates. The results indicate that the microbial processes would hasten the change of mineral phases in the early stage of rock weathering. The experiment with sulfur-oxidizing culture provided data showing a difference in the release time of Ca-Mg and the metals Mn, Fe, and Zn compared to Al. Aluminum seems to be more similar to magnesium release than the other metals. The data suggest a sequence of events in the weathering of the rock: first carbonate (Ca, Mg), then silicate (Al), and then oxides or sulfides (Mn, Fe, and last Zn). These findings do not only contribute to the understanding of microbial processes in weathering in general, but can also be pertinent to special issues such as weathering of building stone.

The leaching efficiencies observed in our study suggest, that under conditions of abundant substrate availability, chemolitho-

trophic activity on catchment rock can contribute to the increase in soluble Ca, Mg, and Mn in Lake Rasass waters as observed by Thies et al. (2007).

Although element release data are subject to source mineralogy and experimental conditions, the results contribute to our understanding of the relation between bacterial activity on catchment rock and Lake Rasass water chemistry (e.g. enrichment of anions and metals in runoff or percolating water). Research in this area is significant, since climate warming increases areas of mineral substrates exposed to the atmosphere by retreat of glaciers and permafrost and enhances weathering processes. In field experiments, quantification of microbial mobilization of major and trace elements from rock remains difficult to achieve. However, on a catchment and global scale, and over extended time periods, quantities of elements released by microbial activity can approach significant amounts.

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