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Functional Groups and Activities of Bacteria in a Highly Acidic Volcanic Mountain Stream and Lake in Patagonia, Argentina

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ABSTRACT

Acidic volcanic waters are naturally occurring extreme habitats that are subject of worldwide geochemical research but have been little investigated with respect to their biology. To fill this gap, the microbial ecology of a volcanic acidic river (pH $\approx$ 0–1.6), Rio Agrio, and the recipient lake Caviahue in Patagonia, Argentina, was studied. Water and sediment samples were investigated for Fe(II), Fe(III), methane, bacterial abundances, biomass, and activities (oxygen consumption, iron oxidation and reduction). The extremely acidic river showed a strong gradient of microbial life with increasing values downstream and few signs of life near the source. Only sulfide-oxidizing and fermentative bacteria could be cultured from the upper part of Rio Agrio. However, in the lower part of the system, microbial biomass and oxygen penetration and consumption in the sediment were comparable to non-extreme aquatic habitats. To characterize similarities and differences of chemically similar natural and man-made acidic waters, our findings were compared to those from acidic mining lakes in Germany. In the lower part of the river and the lake, numbers of iron and sulfur bacteria and total biomass in sediments were comparable to those known from acidic mining lakes. Bacterial abundance in water samples was also very similar for both types of acidic water (around 10^5 mL^{-1}). In contrast, Fe(II) oxidation and Fe(III) reduction potentials appeared to be lower despite higher biogenic oxygen consumption and higher photosynthetic activity at the sediment–water interface. Surprisingly, methanogenesis was detected in the presence of high sulfate concentrations in the profundal sediment of Lake Caviahue. In addition to supplementing microbiological knowledge on acidic volcanic waters, our study provides a new view of these extreme sites in the general context of aquatic habitats.

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Introduction

Anthropogenically acidified lakes and rivers have received much attention during recent years. The search for appropriate remediation strategies for water bodies affected by acid mine drainage and the use of microbial leaching for exploitation of low-grade ores have led to considerable knowledge about microbial processes in such environments [e.g., 71, 42, 13]. However, not much is known about the microbial ecology of naturally acidic rivers and lakes, especially volcanic acidic water systems. Extensive recent studies on their geochemistry [10, 11, 67] classify them as highly dynamic extreme habitats that pose a challenge both to researchers and to organisms that live there. Despite this, chironomids [74] as well as rotifers and algae [49] have been found in acidic volcanic waters. However, at many sites that have been studied with respect to their biology, the volcanic activity has ceased long ago and the crater lakes are no longer acidic [e.g., 3, 58]. Bacterial communities in acidic volcanic waters have rarely been studied, exceptions being the work of Satake and Saijo [62] and Takano and co-workers [65]. The scarcity of microbiological studies might be due to the fact that volcanic waters are inherently difficult to investigate because of their often poor accessibility which is critical for approaches that do not allow conservation of samples. Therefore, the main goal of the present study was to extend the knowledge on the microbiology of aquatic ecosystems influenced by volcanic activities with respect to community composition, biomass, respiratory activity, and Fe cycling. The acidity gradient present in a volcanic stream with several weakly mineralized tributaries could provide some insight into the reactions of the microbial community to pH changes.

The other goal was to find out if chemical similarities of anthropogenic and natural acidic waters (mainly high sulfur and iron content) were reflected in their microbiology. Man-made acidic waters are usually not very old (hundreds of years or less). In contrast, natural acidic lakes and rivers influenced by volcanism or hydrothermal waters, though geologically young and subject to fluctuating environmental conditions, can have a long history (thousands of years) which enables the evolution of specially adapted microbial communities. Extended knowledge of similarities and differences in the microbial ecology of natural and anthropogenic acidified waters might help in the search for effective remediation strategies for acidic mining lakes and rivers.

Materials and Methods

Location and Sampling

The study was carried out at a volcanic river and a lake fed by that river in Patagonia, Argentina. The area of Copahue-Caviahue is located in the volcanic Plio-Holocene Region of the Andes between 37 and 38°45′ S. Copahue Volcano (2965 m a.s.l.) is a predominantly andesitic stratovolcano which contained a small volcanic crater lake in 2000. Rio Agrio originates at the eastern slope of the volcanic cone (2740 m a.s.l.) as a $\approx$ 1 M hydrothermal solution. It flows downstream with an average rate of 2.24 m^3 s^{-1} [54] on a length of 13.5 km (slope 8.37%) before entering the north basin of Lake Caviahue. The mean width of the river is 5 m (km 5.5 to 11.2) and 8 m (km 11.2 to 13.5) [Baffico et al., manuscript in preparation]. Lake Caviahue is situated at 1600 m a.s.l. and is horseshoe-shaped with a north and a south basin. Its morphometric characteristics are as follows: maximum length 9.75 km, maximum width 4.72 km, Z_{max} 95 m, Z_{mean} 51.4 m, area 9.22 km^2, volume 0.474 km^3, T_w 2.6 years [54]. More detailed information including water chemistry, plankton, and trophic status of the lake are found in [49]. All samples were taken in April 2000; for characteristics of sampling sites see Table 1. We sampled three points along the course of Rio Agrio: The "upper Rio Agrio" sampling point was located about 1 km below the source, the "middle Rio Agrio" point was located some kilometers downstream where the river enters a more shallow grassland, and third sampling point was located just above the inflow of Rio Agrio into Lake Caviahue. In Lake Caviahue we sampled two points in the north basin: the "profundal" sample at 83 m water depth and a "littoral" sample at 0.5 m water depth near the mouth of Rio Agrio. River sediment was either sampled by collecting surface sediment with a spoon into sterile polypropylene vials or by coring with a hand-held Plexiglas tube (diameter 3.4 cm, length 20 cm). The latter was also used to obtain littoral sediment from Lake Caviahue. Profundal sediment was sampled with a gravity corer (Uwitec, Mondsee, Austria; diameter 6 cm). Spoons and Plexiglas tubes were rinsed with sample water before use. If not stated otherwise, surface sediments (upper 3 cm) were used for MPN and process-rate determinations. Water samples were obtained with a Limnos sampler or by directly holding sample bottles into the river water.

Oxygen Consumption

To measure oxygen consumption, fresh samples of water and sediment (about 2 g sediment filled up with water) were filled in small (2.5 mL) glass flasks. Oxygen was measured in the sample by a microoptode (Presens, Neuburg, Germany) and then the vessels were sealed bubble free. After about 1 day incubation at 16.5 ± 1°C the flasks were opened and oxygen was measured again. The samples were dried and the oxygen consumption was calculated from the difference between the two oxygen measurements related to the dry weight. All measurements were done in triplicate.

Table 1. Position and water characteristics of sampling sites[a]

Sampling site	Rio Agrio			Lake Caviahue	
	Upper	Middle	Lower	Littoral	Profundal
Position	37°51′24″ S 71°09′05″ W	37°52′10″ S 71°06′34″ W	37°52′42″ S 71°03′19″ W	37°51′24″ S 71°01′01″ W	37°52′17″ S 71°01′10″ W
Approx. stream length [km]	1	5	12		
pH	0.56	1.0	1.45	2.2	2.4–2.7[c]
Temperature [°C]	36	17	17	12	11.5 (surface) 9 (35 m) 5 (≥50 m)
Dissolved Fe [mmol L^{-1}]	25.93	7.18	3.02	Nd	0.39
Al [mmol L^{-1}][b]	112.67	24.83	4.63	Nd	1.42
Sulfate [mmol L^{-1}][b]	557.76	112.43	22.80	Nd	20.16

[a] nd = not determined.
[b] Unpublished data of the Caviahue scientific team.
[c] Friese, pers. comm.

In Situ Oxygen Fluxes

Microprofiles of oxygen were measured with O_2-microoptodes with a tip diameter of 50 μm (Presens Neuburg, Germany). The sensors were moved by a manual micromanipulator (Märzhauser, Wetzlar, Germany) which was fixed to a tripod. Measurements in the river were done directly in the field. Measurements in lake sediments were done in fresh sediment cores in the field laboratory immediately after sampling. After the measurements the porosity of the sediment was determined in centimeter intervals.

From concentration gradients at the sediment surface fluxes were calculated using Fick's first law of diffusion:

$$J_D = -\Phi D_s (dC/dz) F$$

where J_D = diffusive flux (μmol m^{-2} h^{-1}), Φ = porosity of the sediment, D_s = sediment diffusion coefficient (cm^2 s^{-1}), dC/dz = the measured concentration gradient (μmol L^{-1} cm^{-1}), and F = unit conversion factor (36,000 L cm^{-1} m^{-2} s h^{-1}). The bulk sediment diffusion coefficient D_s was calculated from the molecular diffusion coefficient ($D_{oxygen} = 2.3 \times 10^{-5}$ cm^2 s^{-1} [41] by correction for porosity and tortuosity using the empirical equation $D_s = D\,\Phi^2$ [41]. The resulting fluxes are mean values obtained from at least three separate profiles. Photosynthetic activity in the sediment was measured with O_2 microelectrodes by the light-dark shift technique [55]. Sediment cores (diameter 3.4 cm, length 20 cm) were manually taken from the lake and incubated in a bucket containing lake water. The water was continuously aerated and mixed by an aquarium air-pump. The microelectrodes (tip diameter <30 μm, MASCOM, Bremen, Germany) were positioned using a micromanipulator and a dissection microscope. A cold light source (KL200, Zeiss) was used for illumination. The light intensity was about 300 μE s^{-1} m^{-2}. The immediate decrease of the oxygen concentration after shading the sample was recorded by a strip chart writer and converted to a photosynthetic rate in mmol dm^{-3} h^{-1}.

Light was measured by a LICOR quantum sensor (LI190SB). All light measurements were made outside the incubation vessel. Laboratory measurements were carried out at 17°C which was a typical *in situ* temperature in the afternoon.

Field Tests

Fe(II), Fe(III), and sulfide in water samples were analyzed with commercial test kits (LCK 320, LCW053, Dr. Lange, Düsseldorf, Germany). Dissolved Fe(II) and Fe(III) were measured immediately in the field using the LCK 320 test kit and a LASA-100 field photometer (Dr. Lange, Düsseldorf, Germany). Samples were prediluted with distilled water to provide pH values and Fe concentrations within the analytical range of the test.

Laboratory Analyses

Direct Microscopic Counts. Water samples were fixed with 2% glutaraldehyde in the field, filtered onto 0.2 μm polycarbonate filters and stained with acridine orange according to standard procedures [26]. Bacterial abundance and biovolumes were determined by epifluorescence microscopy using 1000× magnification and a Porton grid. Spheres in the Porton grid were used to measure the length and width of 50 individual cells per sample for biovolume (V [μm^3]) determination. Bacterial biomass was calculated applying a nonlinear equation derived from [64].

$$\underline{\text{Biomass}}\ [\underline{\text{fg}}\ \text{C}] = V^{0.59} \cdot 88.6 \cdot 1.0483$$

Most Probable Number (MPN) Counts. MPN counts were performed for different functional groups of bacteria using media that were successfully applied for these organisms in other acidic environments. With respect to pH we gave priority to comparability and optimal conditions for known bacteria over simulating *in situ* conditions in the medium. Samples for MPN counts were filled into sterile flasks or tubes and stored cool (4–6 days) until further processing in Germany. Media for iron-oxidizing bacteria (FeOB), sulfur-oxidizing bacteria (SOB), and sulfate-reducing bacteria (SRB) were as described by Meier (Ph.D. thesis, University of Bonn, 2001). The compounds of the basal mineral medium for FeOB, SOB, and SRB were (in g L^{-1}) 0.1 KH_2PO_4, 0.1 NH_4Cl, 0.1 NaCl, 0.1 KCl, 0.2 $CaCl_2 \cdot 2H_2O$, and 0.2 $MgCl_2 \cdot 6H_2O$, and 1 mL L^{-1} trace element solution SL 12B [72]. Additions for FeOB were

$FeSO_4 \cdot 7H_2O$ (80 mM) and vitamin B_{12} (40 μg L^{-1}); medium pH was 1.6–1.8. Additions for SOB were $Na_2S_2O_3$ (10 mM), vitamin B_{12} (40 μg L^{-1}), and $FeSO_4 \cdot 7H_2O$ (1 mg L^{-1}). The medium was phosphate buffered (6.7 mM KH_2PO_4) and adjusted to pH 4.5. Bromophenol blue (5 mg L^{-1}) was used as pH indicator. Additions for SRB were $MgSO_4 \cdot 7H_2O$ (15 mM), selenite–tungstate-solution (1.2 μg L^{-1} $Na_2SeO_3 \cdot 5H_2O$ and 1.6 μg L^{-1} $Na_2WO_4 \cdot 5H_2O$ according to [73]), vitamin solution [51] (1 mL L^{-1}), sodium lactate (5 mM), sodium acetate (5 mM), and $FeSO_4 \cdot 7H_2O$ (0.05 g L^{-1}). The medium was carbonate buffered (20 mM $NaHCO_3$) and adjusted to pH 7. The medium for Fe(III) reducing bacteria (FeRB) was adapted from a medium for *Geobacter metallireducens* (DSM no. 579). It contained (in g L^{-1}) 13.7 ferric citrate, 1.5 NH_4Cl, 0.6 NaH_2PO_4, 0.1 KCl, 1.6 sodium acetate, 0.05 yeast extract, 0.00025 $Na_2WO_4 \cdot H_2O$ and 10 mL L^{-1} trace elements solution [44]. The medium was buffered with 10 mM MES (2-morpholinoethanesulfonic acid) and poised at pH 6. For counting acidophilic FeRB, the medium of Küsel et al. [37] with a pH of 2.3 was used. The media for SRB and FeRB were cooled under oxygen-free nitrogen after autoclaving. Dithionite was used as reducing agent for the SRB medium. Cultures for iron and sulfur bacteria were prepared in deep multiwell plates (8 parallels). Anoxic conditions for anaerobes were generated by placing cultures in sealed bags with gas generators (Merck Anaerocult A).

The medium for fermentative bacteria was prepared in culture tubes with Durham tubes and butyl rubber septa. It consisted of (g L^{-1}): NaCl 1.0, KCl 0.5, $CaCl_2 \cdot 2H_2O$ 0.1, $MgSO_4$; $7H_2O$ 0.05, $MgCl_2 \cdot 6H_2O$ 0.4, NH_4Cl 1.5, yeast extract 0.25, tryptone 0.25, peptone 0.25, fructose 0.25, Tween-80 0.25, $KH_2PO_4 \cdot H_2O$ 0.6, $Na_2MO_4 \cdot 2H_2O$ 0.00015 mM, trace elements solution 1 mL $Na_2S \cdot 9H_2O$ 1 mM (modified from Detmers, pers. comm.). Resazurin was used as redox indicator, the pH was 2.0. Three parallels and 5 subsequent 10-fold dilutions were prepared per sample. All MPN cultures were incubated at 28°C in the dark for 6 weeks. Indicators for growth were formation of orange or brown precipitates for FeOB, color change from blue to yellow for SOB, black precipitates for SRB, and gas formation for fermenters. Growth of FeRB was judged from accumulation of Fe(II) determined in a microtiter format ferrozine assay. MPN and their confidence limits were calculated with the program of Klee [32].

Phospholipid Phosphate Content. Since we experienced difficulties with microscopic examination of acidic sediments (autofluorescence, mineral particles), determination of the phospholipid phosphate content was chosen as an estimate of total viable microbial biomass in the sediments. The method of [17] was applied with the modifications suggested by Neumann (Ph.D. thesis, University of Marburg, 1995). Briefly, duplicates of freeze-dried sediment were subjected to chloroform–methanol extraction and the extracts were evaporated. The release of P from phospholipid was achieved by incubation at 510°C for 5 h in a muffle furnace. Samples were then treated with sulfuric acid and ammonium molybdate, and 60 min after addition of malachite green solution photometric measurements at 610 nm were taken. When tested for acidic mining lake sediment, this method yielded a relative standard deviation of less than 10%.

Methane. To measure the methane concentration in the water or in the porewater, samples were rapidly filled in small glass vessels (16.6 mL) amended with 4 mL of water containing 1 mM $HgCl_2$. A headspace of about half of each flask's volume was left. The samples were closed with butyl rubber stoppers and stored cool until analysis in Germany. The methane concentration in the headspace was measured by a gas chromatograph equipped with a FID, and the porewater concentration was calculated using a Bunsen solubility coefficient of 0.035 [39].

Fe Extraction from Sediment. Reactive Fe, including HCl-soluble Fe(II) and hydroxylamine-reducible Fe(III), was determined in triplicates using ferrozine [45]. Samples were centrifuged (16.000 *g*, 10 min) instead of filtration before photometry.

Fe Reduction and Oxidation Assays. Potential Fe(II) oxidation and Fe(III) reduction were determined using Fe(II) and Fe(III) sulfates as iron sources. One volume of a 125 mmol/L stock solution (pH 1.7) was combined with 4 volumes of sample in a glass vial (8 mL). Vials for Fe(III) reduction were completely filled and closed bubble-free with rubber stoppers. Vials for Fe(II) oxidation were filled to half of their volume and incubated horizontally to provide aeration. Abiotic controls were prepared by 0.2 μm filtration (water samples) or by addition of $HgCl_2$ to a final concentration of 1 mmol/L (sediment samples). Assays were incubated at 16.5± 1°C in the dark. After 3 weeks, the vials were opened and Fe(II) was measured [45]. Biological iron transformation were assumed when Fe(II) concentrations differed from those of sterile controls.

Carbon and Sulfur in Sediments. To determine total carbon and sulfur in sediments, material was dried and homogenized. Aliquots were analyzed with a CNS elemental analyzer (Vario EL, Elementar).

Short-chain organic acids were analyzed by HPLC (Thermo Separation Products) with an organic acid resin column 300 × 8 mm, eluent 0.05 M H_2SO_4, flow rate 0.6 mL min^{-1}, and a column temperature of 30°C. A diode array detector (Spectra System UV6000LP) was used as a UV detector at 206 nm.

Statistical Analyses. Statistical analyses were performed using SigmaStat™ for Windows V2.03.

Results

Bacterial Abundances

Acridine orange direct counts from water samples were around 2×10^5 mL^{-1} in all samples. However, because of larger cells, bacterial biomass was higher in Rio Agrio than in Lake Caviahue water (5.2–6.7 and 2.2–3.9 mg C m^{-3}, respectively). This difference was statistically significant

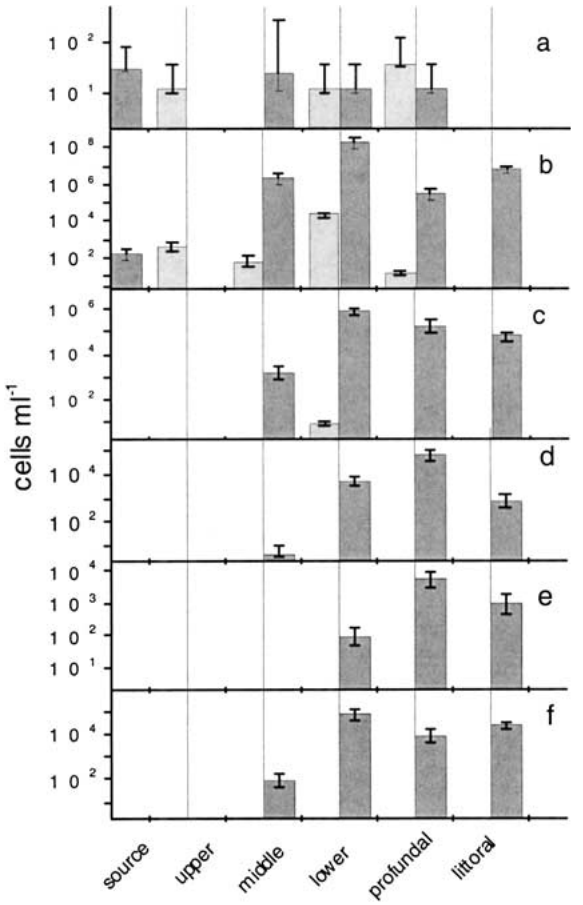

Fig. 1. MPN counts in water (light bars) and sediment (dark bars) in cells mL^{-1} for fermenters (a), S oxidizers (b), Fe oxidizers (c). sulfate reducers (d), Fe reducers at pH 6 (e), and Fe reducers at pH 2.3 (f). The locations are the source, the upper, middle, and lower part of Rio Agrio, and the littoral and profundal of Lake Caviahue.

when comparing all individual biomass values (P = <0.001, Mann–Whitney rank sum test).

The results of the MPN counts show that few culturable mesophilic bacteria were present in the upper part of Rio Agrio (Fig. 1). Only sulfur oxidizers and fermenters were detected in low numbers. From the middle part of Rio Agrio to Lake Caviahue, iron and sulfur oxidizers, FeRB, SRB, and fermenters were present. FeRB and SRB were only found in sediments. The numbers of acidophilic FeRB were higher and they were more widely distributed than FeRB growing at pH 6. It is not clear from our study if these are overlapping or separate populations.

The estimation of total viable microbial biomass in sediments as phospholipid phosphate (given as nmol P g-dw^{-1}) showed some correspondence with cultured bacterial variety and numbers, but only FeRB at pH 6 and SRB

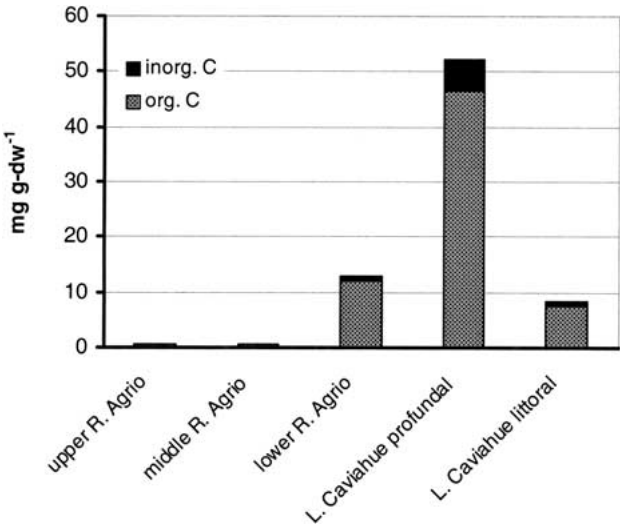

Fig. 2. Anorganic (black bars) and organic carbon (gray bars) in the sediment.

were correlated with phospholipid phosphate content (Pearson product moment correlation coefficient of 0.967). In upper and middle Rio Agrio, biomass was not detectable by our method. Lower Rio Agrio and Lake Caviahue littoral contained 63 and 53 nmol P g-dw^{-1}, respectively, whereas Lake Caviahue profundal contained 231 nmol P g-dw^{-1}. The differences may be partly due to the different texture of the sediments (sandy vs very fine-grained material and a resulting higher surface-to-volume ratio). Assuming an average conversion factor of 100 µmol P per g microbial carbon [12], these sediments would contain between 0.5 and 2.3 mg biomass carbon per g dry weight. However, application of general conversion factors to extreme ecosystems should be interpreted with caution.

Total and organic carbon in the sediments showed a corresponding trend with the microbiological data (Fig. 2, Pearson product moment correlation coefficients of 0.997 and 0.996 with phospholipid P, respectively). Carbon concentrations in upper and middle Rio Agrio sediments were at the detection limit. In all samples between 89 and 94% of the total carbon as organic. Using the biomass values described above, microbial biomass made up between 3 and 7% of the total organic carbon. No free short-chain organic acids could be detected by HPLC. Total sulfur values were between 25 and 5 mg g-dw^{-1} with a decreasing trend downstream (data not shown).

Microbial Activities

Oxygen consumption rates determined by batch incubations were always higher in the sediment than in the water

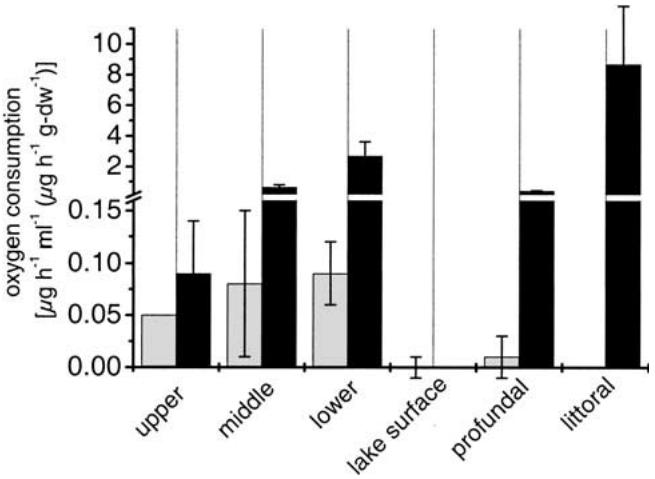

Fig. 3. Oxygen consumption measured in samples of water (light bars) and sediment (dark bars) at the upper, middle and lower part of Rio Agrio and the littoral and profundal of Lake Caviahue.

(Fig. 3). Rates in the water were at the detection limit of our analytical system. The rates were increasing downstream the Rio Agrio. Significant oxygen consumption above 1 µg h^{-1} g-dw^{-1} was observed in the sediments from the middle and lower Rio Agrio and in the lake sediments. In a control experiment addition of $HgCl_2$ to sediment from the mouth of Rio Agrio inhibited more than 80% of the oxygen uptake (data not shown). Thus, the oxygen consumption was predominantly biogenic.

The measurements of oxygen microprofiles showed a deep penetration of oxygen into the sediment at the middle Rio Agrio (Fig. 4a,b). Because of the high flow velocity of the water and the coarse texture of the sediment, we surely have porewater advection, which leads to oxygen saturation in the upper centimeters of the sediment. Thus, it was not possible to calculate a reasonable diffusive flux of oxygen. The decrease of oxygen in the deeper sediment shows, however, that oxygen was consumed at low rate in the sediment. The highest oxygen flux (0.041 nmol cm^{-2} s^{-1}) was observed at the lower Rio Agrio (Table 2). The sediment was anoxic below 1.2 mm depth (Fig. 4c). A major part of the sediment of the Rio Agrio is covered by stones. We also measured oxygen consumption of biofilms at the surface of stones. The oxygen uptake rate

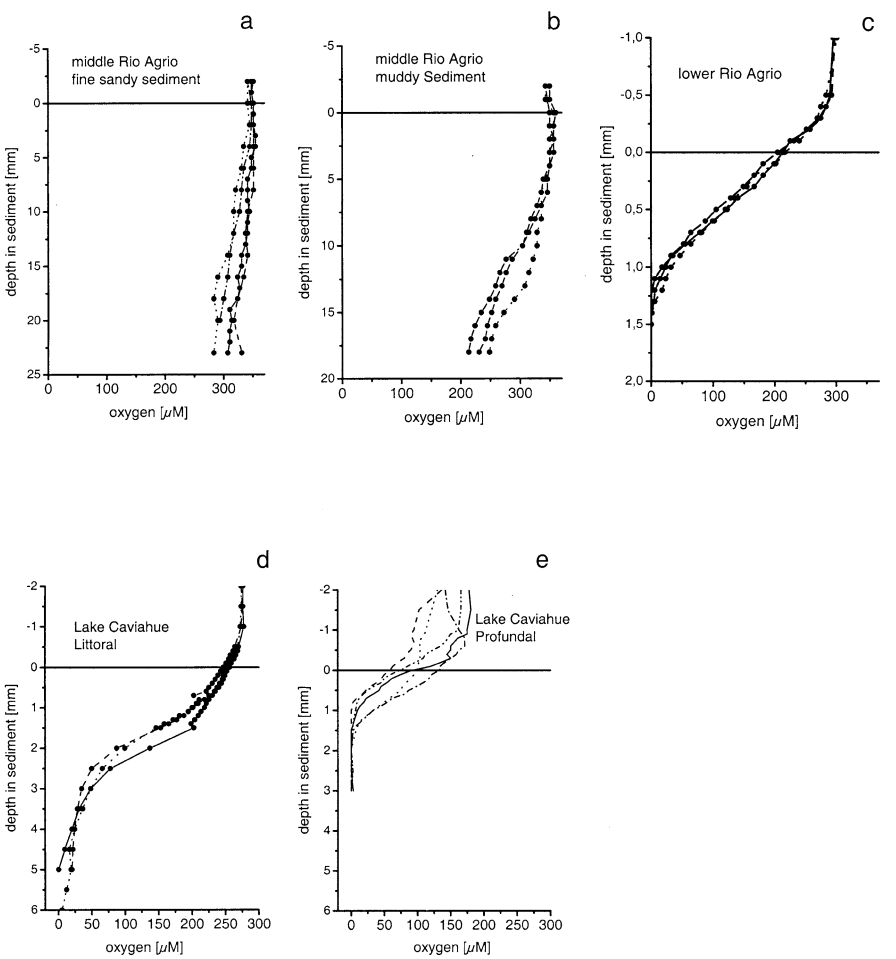

Fig. 4. Microprofiles of oxygen measured *in situ* (Rio Agrio) or in sediment cores (Lake Caviahue). Note the different scales.

Table 2. Oxygen fluxes calculated from oxygen microprofiles (nmol cm^{-2} s^{-1})

Site	Oxygen flux (nmol cm^{-2} s^{-1})	Reference
Middle Rio Agrio	0	This study
Lower Rio Agrio	0.041 ± 0.002	This study
Lake Caviahue Littoral	0.006 ± 0.0004	This study
Lake Caviahue Profundal	0.018 ± 0.011	This study
Mining Lake 111	0.004	Koschorreck, unpublished
Lake Fuchskuhle	0.014	[36]
Lake Vilhelmsborg (Denmark)	0.05	[27]
Estuary Denmark	0.012–0.079	[55]
Biofilm trickling filter	0.11	[35]

of stone biofilms was one order of magnitude lower than sediment uptake rates (Baffico et al., manuscript in preparation). Oxygen fluxes from the water into the sediment were also observed in the lake sediments (Table 2). Penetration of oxygen was higher and the flux was lower at the littoral site compared to the profundal (Fig. 4d,e). Outgassing caused problems during the microsensor measurements in the profundal sediment, leading to a high scatter of the profiles. The profundal sediment was anoxic below 1.5 mm depth (Fig. 4e).

A reason for deeper oxygen penetration and lower oxygen flux may be photosynthetic oxygen production at the littoral site. To test this hypothesis, we measured photosynthetic oxygen production by the light–dark shift method in another sediment core from the littoral (Fig. 4). We saw a clear difference between light and dark incubated sediments and measured an integrated gross photosynthesis of 0.22 nmol cm^{-2} s^{-1} (Fig. 5).

Concentrations of reactive iron were high in Rio Agrio and decreased from the source to the inflow into Lake Caviahue (Fig. 6). At the source and upper Rio Agrio, Fe concentrations in the water were higher than those in wet sediment. Fe(II) was the predominant form as is typical for hydrothermal fluids [56]. However, some Fe(III) was also present in most samples, and at the low pH values *in situ* it was most likely the result of biological oxidation.

From the source to middle Rio Agrio, neither biological iron oxidation nor reduction was detected in our assays. Biological Fe(II) oxidation was found in water of lower Rio Agrio and Lake Caviahue profundal. Fe(III) reduction was detected in sediment from lower Rio Agrio and from Lake Caviahue profundal and littoral (Fig. 7). This indicates biological cycling of Fe in the lower part of this water system. Oxidative processes took place in the water while reductive processes were located in the sediment, as could have been expected from the availability of oxygen *in situ*. Hydrogen sulfide was not detected in any of the samples either by smell or by the field test kit. However, we found that high Fe concentrations interfered with the assay, resulting in underestimation of sulfide concentration. So we cannot totally exclude either the presence of sulfide or very low sulfate reduction rates. Black iron sulfide precipitates were not observed and are unlikely to form at pH < 3.

The profundal of Lake Caviahue was the only location where we detected elevated methane concentrations

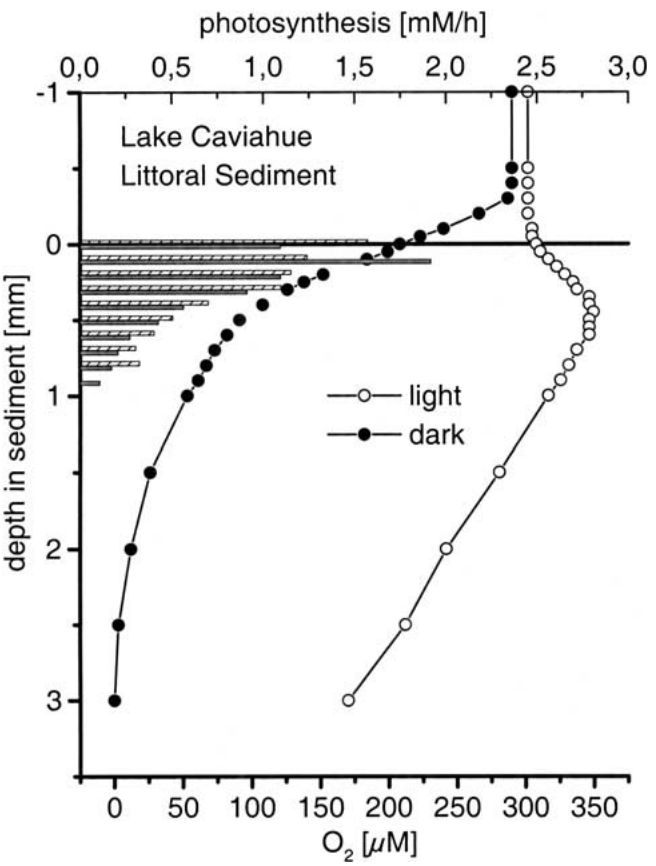

Fig. 5. Oxygen microprofiles (lines) and rates of gross photosynthesis (columns) measured in a sediment core from the littoral of Lake Caviahue. Two different columns indicate replicate measurements.

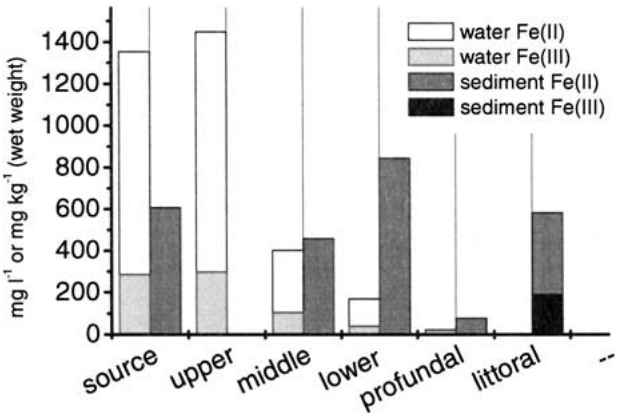

Fig. 6. Reactive iron in water and sediment.

(Table 3). The formation of gas bubbles accompanied by highly increased methane concentrations was observed in a sediment core from the profundal incubated overnight in the laboratory, indicating that methanogenesis was active in the sediment.

Table 3. Concentration of methane in the water and porewater of samples from Lake Caviahue

Sample	Methane (μM)
Surface water	0
Bottom water	53
Sediment 0–10 cm	185
Sediment 0–2.5 cm	167[a]
Sediment 2.5–5 cm	416[a]
Sediment 7–9 cm	5281[a]

[a] Measured after 1 day in the laboratory.

Discussion

Microbial Life in the Rio Agrio and Lake Caviahue

The upper and middle part of Rio Agrio appeared to be poorly colonized by biota and showed low biological activity. This view is supported by similar findings from very different approaches of investigation. The extreme geochemical conditions seem to be an obvious explanation. However, acidity alone is unlikely to be responsible, since high microbial biomass and activity have also been found in biofilms in caves at pH values below 1.0 [70] and in abandoned metal mines with pH 0.5–2.5 [13]. Because of the high flow velocity there is not enough time for organisms to develop in the running water from the source down to the middle Rio Agrio. Given the mean velocity and width of the stream, water will flow 3.6 km h^{-1}; thus passing the whole stream in 3.75 h. Bacterioplankton would thus need very low doubling times to multiply significantly before entering Lake Caviahue, so organisms have to be attached to surfaces. Abundances of culturable bacteria in the free water were always low or not detectable (Fig. 1), indicating that the microscopically visible cells in the water were not culturable with our methods. The sandy and muddy sediments are difficult to colonize, because

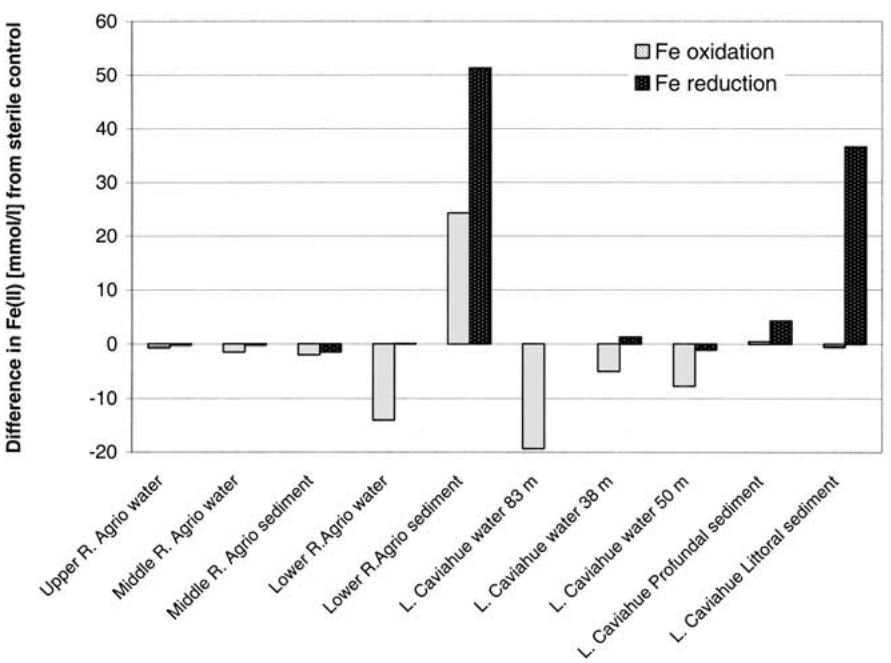

Fig. 7. Fe(II) oxidation and Fe(III) reduction, expressed, as difference in Fe(II) concentration between live and sterile assays after 3 weeks incubation.

they are continuously turned over by the water current. However, muddy and sandy sediments are rather untypical habitats in the upper river. Most of the river bed is covered by stones. The ubiquitous presence of fermenters in low numbers does not necessarily indicate their activity or proliferation in this water system, but can also be due to import of spores. In contrast, sulfide oxidizers might have been active under the prevailing conditions. They have been found to be widespread in volcanic water systems, and sulfide oxidation activity down to pH 1 has been demonstrated [65]. The low availability of both organic and inorganic carbon (Fig. 2) is an additional explanation for low bacterial abundances and activities in the upper and middle Rio Agrio. Because of the hydraulic regime the water as well as the upper sediments and the interstitial at the upper and middle Rio Agrio were completely oxic, excluding anaerobic microbial processes. Oxygen penetration down to a sediment depth of 30 cm was also found in a German mountain stream [53]. The resulting oxygen fluxes at the sediment–water interface of lower Rio Agrio and Lake Caviahue were in the same range as reported for "normal" habitats (Table 2). The sediment respiration rate at the lower Rio Agrio site was virtually the same as in a temperate blackwater stream [19]. This uniformity of oxygen fluxes might be due to diffusion limitation of such fluxes rather than due to a similar heterotrophic potential in different ecosystems. Oxygen consumption rates as well as the extension of the oxic zone in the sediment might be regulated by diffusional characteristics of the sediment rather than availability of organic substrates or the presence of microorganisms. Moreover, part of the observed oxygen consumption is surely due to lithotrophic sulfide oxidation rather than heterotrophic activity [65]. Increasing oxygen consumption shows that there exists a gradient of increasing life downstream. Starting from a nearly sterile geothermic source, microbial life really begins at the middle to lower Rio Agrio. As found in the lower part of Rio Agrio, epilithic communities might play a major role in the colonization of the river (Baffico et al., manuscript in preparation). Here, and in Lake Caviahue littoral, the phospholipid P concentrations are within the range given for other freshwater stream sediments [12], corresponding to $2.1–2.5 \times 10^{10}$ g^{-1} bacterial cells [2]. However, since microalgae and fungi may have contributed significantly to viable biomass in these samples, bacterial abundance is probably overestimated by this simple conversion. The factors that form the strong gradient of life in Rio Agrio are still to be analyzed by correlating biological and chemical data from the river in the future. López-Archilla and co-workers [43] studied the Tinto River ecosystem by principal component analysis and found it to be determined by pH values, metal concentrations, and biological productivity. Since the Rio Agrio system differs from Rio Tinto in its origin (mining vs volcanism), it will be interesting to clarify if these key factors are identical.

MPN counts of iron oxidizing and reducing bacteria as well as sulfur oxidizers (Table 6) are in the range of literature data from different acidic and neutral environments (Tables 4, 5, and 6, respectively). In contrast to the

Table 4. Abundance of acidophilic iron-oxidizing bacteria in different habitats estimated by MPN[a]

Sample	pH	Cells g^{-1} or ml^{-1}	Reference
Mining Lake 111 sediment	2.6	10^6	Meier, Ph.D. thesis,
	6	10^7	Univ. Bonn, 2001
Mining Lake Niemegk sediment	3	$10^4–10^9$	Wendt-Potthoff, unpublished
Mine tailings	2–4	Max. 2×10^8	[14]
Gold mine tailings	6.5–8.5	$10–10^4$ *T. ferrooxidans* $0–10^2$ *T. thiooxidans* $10^4–10^6$ *T. thioparus*	[8]
Copper bioleaching operations	na	$10–10^8$ *T. ferrooxidans* $1–10^5$ *T. thiooxidans* $0–10^3$ *T. acidophilus* $1–10^6$ *L. ferrooxidans*	[22]
Uranium bioleaching	na	$2 \times 10^3–2 \times 10^6$[b]	[5]
Tinto River, Spain	2.5	$10^6–10^9$	[42]
Stream at Kusatsu-Shirane volcano	2–2.9	$3 \times 10^3–10^4$[b]	[65]
Acidic stream from copper mine	2.2–2.8	$10^3–10^4$[b]	[71]
Rio Agrio/Lake Caviahue	1–2	$10–10^6$	This study

[a] na-not available
[b] Determined as colony-forming units.

Table 5. Abundance of iron-reducing bacteria in aquatic habitats estimated by MPN

Sample	pH of medium	Cells g^{-1} or mL^{-1}	Reference
Mining Lake 111 sediment	6	10^3	Wendt-Potthoff, unpublished
Mining Lake Niemegk sediment	6	10^2–$\geq 10^6$	Wendt-Potthoff, unpublished
Mining Lake 77 sediment	2.3	4×10^3	[37]
	6.7	2.3×10^3	
Activated sludge		10^3–10^6	[48]
Eutrophic lake sediment		1.3×10^4 (littoral)	[30]
		8.2×10^4 (profundal)	
Lacustrine sediment (aquifer)		0–10^4	[46]
Rio Agrio/Lake Caviahue	2.3	0–10^4	This study
	6.7	0–10^4	

acidic Tinto River in Spain [42], but similar to a volcanic water system in Japan [65], numbers of sulfur oxidizers tended to be higher than numbers of iron oxidizers. This has been attributed to the lower pH limit of the latter. The exclusive cultivation of iron-reducing bacteria from some sediments, fits well with the observed iron-reducing activities. The numbers of sulfate-reducing bacteria were low compared to normal aquatic sediments, but in the same range as reported for acidic mining lakes (Table 7). So far no sulfate-reducing bacteria have been isolated capable of growth below pH 4 [24]. However, mixed cultures from an acidic stream reduced sulfate at pH 2.8 [66]. Sulfate reduction was also demonstrated in an acidic volcanic lake in Japan. A ^{35}S tracer measurement proved sulfate reduction in lake water with a pH of 1.8 [63]. In an acidic lake sediment sulfate reduction was measured at a pH as low as 3 [23]. In sediments of acidic mining lakes sulfate reduction was detected at near-neutral pH but not at pH 2.7 (Meier, Ph.D. thesis, University of Bonn, 2001). It was shown that the sulfate reducers counted were predominantly present as spores. SRB cultured from acid streamers in disused copper or pyrite mines also formed endospores [29]. The same might be true for Rio Agrio. Although the lower pH limit for microbial sulfate reduction remains unknown, we can only speculate why so many sulfate reducers were present even in the highly acidic Rio Agrio. Especially in the middle and lower part, we frequently observed animal feces at the river bank. These feces might be a source of bacteria as shown in acidic and oxic mine tailings [15]. Although Lake Caviahue profundal sediment appeared to be organically enriched according to total carbon and to phospholipid P derived microbial biomass [12], it was surprising to find methanogenesis in the sediment of Lake Caviahue since its porewater contained about 450 mg L^{-1} sulfate (Friese, pers. comm.). In the presence of sulfate, methanogenesis normally is inhibited and sulfate reduction is the terminal step of carbon mineralization under anoxic conditions. Thermodynamically, sulfate reducers should outcompete methanogens for electron donors. This is the reason why sulfate reduction dominates in marine systems. However, depending on the quality of organic substrates, methanogens can outcompete sulfate reducers [52] or both processes can coexist [1]. We probably did not use adequate methodology to observe low rates of microbial sulfate reduction since sulfate, organic carbon, anoxic conditions, and sulfate-reducing bacteria were present. From the increase of the methane concentration in the sediment core in the laboratory (Table 3) we can roughly estimate a methane production rate of 0.3 μmol h^{-1} g-dw^{-1}. This rate is high compared to other methanogenic habitats. In slurries of fertilized rice field soil a rate of 0.14 μmol h^{-1} g-dw^{-1} has been reported [33]. Typical rates for wetland soils are around 0.06 μmol h^{-1} g-dw^{-1} [9]. This

Table 6. Abundance of sulfur oxidizing bacteria in aquatic habitats estimated by MPN

Sample	pH	Cells g^{-1} or mL^{-1}	Reference
Mining Lake 111 sediment	2.6	10^7	Meier, Ph.D. thesis, Univ.
	6	10^7	Bonn, 2001
Tinto River, Spain	2.5	10^6–10^9	[42]
Yugama crater lake/seasonally flowing streams	1–3.9	10^2–10^6 [a]	[65]
Rio Agrio/Lake Caviahue	1–2	10^2–10^8	This study

[a] Determined as colony-forming units.

Table 7. Abundance of sulfate-reducing bacteria in aquatic sediments estimated by MPN

Sample	pH	Cells g^{-1} or mL^{-1}	Reference
Mining Lake 111 sediment	2.6	10^3	Meier, Ph.D. thesis, Univ. Bonn, 2001
	6	10^4	
Mining Lake Niemegk sediment	3	10^2–10^4	Wendt-Potthoff, unpublished
Lake Baikal (oligotrophic)	7.9	2.1×10^{5b}	[47]
Lake Constance (mesotrophic)	na	2.4×10^6	[57]
Lake Dagow (eutrophic)	6.6–7.8	1.5×10^6	[60]
Lake Shinhama (eutrophic)	7	2.9×10^{4b}	[18]
Lake Teganuma (hypertrophic)		10^{5b}	[19]
Lake Stechlin (oligotrophic)	7.5	1.2×10^5	[61]
Northeast Atlantic Ocean	na	5×10^{3b}	[4]
Kattegat	na	2×10^{6b}	[28]
Microbial mat (North Sea)	na	10^8	[68]
Rio Agrio/Lake Caviahue	1–2	0–10^4	This study

[a] na-not available
[b] Determined as colony-forming units

implies that upward movement of methane following exposure to atmospheric pressure might have contributed to our measurements, and no rate or exact zonation of either sulfate-reducing or methanogenic activity can be given. The issue of methanogenesis and sulfate reduction in a sulfate-rich and acidic environment remains an interesting point for further studies.

Comparison with Acidic Mining Lakes

One goal of our study was to learn from differences and similarities between a natural acidic system and artificial acidic mining lakes. Because of the different quality of the data we semiquantitatively compared the data presented in this study with data from German acidic mining lakes (Table 8). Since the upper Rio Agrio is subject to strong geothermic influence, its inclusion in this comparison is inappropriate and values are only given for reference purposes. The most striking result of the comparison is that microbial activities differ substantially between the systems while microbial abundances as counted by MPN were remarkably similar. Maybe this reflects methodical limitations connected to the MPN technique rather than real conformity between different habitats. MPN counts

Table 8. Comparison of Rio Agrio and Lake Caviahue with acidic mining lakes[a]

Parameter	Upper R. Agrio	Lower R. Agrio	Lake Caviahue	Mining lakes	Reference
Acidity	+++	++	+	+	[21, 49]
Reactive Fe in water	+++	++	+	++	[25]
Reactive Fe in sediment	++	++	+	+++	[16]
Biomass as phospholipid P	–	+	+++	++	Wendt-Potthoff, unpublished
Total/organic carbon	+	++	++	+++	Nohlen 1999 (thesis), [16]
Total sulfur	+++	++	++	++	[16]
Fe reduction	–	+	+	++	[6, 40, 50]
Fe oxidation	–	+	+	+++	Wendt-Potthoff, unpublished
S-oxidizing bacteria	+	+++	+++	+++	Meier 2001 (Ph.D. thesis)
Fe-oxidizing bacteria	(+)	+++	+++	+++	Meier 2001 (Ph.D. thesis)
SRB	(+)	++	++	++	Meier 2001 (Ph.D. thesis)
FeRB	(+)	++	++	++	[37], Wendt-Potthoff, unpublished
Fermenters	+	+	+	+	Wendt-Potthoff, unpublished
Sulfate reduction	–	–	–	(+)	Meier 2001 (Ph.D. thesis), [6, 7, 34] Wendt-Potthoff, unpublished
Methanogenesis	–	–	+	–	Koschorreck, unpublished
Photosynthesis	?	+++	++	+	[31], Koschorreck and Tittel, unpublished
Oxygen consumption	–	++	++	+	Koschorreck, unpublished

[a] –not detected, (+) very low or only in few samples, + low, ++ medium, +++ high concentration or activity.

usually underestimate the real number of bacteria [59], and we did not distinguish between active cells and resting stages. Differences in activity could also be related to groups of microorganisms known to live in highly acidic water systems but not covered by our MPN approach, e.g., aerobic acidophilic heterotrophs [71, 29, 37] or fungi [62, 63]. From confocal laser scanning microscopy of stone surfaces and river sediment, we have some evidence that fungi are abundant in the Rio Agrio (Baffico et al., manuscript in preparation). However, direct microscopic counts of bacteria in water samples were also very similar and about one order of magnitude lower than in typical mesotrophic lakes [38]. All investigated habitats showed high abundance of iron- and sulfur-oxidizing bacteria. This is not surprising because these are typical acidophilic bacteria, and reduced iron and/or sulfur compounds are present at all investigated sites (Fig. 7, [16]).

Mining lakes typically receive a continuous input of acid mine drainage by either surface or groundwater flow. This leads to permanent precipitation of iron in the lake and the sediments are highly enriched in iron oxides. In the sediments an increase of pH with depth is often observed that is probably due to the flow of groundwater with higher pH. The young age of the lakes did not allow the accumulation of a thick authigenic sediment layer, and the chemistry of the sediment is strongly influenced by the parent rock or sediment. This can be seen in high contents of organic carbon originating from lignite ([16], Nohlen, master's thesis, University of Bayreuth, 1999). The only important source of acidity in the Rio Agrio–Lake Caviahue system is the Rio Agrio. Along the course of the river, iron oxides and other compounds precipitate and accumulate in the sediment while comparably low amounts of iron reach the lake. Since the proportion of surface inflow is high compared to mining lake, the groundwater probably has less influence on the chemistry of the sediments of Lake Caviahue. This is reflected in the lower iron content of the Lake Caviahue sediments and consequently lower Fe-turnover activities. In summary, the physico-chemical conditions in the sediment of Lake Caviahue differ from those of acidic mining lakes because of different paths and intensities of iron and acidity inflow.

Oxygen consumption was higher in the Rio Agrio and Lake Caviahue than in mining lakes, which probably reflects differences in the quality of organic substrates. The main source of organic substrates in the Rio Agrio is certainly allochthonous input, supplemented by primary production of lithotrophic bacteria. The lower Rio Agrio also receives a small tributary, Rio Jara, which has little influence on pH and chemistry (Baffico et al., manuscript in preparation), but might be a qualitatively important source of organic carbon. The main sources of organic material in Lake Caviahue are the inflow from the rivers, pelagic production, and the input of domestic sewage waters of Caviahue village (350 permanent inhabitants [49]). These sources contain high amounts of readily available organic carbon (which is supported by the tight correlation between organic carbon and bacterial biomass) while the carbon in mining lakes is predominantly in the form of recalcitrant lignite degradation products (Nohlen, master's thesis, University of Bayreuth, 1999). A recent estimate is that aquatic organic matter accounts for only 5–14% of total organic carbon but for 45–75% of total carbon oxidation rates in mining lake sediments [7]. A better carbon availability in the sediments of Lake Caviahue is also suggested by total biomass values. Lake Caviahue profundal contains 2–3 times more viable biomass as typical acidic mining lakes despite equal total organic carbon content. If mining lake sediment is supplied with easily degradable carbon sources, biomass increases to the same level we found in Lake Caviahue [Wendt-Potthoff, Unpublished]. The better availability of organic substrates might also be the reason for the exclusive occurrence of methanogenesis in Lake Caviahue. Because of better light conditions the contribution to carbon fixation by benthic algae might be more important in the river compared to Lake Caviahue and acidic mining lakes.

Conclusions

The studied water system shows a gradient in colonization and microbial activity from the nearly sterile geothermal source to its still extremely acidic (pH < 2) lower part. Populations of culturable SOB and FeOB are similar to those in a volcanic acidic system in Japan and are probably ubiquitous in acidic volcanic water systems. Anoxic degradation of organic matter does not play a significant role in the upper and middle Rio Agrio, but does so in the lower river and in the lake. The similarity of oxygen fluxes and consumption between the Rio Agrio–Caviahue system and non-extreme habitats show that physical factors (stream flow, sediment texture, light availability) might have a stronger influence on biological activity parameters than does acidity. The role of benthic algae deserves more attention in future studies.

Comparison of the less geothermally influenced part with acidic mining lakes in Germany yielded substantial similarity in total bacterial abundance and culturable populations of iron and sulfur bacteria. Contrasting features of these systems are iron onflow and the different organic carbon sources which result in different availability. Not much is known about the degradation of particulate organic matter. Whereas in mining lakes degradable organic carbon limits heterotrophic processes, the availability of reducible ferric iron probably limits microbial iron reduction in Lake Caviahue. Under conditions when both iron and sulfate reduction are limited, methanogens may successfully compete for organic substrates even under highly acidic conditions.

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