Geomicrobiological investigations in subsaline maar lake sediments over the last 1500 years

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Abstract

Living microorganisms inhabit every environment of the biosphere but only in the last decades their importance governing biochemical cycles in deep sediments has been widely recognized. Most investigations have been accomplished in the marine realm whereas there is a clear paucity of comparable studies in lacustrine sediments. One of the main challenges is to define geomicrobiological proxies that can be used to identify different microbial signals in the sediments. Laguna Potrok Aike, a maar lake located in Southeastern Patagonia, has an annually not stratifying cold water column with temperatures ranging between 4 and 10 °C, and most probably an anoxic water/sediment interface. These unusual features make it a peculiar and interesting site for geomicrobiological studies. Living microbial activity within the sediments was inspected for the first time in a sedimentary core retrieved during an ICDP-sponsored drilling operation. The main goals to study this cold subsaline environment were to characterize the living microbial consortium; to detect early diagenetic signals triggered by active microbes; and to investigate plausible links between climate and microbial populations. Results from a meter long gravity core suggest that microbial activity in lacustrine sediments can be sustained deeper than previously thought due to their adaptation to both changing temperature and oxygen availability. A multi-proxy study of the same core allowed defining past water column conditions and further microbial reworking of the organic fraction within the sediments. Methane content shows a gradual increase with depth as a result of the fermentation of methylated substrates, first methanogenic pathway to take place in the shallow subsurface of freshwater and subsaline environments. Statistical analyses of DGGE microbial diversity profiles indicate four clusters for Bacteria reflecting layered communities linked to the oxidant type whereas three clusters characterize Archaea communities that can be linked to both denitrifiers and methanogens. Independent sedimentary and biological proxies suggest that organic matter production and/or preservation have been lower during the Medieval Climate Anomaly (MCA) coinciding with a low microbial colonization of the sediments. Conversely, a reversed trend with higher organic matter content and substantial microbial activity characterizes the sediments deposited during the Little Ice Age (LIA). Thus, the initial sediments deposited during distinctive time intervals under contrasting environmental conditions have to be taken into account to understand their impact on the development of microbial communities throughout the sediments and their further imprint on early diagenetic signals.

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1. Introduction

Microbial activity in both water column and most recent sediments is one of the dominant factors ruling organic matter reworking (Meyers and Ishiwatari, 1993). Carbon consumption by microbes in the sediments takes place during early diagenesis resulting in the transformation of the organic fraction through successive steps (Nealson, 1997). As the potential of metabolic reactions (i.e. ΔGibbs free energy) tends to decrease with the depletion of available oxidants and through the organic matter degradation chain (Konhauser, 2007), microbial communities respond to substrates differentiation and further adapt through depth (Boschker and Middelburg, 2002) by forming layered-type communities (Nealson and Stahl, 1997). Organic matter diminution, C/N ratio changes and even stable carbon isotopic fractionation (Lehmann et al., 2002) are often post-depositional effects. They can be attributed to the influence of sedimentary microbial
colonies via their nutrient consumption and associated mineralization and remineralization processes (Meyers, 1997; Arrigo, 2005). Denitrification and/or methanogenesis are depth-related examples of such non-steady state processes generating substrate modifications (Freudenthal et al., 2001).

The evolution of anaerobic respiration types and nutrient availability throughout depth in an aquatic system would imply a quick and gradual diminution of microbial activity in the sediments with methanogenesis as the final degradation process. Additionally, other parameters often related to initial depositional conditions have remarkable effects on the structure of microbial communities. Time intervals of variable environmental conditions can be reflected in the lacustrine sedimentary record as changes in sedimentation rate, trophic level and productivity. However, several physicochemical features related to these changes such as OM content, oxygen penetration and pH have a non-uniform preservation which can be affected by post depositional microbial activity (Meyers and Lalier-Vergès, 1999; Zeng et al., 2009). Hence, quantification and characterization of microbial communities are necessary when using organic matter proxies for paleoenvironmental reconstructions. Analogously, constraining microbial influence in lacustrine sediments requires palaeoecological studies to identify organic substances in which microbes preferentially settled. The latter is highly dependent of the different sources of OM (i.e. allochthonous or autochthonous sources such as vegetation from the catchment and/or water column production, respectively).

Initial sampling conditions are fundamental in geomicrobiological investigations in order to avoid potential sources of contamination. In this study a specially tailored field sampling methodology was first applied to recover sediments under as much sterile conditions as possible (Vuillemin et al., 2010). A multi-proxy methodological approach including grain size, water content, loss on ignition, elemental analyses and calcimetry was used to infer primary sources as well as consumption of specific fractions of the organic matter. These results were further compared to geo-microbiological datasets that included in situ ATP (adenosine 5′-triphosphate) measurements, DAPI cell counts, DGGE gel patterns analyses, and methane content obtained via the headspace technique. ATP is a molecule only produced by living organisms and is used to assess microbial activity (Bird et al., 2001). DAPI (4′,6-diamidino-2-phenylindole) is a fluorochrom that binds to DNA allowing to count microbial cells under fluorescence microscopy and further quantify them (Haglund et al., 2003). DGGE, a 16S rRNA fingerprinting technique, indicates microbial diversity and richness (Muyzer and Smalla, 1998; Schäfer and Muyzer, 2001).

The dataset presented in this contribution has been produced from a 1 m long gravity core retrieved in 2008. The comparison of these data with a previous study of the Laguna Potrok Aike climatic record covering the last 1500 years (Haberzettl et al., 2005) allowed us to establish a link between the behavior of microbial communities and climate.

2. Site location

Laguna Potrok Aike (52′S) is a maar lake located in the Pali Aike volcanic field in southern Patagonia, Argentina (Zolitschka et al., 2006). The lake is under the influence of confluent air masses making this presently perennial system a crucial site for the reconstruction of former fluctuations of the westerlies as well as the waxing and waning of the Patagonian ice caps (Hulton et al., 2002; Haberzettl et al., 2007a; Mayr et al., 2007a). It has already been the focus of many investigations dealing with its geometry and stratigraphy (Anselmetti et al., 2008; Gebhardt et al., 2011) as well as its record of changing organic sources and microfossil assemblages (Wille et al., 2007; Mayr et al., 2009; Recasens et al., in press).

Table 1

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>pH</th>
<th>Salinity (g/L)</th>
<th>NO₃</th>
<th>SO₄²⁻</th>
<th>Fe (µM)</th>
<th>Mn (µM)</th>
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<tbody>
<tr>
<td>4–12</td>
<td>8.8</td>
<td>2.31</td>
<td>46.15 (µM)</td>
<td>279.86 (µM)</td>
<td>0.29 (µM)</td>
<td>0.04 (µM)</td>
</tr>
</tbody>
</table>

A multi-proxy record based on five different cores retrieved through the PASADO project has established lake level fluctuations and provides a paleoecological reconstruction for the Late Pleistocene and the Holocene (Haberzettl et al., 2007b). Table 1 summarizes several water column parameters that have been monitored in the modern lake (Zolitschka et al., 2006; Mayr et al., 2007b) and selected results from previous investigations that according to Fenchel (1999) are relevant for this study. Our data suggest that oxygen penetration in the sediments is very limited generating dysoxic conditions. However, previous investigations based on bulk elemental ratios by Haberzettl et al. (2007b) indicated prevalent oxic to suboxic conditions at the water/sediment interface. Only a direct monitoring of this parameter will provide the necessary dataset to clarify this question.

Gravity core 5022-1J was retrieved from the center of the lake at 100 m water depth (Fig. 1) during the PASADO drilling campaign and immediately sampled in the field for geomicrobiological studies. Core PTA 02/4 was previously retrieved in a comparable field for geomicrobiological studies. Core PTA 02/4 was previously retrieved in a comparable location from the deep central basin of Laguna Potrok Aike (Fig. 1, Haberzettl et al., 2005) providing a well-dated paleoclimatological record. An excellent correlation between the latter and core 5022-1J was accomplished using cluster analyses on pollen and diatoms and DGGE profiles, respectively, allowing a direct comparison of paleoclimatic and geomicrobiological proxies.

3. Sampling and methods

3.1. Field sampling and handling

Two cm long and three cm wide windows were precut in a core liner every 5 cm and subsequently closed with strong adhesive tape prior to coring (Fig. 2). Samples were numbered S1 to S19 and regularly spaced corresponding from 5 to 95 cm sediment depth.

Fig. 1. Bathymetric map of Laguna Potrok Aike (modified after Zolitschka et al., 2006) showing the positions of the two gravity cores studied in this paper.
They facilitated a quick and aseptic sampling for geomicrobiological investigations at high resolution. After retrieving the sediment with a gravity core device, the liner was brought back to the laboratory where the windows were successively opened and sampled following the specially designed protocol (Fig. 2). Sediments were sampled using sterile end cut syringes and divided into aliquots to be conditioned for each technique. Samples for methane analyses were taken first to avoid any possible methane escape from the sediment. Spatulas were systematically cleaned with alcohol and burned to increase the grade of disinfection.

The sequence of sampling (Fig. 2, photos 1–5) was as follows: 3 ml of sediment were extracted for methane headspace analyses with a sterile end cut syringe immediately after the aperture of the window and stabilized with 10 ml NaOH (2.5%) in a glass vial crimped with a septum cap; additional 5 ml sediment were extracted with another sterile end cut syringe and after removal of the oxidized top part of the sediment, 1 ml sediment was set in an Eppendorf tube and frozen for DNA extraction; a second 1 ml volume of the sediment was fixed in 2 ml formaldehyde (3%) for DAPI cell count; an additional 1 ml sediment sample was mixed with sterile deionized water for in situ ATP assessment. Aqua-Trace water testers were submerged in the supernatant after 5 min centrifugation and measured with a Uni-Lite NG luminometer (BioTrace®). The water-testers contain a solution of luciferase that reacts with ATP allowing the Uni-Lite NG luminometer to measure the intensity of the luminescence produced during the reaction. Although non-quantitative, this device gives a relative luminescence index (RLU) that provides a good assessment of ATP content (e.g., Nakamura and Takaya, 2003; Vuillemin et al., 2010).

The syringe containing the remaining of the sample was wrapped with plastic foil and stored in a bag flushed with N₂ prior to its hermetic sealing (Fig. 2, photo 6) using a heating device (Super Cello Audion Elektro®) to keep it under anoxic conditions to eventually culture them in the laboratory. Once this sampling was finished the window was sealed with strong adhesive tape and the same procedure was applied to the next window.

### 3.2. Laboratory analyses

The vials sampled in the field were first sonicated to homogenize the sediments within the solution prior to methane headspace determinations. The gas fraction was sampled using an HP7694 chromatograph with a Headspace Sampler and separated by molecular weight in an ionizing column in order to be transported differentially with argon as the carrying gas to a coupled mass spectrometer (Agilent 6850 Series GC System). Methane peaks were detected on the chromatogram at 2 min 11 s whereas methane spikes were used to calibrate and transform detection intensities (pA) into volumes. Traces of nitrous and sulfide gases were detected in the samples, but not measured any further.

CHNS elemental analyses were run in 10 mg of previously oven-dried and powdered samples using a FlashEA 1112 Series elemental analyzer. Detection thresholds lie below 0.1% for carbon, hydrogen and nitrogen, while sulfur detection on this device is considered semi-quantitative with a detection threshold around 0.5%.

Bulk carbonate contents were measured with a CO₂ detector (AC-280 Automated Calcimeter) using 0.3–0.5 g of sediment and 10 ml HCl (3.2%). The CO₂ degassing from this reaction was automatically converted into carbonate content.

Grain size analyses using a CILAS 1180 device were run in samples previously treated with hydrogen peroxide to avoid particle flocculation with organic matter. One ml of sediment was mixed with 2 ml of H₂O₂ (30%) and 2 ml deionized water, vortexed and allowed to react for 24 h. The remaining slurries were diluted in distilled water, centrifuged, dried, powdered, sieved to 2 mm and measured.

An extraction protocol adapted from Harwood et al. (1969) and Williams et al. (1976) was followed to determine phosphorus speciation. Similar procedures have been successfully used to reconstruct productivity and nutrient cycling in recent lacustrine sediments (Loizeau et al., 2001) as well as in Mesozoic marine sediments (Mort et al., 2007). The different forms of phosphorus were separated through successive measurements of total (TP), inorganic (IP), organic (OP), non-apatitic inorganic (NAIP) and apatitic phosphorus (AP) contents. Three series of 50 mg of sediment per sample were used: The first series for TP extractions, the second for IP and OP extractions, and the third one for NAIP and AP extractions. Samples for TP were calcinated up to 550 °C, subsequently mixed with 5 ml HCl (1 N), sonicated overnight and finally centrifuged at 4000 t/min for 20 min. Samples for IP were mixed with 5 ml HCl (1 N), sonicated overnight, and centrifuged. Supernatants were removed. The remnant sediments were rinsed with 3 ml micropure H₂O and dried overnight in stove prior to OP extractions. The next day, the sediments were calcinated up to 550 °C, mixed with 5 ml HCl (1 N) overnight, sonicated and centrifuged. Samples for NAIP were mixed with 5 ml NaOH (1 N), sonicated overnight and centrifuged. Aliquots of 2.5 ml were
removed and 1 ml HCl (3.5 N) per aliquot was added to the remaining sample in order to precipitate humic acids. Aliquots were vortexed and centrifuged after settling overnight. NaOH remnants of NAIP samples were discarded and the remaining sediments rinsed with 3 ml NaCl (1 N) for AP extractions, subsequently mixed with 5 ml HCl (1 N), sonicated overnight and centrifuged. Dilutions were performed with an autosampler dilutor Gilson 401. Aliquots were diluted 50 times for TP and IP, 25 times for AP, 12.5 times for NAIP and 10 times for OP in micropure H2O for final volume of 5 ml. 200 μl ascorbic acid molybdate blue per sample were used as the colorimetric reagent (Rand et al., 1975) and absorbance was measured at 875 nm.

Aliquots were added to all PCR sets with 1 µl of reaction mixture with molecular grade water. Negative controls were added to all PCR sets with 1 µl of molecular grade water as template to provide a contamination check. Aliquots were mixed with 5 ml HCl (1 N), sonicated overnight and centrifuged. The supernatant was discarded after settling overnight. NaOH remaining sample in order to precipitate humic acids. Aliquots were finally resuspended in 1 ml 1×PBS-ethanol (1:1) for storage. For dyeing, the complete aliquots were added to a solution of 2 ml 1×PBS and 20 µl DAPI in a filtration column and incubated in the dark at ambient temperature for 7 min. Samples were filtered onto a 0.2 µm pore-size black polycarbonate filter (Millipore Ø 25 mm) backed by a supporting filter (Schleicher and Schuell BA85, Ø 25 mm, 0.45 µm pore-size), and rinsed afterward with 2 ml 1×PBS. Filters were left to dry and then were mounted on smear slides with a non-fluorescent immersion oil (Leica n23 = 1.518, ν2 = 46) and examined under epifluorescence microscope. Filters were pictured for cell counting avoiding the edges using a digital camera. Scaling was accomplished by further adding grids to the pictures which were then counted up to a minimum of 300 cells.

3.3. DNA extraction and PCR amplifications

Sediment DNA extractions and purifications were performed using the commercial DNA extraction kit Mobio PowerSoil™ Isolation kit. The methodology was applied as recommended in the manufacturer’s instructions.

First PCR amplifications were performed for Bacteria and Archaea with 3 µl DNA template, 1×PCR-buffer (Takara), 0.4 µm/L of each of the primers, 200 µm/L of each of the desoxynucleotide triphosphates, 1.25 units Ex-Taq polymerase (Takara) in a 50-µl PCR reaction mixture with molecular grade water. Negative controls were added to all PCR sets with 1 µl of molecular grade water as template to provide a contamination check.

First amplifications of the 16S rRNA genes from Bacteria were performed using the bacterial universal primer pair 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GTT ACC TAC TTT GAC TAC TT-3′). Reaction mixtures were held at 95 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 90 s plus 1 s per cycle, with a final extension step of 5 min at 72 °C (Webster et al., 2003). For Archaea genes amplifications, a nested PCR approach with overlapping forward primers was selected to avoid an enrichment step by cultures (Vissers et al., 2009). 4F (5′-TCT CTT TTA TCG TAT GAC TT-3′) with Univ1492F (5′-CCG GTA TGG TAT CTC AGC T-3′) were used as the initial primer pair (Dong et al., 2006). Archaea PCR amplifications were performed as follows: 94 °C for 5 min, 30 cycles at 94 °C for 1 min, 53 °C for 1 min, 72 °C for 2 min, and a final extension step at 72 °C for 5 min (modified after Ye et al., 2009). 2 µl of PCR product were subsequently used in the second PCR round with the overlapping forward primer 3F (5′-TCC CGG TTG ATC CTG CCG GA-3′) associated with 9R (5′-CCG GCC AAT TCC TTT AAG TTG C-3′) as the reverse primer. A final nested PCR round was then performed on both bacterial and archaeal products to fix the GC clamp (5′-CGC CGG CGC GGC GGC GGC GGG GGC GGG GCA GCG G G-3′), as necessary in a DGGE approach, and also to shorten the PCR product sequences to 150 bp, further allowing a better denaturation in the gradient gel.

Primers 537F-GC (GC clamp + 5′-CCT AGC GGA GGC AGC AGC-3′) with 518R (5′-ATT AGC GCG GCT GCT GG-3′) were used for Bacteria and A344F-GC (GC clamp + 5′-AGC GGG AGC AGC AGG CCG GA-3′) with W31 (5′-TTC CGG TCC TGC TGG CAC-3′) for Archaea. Nested PCR rounds were performed with 2 µl PCR products following the identical cycles mentioned above for Bacteria and Archaea, respectively.

3.4. DGGE analyses of bacterial and archaeal diversities

DGGE was carried out on the nested PCR products to check for bacterial and archaeal diversities (Dong et al., 2006; Zhao et al., 2008). 7.5% polyacrylamide gels were prepared with a linear gradient of the denaturants urea and formamide increasing from 30% at the top to 70% at the bottom, in which 7 μl PCR product with 7 μl blue stain were loaded for each sample. Standards, made of 5 μl PCR product from 11 bands picked from a previous DGGE gel and 5 μl blue stain, were also loaded in the middle and on each side of the gels. Electrophoreses were run at 80 V for 15 h at 60 °C in 1×TAE buffer. After electrophoresis, polyacrylamide gels were stained with 2 μl SYBRGold nucleic acid gel stain and 20 ml molecular grade water in a closed plastic bag, and slowly agitated on a rocking plate for 30 min. The gels were then viewed and photographed under UV light.

Statistical analyses were performed on gel pictures using the GelCompare® software (BioNumerics). Calculated indexes included Pearson correlation with a likelihood function, Shannon-Weaver index and range-weighted richness (Fromin et al., 2002; Marzorati et al., 2008).

4. Results

4.1. Sedimentology

Previous investigations from cores retrieved in the same area of the lake have shown quite homogeneous sediments with minor lithological changes at naked eye (Haberzettl et al., 2005). Fig. 3A represents standard parameters that are useful to first characterize different lithologies and to estimate primary productivity. Grain size results show an average composition of 20% clay, 60% silt and...
20% sand corresponding to pelagic and hemipelagic sediments, with a comparatively coarser horizon at 70 cm depth. Water content displays a mean value of 60% with a slight decreasing trend throughout depth due to clay compaction. It reaches a maximum of 72% between 30 and 35 cm sediment depth. Organic matter content estimated through loss on ignition (LOI) ranges from a minimum of 6% at 70 cm depth to a maximum of 16% at 30 cm depth with an overall mean value of 9.4%. The age model was obtained using a binomial regression curve based on four radiocarbon ages from different depths of core PTA 02/4 (Haberzettl et al., 2005), a gravity core retrieved from a site further north (see Fig. 1). The correlation between the core presented here and core PTA 02/4, retrieved at identical bathymetric positions, allows the extrapolation of its chronology. Thus this age model implies that core 5022-1J encompasses both the Medieval Climate Anomaly (MCA) and the Little Ice Age (LIA).

4.2. Bulk sediment analyses

Fig. 3B displays results for elemental analyses of the bulk sediments such as total carbon, nitrogen, hydrogen and sulfur. The profiles of the three first elements are similar to LOI with maximum values between 30 and 35 cm depth. Total carbon fluctuates between 1% and 5%, displaying three maxima at 85 cm, 50 cm and 35 cm depth, to gradually increase over the upper 20 cm from less than 1% to more than 2%. Total carbon dataset includes carbonates that are still present in the bulk sediment. The total nitrogen shows a steady content of 0.1% from 95 cm up to 40 cm depth where it increases to reach a maximum content of 0.35%. As for total carbon, it subsequently decreases after 35 cm depth to increase again gradually over the upper 20 cm of sediment. The hydrogen dataset does not differ much from total nitrogen although it fluctuates within less than 1% with a maximum value at 35 cm depth. Sulfur could not be detected in every sample, but gaps in the dataset were ignored in order to extract an overall trend. Sulfur content displays a slight increase between 75 and 60 cm depth whereas nitrogen and hydrogen remain steady. It further increases to reach a maximum of 5% at 30 cm depth followed by a decrease and finally falls below detection for the upper 20 cm of sediment.

Thus, C, N, H and S contents in bulk sediments appear to reflect the total organic content (LOI) showing their maxima between 30 and 35 cm depth. However, a more careful observation of each parameter independently indicates that they display a more autonomous behavior.

Fig. 3C displays the phosphorus speciation results. Total phosphorus (TP) and inorganic phosphorus (IP) show similar variations, OP content being the only difference between them. TP content varies from 700 up to 1000 ppm and IP content between 600 and 800 ppm. Significantly lower values are observed at 75, 70, 60 and 40 cm depth while high values are observed at 95, 80, 65 and 15 cm depth. AP and NAIP (Fig. 3C) show opposite trends since AP peaks correspond to NAIP low values at 80, 65 and 15 cm depth. Conversely, NAIP peaks correspond to AP low values at 90, 75, 35 and 10 cm depth with values ranging from 50 to 100 ppm and 600–700 ppm, respectively. NAIP values increase between 40 and 30 cm while AP appears low and steady. OP content varies from 60 to 200 ppm. Three intervals can be distinguished: low values characterize the intervals between 80 to 70 cm and 60 to 50 cm depth whereas an increasing trend is observed from 25 to 10 cm. OP and NAIP display opposite trends at the bottom of core 5022-1J. Once the AP content has been removed, OP and NAIP profiles are similar to the CNHS results. Unfortunately, the gap for OP analyses from 35 to 25 cm prevents a full comparison.

Non-apatitic inorganic phosphorus (NAIP) and organic phosphorus (OP) are the only two forms that are potentially bioavailable in the water column and sediments while apatitic phosphorus, part of the apatite mineral structure, is insoluble and generally of detrital origin (Zhou et al., 2001). Thus, AP and NAIP contents allow to discriminate between detrital and autochthonous inputs, while OP would reflect productivity if this form of phosphorus was not easily degraded. For the surficial sediments of Laguna Potrok Aike, AP represents the dominant form of phosphorus while OP and NAIP are significantly lower. Despite the gaps in the OP dataset, a comparison between TN and OP normalized to the Redfield ratio (16/1) suggests total nitrogen as the limiting nutrient (Hekely et al., 1993; Arrigo, 2005).

4.3. Products of biological activity

Fig. 3D shows results for the carbonates and methane fractions which origins can either be biological or chemical. Due to its limited presence in the catchment, the carbonate content in sediments can be the result of either water column saturation (chemical factor) or productivity (biogenic factors). Average values of 10% characterize the Laguna Potrok Aike sediments occasionally peaking up to 20% at certain depths such as at 85, 50 and 40 cm. Methane content suggests that this gas is produced within the first meter of sediment and associated to microbial activity. A decreasing linear trend of gas volume from 5% to 2.5% (based on the initial sample volume) indicates sediment anoxia and increasing methanogenesis throughout depth.

The quantification of microbial activity was achieved by in situ ATP measurements (Fig. 3D). These values range from 53 RLJ to 158 RLJ with several peaks at 70, 55, 40 and 20 cm depth. A more complete quantification of microbial population was performed by DAPI cell counts. The comparison between these counts and the in situ ATP measurements exhibits relatively similar trends except at 70 cm and 30 cm depth. This discrepancy might be related to the accumulation of dead cells that is obviously not taken in account in the ATP determination, although dead cells are often subject to high turnover rates within sediments (Haglund et al., 2003; Manini and Danovaro, 2006). Such discrepancy could thus reflect the initial colonization of the substrate by microbes to varying degrees. The age model indicates that these two horizons – 70 and 30 cm – correspond to sediments of the MCA and LIA intervals, respectively.

4.4. Microbial organization

Using bacterial universal primers, samples S3, S8 and S16 show only light bands after the PCR first round (see Fig. 2 for sample location). For Archaea, samples S1, S3, S8 and S10 did not show any positive PCR products at all, though nested PCRs were performed. The inner organization of microbial communities was then characterized by statistical analyses of DGGE gel-band patterns and further cluster analysis (Fig. 4A–C). DGGE bands, Shannon–Weaver index (Fromin et al., 2002) and range-weighted richness (Marzorati et al., 2008) are displayed on Fig. 4A. These parameters are described here from top to bottom following the natural microbial trend evolving from surficial sediments downwards. Archaea number of bands and Shannon-Weaver index show a substantial increase of diversity from 20 cm to 30 cm depth. Relatively stable values characterize the underlying sediments with minimum values at 40, 50 and 80 cm and a final maximum value at 85 cm depth. Range weighted richness points toward a low diversity and grouping of Archaea bands on a short portion of the DGGE gel. Remarkable minima at 15, 40, 50 and 80 cm depth are clear in these indexes for Bacteria.

Fig. 4B and C show the results of cluster analyses and dendrograms that have been separately performed for Archaea and
Cluster delimitation was accomplished using an empirical Pearson index value of 70 in order to have a precise characterization of the different microbial populations. The likelihood function, inducing statistical inference, added a certain stratigraphic consistency to the outcomes, as exemplified by Archaea samples without PCR products not clustered all together. As a result Archaea band patterns were separated into three clusters, respectively. Boundary depths of the clusters are located at 20/25 and 50/55 cm depth for Archaea and 20/25, 35/40 and 65/70 cm depth for Bacteria.
4.5. Microbial communities and paleoenvironment

The main goal of this contribution is to investigate the potential influence of different paleoenvironments on the distribution of microbial communities. Fig. 5 displays a comparison between contrasting climatic zones derived from paleoclimatic proxies (Haberzettl et al., 2005) and microbial units derived from cluster analyses of geomicrobiological proxies. Arrows indicate points of coincidence between cluster boundaries and climatic zones. All three arrows, placed at 20/25, 35/40 and 65/70 cm depth, underline the importance of the Medieval Climate Anomaly (MCA) and the Little Ice Age (LIA) periods marked by climatic units P-6 (dry MCA), P-5 (wet MCA) and P-3 (LIA). This correspondence between paleoclimate and geomicrobiology has been previously mentioned in Section 4.3 since it is also obvious in the DAPI cell counts.

5. Discussion

5.1. Microbial behavior in the sediments

According to Fenchel (1999), denitrification processes are the first taking place in the sediments followed by Mn and Fe reductions. Sulfate reduction normally comes after engendering reduced forms of sulfur that remain and accumulate within the sediment, often as iron sulfides. Following Nealson & Stahl’s (1997) interpretation of a common microbial stratification, the diminution of carbon and nitrogen contents in the first 25 cm of core 5022-1J that is not evident neither in organic matter nor in hydrogen contents, suggests a preferential microbial uptake of nutrients. This interpretation is supported by the sharp decrease in organic phosphorus and non-apatitic inorganic phosphorus. Previous investigations have shown that organic compounds can often diffuse from the sediments to the water column (Wang et al., 2007). Total sulfur, however, shows a different behavior as it can play the roles of electron donor and oxidant. In Laguna Potrok Aike sediments, it is first found as sulfate in the pore water as indicated by its absence in the uppermost 20 cm of these sediments. The sharp peak observed at 30 cm depth corresponds to the S that is mainly adsorbed on both organic matter and Fe. Thus, the initial organic matter content appears as responsible not only for the observed peaks of C and N, but also for S. The final slight rebound from 60 to 75 cm depth may indicate a subsequent accumulation of reduced forms of sulfur via sulfate reduction.

Regarding phosphorus, the steep decrease in OP and NAIP throughout the first 25 cm clearly indicates a preferential degradation of P organic compounds (Anderson et al., 2001), with diffusion to the water column as another potential explanation. Increased contents in organic matter tend to diminish the sorption capacity of the sediments (Wang et al., 2007) resulting in higher P release possibilities, meaning that in the specific interval between 20 and 40 cm, carbon and nitrogen would be adsorbed while bioavailable forms of phosphorus would be released and absorbed by microbes and further remineralized as NAIP. According to Wilson et al. (2010), microbes under anoxic conditions contribute to the short time-life of phosphorus forms within the sediments as P adsorbed on Fe(OH)3 tends to be released by the precipitation of iron sulfides. Increased NAIP content visible in the interval between 20 and 40 cm depth can be linked to such adsorption onto Fe(OH)3 and can thus be considered above the sulfate reduction zone. The use of Mn and Fe as oxidants followed by sulfate reduction can then enhance the fluctuations in NAIP between 40 and 80 cm depth. A measuring gap in the OP profile prevent determining if microbes are numerous enough to play a role in the OP biomass record or if they are active enough to significantly degrade OP sources. Organic phosphorus can also be consumed by microbes and further remineralized to be released and adsorbed into the sediments. In that case they will be recorded as non-apatitic inorganic phosphorus as suggested by the opposite trends of OP and NAIP in the bottom of the core. Additionally, a further release of NAIP by reducing metals might be microbially triggered.

Small peaks of in situ ATP measurements (Fig. 3C) are possibly corresponding to interfaces between different microbial metabolic layers as previously proposed by Nealson (1997) and Fenchel (1999). Transitions in microbial respiration types show higher activities due to the fact that elemental cycling is stimulated by upward diffusion of reduced components triggering the regeneration of their oxidized counterparts (Konhauser, 2007). The slight overall decrease of maxima values also argues for shifts in the respiration process linkable to each oxidant ΔG free energy (Konhauser, 2007). In Laguna Potrok Aike sediments, the organic content appears to be the prevailing factor exerting control over microbial growth rather than oxidant availability. Thus, several microbial respiration-type transitions in the sediments can be identified as follows: from denitrification to Mn reduction at 20 cm depth; from Mn reduction to Fe reduction at 40 cm depth; from Fe reduction to sulfate reduction at 55 cm depth; and finally from sulfate reduction to CO2 reduction at 70 cm depth. Analogously, remarkable minima for Bacteria on DGGE number of bands, Shannon-Weaver index and range-weighted microbial richness (Fig. 4A) can be partially correlated with transitions in metabolic types as mentioned above. In oxygenated lacustrine sediments these transitions normally occur above the first 50 cm (Nealson and Stahl, 1997). The prevailing low temperatures of both sediments and water column (4–5 °C on the sampling day) associated with an anoxic water/sediment interface can explain these comparatively deeper transitions as under such conditions, metabolic rates and degradation processes slow down considerably, especially in the
absence of oxic respiration, while the sedimentation rate is kept constant. Additionally, the precipitation of mainly oxidized forms of Mn and Fe engenders very low concentrations in Laguna Potrok Aike surface waters (see Table 1).

Previous studies have interpreted microbial clusters based on investigations using similar DGGE approaches (Banning et al., 2005; Dong et al., 2006; Ganzert et al., 2007; Ye et al., 2009) as oxidant-related types of metabolism (Barns and Nierzwicki-Bauer, 1997; Nealson and Stahl, 1997). Fig. 4C shows intervals for Bacteria clusters related to types of metabolism (Barns and Nierzwicki-Bauer, 1997; Conrad, 1999) as follows: denitrification (B1), Mn reduction (B2), Fe reduction shifting to sulfate reduction (B3) with Fe reduction weakly defined in samples S8 to S10 (see Fig. 2 for sample location) between 40 and 50 cm sediment depth (B3), and finally CO2 reduction (B4). This succession looks consistent and in turn helps to differentiate signals reflecting activity, density and diversity of microbial populations and their relationships to other proxy datasets. Concerning Archaea distribution, denitrification is expected to be limited to very surficial sediments appearing as a reasonable cause for cluster A1 delimitation. This is also suggested by the specific band positions of samples S2 and S4 not visible any more in DGGE gel patterns after these samples. The next two very similar clusters A2 and A3 encompass different methanogens as suggested by the condensation of their cDNA sequences, possibly produced by different genera. The absence of DNA sequences, any species attribution is only alleged.

Methanogenesis can be further quantified using the methane content in the sediments (Fig. 3D). It shows a linear increasing trend with depth, due to the ability of methanogens to ferment methylelated substrates, which is a non-competitive substrate compared to carbonate reduction and acetate fermentation (Barns and Nierzwicki-Bauer, 1997). This metabolism is considered to take place in lacustrine sediments as soon as oxygen is depleted and down to 2 m depth (Whiticar, 1999). As methanogens tolerate low nitrate and sulfate concentrations (Conrad, 1999), methane production takes place readily below the 70 cm CO2 reduction transition as mentioned above. This latter process would further lead to syntrophy between fermenters and H2 consumers, like methanogens. There are, however, some limiting factors, such as the low temperature of the environment that tends to slow down their metabolic processes and the competition with other CO2 reducers once methylated substrates have been depleted. A slight decrease in methane content can already be noticed below 80 cm depth. At this point, CO2 reducers are more difficult to track than the ones using other oxidants, because the reduced forms of CO2 do not accumulate in the bulk sediment or only as alcohols, organic acids or volatile fatty acids (Wüst et al., 2009). All these chemical forms are indistinguishable from the initial organic matter with standard elemental analyses. Acetogens, for example, are known to outcompete methanogens in cold habitats for carbonate reduction (Kotsyurbenko et al., 2001) and establish a more favorable syntrophy with fermenters (Schink, 2002).

Hence, acetogenesis and acetoelastic methanogenesis can be expected as typical competitive processes in cold environments alike for Laguna Potrok Aike with its deep water column and anoxic water/sediment interface (Kotsyurbenko et al., 2001; Sattley and Madigan, 2007). These processes are interesting since they can be the source of much deeper lacustrine microbial activity via acetogenesis (Sattley and Madigan, 2007), acetoelastic fermentation (Schink, 1997; Nozhevnikova et al., 2007), Archaea adaptation to cold conditions (Cavicchioli, 2006), and even acetate-oxidizing syntrophy (Conrad, 1999; Hattori, 2008). Ammonia and methane anaerobic oxidation (Strous and Jetten, 2004) are probably also taking place, but until now putative.

All the processes described above point toward a correlation between depositional environments and microbial populations without excluding potential post depositional effects on the sediments.

5.2. Paleoenvironmental settings and microbial assemblages

According to Moy et al. (2008), the MCA in southernmost Patagonia was characterized by comparatively drier conditions whereas the LIA was substantially wet. The comparatively low values of water content and organic matter (LOI) for Laguna Potrok Aike record along with coarser grain size at ~ 70 cm sediment depth (Fig. 3A) can be related to low precipitation in the catchment and low primary productivity as a result of the prevalent climatic conditions during the MCA. Conversely, higher values of water content and LOI around 30 cm depth can be linked to both higher precipitation and higher productivity during the LIA. High AP and low OP and NAIP contents in the bottom core sediments also indicate intervals of higher detrital input and lower productivity during the MCA. Opposite signals characterize the upper section of the core corresponding to the LIA. Although in both cases the observed behavior of these parameters can partially result from different degrees of OM preservation, the collective geo-microbiological evidence points toward an initial microbial-environmental relationship. In fact, minerals from the clay fraction were broadly identified for the first meter as 59% smectite, 13% chlorite, 18% illite and 10% kaolinite (Nuttin et al., 2013). Soil leaching during the LIA wetter period would have produced an increased fraction of smectite. Such mineral retains water and tends to adsorb organics (Stamatakis and Koukouzas, 2001), thus favors at the same time microbial settlement and organic matter preservation.

The uppermost part of the Potrok Aike sedimentary record was described by Kliem et al. (2013) as pelagic laminated silts with a relatively high amount of calcite crystals. Indeed, total carbonate content (Fig. 3D) exhibits three clear peaks at 85, 50 and 40 cm depth. In Laguna Potrok Aike they either reflect water column saturation (physical factors) or productivity (biogenic factors). Oehlerich et al. (2013) have shown that sedimentary calcite present in Potrok Aike sediments is mainly a pseudomorph after ikaite, a mineral that is still being precipitated inorganically today due to the specific cold and subsaline conditions of the water column. Gastropods shells acting as a potential source of organic carbonate were found in the deep basin in association to redepositional events (Kliem et al., 2013), and can be excluded from the sedimentary record in the upper 7 m. Haberzettl et al. (2005) interpreted the TIC content as reflecting the changing water balance of the lake as a result of lake level fluctuations. Thus, these three peaks may indicate lower lake levels prior to the LIA and their eventual influence on photosynthetic organisms due to lower cloudiness during the MCA interval. Regarding the microbial influence, cyanobacteria could play a significant control triggering carbonate precipitation, but cannot really be considered as early diagenetic agents within the sediment since their activity ceases beyond the photic zone. Moreover, cyanobacteria have little chance of being preserved while settling throughout the oxygenized water column, and were thus not taken into account any further in this study.

A fairly good correlation can be observed between DAPI cell counts and the MCA and LIA since both climatic intervals display different organic matter productivity and preservation rates. OP and NAIP release, as previously defined (see Subsection 5.1) are possible triggers of microbial development in the LIA organic rich layer. A comparison between paleoclimatic and geomicrobiological proxies (Fig. 5) shows that Bacteria diversity is sensitive to...
paleoenvironmental conditions while Archaea diversity appears to remain fairly independent.

The present comparison indicates that geomicrobiological proxies reflect two trends that are on one hand linked to the oxidant-type layered microbial communities, and on the other hand to the diversity of initial substrates. The latter is resulting from the variability of paleoenvironmental and synsedimentary conditions triggering the development of different microbial populations. Nelson et al. (2007) have demonstrated that the relationship between bacterial diversity and paleoenvironmental settings was mainly a function of sedimentation rate and surrounding vegetation evolution as they likewise reflect both nutrient contents and organic matter refractoriness. These two attributes tend to evolve with sediment depth. Analogously, paleotemperature and paleosalinity are also key factors ruling microbial productivity in the water column and once the sediments are deposited through the activity of endogenic microbial communities via sinking and accumulation of organic matter (Hollander and Smith, 2001).

Dong et al. (2010) has shown that wet and warm climates correlate well with comparatively higher bacteria abundance and diversity, whereas cold and dry climates resulted in lower abundance and diversity. Results of DAPI cell counts and the comparison of Bacteria and Archaea clusters with paleoenvironmental reconstructions for Laguna Potrok Aike confirm this assumption for both MCA and LIA sediments as previously defined for Patagonia by Moy et al. (2008) and Piovano et al. (2009). Thus, DGGE gel-pattern-clusters reflect not only an initial microbial link between microbes and contrasting environmental settings but also their inner organization and adjustment to post depositional substrate evolution and nutrient depletion. The relative dominance of each of these aspects has to be taken into account in order to decide which have conditioned microbial assemblages throughout time and which are dominating or superimposing the final signal.

5.3. Early diagenetic signals

Depending on the authors, the time span of early diagenesis is often considered to cover 10 ka, consequently a 1.5 ka sedimentary record remains very limited to study the microbial influence on organic signals. Previous studies by Mayr et al. (2009) have inferred that the upper 3 m of Laguna Potrok Aike sediments were not affected by any diagenetic alteration, and hence that C/N ratios and stable isotopes were still reflecting the initial sedimentary sources. Results of our geomicrobiological study have shown that although fermentation processes and methanogenesis are already taking place in surficial sediments, it seems that such processes did not affect the bulk sediment signal down to 3 m depth. The latter raises the question of how deep microbial communities can be active and which metabolisms are capable of modulating organic refractory fractions, thus superimposing their secondary production on the bulk initial signatures (Freudenthal et al., 2001; Lehmann et al., 2002). Previous studies on specific fractions, such as gases (Whiticar et al., 1986), dissolved short organic molecules (Heuer et al., 2006 and, 2010), and pore water chemistry (Emerson, 1976; Berner, 1981) have clearly shown the capacities of microbes to degrade and fractionate organic elements as well as to balance the chemistry of interstitial waters triggering authigenic precipitates. Syntrophic co-culture experiments (Tabassum and Rajoka, 2000) have also shown anaerobic conversion of substrates to be highly effective on the organic matter refractory fraction. Previous environmental studies (Rothfuss et al., 1997; Miskin et al., 1998) have demonstrated that Bacteria and Archaea remain active during long time in subsaline sediments. Thus, microbial activity might be sustained deeper in the Laguna Potrok Aike sediment column if 10 ka is considered as the minimum time span for early diagenesis.

6. Conclusions

The geomicrobiological investigations at Laguna Potrok Aike presented here provide a first dataset to start fulfilling the existing lack of this kind of studies in lacustrine sediments. These results show a correlation between paleoenvironmental (depositional) conditions and the presence of living microbial communities. While microbial activity and diversity are significantly lower during dry conditions, they both are higher during comparatively wetter conditions. DAPI cell counts emerge among the geomicrobiological proxies defined here. It strongly reacts to the contrasting paleoenvironmental conditions that characterize both MCA and LIA showing a comparatively lower and much higher microbial population during these intervals, respectively. Clusters defined through the statistical analysis of DGGE gel patterns match well with these two paleoclimatic intervals, showing that microbial organization is also reflecting initial environmental settings. Hence, there is a clear dependence between predominant environmental conditions of deposition — such as water column salinity and temperature, and organic sources — as well as microbial settlement and their further development and final organization.

Microbes have to also handle the evolution of their original substrate even if the sorption of nutrients seems to be affected by their influence on the sediment eH. The geomicrobiological proxies used in the present case study could not completely enlighten the impact of microbes over their habitat. Although microbes can quickly dominate eutrophicated and saturated environments and superimpose to the deposited sediments an early diagenetic signal, it appears that this is not the case in the most recent sediments of subsaline Laguna Potrok Aike where the identified microbial metabolisms do not mask the initial signals. Depending on the conditions, a microbial prolonged food web could partially alter this initial signal through a stepwise degradation. Further geo-microbiological proxies on specific fractions would be then required. However, the present cold temperature and most probably anoxic water/sediment interface of Laguna Potrok Aike suggest that methanogenesis takes place at a very slow rate and is likely to be outcompeted by acetogenesis when CO2 becomes the main available oxidant. Subsequent acetoclastic fermentation would act as a source of highly fractionated methane. Thus, different microbial growth rates with various syntrophic assemblages are conceivable in deeper sediments of Laguna Potrok Aike through a prolonged nutrient cycle. Conversely, it appears that paleoenvironmental settings remain the dominant signal in the first sediment meter of this subsaline lake as recorded in some of the geomicrobiological proxies presented here. The latter is critical in order to develop a set of indicators that could be further applied to this and other lacustrine records at various temporal and geographical scales.

Finally, combining paleoenvironmental and geomicrobiological proxies can help to elucidate the microbial influence on other sedimentary, geochemical and biological proxies and to disentangle their imprint on the sediments.

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Moy, C.M., Dunbar, R.B., Moreno, P.I., Francois, J.-P., Villa-Martinez, R.,


