

# Paleoenvironmental conditions define current sustainability of microbial populations in Laguna Potrok Aike sediments, Argentina

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**Abstract** Rainfall and geology of the catchment exert a dominant control on the trophic state of endorheic basins. River inflows and runoff provide nutrients, influencing primary productivity in the water column. Through time, paleoenvironmental conditions are recorded as variations within the sedimentary organic fraction. Thereafter, microbial populations settle and develop within sediments and lead to degradation processes as long as they remain active. However, their presence is generally not considered in Quaternary studies. The present study is based on the sedimentary record of the maar lake of Laguna Potrok Aike, southern Patagonia. We investigate the relationship between paleoenvironmental conditions and colonization of the corresponding sediments by microbes.

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Microbiological and geochemical analyses were combined to determine factors allowing microbes to sustain their activity over time. The study of Holocene sediments, containing dense and active microbial communities, provided means to evaluate the potential of microbial communities as agents of early diagenesis. We show that phosphorus released during organic matter degradation is essential for microbial growth. In highly colonized sediments, microbial communities appear capable of recycling the excreted ammonium, thus accounting for nitrogen fractionation toward high values in bulk sediment. Microbial activity in Laguna Potrok Aike still persists in 30 ka old sediments. Thus, we proposed that future lacustrine studies should include some microbial indicators to assess their impact in diagenetic processes.

**Keywords** Nutrients · Phosphorus · Microbial activity · Paleoclimate · Trophic state · Early diagenesis · PASADO

## Introduction

Nitrogen (N) is often considered the limiting factor in the marine realm (Doering et al. 1995; Hecky et al. 1993), whereas phosphorus (P) is frequently the limiting nutrient of primary production in modern freshwater environments. In lacustrine closed-basins, the behavior of nutrients is highly complex due to the different internal conditions that modulate their concentrations (Redfield 1958; Smith 1990). For example, external inputs by rivers and runoff represent sporadic loadings of N and P to the basin (Howarth et al. 1988; Vitousek and Howarth 1991). Planktonic assimilation of nutrients depends on turbidity and light penetration (Havens et al. 2003). Long lake water residence time can

lead to nutrient exhaustion within the water column (Howarth and Marino 2006) and water column turbulence prevents quick sinking of particulate and dissolved organic matter (OM), thus enhancing their recycling. Under oxic conditions, N can be recycled from sinking particles, whereas dissolved P precipitates an inorganic phase (i.e. authigenic) by adsorption onto metal oxides (Loizeau et al. 2001). Conversely, anoxic conditions decrease the dissolved inorganic N pool via denitrification while maintaining P in solution. Thereafter in sediments, nutrient sorption still depends on oxygen penetration (Gächter and Müller 2003), OM content (Wang et al. 2007), presence of metal oxides (Hupfer et al. 1998; Kopacek et al. 2005) and microbial activity (Gächter et al. 1988).

Hence, environmental conditions result in varying quantity, type and preservation of nutrients buried as sedimentary organic matter (SOM; Balzer 1984). Such SOM characteristics are considered as physical indices of the past (i.e. proxies) and are frequently used as paleoenvironmental indicators (e.g. Meyers and Lallier-Vergès 1999), assuming that the OM initial features are preserved during microbial diagenesis (Meyers 1997; Meyers and Teranes 2001). However, studies of the water column (Chen et al. 2008) as well as of sediments (Freudenthal et al. 2001; Lehmann et al. 2002) have shown that the microbial use of nutrients results in modifications of the bulk OM original signal. The bulk organic carbon ( $C_{\text{org}}$ ) content,  $C_{\text{org}}/N$  ratio and stable isotopic signatures are generally preserved in the refractory OM that is mainly left aside by microbes (Macko et al. 1993; Giani et al. 2010). However, partial degradation of refractory OM can provide substantial amount of P to microbes in sediments (Wilson et al. 2010) and help them sustain activity during diagenesis (Amon and Benner 1996).

Up to now, geomicrobiological studies in the lacustrine realm have mainly focused on the water column (Humayoun et al. 2003) and very shallow sediments (Zhao et al. 2007). Thus, there is a need to document microbes living in recent and old lake sediments, determine the lacustrine conditions promoting microbial colonization of the sediment and define any related diagenetic influence. Therefore, we traced microbial activity along the complete sedimentary column of Laguna Potrok Aike, an endorheic basin of southern Patagonia, Argentina. Our study combined geochemical and microbiological indices to investigate sediment characteristics resulting from paleoenvironmental conditions that led to microbial colonization and sustainable growth. The inspection of microbial communities in sediments from the Holocene and Last Glacial periods helped disentangle SOM characteristics resulting from paleoenvironmental conditions in the study site and those related to microbial growth. Such investigations are essential to a better interpretation of the bulk SOM signals in terms of climate and diagenesis.

## Site description

The case study presented here is based on the sedimentary record of the maar lake Laguna Potrok Aike, which is located in southern Patagonia, Argentina (Zolitschka et al. 2006). This endorheic basin represents a crucial site to reconstruct the position of the Westerlies, which are the major climatic driving force within the area (Mayr et al. 2007). The basin geometry, OM and microfossil records of Laguna Potrok Aike have been addressed extensively in previous publications (Mayr et al. 2009; Gebhardt et al. 2012; Recasens et al. 2012). Although its water column has a maximum depth of 100 m, the lake is polymictic due to the persistent overturn caused by the Westerlies (average wind speed: 7.4 m/s). Dissolved oxygen normally shifts from oxic to suboxic conditions at the water/sediment interface (Zolitschka et al. 2006) and oxygen penetration within surface sediment is restricted (Vuillemin et al. 2013). Presently, subsaline conditions along with very low  $\text{NO}_3^-$  contents restrict primary productivity within the water column. Due to the semi-arid climatic conditions (site annual precipitation 200–300 mm), limited and sporadic terrestrial inflows occur, further reducing nutrient input to the Laguna Potrok Aike basin.

## Methods

### Field sampling and treatment

The investigations presented here were carried out in two hydraulic piston cores 5022-1A and 5022-1D of 65 mm diameter, with respective lengths of 87 and 97 m (Ohlen-dorf et al. 2011). They were retrieved from the centre of the maar at 100 m water depth (Online Resource 1) in autumn 2008 during the PASADO (Potrok Aike Maar Lake Sediment Archives Drilling Project) campaign.

Core 5022-1D was sampled in the field for geomicrobiological analysis (Vuillemin et al. 2010). After retrieval, the core sections were shuttled every 2 h from the platform back to the field laboratory where a detailed protocol was applied to minimize contamination and optimize sample conditioning (Vuillemin et al. 2013). Prior to opening, the liners were disinfected with alcohol and sampling windows of  $2 \times 3$  cm were cut at regular intervals using a portable circular saw. Once opened, they facilitated a quick and aseptic sampling. Spatulas were systematically cleaned with alcohol and burned for disinfection prior to retrieving new material from each sample window. A total of 60 windows were sampled throughout the core 5022-1D for an overall sediment depth of 93 m. Autoclaved syringes, whose ends were cut, were used to sample the sediment by avoiding obvious macro remains. Thereby, 5 ml sediment

were extracted and the oxidized capping removed before being distributed into separate aliquots for each technique. Details of the process are as follows: (1) 1 ml sediment was transferred to an Eppendorf tube and immediately frozen at  $-10\text{ }^{\circ}\text{C}$  for DNA extraction, (2) a second 1 ml volume of sediment was fixed in 2 ml of formaldehyde (3 %) for 4'-diamidino-2-phenylindole (DAPI) cell count, and (3) an additional 1 ml sediment sample was mixed with 1 ml micropure  $\text{H}_2\text{O}$  and centrifuged for 5 min for immediate adenosine-5'-tri-phosphate (ATP) assessment. Once this sampling was finished, the windows were sealed with strong adhesive tape.

Core 5022-1A was sealed and stored at  $4\text{ }^{\circ}\text{C}$  and sampled for pore water analyses in July 2009. Small holes were drilled in the core liners at regular intervals to retrieve pore waters introducing the Rhizon<sup>®</sup> soil moisture samplers (Eijkelkamp<sup>®</sup>) into the sediment. Up to 12 ml of pore water was retrieved by suction with syringes screwed to the samplers and maintained under vacuum. To avoid any shifts in water chemistry, the recovered samples were immediately flushed with helium gas after sampling. The transfer of pore water samples into sealed vials was performed under a protective atmosphere using a small chamber filled with  $\text{N}_2$  gas.

#### Laboratory analyses

The same methods as in Vuillemin et al. (2013) were used for grain size analysis, chemical separation of different P forms (i.e. P speciation), DAPI cell counts, DNA extractions, archaeal/bacterial DNA polymerization, and denaturing gradient gel electrophoresis (DGGE).

Pore water samples were split for cation and anion analysis and samples for cation analyses were acidified with  $100\text{ }\mu\text{l}$   $\text{HNO}_3$  (65 %). Cation concentrations in pore water (i.e.  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Si}^{4+}$ ,  $\text{Fe}^{2+}$ ) were determined by inductively coupled plasma mass spectrometry (ICP-MS), and anions (i.e.  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-/\text{NO}_3^-$ ) by ion-chromatography.

Sediment samples for carbon isotopic compositions were treated for decalcification with  $\text{HCl}$  (5 %) at  $50\text{ }^{\circ}\text{C}$ , sonicated overnight, repeatedly rinsed with deionized water, centrifuged to discard water and freeze-dried. Stable isotope compositions of organic carbon ( $\delta^{13}\text{C}_{\text{org}}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) of the bulk OM were analyzed from homogenized decalcified ( $\delta^{13}\text{C}_{\text{org}}$ ) or untreated ( $\delta^{15}\text{N}$ ) samples, using an elemental analyzer (EuroVector<sup>®</sup>, Euro EA<sup>®</sup>) linked to a continuous flow isotope-ratio mass spectrometer (Micromass, Isoprime<sup>®</sup>). Isotope ratios are reported in  $\delta$ -notation in per mil according to the following equation:  $\delta = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1,000$ , where R is the measured ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  of the sample and of an international standard (V-PDB and AIR,

respectively). Analytical precision was better than 0.10 % ( $1\sigma$ ). Total organic carbon (TOC) and total nitrogen (TN) were calculated from the yield of  $\text{CO}_2$  and  $\text{N}_2$  after sample combustion in the elemental analyzer. Analytical precision was  $\pm 3\%$  ( $1\sigma$ ) for  $\text{C}_{\text{org}}$  and  $\pm 2\%$  ( $1\sigma$ ) for N. TOC was recalculated to the content of the whole sample and results are presented in dry mass %. TOC and TN values were further used to calculate molar  $\text{C}_{\text{org}}/\text{N}$  ratios.

ATP detections were obtained on the field with Aqua-Trace water testers submerged in the supernatant and measured with a Uni-Lite NG luminometer (BioTrace<sup>®</sup>). Although non-quantitative, this device provides a value for relative luminescence units (RLU) corresponding to the light produced during the reaction of ATP with luciferase (Vuillemin et al. 2010). It provides a good assessment of ATP content (Nakamura and Takaya 2003). It is presently used as an index of microbial activity within sediments. Background values measured on micropure  $\text{H}_2\text{O}$  ranged between 25 and 30 RLU. Thus, a value of 30 was systematically subtracted from the readings for background correction.

DGGE gel pictures were aligned using the GelCompare<sup>R</sup> software (BioNumerics) and the positions and number of DGGE bands were extracted for each sample. The index of range-weighted richness ( $R_r$ ), which is used to describe the total diversity in relation to the viability of the environmental settings (Fromin et al. 2002; Marzorati et al. 2008), was calculated as follows:  $R_r = (\text{N}^2 \times \text{D}_g)$ , where N represents the total number of bands in the pattern,  $\text{D}_g$  the denaturing gradient comprised between the first and the last band of the pattern. Gel pictures and respective sample depths are shown in the Online Resource 2. A tree clustering approach, based on the number of bands and their respective positions on the gels, was applied using Euclidean distances with the unweighted pair-group average algorithm. The resulting tree is currently used to infer ecological distances between microbial populations from different depths (Marzorati et al. 2008).

Two 3D scatterplots were established. The first one displays DAPI as the x-axis, total number of DGGE bands as the y-axis and ATP as the z-axis in order to clarify relationships between microbial indicators. The second one displays TOC as the x-axis, OP as the y-axis and DAPI as the z-axis to define the influence of nutrient concentrations on microbial populations. A surface plot was also established with these three indices. A multivariate statistical approach based on a principal component analysis (PCA) was then applied to the multiproxy dataset to discern relationships between geochemical and microbiological variables and pinpoint any influence of microbes on the bulk OM signal (Fromin et al. 2002). The PCA included the standardized variables clay content, TOC, TN, organic phosphorus (OP), non-apatitic inorganic phosphorus

(NAIP),  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , ATP measurements, DAPI cell counts and total number of DGGE bands. All analyses were performed using STATISTICA® (data analysis software system) version 7.0 (StatSoft, Inc.).

Correlation between cores 5022-1A and 5022-1D was achieved by using magnetic susceptibility profiles and supported by visual characteristics on the split cores (Recasens et al. 2012). The present age model (Fig. 1c) was attained combining tephrochronology, radiocarbon dating and geomagnetic relative paleointensity (Kliem et al. 2013).

## Results

### Grain size and pore water chemistry

Specific horizons along the record reflecting different sedimentary regimes and associated lake level variations were defined by the relative distribution of clay and silt versus sand (Fig. 1a). A silt/clay-dominated horizon occurs between 80 and 70 m, a clay-dominated layer at 37 m, while many sand layers punctuate the deep Glacial interval (ca. 82, 70, 60, 50 and 40 m depth). During the Last Glacial Maximum (LGM), the sediment varies from silt- to sand-dominated textures which possibly represent redeposition events from the maar flanks associated with lake level fluctuations (Kliem et al. 2013). At the Late Glacial transition, sedimentation shifts to a pelagic regime as denoted by the dominance of silt and clay. Some sandy event layers occur along the Holocene record that can be associated with lake-level oscillations (Anselmetti et al. 2009).

Pore water analyses indicate that both salinity and alkalinity of the water column have experienced substantial shifts through time (Fig. 1a). Chloride displays a mean value of 80 ppm along the deep Glacial record (from ca. 90–30 m depth), with the exception of a peak (300 ppm) at 74 m depth coinciding with finer grain size (Fig. 1a). During the Glacial to Holocene transition (ca. 25–12 ka BP), the lake shifts toward subsaline conditions as shown by the chloride concentrations increasing from 120 to 780 ppm. Salinity decreases then slightly to 600 ppm within Holocene sediments. Nitrite/nitrate exhibits values lower than 1 ppm without any relevant maxima or minima (Fig. 1a). Phosphate concentrations are mostly zero in the lower part of the record showing only minor increases at 85, 55 and 45 m, whereas the concentration ranges between 6 and 10 ppm along Holocene sediments (Fig. 1a). Sulfate displays three sharp peaks (ca. 1,500, 1,200 and 900 ppm) around 50, 40, and 28 m depth corresponding with sandy layers (Fig. 1a). Concentrations for the rest of the sediment column average 300 ppm with

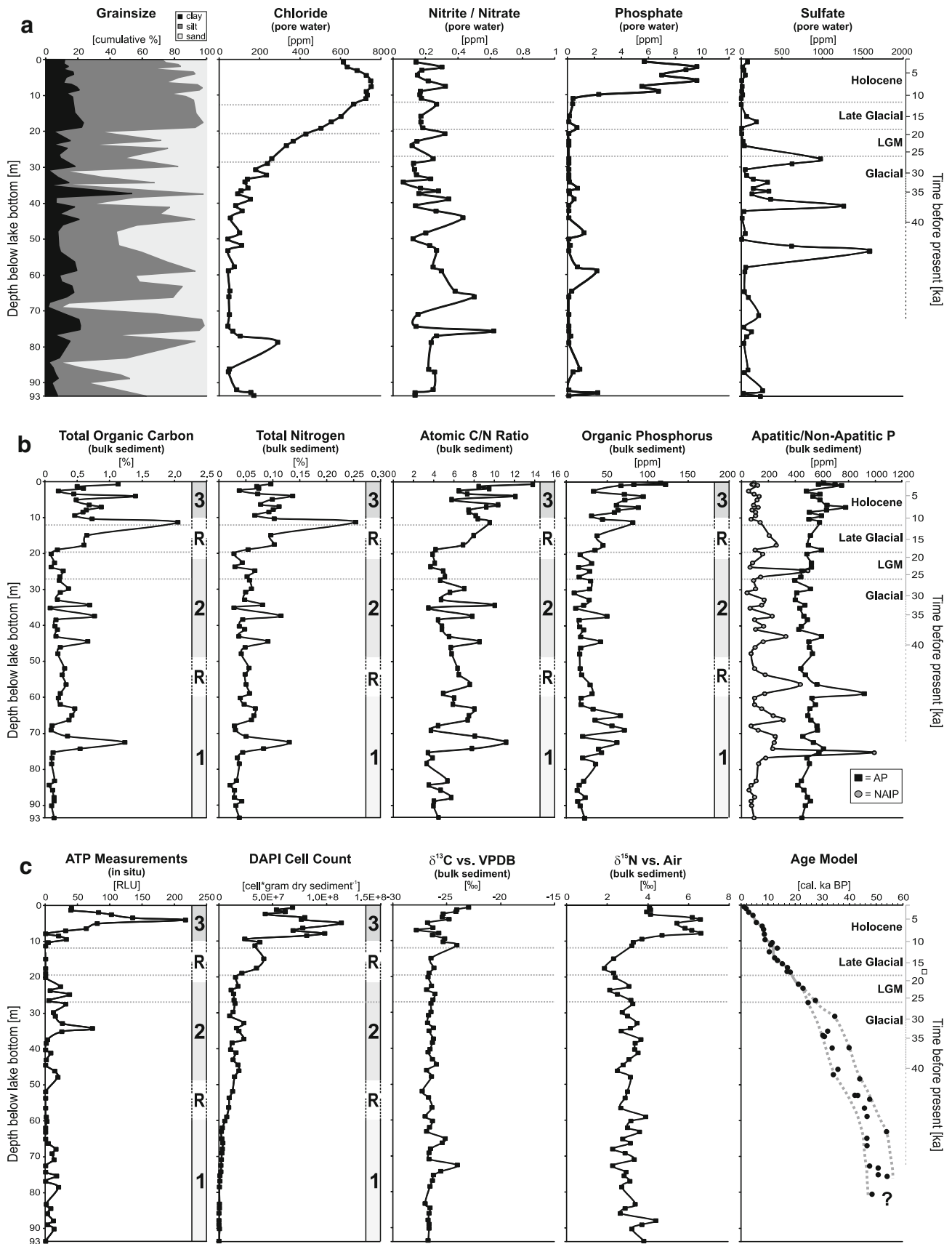
**Fig. 1** Geochemical and microbiological proxies of core 5022-1D along with pore water chemistry from core 5022-1A. **a** From left to right: Grain size; chloride; nitrite and nitrate; phosphate; sulfate. **b** From left to right: Total organic carbon; total nitrogen; molar  $C_{\text{org}}/N$  ratio; apatitic phosphorus (AP) and non-apatitic inorganic phosphorus (NAIP); organic phosphorus (OP) [ppm]. **c** From left to right: In situ ATP measurements in RLU; DAPI cell counts;  $\delta^{13}\text{C}_{\text{org}}$  of bulk sediment;  $\delta^{15}\text{N}$  of bulk sediment; the age model for Laguna Potrok Aike sedimentary column (after Kliem et al. 2013). Dashed lines indicate successively transition depths to intervals corresponding to the Last Glacial Maximum (LGM), the Late Glacial and the Holocene. Shaded intervals numbered from 1 to 3 refer to microbial groups defined in Fig. 3a. The letters R (i.e. regression) indicate intervals related to lake level drops

particularly low values from the Late Glacial to the uppermost sediments. For comparison, lake surface water samples measured from 2002 to 2004 display mean values of 632 ppm for chloride, 1.6 ppm for nitrate, 2,384 ppm for total phosphorus (TP) and 26.9 ppm for sulfate (Zolitschka et al. 2006), thus corresponding to N-limiting subsaline conditions.

### C and N contents, P speciation and stable isotopes

The average TOC content is very low in deep Glacial sediments, often with values below 0.3 % (dry mass). However, TOC displays two main peaks at 74 and 37 m, whereas the highest TOC content (2 %) is observed around 10 m depth and corresponds to the Late Glacial/Holocene transition (Fig. 1b). Thereafter, three other peaks occur along the Holocene record. TOC peaks can all be associated with horizons of finer grain sizes. TN behaves similarly to TOC but with lower average values reaching a maximum of 0.25 % at the Late Glacial/Holocene transition (Fig. 1b). The molar  $C_{\text{org}}/N$  ratio displays very low values along the Glacial record, with the exception of two values over 10 at 74 and 37 m depth (Fig. 1b). The molar  $C_{\text{org}}/N$  ratio rises from 4 to 10 during the Late Glacial period, and fluctuates between 6 and 12 within Holocene sediments, reaching its maximum value in the uppermost part of the record.

P speciation is the separation of P into its different chemical forms. Total phosphorus (TP) is equal to the sum of inorganic (IP) and organic (OP) phosphorus, while IP is equal to the sum of apatitic phosphorus (AP) and non-apatitic inorganic phosphorus (NAIP; Loizeau et al. 2001). AP is actually the dominant form of P in Laguna Potrok Aike sediments with an average concentration of 500–600 ppm along the sediment column (Fig. 1b). AP increases can be associated with coarse grain sizes with maximal values located around 60, 7 and 1 m depth. NAIP shows frequent peaks along the Glacial record, often with synchronous AP increases (ca. 75, 66, 56, 43 m depth), but these two profiles differ in Late Glacial and Holocene sediments. OP displays a similar trend to TOC and TN





(Fig. 1b) with peaks at 74 and 37 m depth in the deep Glacial record, values increasing along the Late Glacial transition and fluctuations in the Holocene record. Its maximum value is located in uppermost sediments. Interestingly, OP content shows an opposite tendency to NAIP from the Late Glacial transition to the top of the sedimentary record.

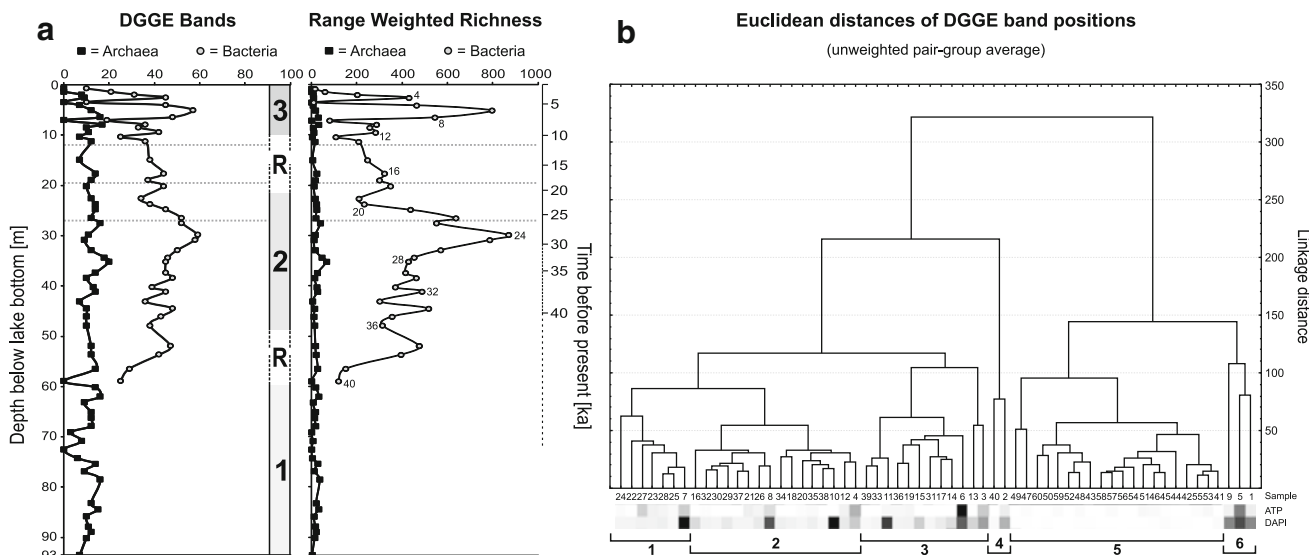
The  $\delta^{13}\text{C}_{\text{org}}$  values in Glacial sediments average about  $-26.5\text{‰}$  with only minor excursions at 74 and 68 m depth (Fig. 1c). Changes in  $\delta^{13}\text{C}_{\text{org}}$  occur at the Late Glacial/Holocene transition and are limited to the Holocene interval where their gradual decrease (from  $-24.1$  to  $-27.8\text{‰}$  at 11–7 m depth) is followed by a gradual increase, reaching the maximal value ( $-23.0\text{‰}$ ) in the uppermost part of the record. The  $\delta^{15}\text{N}$  values average  $3.0\text{‰}$ , showing minor variations below the transition to the Late Glacial (Fig. 1c). At the Holocene transition,  $\delta^{15}\text{N}$  values increase to maxima of  $6.6\text{‰}$  at 8 and 4 m depth, where they decrease sharply to  $4\text{‰}$  and remain constant up to the surface. Along the Holocene record,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}_{\text{org}}$  compositions display opposite trends.

#### Microbial characteristics and PCA

In situ ATP measurements (Fig. 1c) show small peaks of activity in specific layers of deep Glacial sediments (ca. 49, 34–29 m depth) with some residual activity at the base of the record (ca. 80, 70 m depth). Along the Holocene record, ATP values display a rapid increase (from 8 to 4 m depth) with the peak of microbial activity (220 RLU) located at 4 m depth. ATP values decrease then gradually to 40 RLU in the uppermost sediments.

DAPI cell counts (Fig. 1c) indicate that there are practically no microbes in the lowermost part of the deepest Glacial record. From 60 to 40 m depth, cell counts increase slightly reflecting a microbial population of low abundance. A small peak can be noticed at 34 m. Cell counts then increase gradually along the LGM and Late Glacial intervals (20–10 m depth). Values increase sharply in Holocene sediments where they fluctuate according with the grain sizes. However, DAPI staining does not allow the distinction between active, inert or dead cells that possibly accumulated in the sediment. For this, DAPI and ATP profiles can be compared in order to assess the living biomass associated with the presence of microbes (Bird et al. 2001; Nakamura and Takaya 2003). These two indices provide evidence of active microbes within two sedimentary intervals. The first one corresponds to deep Glacial and LGM sediments (ca. from 50 to 22 m depth), and the second to the uppermost 10 m of Holocene sediments. These Glacial and Holocene intervals display respectively low and high microbial population activities and densities. Microbial populations within the Late Glacial interval can be considered inactive, if not dead.

DGGE gel features can be used as indicators of relative microbial diversity, at least in terms of taxonomic units, with the restriction that some bands may be derived from extracellular DNA preserved within fine anoxic sediments, especially resistant archaeal sequences. Archaea display a reduced but constant number of DGGE bands along the complete record, while Bacteria display clear maximal values around 30 and 5 m depth (Fig. 2a). The number of DGGE bands emphasizes anyway the dominance of Bacteria over Archaea, although PCR products could not be



**Fig. 2** Statistical indices obtained from DGGE gel features. **a** Number of DGGE bands for Archaea (black squares) and Bacteria (grey dots); range-weighted richness. **b** Euclidean distance clustering tree

for DGGE band positions. ATP and DAPI values are indicated in grayscale at the base of the tree. Shaded intervals numbered from 1 to 3 refer to microbial groups defined in Fig. 3a

obtained for Bacteria below 60 m depth even after a second run of PCR. Moreover, maximal values for Bacteria correspond with the two intervals where microbial populations appear as active.

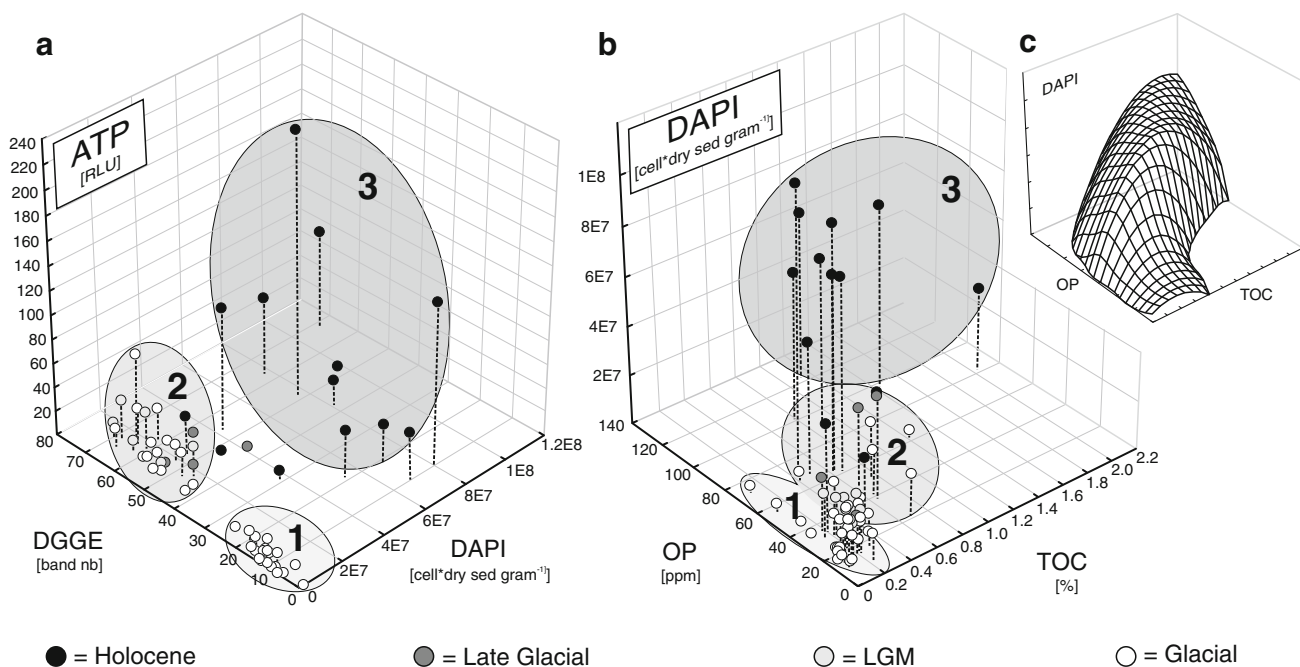
This pattern becomes even clearer on the *Rr* index. The percentage of denaturing gradient used to describe the total number of bands (see EA-2) per sample can be reported to characteristics of the hosting environment. The *Rr* index currently serves as an indicator of habitable and adverse conditions (Marzorati et al. 2008), possibly resulting from variable paleoclimatic conditions and sedimentary regimes. The *Rr* profile for Bacteria (Fig. 2a) displays two maxima located around 5 and 30 m depth. They indicate favorable conditions for microbial colonization in sediments relating to the lake paleoenvironment 30 and 5 ka ago.

The Euclidian distance tree based on DGGE band positions of each sample gel (Fig. 2b; EA-2) is presently used to infer ecological distances between populations (Marzorati et al. 2008). It allows the distinction of six different clusters. The broadest cluster actually corresponds to the lowermost part of the record where activity, density and diversity of the populations demonstrate completely inert cells. The other clusters regroup samples from different depths with variable microbial activity and density. Changes in microbial organization can be addressed in terms of varying sedimentary and geochemical conditions (e.g. grain size, OM content, pore water chemistry).

However, species identification is required to fully understand the present tree in term of microbial ecology.

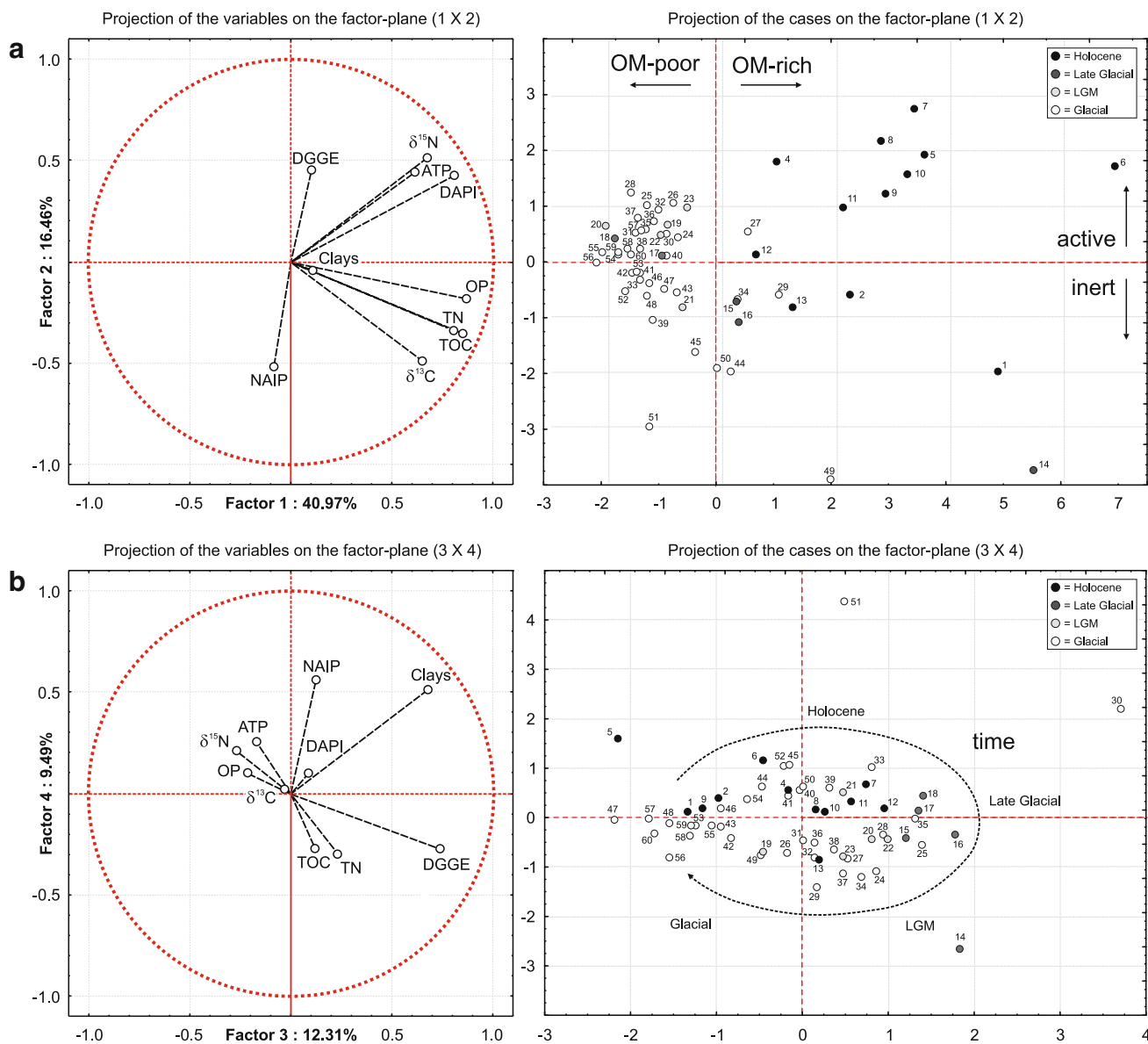
The first 3D scatterplot is based on microbial characteristics (i.e. DAPI, DGGE and ATP) and regroups samples in three distinct clusters (Fig. 3a). The first one corresponds to deep Glacial sediments, the second to LGM and Late Glacial sediments and the third one to Holocene sediments. Overall, these three groups seem to correspond to microbial populations being inert, surviving and active. The second 3D scatterplot displays TOC and OP against DAPI (Fig. 3b), and indicates a similar repartition of samples. The corresponding surface plot (Fig. 3c) shows that three variables vary jointly. This result indicates a direct relationship between organic content and the amount of microbial biomass present in the sediment. It can also imply a decrease in nutrient concentrations with depth.

Two projection plots based on four principal components (PC) with eigenvalues of 40.97, 16.46, 12.31 and 9.49 %, were established (Fig. 4a, b). A correlation between ATP detection and DAPI cell counts is obvious on the first PCA plot (Fig. 4a), while the number of DGGE bands appears inversely correlated with NAIP. Regarding SOM signatures,  $\delta^{13}\text{C}_{\text{org}}$  correlates with TOC, TN and OP, while  $\delta^{15}\text{N}$  is plotted next to ATP and DAPI indices. Thus, the first PC can be interpreted in terms of organic content and the second PC in terms of microbial activity. The



**Fig. 3** 3D scatterplots displaying microbial characteristics altogether and microbial density in function of organic content. **a** Scatterplot for DAPI, DGGE and ATP used to indicate the relationship between microbial activity, density and diversity. **b** Scatterplot for TOC, OP and DAPI highlighting the direct relationship between organic

content and microbial biomass within sediment. **c** Surface plot for TOC, OP and DAPI, as inset, showing a linear trend between the three variables. Groups 1 to 3 (grey circles) indicate inert, surviving and active microbial populations. These assemblages appear depth-dependent on Figs. 1 and 2



**Fig. 4** Principal Components Analysis (PCA) projection plots of variables (*left*) and cases (*right*). **a** Factors 1 and 2. **b** Factors 3 and 4

projection plot of cases (Fig. 4a) separates Holocene and Glacial samples from each others. Unexpected results concern microbially inactive organic-rich sediments, which are mainly linked to the Late Glacial period, and microbially active but organic-poor sediments, mainly related to the LGM. The second PCA plot emphasizes cases n° 5, 14, 30 and 51, which are clearly eccentric on the projection plot (Fig. 4b). They correspond to maximal values in ATP,  $\delta^{15}\text{N}$ , TOC, TN, clay content and NAIP. Leaving these eccentric values aside, a clockwise distribution of cases can be noticed which follows depth and age of samples (Fig. 4b). In summary, PC1 (41 %) accounts for OM content, PC2 (16 %) for the presence of microbes and PC3 (12 %) for depth and/or time span.

## Discussion

### Sedimentation type and nutrient availability

Gravity and clastic events in the basin are frequent in Laguna Potrok Aike during glacial times as the lake level oscillated with variable river inflows and eroded the shores (Kliem et al. 2013; Zolitschka et al. 2013). Run-off brings mostly refractory OM from terrestrial sources which tend to be better preserved in the SOM (Henrichs 1993). Important SOM preservation along the Glacial record is observed around 74 and 37 m depth (Fig. 1b). However, high sedimentation rates during the Glacial period are mostly associated with low TOC contents. Clastic material



derived from the volcanic catchment of Laguna Potrok Aike (Ross et al. 2010; Coronato et al. 2013) can affect P chemical forms in various ways.

Because authigenic apatite is known to form in the marine environment only (Compton et al. 2007), AP represents the detrital mineral apatite which is insoluble and sinks quickly to the bottom (Zhou et al. 2001), thus recording sporadic external inputs (Fig. 1b). NAIP, normally dissolved in the water column, precipitates in fine particles when adsorbed onto smectites and metal oxides (Anderson et al. 2001; Stamatakis and Koukoulas 2001). Main NAIP peaks in the Glacial record (Fig. 1b) correspond with the presence of iron phosphates that were documented among smectites (Nuttin et al. 2013). OP is only associated with SOM, and its record greatly depends on OM sources and degradation processes. Oxygenized conditions and particle resuspension during important mixing of the water column give little chance for the preservation of labile OM, such as microalgal OM (Amon and Benner 1996). Refractory OM gets only hydrolyzed to humic and fulvic acids during sinking, and thus represents a preferential OP sink to the sediments (Giani et al. 2010). Due to these different sedimentary behaviors, AP, NAIP and OP record variations in organic sources and sedimentary regime. However, the P sedimentary record is subject to alterations. Under suboxic conditions at the water/sediment interface, NAIP can diffuse from the sediment back to the hypolimnion (Haberzettl et al. 2007). Within the sediment,  $Mn^{4+}$  and  $Fe^{3+}$  microbial reduction releases NAIP (Gächter et al. 1988; Gächter and Müller 2003), while microbial degradation of labile OM affects OP content over time (Jones 1985). Below the diffusion boundary layer of the sediment (Balzer 1984), soluble fractions are retained in pore water and available to microbes.

In Holocene sediments, dissolved P concentrations mainly derive from OM degradation and can be associated with microbial processes (Zhou et al. 2001; Smith and Prairie 2004). Along the Late Glacial record, the sudden P depletion of pore water, low OP content and synchronous NAIP increase (Fig. 1a, b) indicate P remineralization processes (Smith and Prairie 2004). In the LGM record, OP content and phosphate concentration are too low to discriminate P sorption processes (Fig. 1a, b). Deep Glacial sediments contains vivianite (i.e.  $Fe_3(PO_4)_2 \cdot 8H_2O$ ), a mineral frequently documented in cold and dry climatic zones (Fagel et al. 2005). Vivianite is an important P sink in the water column and sediments (Sapota et al. 2006) making it unavailable to algae and microbes, and limiting not only primary productivity, but also microbial development within sediments. Thus, P availability clearly decreases throughout depth leading to starvation of microbial populations (Figs. 1c, 3a).

Paleoenvironmental conditions and microbial colonization

Pelagic conditions promote microbial settlement in shallow sediments (Deming and Baross 1993), whereas sudden loadings of sediment (Macko et al. 1993) disrupt layered microbial communities (Nealson 1997; Konhauser 2007). A regression phase was inferred in the Late Glacial record at Laguna Potrok Aike (Gebhardt et al. 2012; Zolitschka et al. 2013). The corresponding interval displays multiple sandy layers poorly colonized by microbes currently inactive (Figs. 1a, c, 2). Another important regression phase occurred during the Early mid-Holocene (ca. 8.7–7.3 ka BP; Anselmetti et al. 2009) that led to another disruption of microbial communities (8.4 m depth; Fig. 1). On the contrary, microbial activity increases in Late mid-Holocene sediments (ca. 7.3–6.3 ka BP), corresponding with a transgression phase and moister conditions (Haberzettl et al. 2007; Zolitschka et al. 2013).

Globally, the *Rr* index (Fig. 2a) highlights two sedimentary horizons (ca. 5 and 30 m depth) representing favorable habitats for microbes (Fromin et al. 2002; Marzorati et al. 2008). Microbial populations grow in Holocene sediments and maintain a low activity in Glacial sediments (Figs. 1c, 2). Both intervals coincide with periods of positive temperature excursions in South Patagonia (Kilian and Lamy 2012) during Glacial (ca. 50–30 ka BP) and Holocene times (ca. 12.5–4 ka BP), with generally active inflows into the Laguna Potrok Aike basin (Haberzettl et al. 2007; Zolitschka et al. 2013). Thus, periods of lake level drop (ca. 44–38, 16–12 and 9–7 ka ago; Zolitschka et al. 2013) correspond with a microbial colonization restricted by redeposition events. Periods of lake level rise (ca. 38–33 and 7–1 ka ago; Zolitschka et al. 2013) correspond to high microbial colonization of the sediment associated with a return to pelagic conditions and important nutrient fluxes into the Laguna Potrok Aike closed-basin. The recovery of primary productivity (Recasens et al. 2012) also led to OM sources favorable to microbial development.

Indeed, sediments from different climatic periods are clearly separated in the PCA projections (Fig. 4a, b). The first two PCs (Fig. 4a) show that samples are distributed according to their OM content and the presence of microbes. Overall, the Holocene, Late Glacial, LGM and deep Glacial records can be characterized as organic-rich and microbially active, organic-rich but microbially inactive, organic-poor but microbially active and organic-poor and microbially inactive sediments. The third and fourth PCs (Fig. 4b) point toward an influence of age/depth on the dataset that can be associated with decreasing OM preservation and microbial survival over time. Similarly, an evolution from growth to survival and inert state appears in the three groups defined by microbial indices (Fig. 3a, c).

The present results pinpoint the important influence of paleoclimatic conditions on the colonization of sediments by microbes (Vuillemin et al. 2013); wet conditions promoting microbial development, dry conditions leading to the reduced presence of microbes in the corresponding intervals. In addition, the steep maar geometry of Laguna Potrok Aike exerts a significant control on the sedimentary regime (Gebhardt et al. 2012) leading to the frequent disturbance of microbial communities by gravity-induced sedimentary events during lake-level changes.

#### Microbial organization and sustainability

In the sediments, chemoheterotrophs are mainly dependent on nutrients released during OM degradation (Zhou et al. 2001; Smith and Prairie 2004), and thus on the type of sources present in the SOM. Labile OM, which is already easily degraded in the water column, may be available to bacteria only in minor amounts within sediments. The turnover of refractory compounds is demanding and tends to slow down microbial growth (Jones 1985). Under slow degradation rates, refractory OM can release ester-bonded P, but not carbon-bonded N (Vitousek and Howarth 1991). Pore water concentrations along the Holocene record (Fig. 1a) attest of a preferential P release during SOM degradation (Villar et al. 1999), whereas the nitrite/nitrate depletion may point ammonium as an alternative source of N for microbes (McGoldrick et al. 2008).

Freely available dissolved phosphate strongly stimulates microbial growth (Smith and Prairie 2004), as shown by the important presence of active microbes within Holocene sediments (Figs. 1c and 2a), but microbial development also depends greatly on the presence of  $e^-$  acceptors (i.e.  $\text{NO}_3^-$ ,  $\text{Mn}^{4+}$ ,  $\text{Fe}^{3+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_2$ ; Nealson 1997; Konhauser 2007). The *Rr* index (Marzorati et al. 2008) provides evidence for two habitats favorable to microbial colonization at 5 and 30 m depth (Fig. 2a). In Holocene sediments,  $\text{CO}_2$  is largely available as a byproduct of fermentation and can be used in  $\text{CO}_2$  reduction processes, such as acetogenesis (Heuer et al. 2010) and methanogenesis (Whiticar 1999). In Glacial sediments, sulfate, probably derived from basaltic tephra reworked from the catchment (Ross et al. 2010), is available as an oxidant (Fig. 1a) and may allow related metabolic processes. The Euclidian distance tree potentially reflects ecological distances (Fig. 2b) resulting from the use of different oxidants and different OM and mineral sources, addressing the relationship between sediment geochemical conditions and microbial organization.

Methane and acetate (Bastviken et al. 2003; Wüst et al. 2009) represent substantial sources of labile  $\text{C}_{\text{org}}$  which can be made available to microbes via anaerobic oxidation processes. Sulfate reducers are known to perform anaerobic

oxidation of methane and acetate at minimal metabolic costs (Nüsslein et al. 2001; Strous and Jetten 2004). In addition, the OP required to produce ATP can potentially derive from the turnover of dead cells which is known to provide sufficient P amounts to sustain microbial populations in deep sedimentary environments (Dell'Anno and Danovaro 2005; Corinaldesi et al. 2011). Moreover, microbial indices (Figs. 1c, 3a) indicate living microbes in these Glacial sediments (ca. 30 m depth), even if phosphate is not readily available in pore water (Fig. 1a, c). Thus, the present deep lacustrine sediments support long-term microbial activity.

#### Microbial signal in OM signatures

Growth and activity of microbial populations (Fig. 3b) involve degradation and recycling of the SOM, leading microbes to colonize preferentially organic-rich sediments such as those of the Holocene record (Figs. 1c, 3b). Moreover, microbial biomass appears proportional to the sediment organic content (Fig. 3b, c). However, OM sources exert an initial control on microbial development (Rothfuss et al. 1997; Fenchel 1999) and paleoenvironmental signals tend to prevail during early diagenetic stages (Vuillemin et al. 2013). In a previous study, Mayr et al. (2009) identified soils, aquatic plants, diatoms and cyanobacteria as main OM sources in Potrok Aike sediments, and suggested that their original isotope signatures were preserved. In the absence of denitrification evidences in the water column (Chen et al. 2008),  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}_{\text{org}}$  opposite trends observed along the Holocene record (Fig. 1c) were previously interpreted as an essential contribution of soil among the initial sources (Mayr et al. 2009). However, the water column shifts toward subsaline conditions during that period, as evidenced by the chloride content (Fig. 1a; Branchu et al. 2010). Salinity in the water column increases with evaporation while a long residence time in the water column also leads to increased internal recycling of nutrients (Howarth and Marino 2006), possibly making N-fixing cyanobacteria the major suppliers of organic N to other algae (Marcarelli et al. 2006; Hakanson et al. 2007). Microalgal OM is easily degraded in the water column and may have provided only minor amounts of the N available to bacteria (Hardison et al. 2010), thus engendering N-limiting conditions in the water column and sediments.

In addition, the respective Holocene sediments are solely colonized by active microbes (Figs. 1c, 2). High respiration rates and persistent microbial activity can be therein major contributors to early diagenetic processes (Miskin et al. 1998; Bird et al. 2001; Nelson et al. 2007; Dong et al. 2010). Generally, microbial imprints lead to coupled  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{15}\text{N}$  negative trends (Freudenthal

et al. 2001; Lehmann et al. 2002) as heterotrophic bacteria favor the uptake of light isotopes when growing without substrate limitation (McGoldrick et al. 2008). Under nitrite/nitrate-depleted pore water conditions (Fig. 1a), microbial growth requires the uptake of the heavy N isotope with less discrimination, as in the recycling of ammonium (McGoldrick et al. 2008). This process can act as an important cause of  $\delta^{15}\text{N}$  fractionation of the substrate toward positive values (Hoch et al. 1992; Casciotti 2009).

In parallel, refractory OM, which is often enriched in  $^{12}\text{C}$ , accumulate during diagenesis (Boschker and Middelburg 2002; Lehmann et al. 2002). Humic leftovers mainly contribute to preserving TOC,  $\delta^{13}\text{C}$  and specific biomarkers throughout the sedimentary record (Meyers and Ishiwatari 1993; Giani et al. 2010). Nevertheless, refractory OM can be degraded during advanced diagenetic stages through complex microbial interactions (McInerney et al. 2009; Wüst et al. 2009). The resulting microbial biomass gets gradually enriched in  $^{12}\text{C}$  and  $^{15}\text{N}$  (Freudenthal et al. 2001) and accumulate in highly colonized sediments. Microbial biomass, thus, participates to the  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{15}\text{N}$  opposite trend observed in the bulk (Figs. 1c, 3b). On the contrary, when sediments are poorly colonized, the absence of microbial recycling leads to ammonium adsorption onto clays (Freudenthal et al. 2001) and contributes to low  $\delta^{15}\text{N}$  values. Thus, SOM signals are potentially influenced by prolonged degradation processes, nutrient recycling and the presence of microbial biomass (Macko and Estep 1984).

The SOM signal, as explained by the PCA, reflects in first refractory OM sources, preserving TOC, TN and  $\delta^{13}\text{C}$  (PC1: 40.97 %), and active microbial processes in second, ruling ATP, DAPI and  $\delta^{15}\text{N}$  (PC2: 16.46 %). Moreover, the preferential colonization of organic-rich horizons by microbes can lead to long-term activity, gradually influencing OM signatures. Thus, a modification of the SOM signal can be expected over time (PC3: 12.31 %).

## Conclusion

The present multiproxy-study combining geomicrobiological and geochemical techniques clearly demonstrates that microbial communities settle, are active and productive in the freshwater sediments of this maar. Since nutrient supply depends on climate in the lake catchment, microbial communities first settle in the sediment according to paleoenvironmental conditions. Comparison with paleoclimatic reconstructions showed that microbial colonization of the sediment was systematically higher in time intervals corresponding with warm conditions and active river inflows into the lake basin. High primary production led to high nutrient availability within sediments, and thus microbial activity was sustained within the

corresponding intervals. Conversely, during the Glacial period, sporadic redeposition events associated with lake-level drops prevented a constant settlement of microbial populations within sediments.

However, the ability of microbial communities to survive in deep lacustrine sediments could be demonstrated in specific intervals. Microbial activity was shown to be sustained in 30 ka old sediments and could imply sulfate-reduction processes. In Holocene organic rich sediments, microbial communities were found to be abundant and active. Dissolved phosphate derived from OM degradation was a main factor of microbial growth. In parallel, N-limiting conditions within pore water forced microbes to recycle the excreted ammonium, leading to increased  $\delta^{15}\text{N}$  values. Signals derived from  $\text{C}_{\text{org}}$ , which is rarely a limiting factor in the sediment, appeared less affected. The accumulation of a microbial biomass enriched in  $^{12}\text{C}$  and  $^{15}\text{N}$  is also suspected to have partially influenced bulk SOM signals in highly colonized sediments.

In conclusion, most SOM bulk indicators along the sedimentary record of Laguna Potrok Aike can provide robust paleoenvironmental reconstructions despite partial post-depositional microbial reworking. However, these results point to the persistence of living microbes and their related impact on the SOM to be taken into account when using bulk organic proxies for paleoenvironmental reconstructions in other lacustrine records.

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