

Characterizing the subsurface biosphere in Laguna Potrok Aike sediments (Argentina): A case study

VUILLEMIN, Aurèle

Abstract

Lacustrine sediments are excellent archives of past environments while sheltering active microbial populations. However, up to now, microbiological studies in lakes have been confined to surficial sediments with a poor characterization of the evolution of the hosted populations. Microbial biogeochemical cycles can nevertheless induce specific diagenetic changes on primary environmental signals. Determining microbial interactions in these lacustrine sediments is fundamental to clarify the diagenetic impact of microbes in both the sedimentary organic matter and the mineral fraction. The multidisciplinary approach developed in this thesis clearly indicates a link between initial environmental conditions and the degree of sediment colonization. Biogeochemistry of methane and fatty acids provided evidence of a microbial imprint on organic proxies in the most colonized intervals as methanogenic populations appear to be stratified in Holocene sediments. The substantial role of microbes could also be demonstrated by the formation of phosphate concretions within Glacial sediments.

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UNIVERSITÉ
DE GENÈVE

**CHARACTERIZING THE SUBSURFACE BIOSPHERE
IN LAGUNA POTROK AIKE SEDIMENTS (ARGENTINA)
- A CASE STUDY -**

THÈSE

présentée à la Faculté des Sciences de l'Université de Genève
pour obtenir le grade de Docteur ès sciences, mention Sciences de la Terre

par

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de

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**UNIVERSITÉ
DE GENÈVE**

FACULTÉ DES SCIENCES

**Doctorat ès sciences
Mention sciences de la Terre**

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intitulée :

**" Characterizing the Subsurface Biosphere
in Laguna Potrok Aike Sediments (Argentina)
- A Case Study - "**

La Faculté des sciences, sur le préavis de Messieurs D. ARIZTEGUI, professeur associé et directeur de thèse (Département de géologie et paléontologie), B. ZOLITSCHKA, professeur (Geopolar, Universität Bremen, Allemagne), V. GROSSI, docteur (Laboratoire de géologie de Lyon, Terre, Planètes, Environnement, Université Lyon-I, France) et Madame P. JUNIER, professeure (Institut de biologie, Université de Neuchâtel, Suisse), autorise l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

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N.B. - La thèse doit porter la déclaration précédente et remplir les conditions énumérées dans les "Informations relatives aux thèses de doctorat à l'Université de Genève".

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ABSTRACT

Microbial activity is fully recognized as a major player in lithification processes. Results from sediment cores retrieved during ODP and IODP-sponsored drilling campaigns have shown that these organisms are active even in extreme environments and capable of catalyzing and enhancing diagenetic reactions. The distribution and diversity of microbes in marine sediments have been already studied for several years, but these investigations are still missing for their lacustrine counterparts. As modern lakes hold archives of past environments while sheltering actual and active microbial populations, they represent ideal systems to study early diagenetic processes. Indeed, microbial processes within the sediment can modify the sedimentary recordings. Using physical and chemical features of sediments to which a microbiological approach adds the understanding of the role played by microbes, geomicrobiological studies aim to determine the relationships between climate, hydrological regime, trophic states, microbial colonization and diagenetic reworking of lacustrine substrates. Up to now, these studies in lake basins have mainly focused on either the water column and/or the very recent sediments. Lake sediments retrieved for multiproxy studies, such as those from ICDP-sponsored research programs, provide a unique opportunity to explore and apply recent developments of the marine geomicrobiological field to the lacustrine realm.

In Autumn 2008, a 100-meter long core from Lake Potrok Aike was fully dedicated to a geomicrobiological study. A sampling strategy was specially tailored to avoid contamination enabling the inspection of undisturbed lacustrine sediments and the tracking of ongoing microbial activity throughout depth. Sampling was optimized to avoid any contamination and preserve the sediment initial signatures for further microbiological techniques. Lacustrine proxies allowed defining the variable sedimentation regimes along the last 60 ka. Different microbial substrates were characterized by analyzing bulk organic fractions and pore waters chemistry while variable degrees of sediment colonization were observed in relation to organic sources refractoriness. Living microbes activity in the sediments was assessed using in situ ATP (adenosine 5'-triphosphate) detection, microbial populations further quantified via cell counts, and the degree of substrate colonization globally defined via DGGE (molecular fingerprinting technique). Furthermore, clone libraries allowed the attribution of species, and thus the identification of ongoing metabolic processes. Such phylogenetic approach also pinpointed the evolution of diversity with respect to both sediment depth and dominant lithology.

One main focus was the determination of the microbial consortium influence on organic matter during early diagenesis, and to parallel these results to paleoclimatic interpretations of the Holocene record. In anoxic habitats, a stepwise degradation can convert primary inputs into simpler molecules, which can sometimes mask the initial signals of the bulk organic fraction. We chose to inspect methanogenic populations and assess their possible imprint on organic sources due to substrate fractionation associated with microbial processes. For this, we measured methane content and its respective carbon isotopic composition and established a phylogenetic tree of methanogenic populations that could evidence their stratification throughout Holocenes sediments. Sequence identifications revealed species related to *Methanolinea* and *Methanoregula* as the most abundant, indicating that CO₂ reduction was the major pathway leading to methane production. Methane $\delta^{13}\text{C}$ compositions displayed a high value in shallow sediments (ca. -24 ‰) associated with its diffusion upwards and oxidation,

whereas values at depth were much more negative (ca. -65 ‰ to -68 ‰) indicating a high fractionation during methane production. Methanogenic pathways in the sediments were successively identified as methyl fermentation down to 2 m depth, CO₂ reduction down to 8 m depth and syntrophic oxidation of acetate as a late stage of methane production below 8 m depth. The presence of *Syntrophus*-related sequences suggested syntrophic associations with *Methanoregula*.

Because microbial biomass is often difficult to discern from terrestrial and/or algal inputs, lipid extractions were necessary. The identification of even saturated, unsaturated and branched-chain fatty acids along with their respective isotopic compositions allowed tracing initial organic sources, their reworking by microbes and the production of secondary biomass within the sediments. These results evidenced a substantial microbial resynthesis of medium-chain even saturated fatty acids leading to a $\delta^{13}\text{C}$ fractionation of -3 to -6 ‰, while long-chain even saturated fatty acids underwent a partial alteration of their isotopic compositions in sedimentary horizons highly colonized by microbes. The $\delta^{13}\text{C}$ compositions of microbial fatty acids showed that microbial biomass was mainly derived from algal matter while very ¹³C-depleted compositions indicated compounds produced by methanogens. A phylogenetic tree for Bacteria was established at 5 m depth in a horizon consisting of methane bearing clays and subsaline pore waters displaying maximum microbial density and activity of the entire record. The microbial population therein reflected local geochemical conditions with an adaptation to salinity and different OM degradation abilities. Substantial ongoing nutrient recycling processes and complementarities in the OM degradation chain affecting the signature of total sediments could be demonstrated. We tracked down further signs of microbial activity throughout Glacial sediments and clearly identified a horizon at 30 m depth composed of organic-poor clays overlain by basaltic sands with a high dissolved sulfate content. This specific horizon displayed a low microbial population density, but a sustained activity. Phylogenetic attributions revealed the major presence of sulfate reducers and Actinobacteria along with numerous lithotrophs. Authigenic iron sulfides at similar depths were often found as framboids which suggested anaerobic oxidation of methane, as also inferred by an important increase in the $\delta^{13}\text{C}_{\text{CH}_4}$ composition. Signals of microbial activity faded below 40 m sediment depth while authigenic concretions including vivianite were found increasingly. As our study also aimed at a better understanding of microbe/mineral interactions and the identification of biosignatures, a thoroughly study of these concretions provided information of past trophic states of the lake system and processes undergone by the sediments during former microbial activity.

Overall, the multiproxy signals indicated the sustainability of microbial influence throughout depth. Methanogenesis appeared initially as the dominant process during early diagenesis, but sporadic gravity events associated with lake oscillations of the Potrok Aike maar disrupted microbial populations during the Glacial period. Mafic volcanics reworked from the catchment to the lake basin acted as main supplies of iron, sulfur and phosphorus, thus impacting on primary productivity and arousing additional microbial metabolisms. The Holocene transition corresponds to a shift to a pelagic sedimentation with subsaline conditions which promoted methanogenesis in these sediments.

RESUME

L'activité microbienne est reconnue comme un acteur principal des processus de lithification. Les résultats des forages ODP et IODP ont montré que ces organismes sont actifs même en milieux extrêmes, et capables de catalyser certaines réactions diagénétiques. La distribution et la diversité microbiennes dans les sédiments marins ont déjà fait l'objet d'études depuis plusieurs années, mais ces mêmes investigations manquent pour leurs homologues lacustres. Les lacs modernes, parce qu'ils recèlent les archives des climats passés tout en hébergeant des populations microbiennes actives, représentent des systèmes idéaux pour étudier les processus de diagénèse précoce. A l'aide des caractéristiques physiques et chimiques des sédiments auxquelles une approche microbiologique ajoute la compréhension du rôle joué par les microbes, les études géomicrobiologiques visent à déterminer les relations entre climat, régime hydrologique, états trophiques, et colonisation microbienne des substrats. Jusqu'à présent, ces études en bassins lacustres se sont principalement focalisées sur la colonne d'eau et sur les sédiments très récents. Les sédiments lacustres récupérés lors des programmes de recherche multidisciplinaires de l'ICDP, fournissent une opportunité unique d'explorer et d'appliquer les développements récents de la géomicrobiologie marine au domaine lacustre.

En automne 2008, une carotte sédimentaire de 100 m de long du maar Potrok Aike a été dédiée à la géomicrobiologie. L'échantillonnage spécialement adapté pour éviter toute forme de contamination a permis l'inspection de sédiments lacustres non perturbés et le suivi de l'activité microbienne jusqu'en profondeur. Les échantillons ont été conditionnés pour optimiser, à des fins d'analyses microbiologiques, la préservation de leurs signaux initiaux. Les indicateurs limnologiques ont permis la définition des variations du régime sédimentaire au cours des derniers 60'000 ans. Différents substrats microbiens ont pu être caractérisés par l'analyse des fractions organiques et de la chimie des eaux interstitielles. Différents degrés de colonisation du sédiment ont été observés en relation à la réfractarité des sources organiques. L'activité des microbes a pu être évaluée par une méthode de détection d'ATP, les populations microbiennes quantifiées par comptage de cellules, et le degré de colonisation du substrat globalement défini par DGGE (technique de traçage moléculaire). Par la suite, les techniques de clonage ont permis l'attribution d'espèces et l'identification des processus métaboliques en cours. Cette approche a également précisé l'évolution de la diversité microbienne en fonction de la profondeur et de la lithologie des sédiments.

Notre étude a principalement visé la détermination de l'influence microbienne sur la matière organique au cours de la diagénèse précoce, et la mise en parallèle de ces résultats avec les interprétations paléoclimatiques de l'enregistrement holocène. En milieu anoxique, une dégradation graduelle peut affecter les apports primaires jusqu'à masquer parfois les signaux sédimentaires initiaux. Nous avons ainsi choisi d'étudier les populations méthanogènes pour estimer une possible empreinte du substrat organique suite à un fractionnement associés à des processus microbiens. Pour ce faire, nous avons mesuré les teneurs et compositions isotopiques du méthane, puis établi un arbre phylogénétique des populations méthanogènes, ce qui a mis en évidence une stratification de ces populations au sein des sédiments. Les séquences génétiques ont révélé *Methanolinea* et *Methanoregula* comme les espèces plus abondantes, indiquant la réduction du CO₂ comme voie principale de la production du méthane. Les compositions $\delta^{13}\text{C}$ du méthane ont montré une valeur élevée en surface (ca. -24

‰) associée à la diffusion et l'oxydation de ce gaz, alors que des valeurs bien plus négatives ont été mesurées en profondeur (ca. -65 ‰ à -68 ‰). Les processus méthanogéniques dans les sédiments ont été successivement identifiés comme la fermentation des substrats méthylés jusqu'à 2 m de profondeur, la réduction du CO₂ jusqu'à 8 m de profondeur et, sous les 8 m de profondeur, l'oxydation syntrophique de l'acétate interprétée comme un stage tardif. La présence de séquences de type *Syntrophus* suggère des associations syntrophiques avec *Methanoregula*.

Parce que la biomasse microbienne est souvent difficile à distinguer des apports terrestres et/ou algaires, une extraction des lipides s'est avérée nécessaire. L'identification des divers acides gras et de leurs compositions isotopiques ont permis le traçage des sources organiques initiales, de leur remaniement par les microbes et de la production d'une biomasse secondaire dans les sédiments. Ces résultats ont mis en évidence une resynthèse microbienne des acides gras saturés pairs à chaîne moyenne engendrant un fractionnement de leur $\delta^{13}\text{C}$ de -3 à -6 ‰, un remaniement partiel des acides gras saturés pairs à chaîne longue dans les niveaux fortement colonisés par les microbes. La composition $\delta^{13}\text{C}$ des acides gras microbiens ont montré l'origine principalement algale de la biomasse microbienne et la composition très appauvrie en ^{13}C des composés produits par les méthanogènes. Un arbre phylogénétique des Bactéries a été établi à 5 m de profondeur, dans un horizon argileux méthanique et saumâtre, affichant les densité et activité microbiennes maximales de l'enregistrement sédimentaire. La population microbienne y reflète les conditions géochimiques locales avec une adaptation à la salinité et diverses capacités de dégradation du substrat. Un recyclage actif des nutriments ainsi que des complémentarités dans la chaîne de dégradation de la matière organique sont ressortis comme affectant la signature du sédiment total. Nous avons par la suite traqué l'activité microbienne dans l'enregistrement glaciaire, et identifié un horizon à 30 m de profondeur composé d'argiles peu organiques recouvertes de sables basaltiques à teneur élevée en sulfates. Cet interval spécifique affichait une population microbienne de faible densité mais à l'activité soutenue. Les arbres phylogénétiques ont révélé une majorité de sulfato-réducteurs et d'actinobactéries en présence de nombreux lithotrophes. Des sulfures de fer authigènes sous forme framboïdale ont été identifiés à des profondeurs similaires associés à une augmentation du $\delta^{13}\text{C}_{\text{CH}_4}$ suggérant des processus d'oxydation anaérobie du méthane. Sous 40 m de profondeur, les signes d'activité microbienne ont commencé à disparaître, alors que la vivianite en concrétions est devenue de plus en plus fréquentes. Une étude complète de ces concrétions, visant une meilleure compréhension des interactions entre microbes et minéraux et l'établissement de biosignatures, a révélé des informations quant aux états trophiques du système lacustre passés et aux processus diagénétiques microbiens anciens dans les sédiments.

D'une façon générale, cette étude multiple a indiqué la durabilité de l'influence microbienne dans l'enregistrement. La méthanogénèse est apparue comme un processus dominant de la diagénèse précoce mais les événements gravitaires associés aux oscillations du niveau d'eau du maar ont perturbé les populations microbiennes au cours de la période glaciaire. Le matériel volcanique remanié du bassin versant vers le lac a apporté fer, soufre et phosphore, influençant la productivité primaire et suscitant divers métabolismes microbiens. La transition Holocène correspond à un changement de régime vers une sédimentation pélagique et des conditions saumâtres ce qui a favorisé la méthanogénèse dans ces sédiments.

CHAPTER 1

Introduction

INTRODUCTION

1. Geomicrobiology: Background

Foreword

The Earth is ~4.6 eons old and includes the lithosphere, hydrosphere, and atmosphere, all habitable by microbes to a greater or lesser extent constituting the biosphere. The planet was initially surrounded by an oxygen-deprived atmosphere and primitive life probably arose 0.5–0.7 eons after the Earth formation. The earliest forms of prokaryotes are thought to have evolved from a precellular autotrophic surface-bound metabolism to a detached primitive autotrophic cellular metabolism. The emerging physiological types went broadly from anoxygenic photoautotrophy to aerobically respiring heterotrophy. Except for cyanobacteria, aerobic prokaryotes did not evolve until free oxygen began to accumulate in the atmosphere, and eukaryotic forms did not appear until the accumulated oxygen in the atmosphere attained significant levels. The time at which oxygen began to accumulate is not yet precisely known but estimated as 2.3 eons ago, although it may have been earlier.

The scope of geomicrobiology: A brief explanation of a complex nature

A rapid browse into the recent literature shows a wide variability in the definition of this relatively new and fast evolving field of research. Some examples are as follows:

“Geomicrobiology is the result of the combination of geology and microbiology. The field of geomicrobiology concerns the role of microbe and microbial processes in geological and geochemical processes and vice-versa.”

WIKIPEDIA The Free Encyclopedia (2013)

“Geomicrobiology deals with the role that microbes play at present on Earth in a number of fundamental geologic processes and have played in the past since the beginning of life. These processes include the cycling of organic and some forms of inorganic matter at the surface and in the subsurface of Earth, the weathering of rocks, soil and sediment formation and transformation, and the genesis and degradation of various minerals and fossil fuels.”

H.L. Ehrlich (2009)

“Since their origin, microorganisms have had a profound influence on shaping our planet. From localized niches, that occur on the order of micrometers, to ecosystems as immense as the oceans, microorganisms are intimately involved in transforming inorganic and organic compounds to meet their nutritional and energetic needs. Because the metabolic waste from one type of species nearly always provides substrates for another, there is a continuous recycling of elements throughout the biosphere. This interdependence can exist between species growing in close proximity to one another, where any number of sorption, precipitation, and redox reactions inevitably creates unique community-specific biogeochemical and mineralogical signatures. Alternatively, the communities can be spatially separated, and elemental cycling may take on more complex and convoluted pathways, such as the transfer of metabolites across the sediment–water interface or from the ocean water column to the atmosphere. The latter examples are particularly important for global-scale cycling of carbon, nitrogen, sulfur, and oxygen.”

K. Konhauser (2007)

Geomicrobiology is then an area of science where geology meets microbiology, molecular biology, and geochemistry, combining their techniques to inspect the microbial role in geological and environmental processes. The most familiar topics to geologists mainly cover biogeochemical cycles, microbialites and microbes/minerals interactions. This new evolving field of Earth Sciences addresses stromatolites and black smokers, shallow lagoons and deep-sea methane seeps, biodiversity reserves as well as anthropogenic wastes. Many academic and industrial fields of research are presently developing a geomicrobiological approach. Global climate, water resources, bioremediation, nanotechnology, fuel production, extremophiles, or life detection on other planets are among hot topics.

2. Goals of the thesis: Geomicrobiology in paleoclimatic reconstructions

The distribution and diversity of microbes in marine sediments have been already studied for several years (D'Hondt et al., 2004; Teske, 2005), but these investigations are still missing for their lacustrine counterparts. We decided to look at microbes settled within sediments in a lacustrine basin (subsurface biosphere) for the first time within the framework of an International Continental Drilling Program (ICDP) campaign. Previous studies have mainly focused on the water column and/or very shallow sediments (Humayoun et al., 2003; Zhao et al., 2007). We performed a systematic sampling down to 93 m depth on a hydraulic piston core retrieved from Laguna Potrok Aike in October 2008 (Recasens et al. 2012).

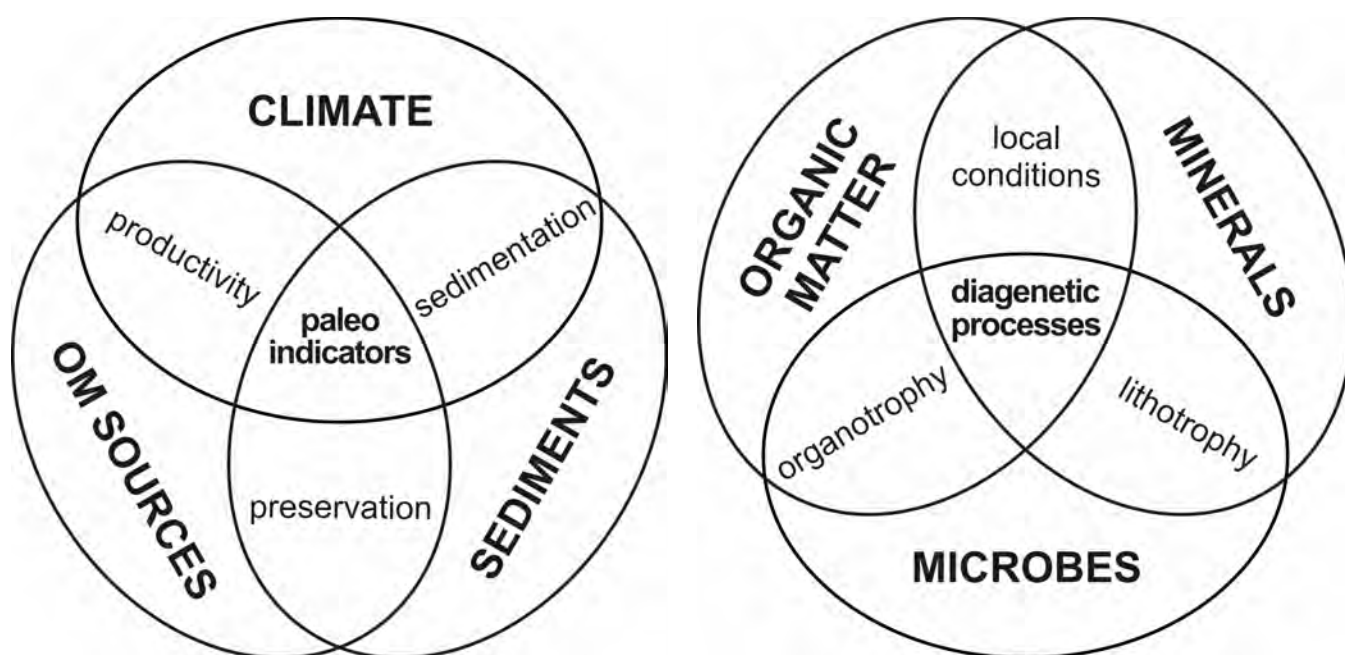


Figure 1 Diagrams showing the different factors and their degree of interaction influencing bulk organic matter proxies **Left:** The prevalent climate in the catchment area of Laguna Potrok Aike defines the primary productivity in the water column as well as the external inputs associated with runoff, and thus exerts control on the organic matter sedimented in the basin. The prevailing climate conditions are also controlling the lake level, and thus the sedimentation regime resulting in variable lithologies and grain sizes. **Right:** The sedimented material represents the initial conditions under which microbes develop leading to variable diagenetic processes.

Since one of the main goals of this dissertation is to determine the impact of microbial activity during early stages of diagenesis, a sampling protocol was specially defined so that initial microbial signals would be preserved. Diverse microbiological techniques were selectively applied in parallel to the development of a multiproxy dataset to establish the preferential microbial colonization of certain sedimentary horizons and their link to the prevailing

paleoclimatic conditions (Fig. 1). This is important since, on a premise basis, the influence of microbial activity on the climatic signal is most often considered negligible (Meyers and Teranes 2001). The latter is generally done without assessing the actual activity of living microbes (Fig. 2). Different degradation and fractionation potentials can be related to microbial growth rates, means of survival, depths of sustainable activity, types of metabolism, and interactions within their populations (Nealson 1997; Rothfuss et al. 1997; Wüst et al. 2009). These factors will further affect the sediment initial conditions over time (Figs. 1 and 2).

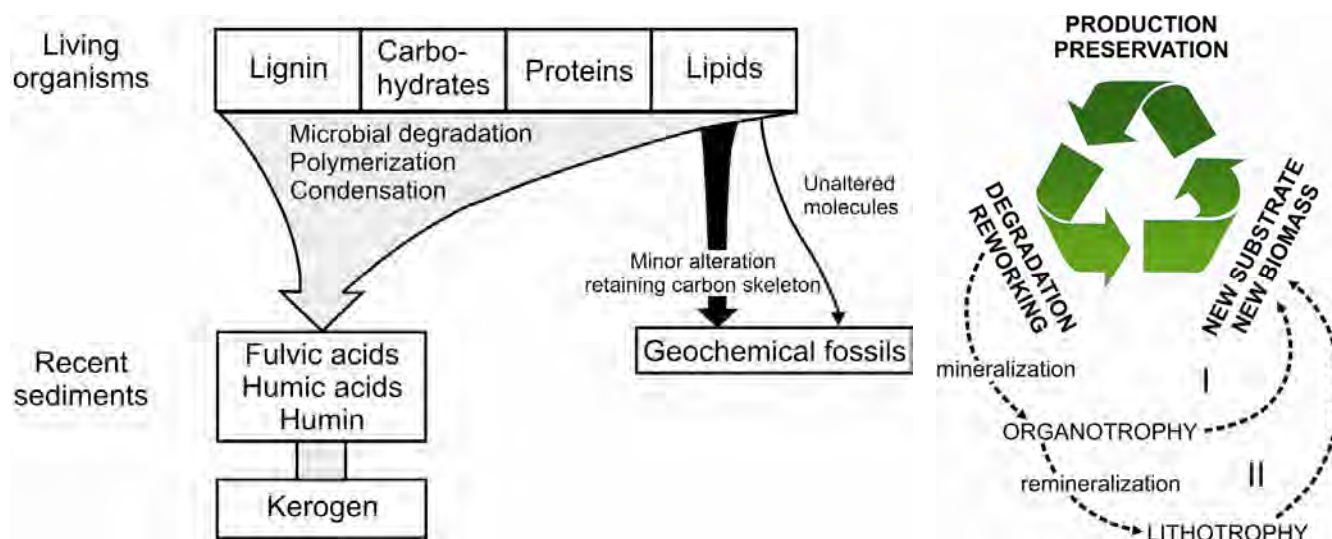


Figure 2 Schemas showing the presence and role of microbes during early diagenesis **Left:** Scheme displaying a minimal microbial degradation since early diagenetic processes preserve lipids as well as humic and fulvic acids, **Right:** Cycle I is characterized by a constant recycling and reworking of initial organic substrates into new biomass by microbes, whereas in cycle II nutrient depletion drives microbial metabolisms towards the use of inorganic compounds issued from remineralization and mineral alteration.

Thus, microbial activity, abundance and diversity need to be investigated throughout depth before any bulk organic proxy can be used as a paleoindicator. Authigenic minerals (Glasauer et al. 2003) and lipid biomarkers (Boschker and Middelburg 2002) are also being inspected in order to define the preservation of signals of both former and ongoing microbial processes.

The final goal of this thesis is to determine the influence of microbial induced processes on sediment biogeochemistry that may change the primary environmental signals. Our results are shedding new light on the interactions between microbes and minerals in freshwater environments. Furthermore, they show clear evidence of microbial diagenetic imprints on sedimentary organic matter proxies calling for caution when they are indiscriminately used for paleoclimatic reconstructions.

3. The PASADO Project

The "Potrok Aike Maar Lake Sediment Archive Drilling Project" (PASADO) is an international research initiative within the framework of the International Continent Drilling Program (ICDP) which addresses several key issues related to climatic and environmental reconstructions over the last glacial cycle. Laguna Potrok Aike is a maar lake located at 52°S within the Pali Aike Volcanic Field, southern Patagonia, Argentina (Zolitschka et al. 2006). This study site (Fig. 3) was chosen due to its location, which is ideal to record changes in the atmospheric circulation of the Westerly winds (Mayr et al. 2007). Previous results of the "South Argentinean Lake Sediment Archives and Modelling" (SALSA) project focused especially on lake level

fluctuations (Anselmetti et al. 2008), microfossil assemblages (Wille et al. 2007), and sources of organic matter (Mayr et al. 2009) provided the first continuous high resolution paleoclimatic reconstruction for southern Patagonia covering the last 16 ka.

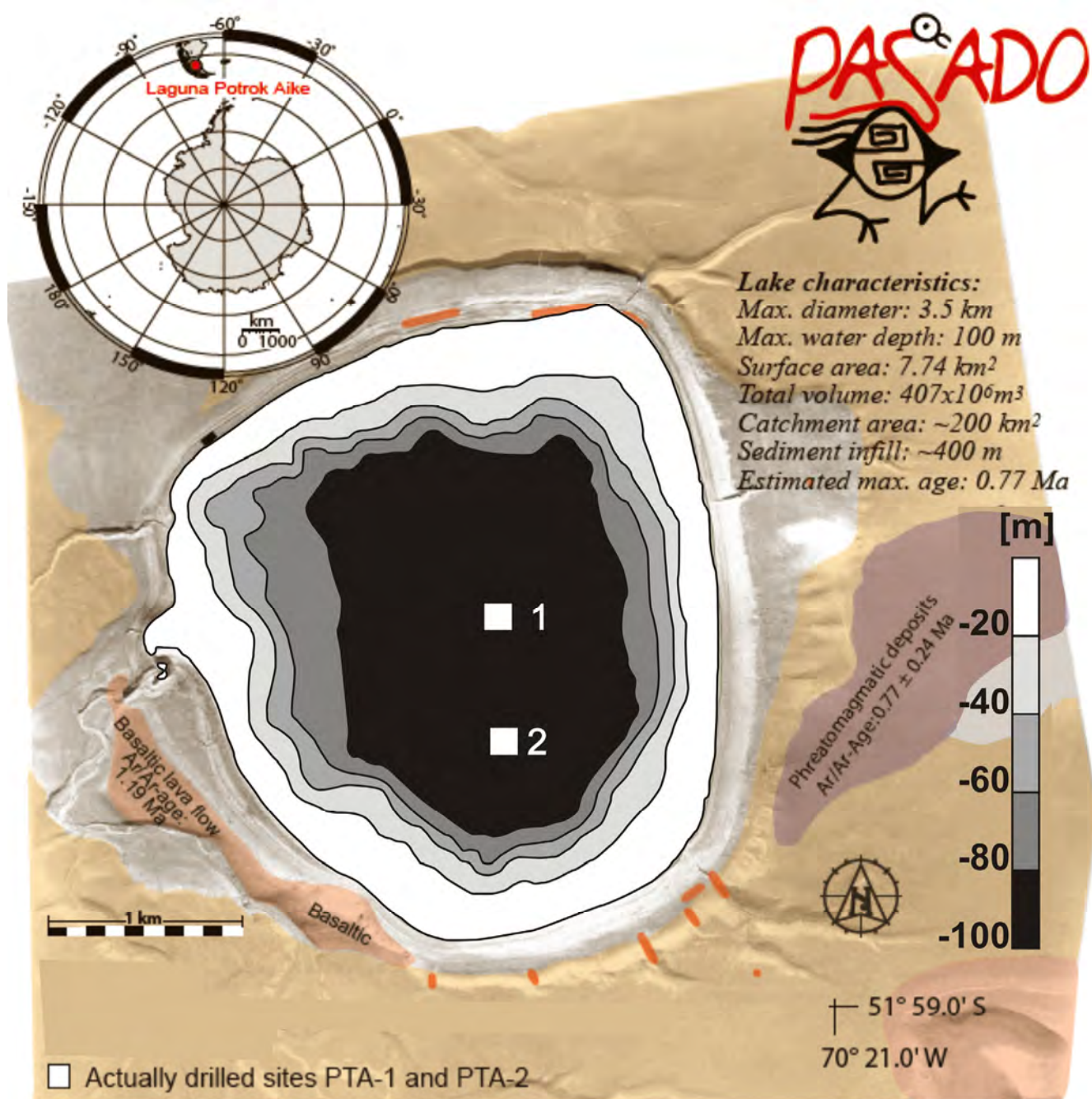


Figure 3 Bathymetric map of Laguna Potrok Aike with the location of the two drilling sites (after Zolitschka et al. 2009). The inset map on top right shows the site location in sub-Antarctic South America.

The drilling operations of the PASADO project were accomplished in November 2008 (Zolitschka et al. 2009) and provided one of the longest lacustrine sedimentary column recovered for the entire southern hemisphere (Recasens et al. 2012). The PASADO Science Team implemented numerous techniques to elaborate a full multiproxy dataset (Fig. 4), allowing accurate paleoenvironmental reconstructions accounting for at least 55 ka (Gebhardt et al. 2012). The sedimentary sequence of Laguna Potrok Aike can be now compared to marine sediment archives and ice cores, enabling geological, environmental and climatic correlations on a global scale (Kilian and Lamy 2012).

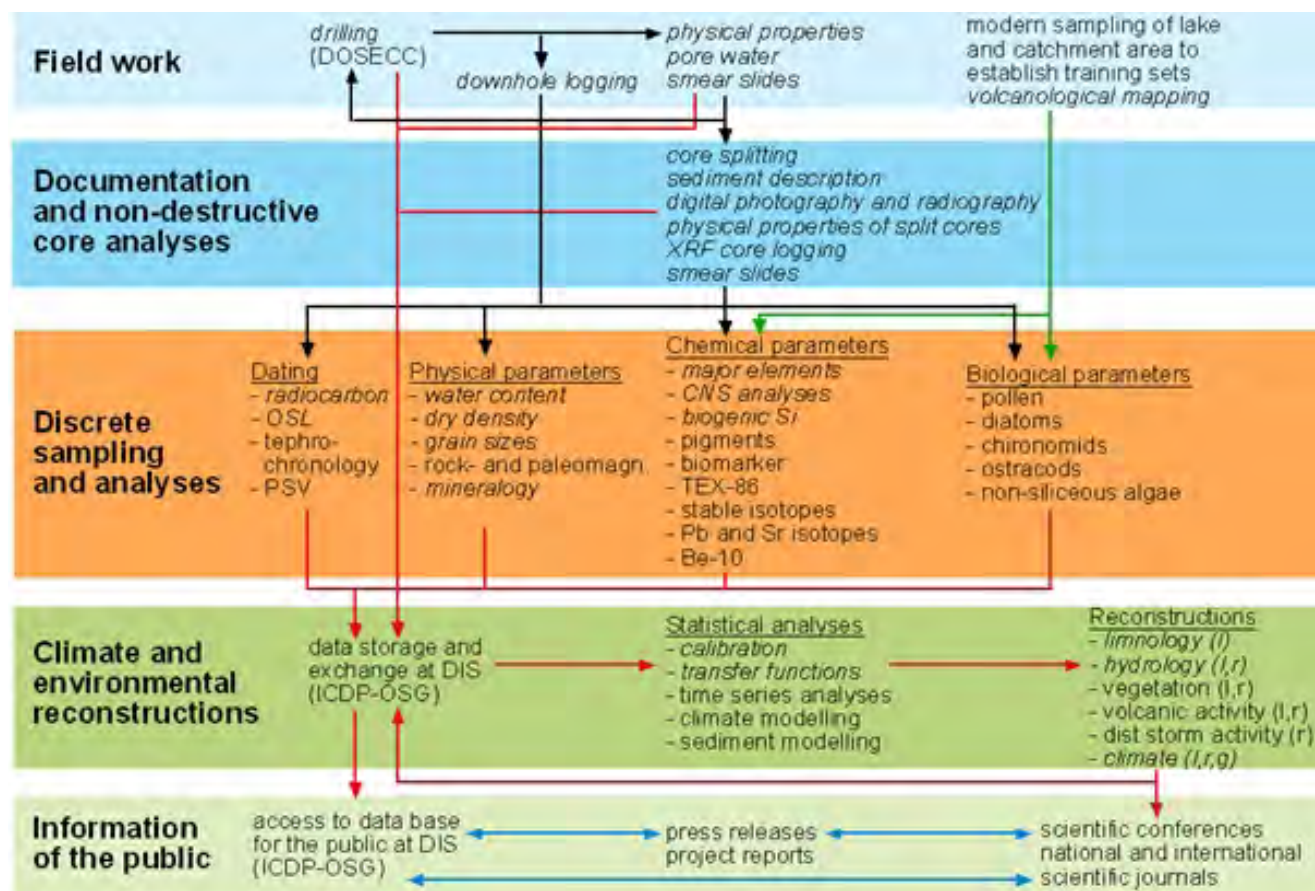


Figure 4 The project design documents the integration of methods and techniques to achieve the overall aims of climate and environmental reconstruction, which are either local (l), regional (r) or global (g) (from www.pasado.uni-bremen.de).

The geomicrobiological investigations that are the heart of this thesis were carried out and compared with the resulting multiproxy record from the PASADO scientific team. The latter allowed defining whether certain geomicrobiological proxies can be used as paleoclimatic and/or diagenetic indicators. Firstly, we reassessed the origin of the variations observed in some commonly used organic proxies in order to identify possible changes of the environmental signal during successive diagenetic stages. Microbiological techniques were fitted to determine active microbial processes within the retrieved sediments, while geochemical proxies from pore water and the organic fraction help to separate the influence of climate and/or diagenesis, respectively.

The results of a careful analysis of these entangled signals are shedding light on the syn- and post-depositional sedimentary processes occurring in lacustrine basins. As a result our pioneer study has set up a sampling strategy and a research approach for investigations in other ICDP projects in the same fashion than in the marine realm.

4. Structure of the thesis

This dissertation focuses on the microbial impact on the sedimentary organic fraction and thus on widely used organic proxies. Each of the following chapters corresponds to a scientific manuscript in press, submitted or in preparation to be submitted to an international journal. Complete datasets were submitted to PANGAEA® Data Publisher for Earth & Environmental Science and are available online at <http://doi.pangaea.de/10.1594/PANGAEA.811523> and [811524](http://doi.pangaea.de/10.1594/PANGAEA.811524). After a general introduction in Chapter 1, the methodology that was developed and used in this study is presented in Chapter 2. Chapters 3 to 7 focus on different sedimentary

fractions, and their use in paleoclimatic reconstructions. Last, chapter 8 contains the main conclusions and an outlook of the investigations.

The second chapter introduces the field conditions and the procedure that were applied during the drilling campaign to minimize contamination risks and condition samples for specific geochemical and microbiological techniques. This chapter is a modified version of a paper published in *Scientific Drilling*.

The third chapter establishes a geomicrobiological multiproxy dataset in the shallowest sediments. Investigations focus on the structural ecology of microbes and its possible link with paleoclimatic and depositional conditions. The role of microbes during early diagenesis is approached. A modified version of this chapter is in press (2013) in *Quaternary Science Reviews*.

The fourth chapter is centered on the microbial sustainability along the complete sedimentary record of Laguna Potrok Aike. Specific geochemical conditions leading to the preferential colonization of certain sedimentary layers by microbes are discussed in terms of sedimentary regimes, trophic states of the water column and primary productivity. The diagenetic impact of microbes is inspected thoroughly. A similar version of this chapter is presently under review in *Aquatic Sciences*.

The foci of the fifth chapter are the authigenic concretions observed along the Glacial record of Laguna Potrok Aike and aims at determining the conditions and depths of their formation. Microbial processes in relation to diagenetic effects are compared with independently determined depositional conditions to define a possible use of mineral authigenic phases within the concretions as paleoindicators and/or microbial biosignatures. A modified version of this chapter is in press (2013) in the *Journal of Paleolimnology*.

The sixth chapter investigates the influence of active methanogenic populations within Holocene sediments and their relative impact on the different organic sources present in the bulk fraction. This is achieved through the analysis of methane and fatty acids, while cloning allows the identification of microbes and their related metabolic pathways. A substantial part of this chapter is presently under review in *Geomicrobiological Journal*.

The seventh chapter dives in complex interactions between microbial species, aiming to define microbial metabolic strategies and active processes within variable lacustrine habitats. Phylogenetics of Bacteria and Archaea are achieved in two horizons displaying different ages, geochemical conditions and trophic states. A modified version of this chapter will be submitted to an international journal.

The final chapter summarizes the main findings of this research and puts into perspective the geomicrobiological approach and its applications to other lacustrine sedimentary records.

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Funding agencies



CHAPTER 2

Field campaign and sampling

A modified version of this chapter has been published as

Vuillemin et al. (2010) in Scientific Drilling

doi:10.2204/iodp.sd.10.04.2010

Establishing sampling procedures in lake cores for subsurface biosphere studies: Assessing *in situ* microbial activity

Introduction

Sub-recent sediments in modern lakes are ideal to study early diagenetic processes with a combination of physical, chemical, and biological approaches. Current developments in the rapidly evolving field of geomicrobiology have allowed determining the role of microbes in these processes (Nealson and Stahl, 1997; Frankel and Bazylinski, 2003). Their distribution and diversity in marine sediments have been studied for some years (Parkes et al., 1994; D'Hondt et al., 2004; Teske, 2005). Comparable studies in the lacustrine realm, however, are quite scarce and mainly focused on the water column (Humayoun et al., 2003) and/or very shallow sediments (Spring et al., 2000; Zhao et al., 2007). Thus, there is a need to determine the presence of living microbes in older lacustrine sediments, their growth and metabolic paths as well as their phylogenies that seem to differ from already known isolates.

During the PASADO (Potrok Aike Maar Lake Sediment Archive Drilling Project) ICDP (International Continental Scientific Drilling Program) drilling, more than 500 meters of sedimentary cores were retrieved from this crater lake (Zolitschka et al., 2009). A 100-m-long core was dedicated to a detailed geomicrobiological study and sampled in order to fill the gap of knowledge of the lacustrine subsurface biosphere.

Here we report the complete *in situ* sampling procedure that aims to recover aseptic samples as well as determining active *in situ* biological activity. Preliminary results demonstrate that these procedures provide a very useful semi-quantitative index that immediately reveals whether there are biologically active zones within the sediments.

The PASADO Project

Laguna Potrok Aike is a 770-ka-old maar lake located at 51°58' S and 70°22' W in the Santa Cruz Province, Argentina, within the 3.8-Ma-old Pali Aike Volcanic Field (Fig. 1; Zolitschka et al., 2006). Although annual precipitation ranging between 200 mm and 300 mm gives a semi-arid character to the area, the lake is presently the only permanently water-filled lacustrine system in the southeastern Patagonian steppe. Today it has a maximum diameter of 3.5 km, a total surface of 7.74 km², and a maximum water depth of 100 m. The lake regime is polymictic, and the water-column is non-stratified with an anoxic sediment-water interphase.

A seismic study of this lacustrine basin showed a thick sedimentary sequence (Anselmetti et al., 2009; Gebhardt et al., in review) that was the target of the PASADO project. This international research initiative had a key objective: quantitative climatic and environmental reconstruction of this remote area through time. The multiproxy study also provides unique material to initiate, for the first time in an ICDP project, a systematic study of the living lacustrine subsurface environment. From a total of 533 meters of sediment cores recovered at 100 m water depth (Fig. 1), a one-meter-long gravity core PTA-1J and the 97-m-long hydraulic piston core PTA-1D were sampled following a newly established strategy to obtain aseptic samples for geomicrobiological studies.

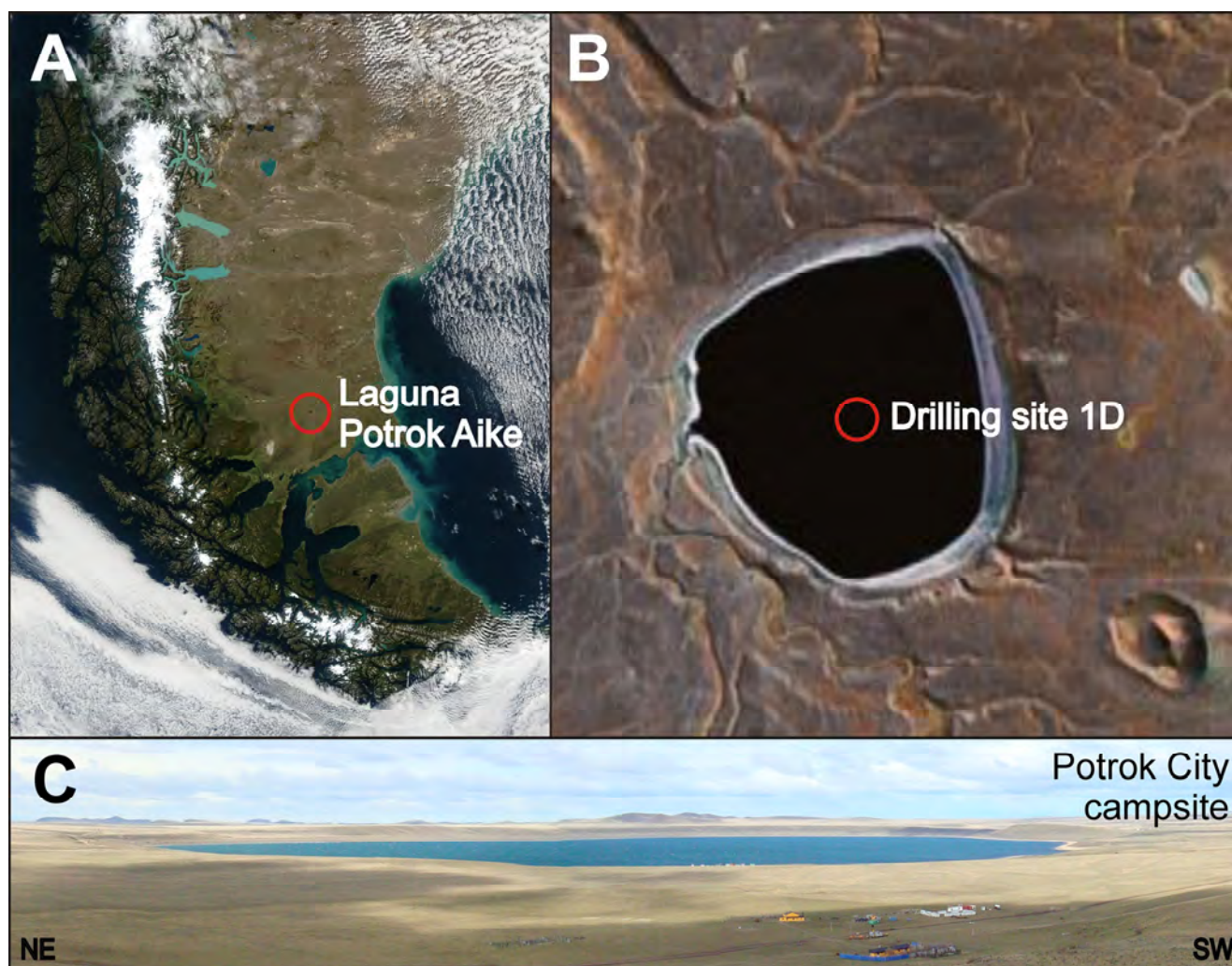


Figure 1 [A] Satellite image of Laguna Potrok Aike located in southernmost continental Patagonia, north of the Strait of Magellan; [B] close-up of the lake showing the position of the drilling site discussed here; [C] panoramic view of the lake site with the field camp in the foreground.

Sampling Procedure

A strategy was designed to minimize contamination risks in the field and laboratory. The size and configuration of the drilling platform prevented the setting up of a sampling laboratory with maximum conditions of asepsis on the platform. Thus, the retrieved cores were transported every 90 min from the platform to a laboratory on the campsite where they were sampled (Figs. 2A to 2C). The liners of hydraulic piston cores were first disinfected with isopropanol and then sprayed with fungicide.

Thereafter, sampling windows were cut in the liner every one or two meters and at higher resolution for the upper 15 m using a portable circular saw (Fig. 3A). Conversely, in the gravity core twenty windows were cut at 5-cm spacing in the empty liner and sealed with strong adhesive tape prior to coring. This latter technique facilitated opening windows and allowed sampling quickly at a higher resolution. Samples from these windows were immediately chemically fixed and/or frozen, optimizing the preservation of their initial conditions for further analyses.

A quick biological activity test, which is commercially available for industrial hygiene monitoring, was applied in order to test microbial activity in the sediments immediately after

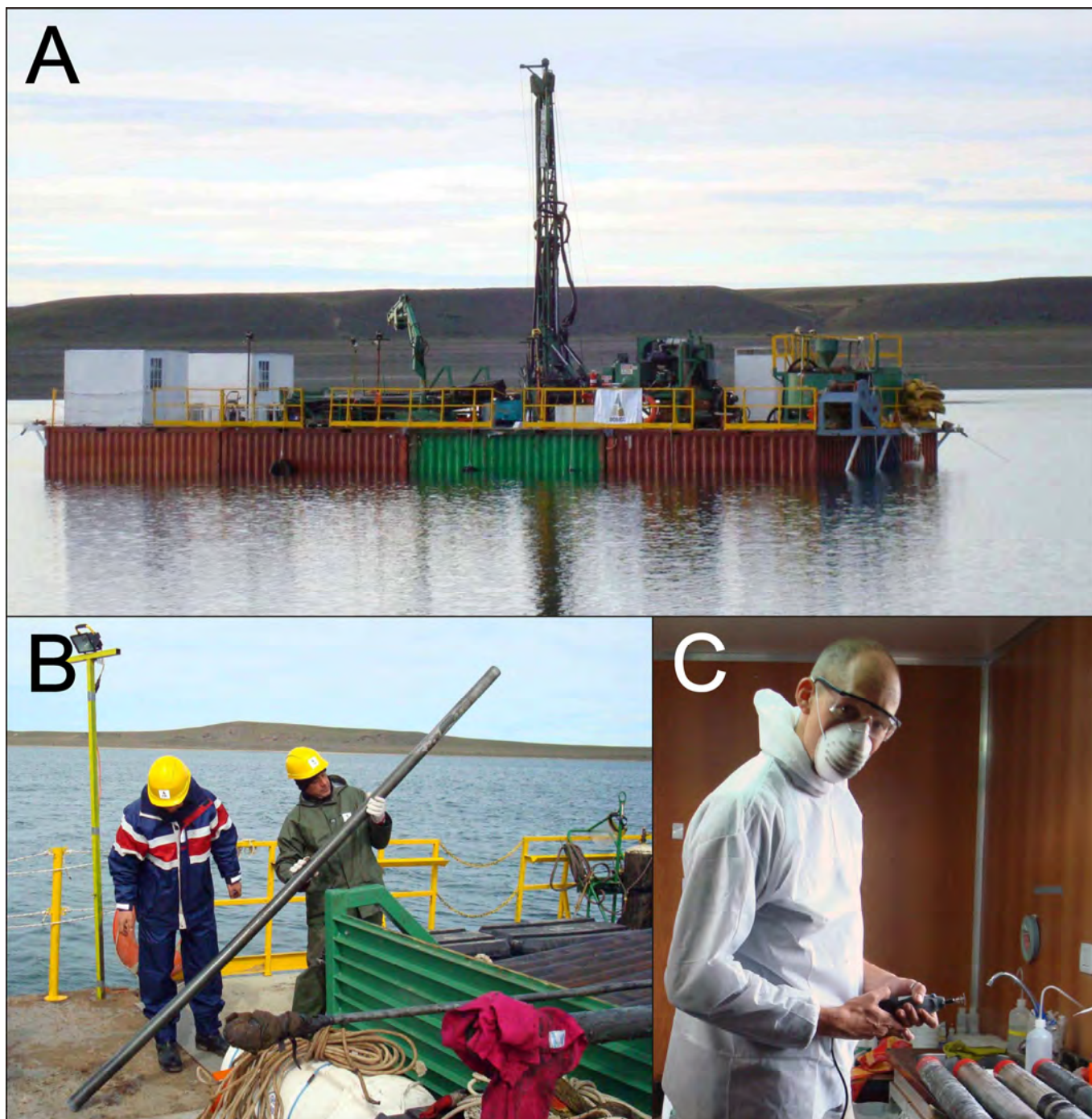
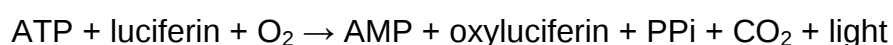


Figure 2 [A] Drilling platform GLAD 800. After retrieval [B], the cores were transported from the platform to the laboratory where they were sampled at once [C].

coring. *In situ* adenosine-5'-triphosphate (ATP) measurements were taken as an indication of living organisms within the sediments. The presence of ATP is a marker molecule for metabolically active cells (Bird et al., 2001), since it is not known to form abiotically. ATP can be easily detected with high sensitivity and high specificity using an enzymatic assay (Lee et al., 2010).



ATP is degraded to adenosine monophosphate (AMP) and pyrophosphate (PPi) while luciferin is being oxidized. Light is emitted as a result of the reaction, which is detected by a photomultiplier. We used the Uni-Lite® NG Luminometer (Biotrace International Plc, Bridgend, U.K.) in combination with the “Clean-Trace” and “Aqua-Trace” swab kits (3M, U.S., Fig. 3E).

The sensitivity of the test is on the order of 10^{-20} moles of ATP per ml of water, corresponding to a standard of 5 cells of *Escherichia coli* as expressed in RLU (relative luminescence units). This handheld device was previously tested at the Geomicrobiology Laboratory, ETH Zurich (Switzerland), where it was determined that this method could be applied on geological material such as rock surfaces and other environmental biofilms. It was also successfully used for fast and accurate measurement of life activity for freshly retrieved cores in lithified sediments of the IODP Expedition 310 in Tahiti (Camoin et al., 2007). The performance of this instrument in fresh sediments was however uncertain, and to our knowledge this is the first time that it was successfully applied to lacustrine sediments. Additionally, the application of this test to water samples can aid in the evaluation of the degree of contamination of the drilling water, which percolates along the inside of the core liner.

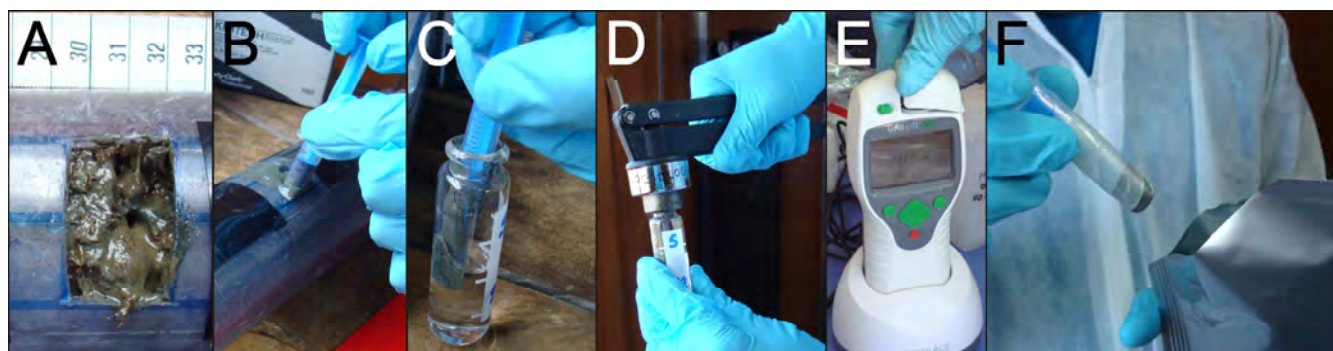


Figure 3 [A] Window cut for sampling; [B-D] sampling for methane headspace determinations; [E] preparation of the sample for *in situ* ATP measurements: sample is mixed with deionized water prior to centrifugation, then tested with the Uni-Lite® NG water tester (shown); and [F] storage of the remaining sediment for cell culture. Refer to text for details.

Figures 3A-3F summarize the sequence and sampling procedures established in this project. Part of the sampling required precise volumes that were obtained using sterile syringes. Thus, samples of 3 mL and 5 mL of sediment were extracted from freshly opened windows using these syringes whose narrow tips were cut off in order to collect “minicores” (Fig. 3B). The first extracted sample was designated for methane analyses because of its immediate release into the environment due to volume expansion when exposed to ambient pressure. Hence, a portion (3 mL) of this first sample was chemically stabilized using 10 mL of 2.5% sodium hydroxide, and then sealed in vials for headspace analysis (Fig. 3C and D). The sediments were further sampled for different techniques using 5-mL syringes and portioned out as follows: the first 1-mL portion of sample was placed in an Eppendorf tube and kept frozen for further DNA extraction; a second 1-mL portion was chemically fixed in formaldehyde (final concentration, 2%) for DAPI (4',6-diamidino-2-phenylindole) cell count; a third 1-mL portion of the sediment was mixed with 1-mL of deionized water in an Eppendorf tube and centrifuged for five minutes. Commercially available water testers (Biotrace International) were carefully submerged in the supernatant, and its ATP content was measured with the Uni-Lite® NG luminometer as an index of *in situ* microbial activity (Fig. 3E). The remaining sediment in the syringe was coated with plastic foil and hermetically sealed into aluminum foil bags (Fig. 3F). These bags were flushed with nitrogen (to prevent oxidation) prior to sealing with a heating device. These samples can be further used for microbial culture experiments back at the home laboratory. Once the sampling was accomplished, the windows were sealed with strong adhesive tape. This sampling procedure was carried out non-stop over a 48-hour period. A comparable sampling procedure for marine sediments can be found by Bird et al. (2001).

Assessing *in situ* Microbial Activity in Sediments

The presence of nutrients as energy sources is critical, promoting an active behavior of the inner microbial communities within sediments. When certain nutrient concentrations are below a threshold, microbial metabolism and population density are lowered progressively as these microbial communities enter in dormant state. Thus, microbial communities installed in deep sediments can be considered as mainly oligotroph and dormant.

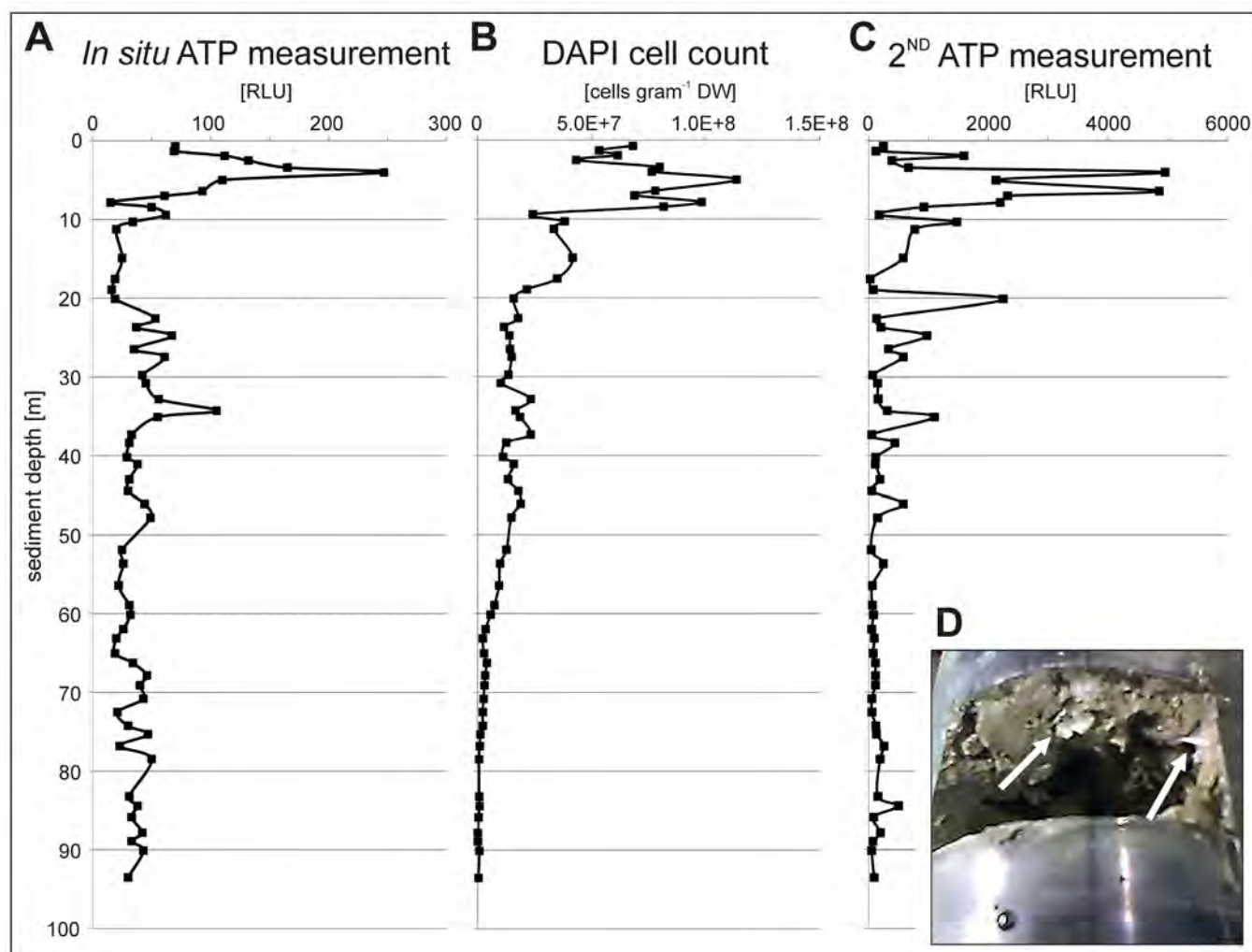


Figure 4 [A] The first ATP measurements were taken in an average of an hour and a half after each core recovery, and are considered as excellent indicators of *in situ* microbial activity. Noise was measured around 30 RLU (relative luminescence unit); [B] DAPI cell count provides a quantification of DNA present in the same samples; [C] second ATP measurements performed ten months later to test for eventual shifts in microbial activity. Although ATP indexes of active layers increased up to 20-fold, the originally nutrient depleted layers remained inactive. Insert [D] shows a picture of mold (white arrows) developed after exposure of the sediments to oxygen and PT ambient conditions. This partially caused the increased ATP values for the second run of measurements.

The 97-m-long sediment core retrieved from Laguna Potrok Aike provided us the opportunity to identify a transition from a weak but active to a dormant state of microbial communities as reflected by *in situ* ATP measurements (Fig. 4A). These results were further compared with those from DAPI counting on the fixed samples carried out several months later in the laboratory (Fig. 4B). The DAPI fluorochrome dyes DNA without distinction—active, dormant, and dead cells, either eukaryote or prokaryote—and it is considered as a semi-quantitative index of cell density within the sediment. ATP and DAPI datasets, however, show an

increasing trend from the sediment surface to ~6-m depth within sediments mainly composed of black mud and subject to gas expansion. The DAPI and ATP trends throughout depth suggest an exponential decrease in microbial activity that is most probably linked to a progressive compaction and gradual nutrient depletion within the sediments. There is, however, detectable microbial activity down to 40–50 m and recoverable DNA down to 60 m sediment depth.

The sediments recovered from Laguna Potrok Aike are dominantly argillaceous but are occasionally interrupted by coarser sandy layers associated to slumps triggered by erosional and/or volcanic activities (Zolitschka et al., 2009). The latter are very important since allochthonous organic matter is harder to degrade, and microbial preservation is highly dependent on grain size. Different sediment features further constrain microbial activity, as they provide colonization niches. Although microbial communities may adapt to trophic changes by shifting either their activity and/or dominant species, they are still highly representative of the lake catchment and their dominating climate. Ongoing multiproxy analyses of these cores will allow characterizing the sedimentary sequence and provide the critical grounds to interpret the results of the observed microbial behavior.

Validating *in situ* ATP Measurements

Metabolic microbial activity can change drastically when exposed to ambient temperature and pressure, light, and oxygen. In order to identify and possibly quantify the magnitude of these metabolic changes, a second set of ATP measurements was produced ten months after cores were retrieved (Fig. 4C). Both results indicate very similar distributions of microbial activity displaying the highest values at the same depths. In spite of the liner disinfection and the sealing of the sampling windows, mold had grown superficially on some windows, as shown in Fig. 4D. The development of mesophilic aerobic microorganisms explains the comparatively higher ATP index of this second dataset. These measurements warn about the omnipresent risks of contamination during sampling and further storage of the samples. They secondarily provide information about the nutrient resources of the sediments and its accessibility and use by microbes. Thus, this comparison between *in situ* and later ATP measurements highlights the relevance of the immediate measurement of microbiological living activity in the field. The comparison presented here between ATP values quickly obtained with a handset device further validates those *in situ* results produced by more established and tedious analyses such as DAPI cell counting of microbial cells.

Future Improvements in Detecting the Living Biosphere in Lake Sediments

Lacustrine systems gather widely diverse water types such as brackish (Banning et al., 2005), acidic (Chan et al., 2002), hypersaline (Cytryn et al., 2000), or alkaline (Jones et al., 1998), among others. Each of them contains very different sediment and associated microbial assemblages. Understanding trophic states within the water columns and the sediments is essential to reconstructing past climates (Nelson et al., 2007) as well as to managing anthropogenic impact on modern lakes (Ye et al., 2009).

The assessment of microbial activity presented here provides information on various ongoing organic matter mineralization processes in the sediments and helps to understand the influence of microbes during early diagenesis. Our procedure can be easily applied as routine,

adding valuable microbiological information that is complementary and relevant to several standard lacustrine proxies such as the stable isotope composition of authigenic carbonates and organic matter. Thus, the Uni-Lite[®] NG ATP tester is an excellent alternative to previously proposed complex ATP extractions (Stoeck et al., 2000; Bird et al., 2001; Nakamura and Takaya, 2003).

We are confident that the sampling protocol proposed here will allow scientists to sample cores in other ICDP projects with minimal contamination risks. It further points towards new research avenues and technical developments to better detect microbial activity and metabolic functions of the subsurface lacustrine biosphere.

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CHAPTER 3

Surficial sediments

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

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 by 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.

1. Introduction

Microbial activity in both water column and most recent sediments is one of the dominant factors ruling organic matter reworking (Meyers, 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. Δ Gibbs 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 & Middelburg, 2002) by

forming layered-type communities (Nealson & 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 postdepositional microbial activity (Meyers, 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 paleoclimatic studies to identify organic substrates 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 multiproxy 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 geomicrobiological 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-phénylindole) 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 & Smalla, 1998; Schäfer & 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; Mayr et al., 2007a; Habberzettl 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., 2012).

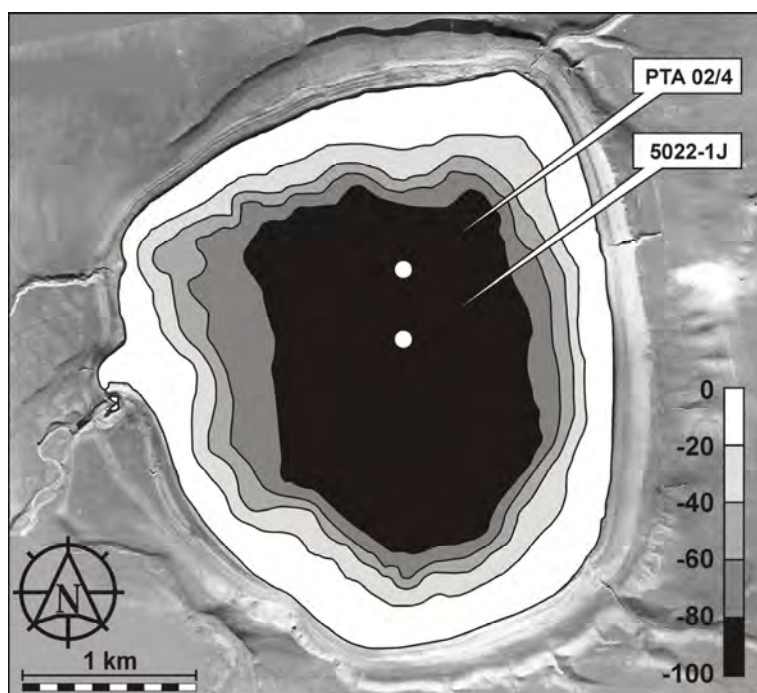


Figure 1 Bathymetric map of Laguna Potrok Aike (modified after Zolitschka et al., 2006) showing the positions of the two gravity cores studied in this paper.

A multiproxy record based on five different cores retrieved through the PASADO project has established lake level fluctuations and provides a paleoclimatic reconstruction for the Late Pleistocene and the Holocene (Habberzettl 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 Habberzettl et al. (2007b) indicated prevalent oxic to suboxic conditions at the water/sediment interphase. Only a direct monitoring of this parameter will provide the necessary dataset to clarify this question.

Table 1 Mean surface water values of pH, temperature, salinity, and nitrates, sulfates, iron and manganese concentrations (from Zolitschka et al. 2006; Mayr et al. 2007). Ion concentrations are determinant for microbial anaerobic respiration pathways.

T°	pH	Salinity	NO ₃ ⁻	SO ₄ ²⁻	Fe	Mn
4-12 (°C)	8.8	2.31 (g L ⁻¹)	46.15 (μM)	279.86 (μM)	0.29 (μM)	0.04 (μM)

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 location from the deep central basin of Laguna Potrok Aike (Fig. 1, Habberzettl 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. 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 field 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.

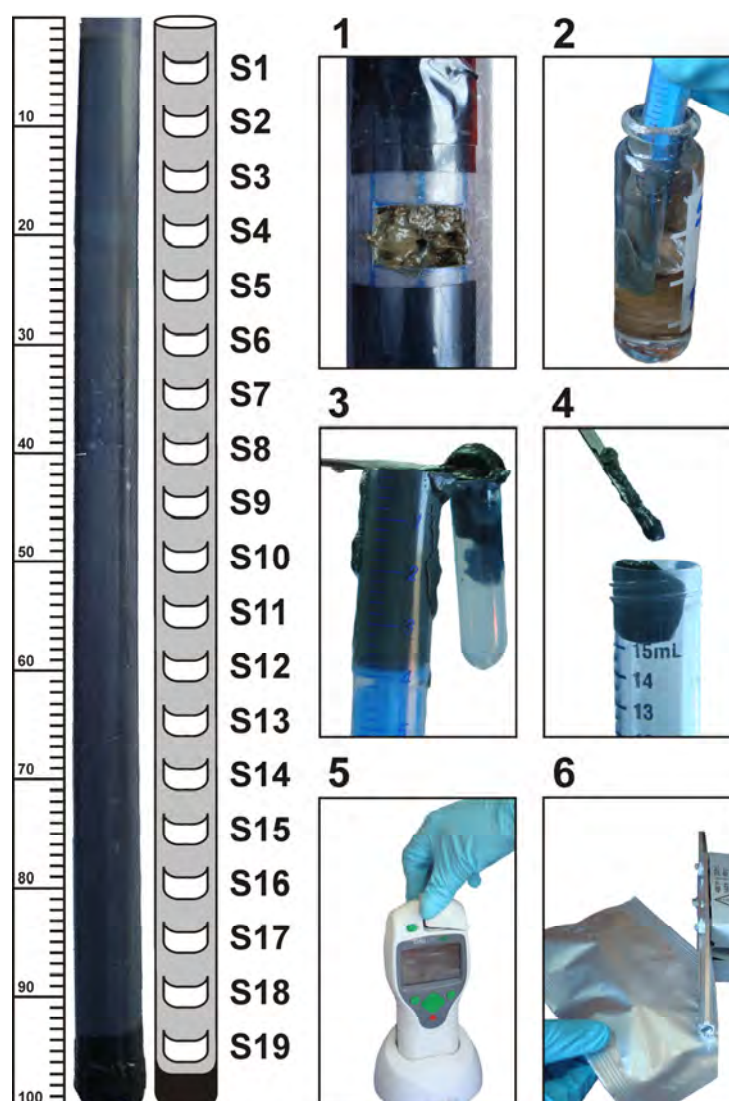


Figure 2 The 1 m long gravity core (5022-1J) retrieved from Laguna Potrok Aike and the liner scheme showing 19 windows that were precut every 5 cm (samples S1 to S19 from top to bottom, left). The sequence of photographs 1 to 6 (right) shows the sampling procedure: **1.** Window aperture; **2.** Methane headspace; **3.** DNA extraction; **4.** Cell count; **5.** *in situ* ATP measurement with a Uni-Lite NG luminometer; and **6.** Syringe storage in a hermetic bag flushed with N₂.

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 minutes 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 & 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 a 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 sec whereas methane spikes were used to calibrate and transform detection intensities (pA) into volumes. Traces of nitrous and sulfidic 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 to 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 hours. The remaining slurries were diluted in distilled water, centrifugated, 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 (1N), sonicated overnight and finally centrifuged at 4000 t/min for 20 min. Samples for IP were mixed with 5 ml HCl (1N), 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 (1N) overnight, sonicated and centrifuged. Samples for NAIP were mixed with 5 ml NaOH (1N), sonicated overnight and centrifuged. Aliquots of 2.5 ml were removed and 1 ml HCl (3.5N) 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 remnant sediments rinsed with 3 ml NaCl (1N) for AP extractions, subsequently mixed with 5 ml HCl (1N), 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 µm with two different spectrophotometers: A Digitana Spectronic 1201, and a Perkin Elmer U-VIS λ25. Accuracy of the method ranges between 5 to 10 % when applied to homogeneous sediments.

For enumeration of microorganisms (Bacteria and Archaea), 1 ml of sediment per sample was fixed in the field laboratory with formaldehyde (final concentration: 2%) for subsequent DAPI staining (4', 6-diamidino-2-phenylindole). The sample slurries were later diluted in 10 ml 1× hexametaphosphate to desorb cells from clays. After centrifugation, aliquots of 2 ml were separated in sterile eppendorf tubes and centrifugated. The supernatant was discarded and aliquots were rinsed twice with 1× PBS and centrifugated. 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 afterwards with 2 ml 1× PBS. Filters were left to dry and were then mounted on smear slides with a non-fluorescent immersion oil (Leica n_e²³ = 1.518, v_e = 46) and examined under epifluorescent microscopy. 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 µmol/L of each of the primers, 200 µmol/L of each of the

desoxynucleotide triphosphates, 1.25 units Ex-Taq polymerase (Takara) in a 50- μ l PCR reaction mixture with molecular grade water. Negative controls were added to all PCR sets with 1 μ l 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'-GGT TAC CTT GTT ACG ACT T-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'-TCY GGT TGA TCC TGC CRG-3') with Univ1492R (5'-CGG TTA CCT TGT TAC GAC TT-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 μ l of PCR product were subsequently used in the second PCR round with the overlapping forward primer 3F (5'-TTC CGG TTG ATC CTG CCG GA-3') associated with 9R (5'-CCC GCC AAT TCC TTT AAG TTT C-3') as the reverse primer.

A final nested PCR round was then performed on both bacterial and archaeal products to fix the GC clam (5'- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG 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 357F-GC (GC clam + 5'-CCT ACG GGA GGC AGC AG-3') with 518R (5'-ATT ACG GCG GCT GCT GG-3 ') were used for Bacteria and A344F-GC (GC clam + 5'-ACG GGG AGC AGC AGG CGC GA -3') with W31 (5'-TTA CCG CGC TGC TGG CAC-3') for Archaea. Nested PCR rounds were performed with 2 μ l 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 μ l PCR product with 7 μ l blue stain were loaded for each sample. Standards, made of 5 μ l PCR product from 11 bands picked from a previous DGGE gel and 5 μ l 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 $\times$ TAE buffer. After electrophoresis, polyacrylamide gels were stained with 2 μ l 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^R software (BioNumerics). Calculated indexes included Pearson correlation with a likelihood function, Shannon-Weaver index and range-weighted richness (Fromin et al., 2002; Marzoratti 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). Figure 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

Figure 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.

Figure 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

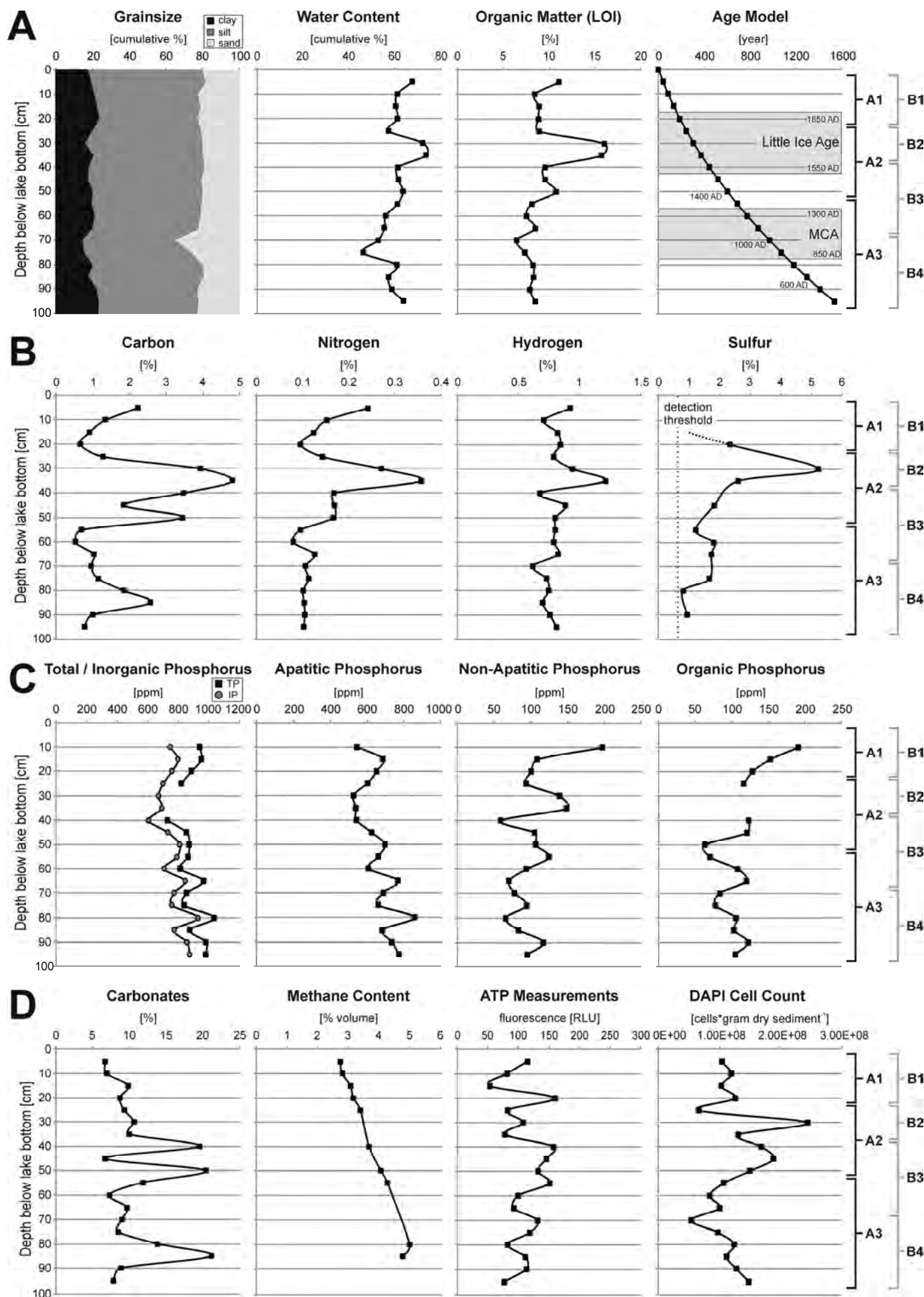


Figure 3 Multiproxy results based on standard and geomicrobiological techniques. Cluster units, discussed in section 4.4, are marked here for reference. **A.** Grainsize, water content and loss on ignition (LOI) throughout depth, and sediment age model calculated on a binomial regression curve based on Haberzettl et al. (2005). **B.** Total carbon, nitrogen, hydrogen and sulfur of bulk sediment samples. Since the S content is very low and close to the equipment detection limit, these values are only considered as semi-quantitative. **C.** Total phosphorus (TP) and inorganic phosphorus (IP), apatitic inorganic phosphorus (AP), non-apatitic inorganic phosphorus (NAIP), and organic phosphorus (OP) in bulk sediment samples. Samples S1, S6 and S7 could not or only partially be measured due to the small volume of available sediment samples. **D.** Total carbonate content; methane content in percentage based on the initial volume of sample (3 cm³); *in situ* ATP measurements in relative luminescence units [RLU]; and DAPI cell counts in cells per gram of dry sediment.

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 to 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 (Hecky et al., 1993; Arrigo, 2005).

4.3 Products of biological activity

Figure 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 RLU to 158 RLU 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 (Haglung et al.,

2003; Manini & 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.

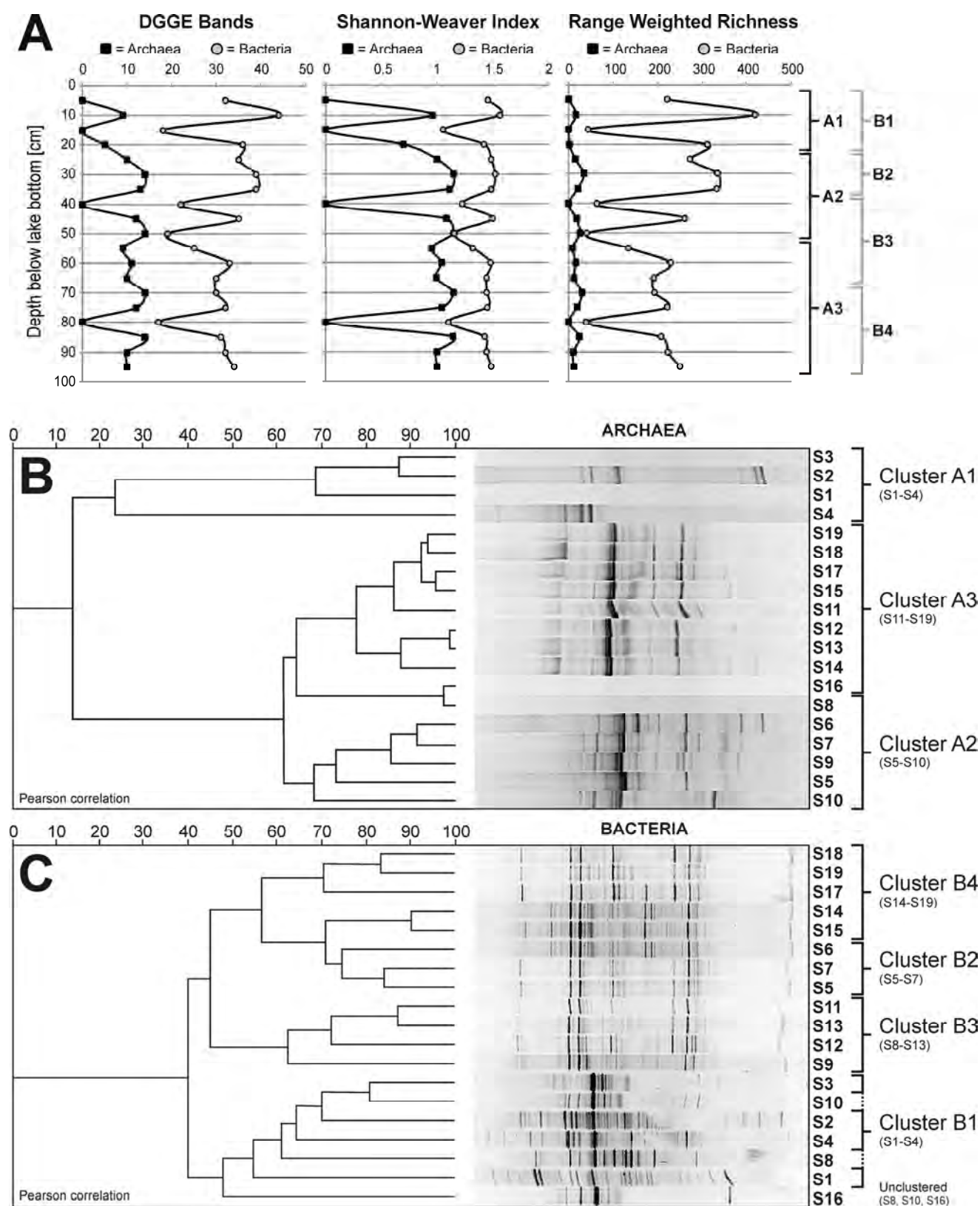


Figure 4: Statistical analyses of DGGE profiles **A.** Variations of the denaturing gradient gel electrophoresis (DGGE) throughout depth for both Bacteria and Archaea. Statistical indexes emphasize the diversity of every sample assemblage with depth. From left to right: Number of bands, Shannon-Weaver index of diversity (H') and range weighted richness (R_r). **B.** and **C.** Dendrograms for Archaea and Bacteria, calculated on the basis of the Pearson correlation coefficient. For both dendrograms, samples from the uppermost part of the core display more diverse and contrasted patterns, while the ones from the underlying samples are more constant.

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 to 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 towards 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.

Figures 4B and C show the results of cluster analyses and dendrograms that have been separately performed for Archaea and Bacteria. 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 and Bacteria band patterns were separated into three and four 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. Figure 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

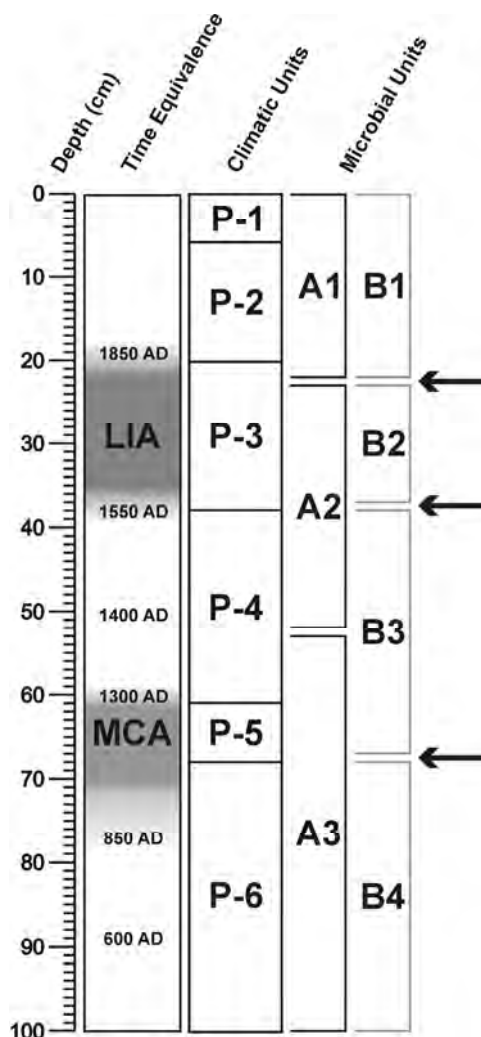


Figure 5 Comparison between cluster analyses of climatic intervals, derived from paleoclimatic proxies (Haberzettl et al., 2005), and Archaea/Bacteria units, derived from geomicrobiological proxies. Arrows indicate concordant limits between climatic units and microbial units and underline the importance of specific climatic periods.

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 to 40 cm, carbon and nitrogen would be adsorbed while bio-available 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 a short half-life of phosphorus within

the sediment, as P adsorbed on $\text{Fe}(\text{OH})_3$ tends to be released by the precipitation of iron sulfides. The increased NAIP content visible in the interval between 20 and 40 cm depth can be linked to such adsorption onto $\text{Fe}(\text{OH})_3$ 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 CO_2 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 & 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 & Nierzwicki-Bauer, 1997; Nealson & Stahl, 1997). Fig. 4C shows intervals for Bacteria clusters 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 CO_2 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 constant increase

in methane content, possibly produced by « *Methanosarcinales* » that is a common order known in freshwater environments that uses methylated substrates as well as formate, H_2/CO_2 and even acetate (Garcia et al., 2000). However, in 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 methylated substrates, which is a non-competitive substrate compared to carbonate reduction and acetate fermentation (Barns & 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 CO_2 reduction transition as mentioned above. This latter process would further lead to syntrophy between fermenters and H_2 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 CO_2 reducers once methylated substrates have been depleted. A slight decrease in methane content can already be noticed below 80 cm depth. At this point, CO_2 reducers are more difficult to track than the ones using other oxidants, because the reduced forms of CO_2 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 acetoclastic 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 & Madigan, 2007). These processes are interesting since they can be the source of much deeper lacustrine microbial activity via acetogenesis (Sattley & Madigan, 2007), acetoclastic 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 & Jetten, 2004) are probably also taking place, but until now putative.

All the processes described above point towards 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 geomicrobiological evidence points towards 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., this issue). 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 & Koukoulas, 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. Gastropod 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 & 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 timespan 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 three meters 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 modifying 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 & 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 geomicrobiological 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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CHAPTER 4

Sustainability of microbial activity

A modified version of this chapter is under review as
Vuillemin et al. in Aquatic Sciences

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

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. 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 in order to assess their impact in diagenetic processes.

1. 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 much 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, 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), content in organic matter (OM; 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_{org}) content, C_{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 an 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 disentangling 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.

2. 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 (Gebhardt et al. 2012; Mayr et al. 2009; 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 NO_3^- contents restrict primary productivity within the water column. Due to the semi-arid climatic conditions (site annual precipitation: 200 to 300 mm), limited and sporadic terrestrial inflows occur, further reducing nutrient input to the Laguna Potrok Aike basin.

3. Methods

3.1 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 (Ohlendorf et al. 2011). They were retrieved from the centre of the maar at 100 m water depth (see annex A-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 analyses (Vuillemin et al. 2010). After retrieval, the core sections were shuttled every two hours 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 × 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: i) 1 ml sediment was transferred to an Eppendorf tube and immediately frozen at -10°C for DNA extraction, ii) a second 1 ml volume of sediment was fixed in 2 ml of formaldehyde (3%) for 4'-6-diamidino-2-phenylindole (DAPI) cell count, and iii) an additional 1 ml sediment sample was mixed with 1 ml micropure H₂O and centrifuged for five minutes 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°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® soil moisture samplers (Eijkelkamp®) 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 N₂ gas.

3.2 Laboratory analyses

The same methods as in Vuillemin et al. (2013) were used for grain size analyses, 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 analyses and samples for cation analyses were acidified with 100 µl HNO₃ (65 %). Cation concentrations in pore water (i.e. Na⁺, K⁺, Mg²⁺, Ca²⁺, Si⁴⁺, Fe²⁺) were determined by inductively coupled plasma mass spectrometry (ICP-MS), and anions (i.e. Cl⁻, SO₄²⁻, PO₄³⁻, NO₂⁻/NO₃⁻) by ion-chromatography.

Sediment samples for carbon isotopic compositions were treated for decalcification with HCl (5%) at 50°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 organic matter were analyzed from homogenized decalcified ($\delta^{13}\text{C}_{\text{org}}$) or untreated ($\delta^{15}\text{N}$) samples, using an elemental analyzer (EuroVector®, Euro EA®) linked to a continuous flow isotope-ratio mass spectrometer (Micromass, Isoprime®). Isotope ratios are reported in δ -notation in per mil according to the following equation: $\delta = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$, 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 σ). Total organic carbon (TOC) and total nitrogen (TN) were calculated from the yield of CO_2 and N_2 after sample combustion in the elemental analyzer. Analytical precision was ± 3 % (1 σ) for C_{org} and ± 2 % (1 σ) 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®). 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 H_2O 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^R 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: $R_r = (N^2 \times D_g)$, where N represents the total number of bands in the pattern, 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 annex A-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 (see annex A-3). 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).

4. Results

4.1 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 to 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 to 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 to 12 ka BP), the lake shifts toward subsaline conditions as shown by the chloride concentrations increasing from 120 ppm 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. 1500, 1200 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 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, 2384 ppm for total phosphorus (TP) and 26.9 ppm for sulfate (Zolitschka et al. 2006), thus corresponding to N-limiting subsaline conditions.

4.2 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_{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_{org}/N ratio rises from 4 to 10 during the Late Glacial period, and fluctuates between 6

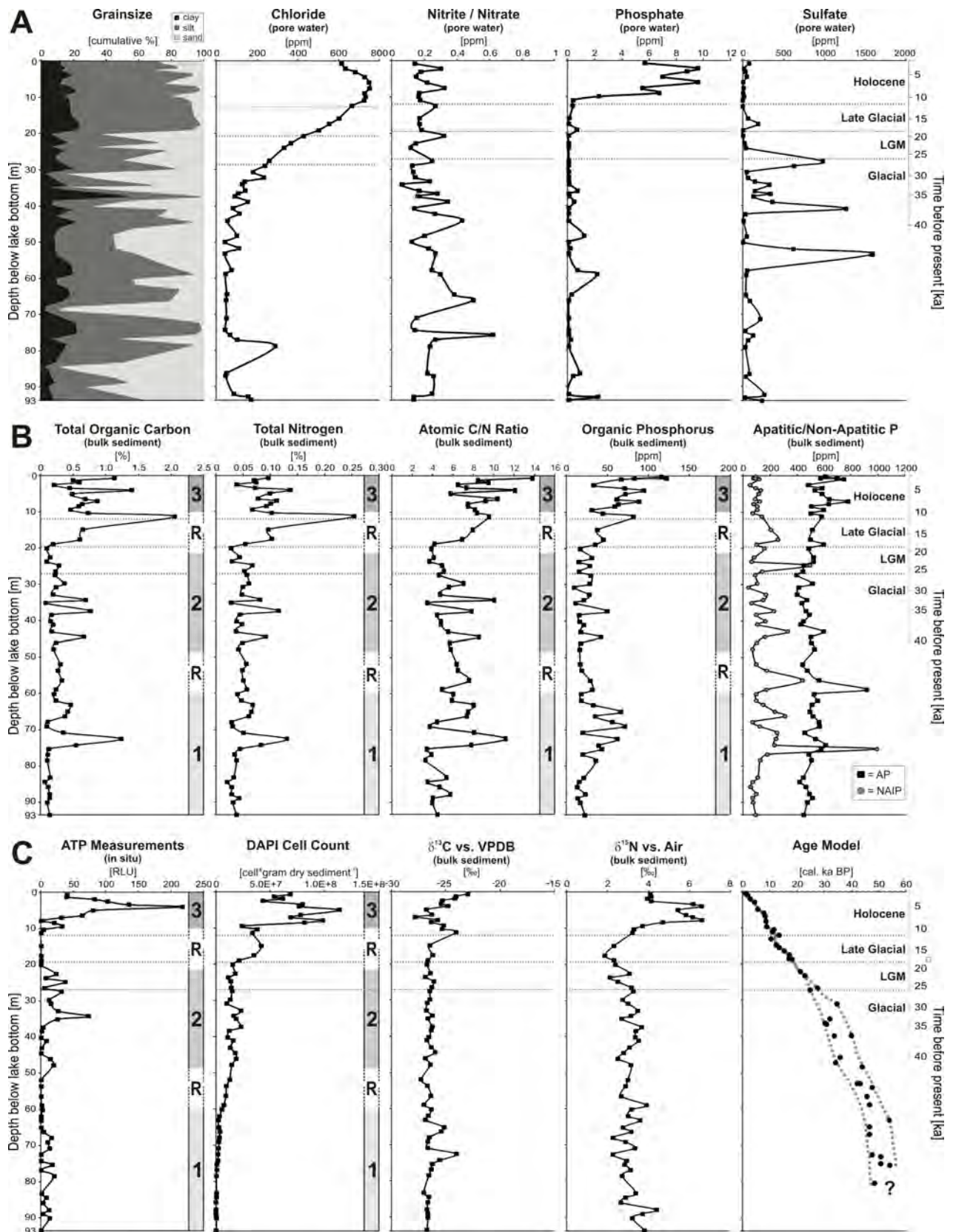


Figure 1 Geochemical and microbiological proxies of core 5022-1D along with pore water chemistry from core 5022-1A. **A)** From left to right: Grain size; chloride; nitrite and nitrate; phosphate; sulfate. **B)** From left to right: Total organic carbon; total nitrogen; molar C_{org}/N ratio; apatitic phosphorus (AP) and non-apatitic inorganic phosphorus (NAIP); organic phosphorus (OP) [ppm]. **C)** From left to right: In situ ATP measurements in relative luminescence units; 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 main lake level drops.

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 to 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 ‰ 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 ‰ at 11 to 7 m depth) is followed by a gradual increase, reaching the maximal value (-23.0 ‰) in the uppermost part of the record. The $\delta^{15}\text{N}$ values average 3.0 ‰, 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 ‰ at 8 and 4 m depth, where they decrease sharply to 4 ‰ 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.

4.3 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 to 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 to 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 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 annex A-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.

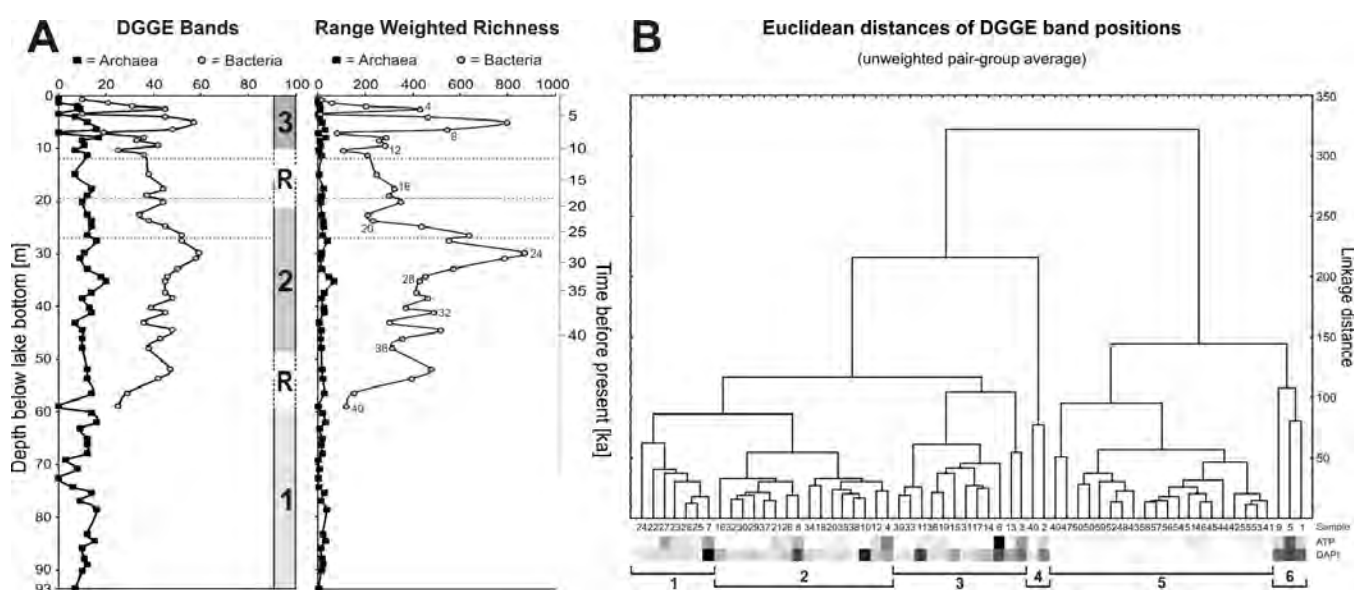


Figure 2 Statistical indices obtained from DGGE gel features. **A)** Number of DGGE bands for Archaea (black squares) and Bacteria (grey dots); range weighted richness. **B)** Euclidean distance clustering tree for DGGE band positions. ATP and DAPI values are indicated in grayscale at the base of the tree. Shaded intervals numbered from 1 to 3 refer to microbial groups defined in Fig. 3A.

The Euclidian distance tree based on DGGE band positions of each sample gel (Fig. 2B; A-2) is presently used to infer ecological distances between populations (Marzorati et al. 2008). It allows the distinction of 6 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.

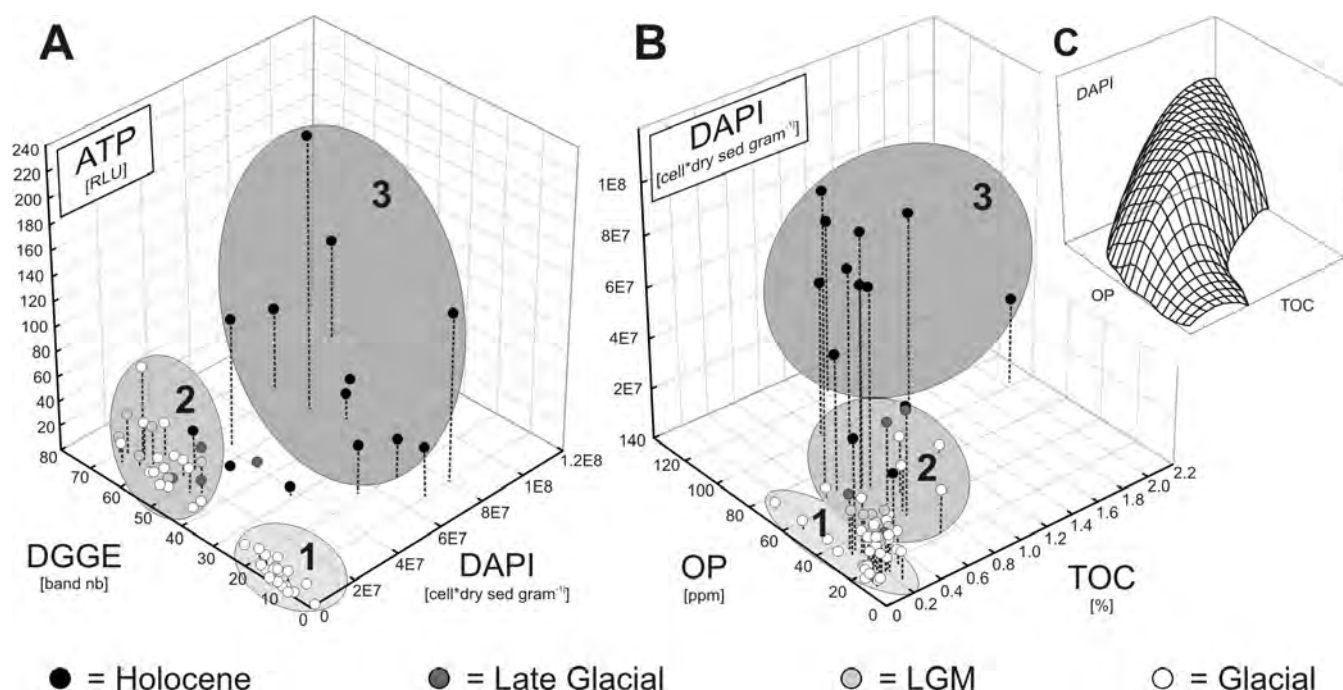


Figure 3 3D scatterplots displaying microbial characteristics altogether and microbial density in function of organic content. **A)** Scatterplot for DAPI, DGGE and ATP used to indicate the relationship between microbial activity, density and diversity. **B)** Scatterplot for TOC, OP and DAPI highlighting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

Two projection plots based on four principal components (PC) with eigenvalues of 40.97, 16.46, 12.31 and 9.49 %, were established (Figs. 4A and 4B). 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 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.

5. Discussion

5.1 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 (Figs. 1A and 1B) 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 (Figs. 1A and 1B). 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 and 3A).

5.2 Paleoenvironmental conditions and sediment 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 et al. 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, 1C and 2). Another important regression phase occurred during the Early mid-Holocene (ca. 8.7 to 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 to 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 and 2). Both intervals coincide with periods of positive temperature excursions in South Patagonia (Kilian and Lamy 2012) during Glacial (ca. 50 to 30 ka BP) and Holocene times (ca. 12.5 to 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 to 38, 16 to 12 and 9 to 7 ka ago; Zolitschka et al. 2013) correspond with a microbial colonization restricted by redeposition events. Periods of lake level rise (ca. 38 to 33 and 7 to 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 (Figs. 4A and 4B). The first two PCs (Fig. 4A) show that samples can be clearly separated 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, survival and inert behaviors appears as groups defined by microbial indices (Figs. 3A and 3C).

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.

5.3 Microbial organization and sustainability

In the sediments, chemoheterotrophs are mainly dependent on nutrient 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).

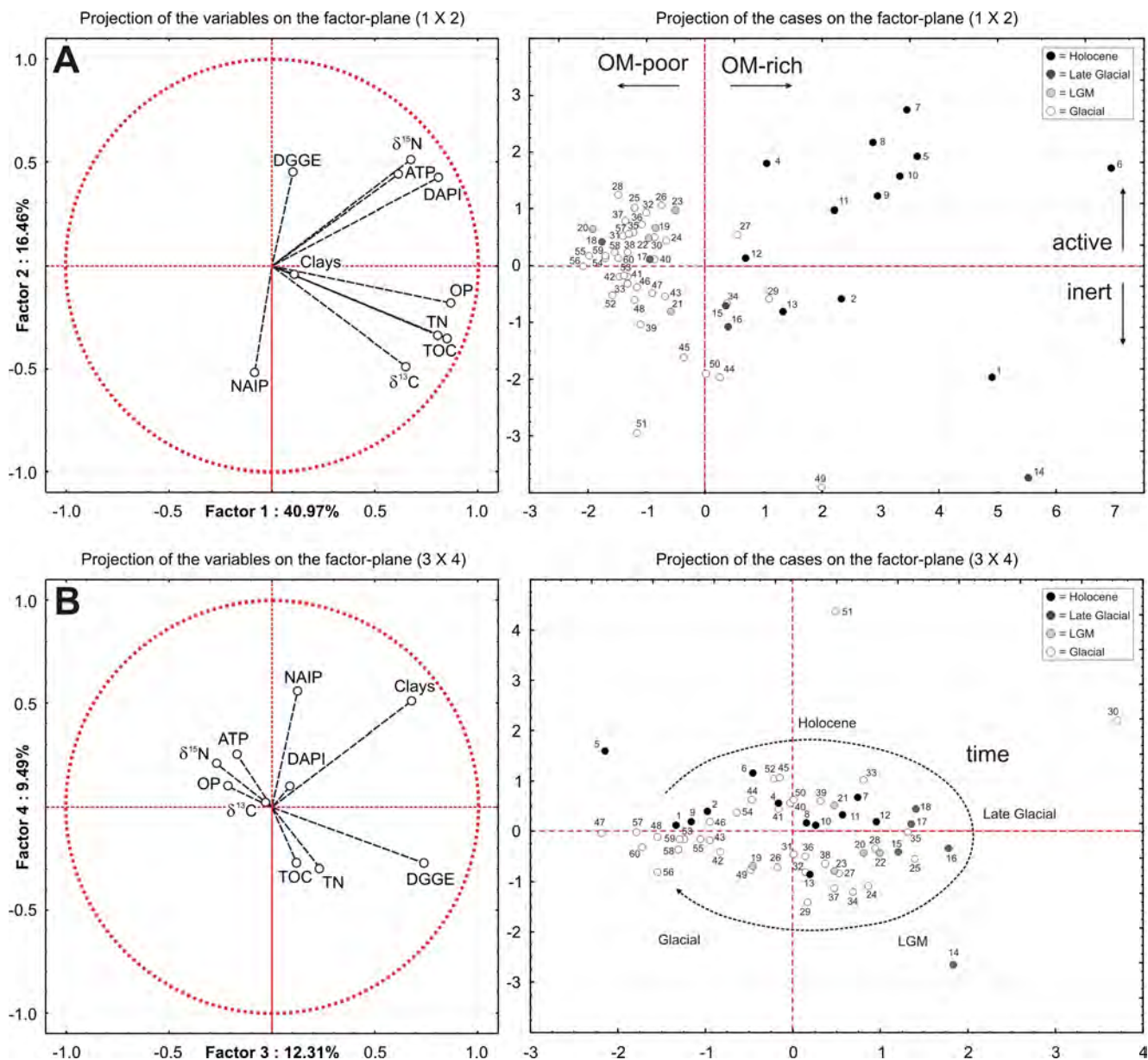


Figure 4 Principal Components Analysis (PCA) projection plots of variables (left) and cases (right). A: Factors 1 and 2. B: Factors 3 and 4.

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 (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, CO_2 is largely available as a byproduct of fermentation and can be used in 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 C_{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 and 3A) indicate living microbes in these Glacial sediments (ca. 30 m depth), even if phosphate is not readily available in pore water (Figs. 1A and 1C). Thus, the present deep lacustrine sediments support long-term microbial activity.

5.4 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 and 3B). Moreover, microbial biomass appears proportional to the sediment organic content (Figs. 3B and 3C). 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), $\delta^{15}N$ and $\delta^{13}C_{org}$ opposite trends observed along the Holocene record (Fig. 1C) were 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; Håkanson 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 sorely colonized by active microbes (Figs. 1C and 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 $\delta^{13}C_{org}$ and $\delta^{15}N$ 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 $\delta^{15}N$ fractionation of the substrate toward positive values (Hoch et al. 1992; Casciotti 2009).

In parallel, refractory OM, which is often enriched in ^{12}C , accumulate during diagenesis (Boschker and Middelburg 2002; Lehmann et al. 2002). Humic leftovers mainly contribute to preserving TOC, $\delta^{13}C$ 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}C and ^{15}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 and 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 %).

6. Conclusions

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 C_{org} , which is rarely a limiting factor in the sediment, appeared less affected. The accumulation of a microbial biomass enriched in ^{12}C and ^{15}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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CHAPTER 5

Searching for biosignatures in deep sediments

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Origin and significance of diagenetic concretions in sediments of Laguna Potrok Aike, southern Patagonia, Argentina

Abstract

Authigenic minerals can form in the water column and sediments of lakes, either abiotically or mediated by biological activity. Such minerals have been used as paleosalinity and paleoproductivity indicators and reflect trophic state and early diagenetic conditions. They are also considered potential indicators of past and perhaps ongoing microbial activity within sediments. Authigenic concretions, including vivianite were described in late glacial sediments of Laguna Potrok Aike, a maar lake in southernmost Argentina. Occurrence of iron phosphate implies specific phosphorus sorption behavior and a reducing environment, with methane present. Because organic matter content in these sediments was generally low during glacial times, there must have been alternative sources of phosphorus and biogenic methane. Identifying these sources can help define past trophic state of the lake and diagenetic processes in the sediments.

We used scanning electron microscopy, phosphorus speciation in bulk sediment, pore water analyses, *in situ* ATP measurements, microbial cell counts, and measurements of methane content and its carbon isotope composition ($\delta^{13}\text{C}_{\text{CH}_4}$) to identify components of and processes in the sediment. The multiple approaches indicated that volcanic materials in the catchment are important suppliers of iron, sulfur and phosphorus. These elements influence primary productivity and play a role in microbial metabolism during early diagenesis. Authigenic processes led to the formation of pyrite framboids and revealed sulfate reduction. Anaerobic oxidation of methane and shifts in pore water ion concentration indicated microbial influence with depth. This study documents the presence of active microbes within the sediments and their relationship to changing environmental conditions. It also illustrates the substantial role played by microbes in the formation of Laguna Potrok Aike concretions. Thus, authigenic minerals can be used as biosignatures in these late Pleistocene maar sediments.

1. Introduction

Formation of authigenic minerals in lacustrine systems has been reported in the water column (Böttcher and Lepland 2000), at the sediment-water interface (Manning et al. 1999), and within sediments (Berner 1981). Such mineral formation can occur as a consequence of saturated ion concentrations (Wilkin and Arthur 2001) and/or early diagenetic processes such as organic matter (OM) decomposition (Berner 1981) and microbial activity (Beveridge et al. 1983). Thus, these minerals poorly reflect their initial sedimentary settings. Indeed, the evolution of reducing conditions in the sediment (Berner 1981) and pore waters (Emerson 1976) is known to control the formation, transformation and preservation of authigenic minerals, as they go through complex stages involving precipitation, amorphous phases (Glasauer et al. 2003) and precursors (Wilkin and Barnes 1997).

In lakes, the formation of iron minerals can be either syngenetic or diagenetic (Wilkin and Barnes 1997; Fagel et al. 2005). Hydrous ferric oxide (HFO) derived from goethite ($\text{FeO}(\text{OH})$) and limonite ($\text{FeO}(\text{OH}) \cdot n\text{H}_2\text{O}$) can be further transformed into diagenetic forms of reduced iron such as magnetite (Fe_3O_4), siderite (FeCO_3) and vivianite ($\text{Fe}^{2+}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) (Zachara

et al. 1998; Konhauser 2007). In freshwater basins, precipitation and dissolution of iron phosphates are important mechanisms that regulate phosphorus concentration in the water column (Nriagu and Dell 1974). Formation of iron phosphates results from phosphorus adsorption and sinking to the sediments (Gächter et al. 1988; Wang et al. 2007), processes that primarily depend on water-column conditions such as salinity, density stratification of the water body, presence of iron oxides (Wilson et al. 2010) and the nature of sedimented clay minerals (Stamatakis and Koukoulas 2001). Phosphorus sorption on HFO often results in the formation of “green rust” which is a main amorphous precursor of iron phosphate minerals (Fredrickson et al. 1998). Nonetheless, reduction of Fe(III) is required to form most of those minerals. In the sediment, where microbes show stronger catalyzing capacity than in the water column (Lovley 1997), dissolved ions (Nriagu 1972), OM mineralization (Emerson 1976) and reactivity linked to nutrient recycling (Anderson et al. 2001) are additional factors that are influenced by microbes and affect authigenic minerals. Especially under eutrophic conditions (Hupfer et al. 1998; Manning et al. 1999), microbial communities (Nealson and Stahl 1997) have been invoked as contributors to early and shallow-burial formation of authigenic minerals. OM decay and microbial activity produce humic and gel-like substances that promote supersaturated conditions in pore waters (Zeliber et al. 1988), resulting in the crystallization of aggregates. Different degrees of hypoxia and various pore water chemistries (e.g. iron, phosphate, sulfide) can be generated by microbial processes, thus leading to specific mineral phases and specific crystal shapes (Postma 1981; Glasauer et al. 2003; Konhauser 2007). For example, in the presence of dissolved Fe(II), methanogenesis and sulfate reduction promote mainly vivianite and pyrite formation. Under reducing conditions, vivianite is the most stable iron phosphate (Emerson 1976; Berner 1981), thus exerting significant control over Fe and P geochemical cycles (Nriagu 1972). Yet production of H₂S via sulfate reduction tends to destabilize and scavenge iron from its structure (Nriagu 1972). Coexistence of pyrite and vivianite in Laguna Potrok Aike concretions requires investigation, as microbial communities commonly develop competitive or synergetic behaviors (Nauhaus et al. 2002). Furthermore, dissimilatory iron-reducing bacteria (DIRB), sulfate-reducing bacteria (SRB) and even methanogens (Zhang et al. 2012) not only oxidize OM while reducing iron (Fredrickson et al. 1998; Zachara et al. 1998), but sometimes alter minerals (Stucki and Kostka 2006) and associated sediment properties (Kostka et al. 1999; Dong et al. 2009).

Previous geomicrobiological studies of Laguna Potrok Aike sediments, southern Argentina, demonstrated the influence of endogenic, layered microbial communities (Nealson and Stahl 1997) on surficial sediments and their ability to recycle nutrients (Vuillemin et al. 2013). The discovery of vivianite concretions in deep, glacial-age sediments (Nuttin et al. 2013) offers the opportunity to infer prevailing bottom-water conditions and inputs to sediments at the time of vivianite formation. It also presents an opportunity to elucidate the possible role of microbes in development of the concretions, during both early diagenesis and following deep burial. Lastly, it provides an opportunity to better define the depths of formation for such minerals and the microbial processes involved in authigenesis. Use of the concretions as paleoindicators or microbial biosignatures can help address aspects of the phosphorus cycle, both in the water column and via microbial activity within the sediments.

This study combines geochemistry, such as chemical separation of different phosphorus forms (i.e. P speciation), total organic carbon (TOC), methane content, carbon isotopes ($\delta^{13}\text{C}_{\text{org}}$, $\delta^{13}\text{C}_{\text{CH}_4}$) and pore water analyses, with microbiological evidence for microbial life, such as *in situ* ATP (adenosine-triphosphate) detection and DAPI (4', 6-diamidino-2-phenylindole) cell

counts. Additionally, scanning electron microscopy (SEM) allowed high magnification imagery of the different mineral phases that were identified using X-ray energy spectroscopy (EDS).

1.1 Site description

Laguna Potrok Aike (52°S/70°W) is a maar lake in the Pali Aike volcanic field of southern Patagonia, Argentina (Zolitschka et al. 2006). Catchment lithology is dominated mainly by mafic volcanics (Fig. 1) whereas carbonates are absent. Today, the lake has a maximum diameter of 3.5 km, an area of 7.74 km² and a maximum water depth of 100 m. Annual precipitation ranges between 200 and 300 mm, reflecting the semi-arid climate of the area. Average wind speed is 7.4 m/s and annual temperature extremes range between 33°C and -16°C. Potrok Aike is one of the few permanent water-filled lakes in the southeastern Patagonian steppe, making its sediment record important for paleoclimate reconstructions (Haberzettl et al. 2007). Its seismic stratigraphy has been extensively studied (Anselmetti et al. 2009; Gebhardt et al. 2011), as have its sediment OM sources (Mayr et al. 2009).

Today the lake is polymictic with a non-stratified water column and oxygenated bottom waters. It displays low productivity because of subsaline conditions and is considered mesotrophic (Zolitschka et al. 2006). Haberzettl et al. (2007) examined bulk element ratios in sediments and suggested that oxic to suboxic conditions have prevailed at the water/sediment interface. Other sediment features, however, such as color and grain size, along with the presence of biogenic methane in very shallow sediments (Vuillemin et al. 2013), point to limited oxygen penetration below the water/sediment interface.

Recently, study of five sediment cores retrieved through the ICDP-sponsored PASADO project enabled inference of past lake-level fluctuations (Kliem et al. 2013; Ohlendorf et al. 2013) and paleoclimate reconstruction for the late Pleistocene and Holocene (Recasens et al. 2012).

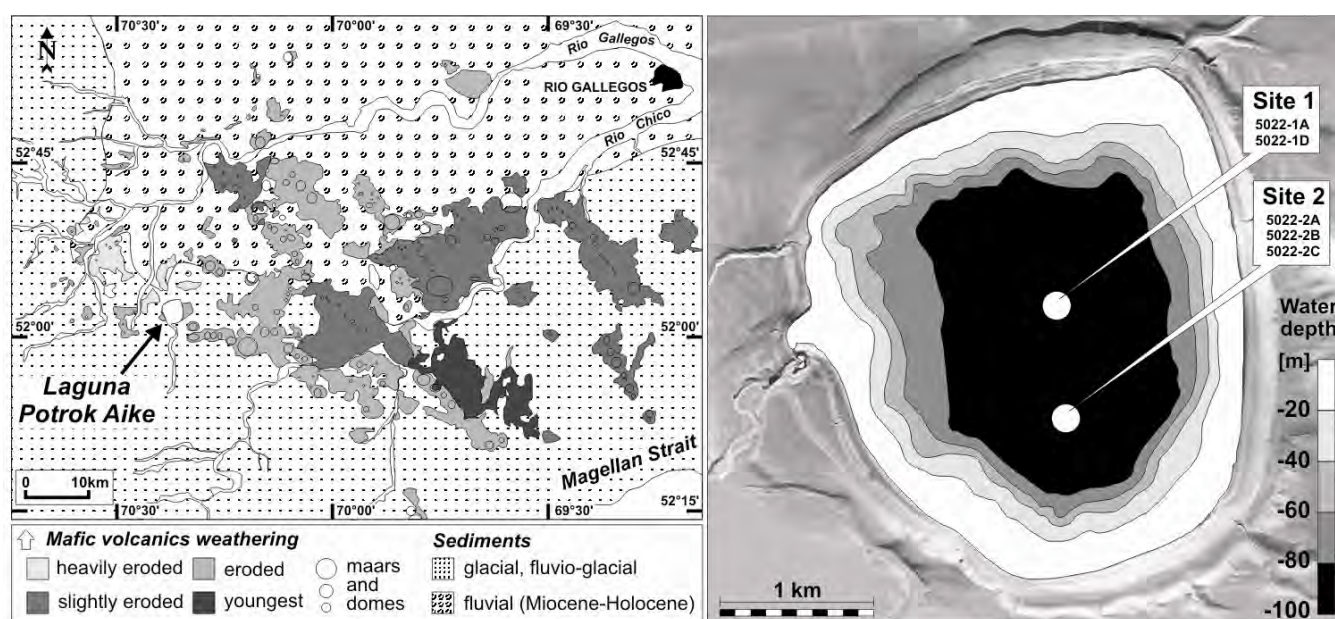


Figure 1 Left: Map of the Pali Aike volcanic field (modified after Ross et al. 2011) displaying the location of Laguna Potrok Aike within its catchment. The catchment area represents some 200 km² (Ohlendorf et al. 2013). Right: Bathymetric map of Laguna Potrok Aike (modified after Zolitschka et al. 2006) showing the two drilling sites and the cores sampled in the present study

2. Materials and methods

2.1 Sampling strategy

For this study, we utilized two hydraulic piston cores retrieved from the center of the lake, at 100 m water depth (Fig. 1). Cores 5022-1A and 5022-1D were 65 mm in diameter, with respective lengths of 87 and 97 m, with a sediment record encompassing at least 55 ka (Kliem et al. 2013). Core 5022-1D was sampled in the field for geomicrobiological studies. A special subsampling protocol was applied to minimize contamination risks and is described in detail elsewhere (Vuillemin et al. 2013). Sampling windows were cut in the core liners to facilitate quick sampling under semi-aseptic conditions in the field laboratory. Specific conditioning was applied to sediments for methane headspace analyses, DAPI cell counts, and *in situ* ATP detection assessment (Vuillemin et al. 2013). Core 5022-1A was sealed and stored at 4°C, and sampled for pore water analyses in Bremen, Germany. Core 5022-1D was resampled for standard bulk analyses. Vivianite concretions (Nuttin et al. 2013) were sampled in three cores from site 2 (Fig. 1). Corresponding depths in cores from site 1 were obtained by correlation using magnetic susceptibility profiles (Recasens et al. 2012). Correlation was confirmed by the presence of similar authigenic minerals at corresponding depths in the core from site 1, documented using digital pictures of opened core 5022-1D (Fig. 2). A complete stratigraphic record of the 5022-1D core and sample depths is available in the annexes (see Annex A-4).

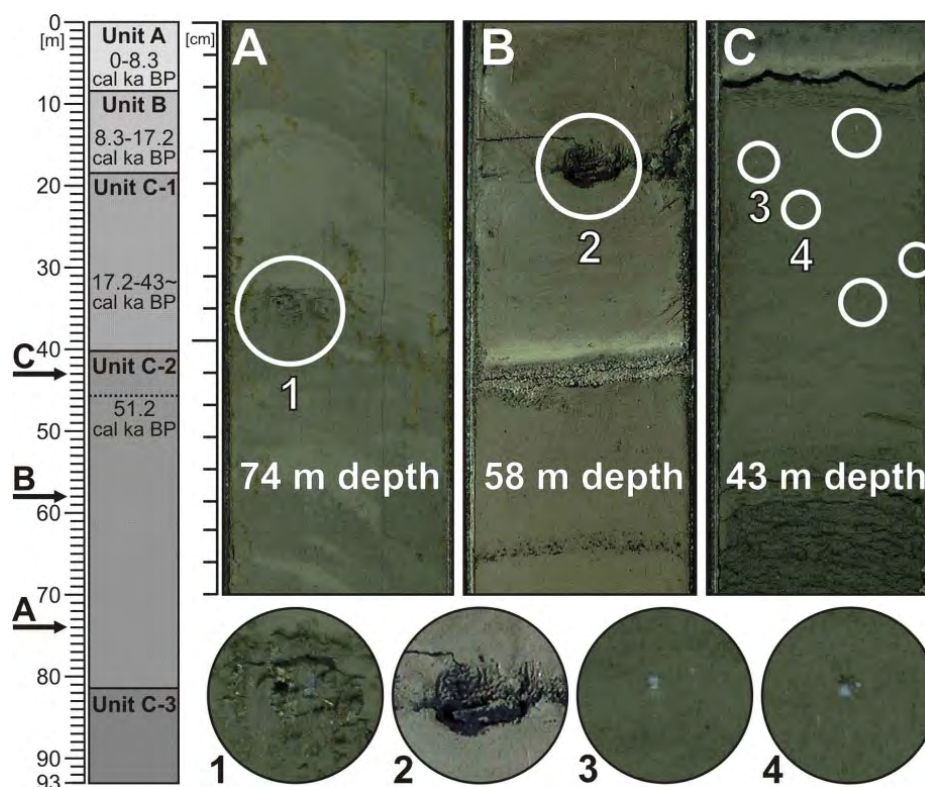


Figure 2 Left: Synthetic log of Potrok Aike sedimentary record (after Kliem et al. 2013) with brief descriptions of the lithostratigraphic units. **Unit A:** Pelagic laminated silts with presence of calcite **Unit B:** Pelagic laminated silts, fine sand layers and gravity events. **Unit C-1:** Pelagic laminated silts, fine sand layers and gravity events (thickness <1 m). **Unit C-2:** Pelagic laminated silts, fine sand layers and several gravity events (thickness up to 1 m). **Unit C-3:** Sand and gravel layers, few pelagic laminated silts, fine sand layers and numerous gravity events (thickness >1 m). Concretions, indicated by arrows, are all located within unit C-2. **Right:** Photographs of the sediment intervals containing authigenic vivianite. Basaltic tephra underlies each of these sequences. **(A)** core 5022-1D at 74 m depth **(B)** core 5022-2A at 58 m depth, corresponding to a depth 53 m for site 1 **(C)** core 5022-1D at 43 m depth. Numbered circles (1-4) indicate the positions of the close-up pictures of the concretions and nuggets of vivianite.

2.2 Analyses

Methods for determination of methane headspace, phosphorus speciation, *in situ* ATP detection and DAPI cell counts were published (Vuillemin et al. 2013).

Total organic carbon (TOC), total nitrogen (TN) and the stable carbon isotope composition ($\delta^{13}\text{C}_{\text{org}}$) of the homogenized bulk organic fraction were analyzed on decalcified and untreated samples, respectively, using an elemental analyzer (EuroVector[®], Euro EA[®]) linked by continuous flow to an isotope-ratio mass spectrometer (Micromass, IsoPrime[®]). Isotope ratios are reported in δ -notation in per mil according to the following equation: $\delta = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$, where R is the measured ratio of $^{13}\text{C}/^{12}\text{C}$ in the sample and Vienna PeeDee Belemnite standard (V-PDB). Analytical precision of isotope analyses was ≤ 0.10 ‰. TOC and TN were calculated according to the yield of CO_2 and N_2 after sample combustion in the elemental analyzer. Analytical precision was ± 3 % (1s) for carbon and ± 2 % (1s) for nitrogen. TOC of the decarbonized sample was back-calculated to the whole sample and results are presented in weight %. TOC and TN values were used to calculate atomic $\text{C}_{\text{org}}/\text{N}$ ratios.

The carbon isotopic composition of methane ($\delta^{13}\text{C}_{\text{CH}_4}$) was determined on the same samples used for headspace chromatography. Duplicate measurements were processed with an IsoPrime[®] mass spectrometer connected to a trace gas preconcentrator. Results are given in standard δ -notation relative to V-PDB.

Pore water samples from core 5022-1A were obtained using soil moisture samplers (Rhizon[®] soil moisture samplers Eijkelkamp[®]) inserted into sediments through small holes drilled in the core liners. Fluids were extracted using syringes screwed to the rhizons and maintained under low pressure. To avoid shifts in water chemistry, recovered samples were split for cation and anion analyses after sampling, and immediately flushed with helium gas. Samples for cation analyses were acidified with 100 μl HNO_3 (65 % suprapure). Transfer of pore water samples into sealed vials was performed under a N_2 atmosphere in a small chamber. Cations were determined by ICP-MS and anions were analyzed by ion-chromatography.

Minerals and matrices of authigenic concretions were observed using a binocular microscope (Nikon SMZ800 equipped with a Go-3 QImaging Digital USB Microscope Camera) and a scanning electron microscope (SEM) (Carl Zeiss EVO[®] 50). For SEM observation, dried samples were mounted on 12.7-mm-diameter aluminum stubs, using double-sided adhesive carbon discs. They were observed under variable pressure mode (10 to 400 Pa), enabling observation of non-conductive samples without metal coating. Such conditions prevent metal contamination and minimize the charge-up on the surface during observation. The Carl Zeiss EVO[®] 50 SEM is equipped with a Variable Pressure Secondary Electron detector (VPSE) for topographic images, a 4-Quadrant Backscattered electron Detector (QBSD) for backscattered electron (BSE) images, which allows viewing images in chemical contrast depending on the mean atomic number of the specimen, and an X-ray Energy Dispersive Spectroscopy (EDS) microanalysis system (model: INCAx-sight EDS detector, Oxford Instruments) for elemental analyses of surficial nanoparticles. The Lithium Drifted Silicon Si (Li) detector has a resolution of 133 eV and can identify elements from beryllium (Z=4) to uranium (Z=92) for concentrations $>1,000$ ppm. It must be operated close to liquid nitrogen temperatures. The accelerating voltage was 20 kV and working distances were 8.5 mm for BSE and 6.5 mm for secondary electron images. Additional imaging was also performed on coated samples with a Jeol JSM 7001F Scanning Electron Microscope. Prior to imaging, samples were mounted on aluminium

stubs with double-sided conductive carbon tape, and an ultra-thin coating (~15 nm) of gold was deposited on the samples by low vacuum sputter coating with a Leica EM SCD 500 metallizer.

3. Results

3.1 Organic carbon in bulk sediment

The average TOC content in glacial sediments is very low with values often ≤ 0.3 % (Fig. 3A). Two horizons within the glacial record, however, display higher TOC values of 1.23 and 0.76 %, at 74 and at 37 m depth, respectively. TOC values along the Holocene record range between 0.3 and 2.0 %, with the highest TOC content around 10 m depth corresponding roughly to the Late Glacial/Holocene transition. Values then fluctuate between 0.3 and 1.5 % in the uppermost 10 m of sediment. TN (not shown) displays identical trends to those of TOC, but has lower average values, with a maximum of 0.25 % at 10 m sediment depth. From the core bottom up to 20 m depth, the atomic C_{org}/N ratio (Fig. 3A) displays very low values, with two exceptional peaks ≥ 10 within the mentioned glacial horizons, at 74 and 37 m depth. In the uppermost 20 m of sediment, the atomic C_{org}/N ratio increases from 4 to 10, and fluctuates between 6 and 12 along the Holocene record, reaching a maximum of 14 at 0.7 m depth. Carbon isotopes of bulk organic matter (Fig. 3A) show variations that are confined to the Holocene sediments. The glacial sediments display a uniform trend, with peaks of -25.1 and -24.0 ‰ around 70 m depth. From 10 to 5 m depth, $\delta^{13}C_{org}$ decreases from -24.1 to -27.8 ‰. From this depth to the top, the isotopic composition displays an increasing trend, reaching -23.0 ‰ in the uppermost sediments.

3.2 Phosphorus speciation

Total phosphorus (TP) equals the sum of inorganic phosphorus (IP) and organic phosphorus (OP), and IP corresponds to the sum of apatite phosphorus (AP) and non-apatite inorganic phosphorus (NAIP). Figure 3A displays results for OP, AP and NAIP, whereas TP is shown in Fig. 3C. The OP content is high within the first glacial horizon at 74 m depth, whereas the highest values are in the uppermost 10 m of sediment. The OP content is greatest at 0.7 m depth, but decreases gradually from the surface down to 20 m. AP appears to be the dominant form of phosphorus, with an average content of 500 to 600 ppm throughout the sediment column. NAIP shows both increasing and decreasing trends, with sporadic peaks in the glacial record (75, 66, 56, 43 and 25 m depth). In the same broad interval, AP displays synchronous increases at about 75, 57, and 43 m depth. TP displays the highest values at these same depths, coinciding with vivianite concretions that were sampled subsequently. Within the uppermost 20 m, where no authigenic concretions were found, AP and NAIP contents differ significantly from one another and NAIP and OP contents are negatively correlated.

3.3 Pore water analyses

Figure 3B displays pore water concentrations for chloride, phosphate, sulfate, calcium and iron. From 30 m to 5 m depth, chloride content increases gradually from 200 to 800 ppm, illustrating the shift of the water column from freshwater to subsaline conditions during the late glacial-Holocene transition. The data also show that pore waters reflect the original lake water composition and were not affected by diffusion of lake water into the sediments. The

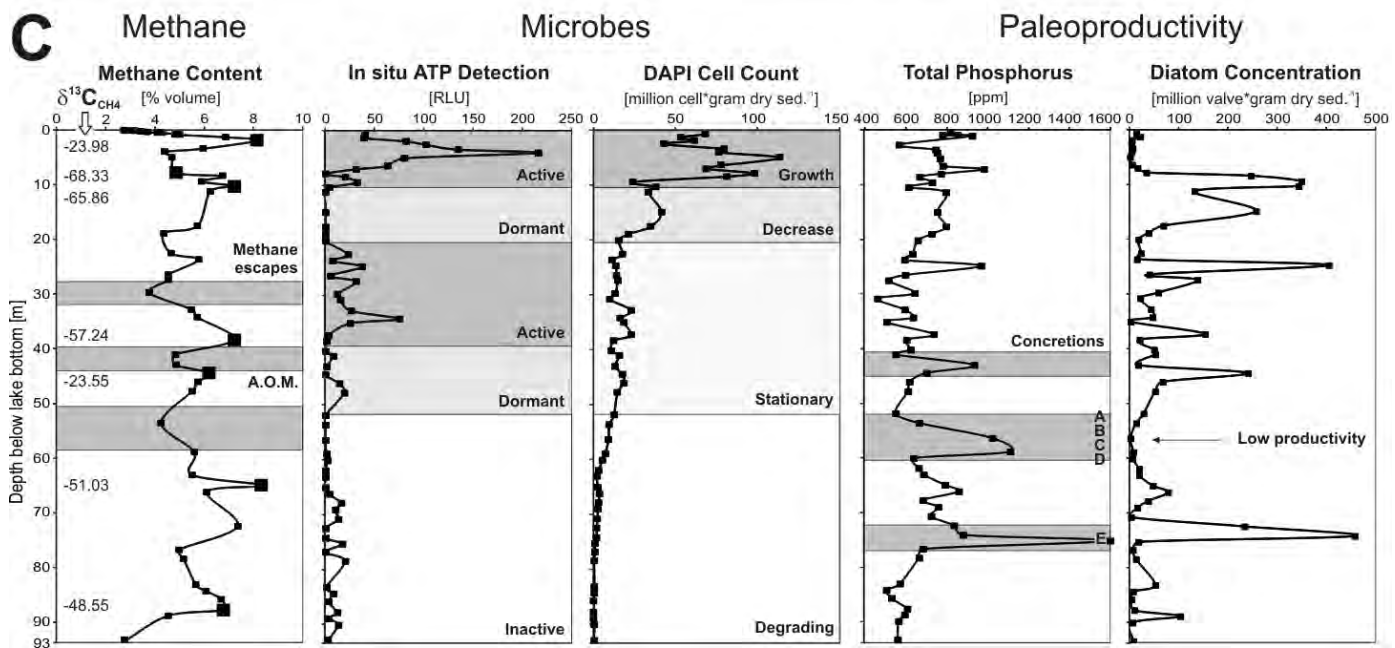
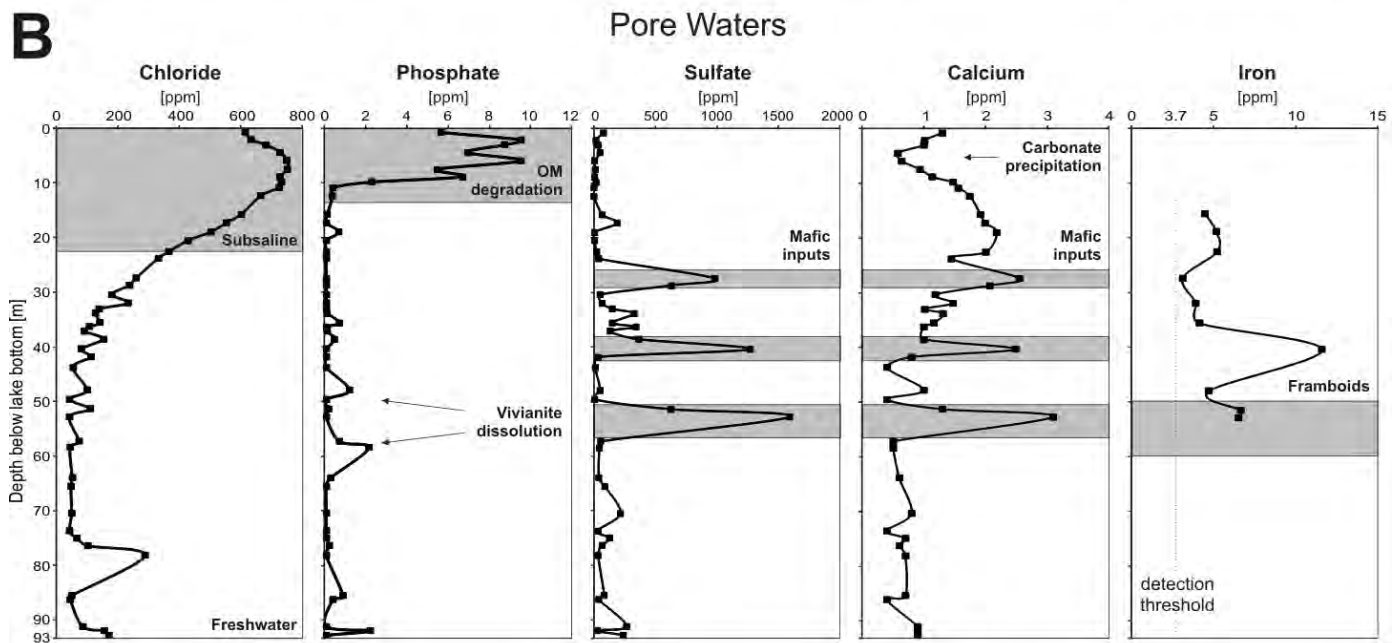
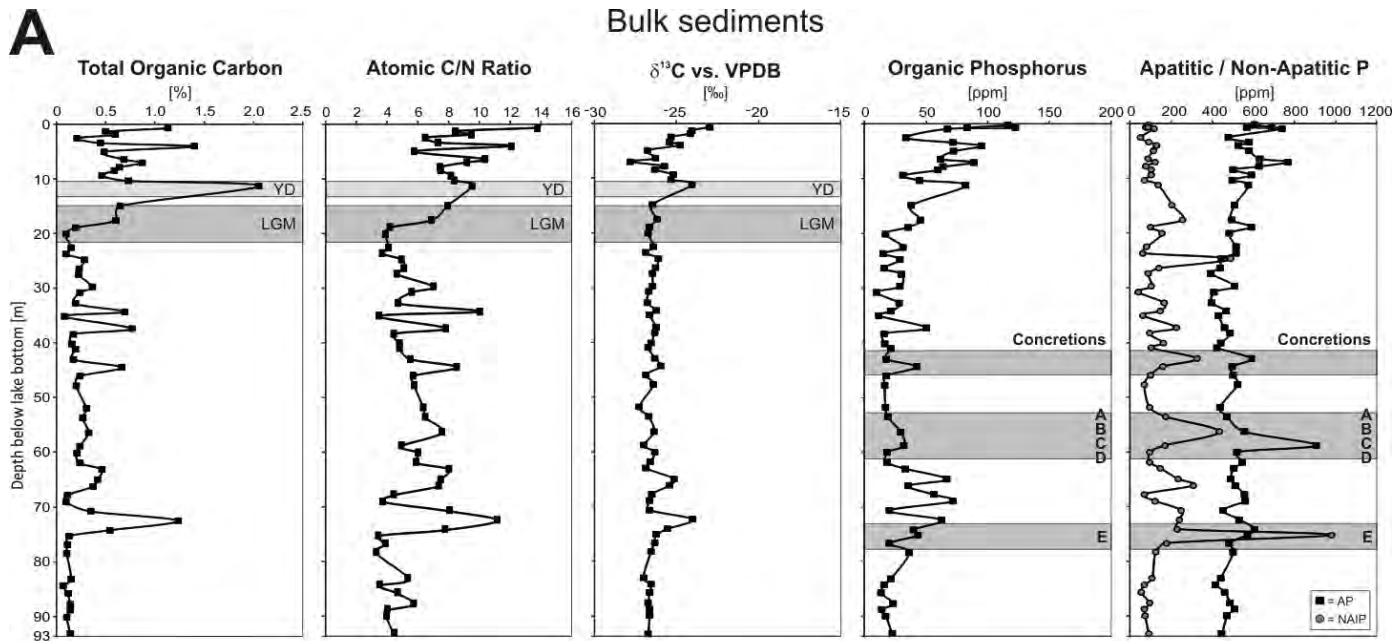


Figure 3 A: Results for OM characterization on core 5022-1D. **From left to right:** Total Organic Carbon (TOC); atomic C/N ratio; $\delta^{13}\text{C}_{\text{org}}$ of the bulk sediment; Organic Phosphorus (OP); Apatite Phosphorus (AP) and Non-Apatite Inorganic Phosphorus (NAIP). Grey shadings highlight successively from left to right: The Late Glacial Maximum (LGM) and Younger Dryas (YD) chronozones, the sedimentary sequences presented in Fig. 2 and concretions labeled A to E from Figure 4. **B:** Results of pore water analyses from core 5022-1A. **From left to right:** Chloride; phosphate: Arrows indicate intervals of vivianite alteration; sulfate; calcium: The arrow indicates ikaite precipitation; iron: The disappearance of dissolved iron is linked to its precipitation as sulfides; Grey shadings highlight from left to right: Subsaline paleoconditions of the water column, the influence of organic matter degradation, mafic inputs related to the reworking of volcanites by gravity events, the interval where framboids were observed. **C:** Results from core 5022-1D for methane, indexes related to microbial population and proxy indicators of paleoproductivity. **From left to right:** Methane content and results for methane carbon isotopes ($\delta^{13}\text{C}_{\text{CH}_4}$) with AOM denoted by a maximum $\delta^{13}\text{C}_{\text{CH}_4}$ value at 44.5 m sediment depth: Larger squares indicate samples on which $\delta^{13}\text{C}_{\text{CH}_4}$ was measured; *in situ* ATP detection used as an index of microbial activity within sediments; DAPI cell counts indicating the density of microbes; total phosphorus of the bulk sediment; diatom concentration (modified after Recasens et al. 2012): The arrow indicates an interval of low algal productivity. Grey shadings highlight from left to right: Methane escapes associated with gravity events, different degrees of microbial activity, the evolution of microbial population density, the sediment sequences and concretions from Figs. 2 and 4

orthophosphate and OP profiles show similar trends, with high concentrations in the uppermost 10 m. In the glacial record, some low phosphate concentrations in pore waters are found at depths where concretions were observed, i.e. at 74, 58 and 43 m depth. Sulfate concentration in pore waters shows three sharp peaks, at 50, 49 and 25 m depth, which can be related to inputs of altered mafic tephra (Kliem et al. 2013). The same peaks can be identified for calcium in accordance with the mafic composition of such sediments. Otherwise, the calcium content decreases from 20 m to 5 m depth, concomitantly with the observed chloride increase. Dissolved iron concentrations in pore waters were often below the detection limit and very low concentrations were observed from 50 to 15 m depth, with a maximum value of 12 ppm at 38 m depth.

3.4 Methane content and $\delta^{13}\text{C}_{\text{CH}_4}$

Figure 3C shows total methane content in percent of the initial sample volume (3 ml = 3 cm³). Large squares indicate samples in which $\delta^{13}\text{C}_{\text{CH}_4}$ was measured and isotope values are listed next to the graph. Surface sediments show high methane content linked to the activity of methanogens. Below the surface, methane content decreases substantially from 2 to 8 m depth and rises again at 9 m depth. Below 20 m depth, methane content is quite variable.

The $\delta^{13}\text{C}_{\text{CH}_4}$ value (Fig. 3C) at 2 m depth is - 23.98 ‰ and decreases to -68.33 ‰ at 8 m depth. At 12 m depth, a peak in methane content coincides with a $\delta^{13}\text{C}_{\text{CH}_4}$ value of - 65.86 ‰. A much higher value (- 23.55 ‰) is observed around 40 m depth at the top of the interval containing framboids.

3.5 Microbial population activity and density

In situ ATP measurements (Fig. 3C) show a sharp increase from the surface down to 4 m depth, followed by a strong decrease down to 8 m depth. Small increases are then seen at 9, 34 and 49 m depth, along with some slightly higher TOC and OP values for the glacial record (Fig. 3A). DAPI cell counts (Fig. 3C) decrease from the surface to 3 m and then increase to a maximum value at 5 m depth. The microbial population density fluctuates below that depth, and decreases dramatically at 9 m. Then it stabilizes at a low density, decreasing gradually down to 60 m, where it almost disappears. A small peak, however, is evident at 34 m.

3.6 Microscopic observations

3.6.1 Concretions

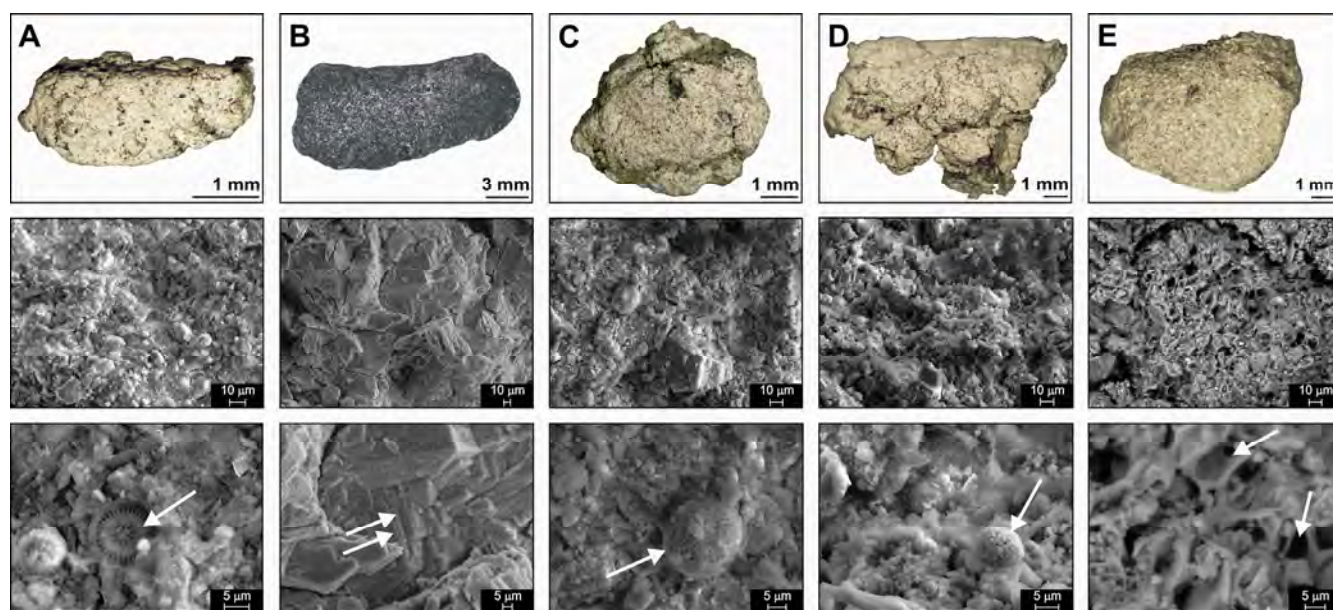


Figure 4 Images of concretions under increasing magnification using a binocular microscope (first row) reveal scattered dark minerals. Successive SEM close-up pictures (second and third rows) show different textures such as heterogenous, massive and porous. **(A)** Concretion showing an heterogenous texture with black - opaque - minerals, diatoms (white arrow), and clays (core 5022-2A; sites 1/2 depth: 53/57 m) **(B)** Block of massive vivianite, pictured after oxidation and fading of its blue color (core 5022-2A; site 1/2 depth: 53/57 m) showing a single composition with perfect cleavage (white arrows) **(C)** Concretion of fine texture showing black minerals, altered clays (microbial ?) and framboids (white arrow) (core 5022-2B, site 1/2 depth: 57/66 m) **(D)** Concretion of fine texture with black minerals, altered clays (microbial ?) and framboids (white arrow) (core 5022-2B; site 1/2 depth : 59/67 m) **(E)** Concretion apparently composed of volcanic vacuolar material (white arrows) from tephra (core 5022-2C; site 1/2 depth: 74/79 m)

Concretions shown in Fig. 4 (A to E) were recovered from clayey layers of sediment sequences, composed of mafic sands at the base, overlain by clays topped with a very thin horizon (Fig. 2) at 56.96, 66.23, 67.14 and 78.58 m depth in the site 2 composite core (Gebhardt et al. 2011), corresponding approximately to 46, 53, 57, 58 and 74 m depth in the site 1 composite core (Recasens et al. 2012). Concretion B is a blue and blocky vivianite mass (Nuttin et al. 2013) that quickly faded away after core opening (Fig. 2 B2). Observation under increasing magnification revealed a massive texture of a single composition. The perfect cleavage of vivianite is even visible under the highest magnification (Fig. 4B, right). Observation of concretions A, C, D and E under reflected light shows they are <1 cm in diameter (Fig. 4, top). SEM images exhibit textural heterogeneities, such as variable sizes of incorporated grains dominantly in fine matrixes, as well as the presence or absence of cementation. The highest SEM magnification provided the most interesting information, as specific components appeared entangled within the concretions. Indeed, concretion A is rich in diatom frustules. Concretions C and D show framboids within foliated clays, also associated with what is most probably exopolymeral substances (EPS). Concretion E is mainly composed of porous volcanic material.

3.6.2 Framboids and precursors

Framboids and their precursors were identified in concretions A, C and D. Numerous microcrystals of iron sulfide are displayed in Fig. 5A, illustrating an initial stage of nucleation

(Wilkin et al. 1996). The different framboids presented in Fig. 5C display a rather homogenous size distribution, with diameters ranging between 10 and 15 μm .

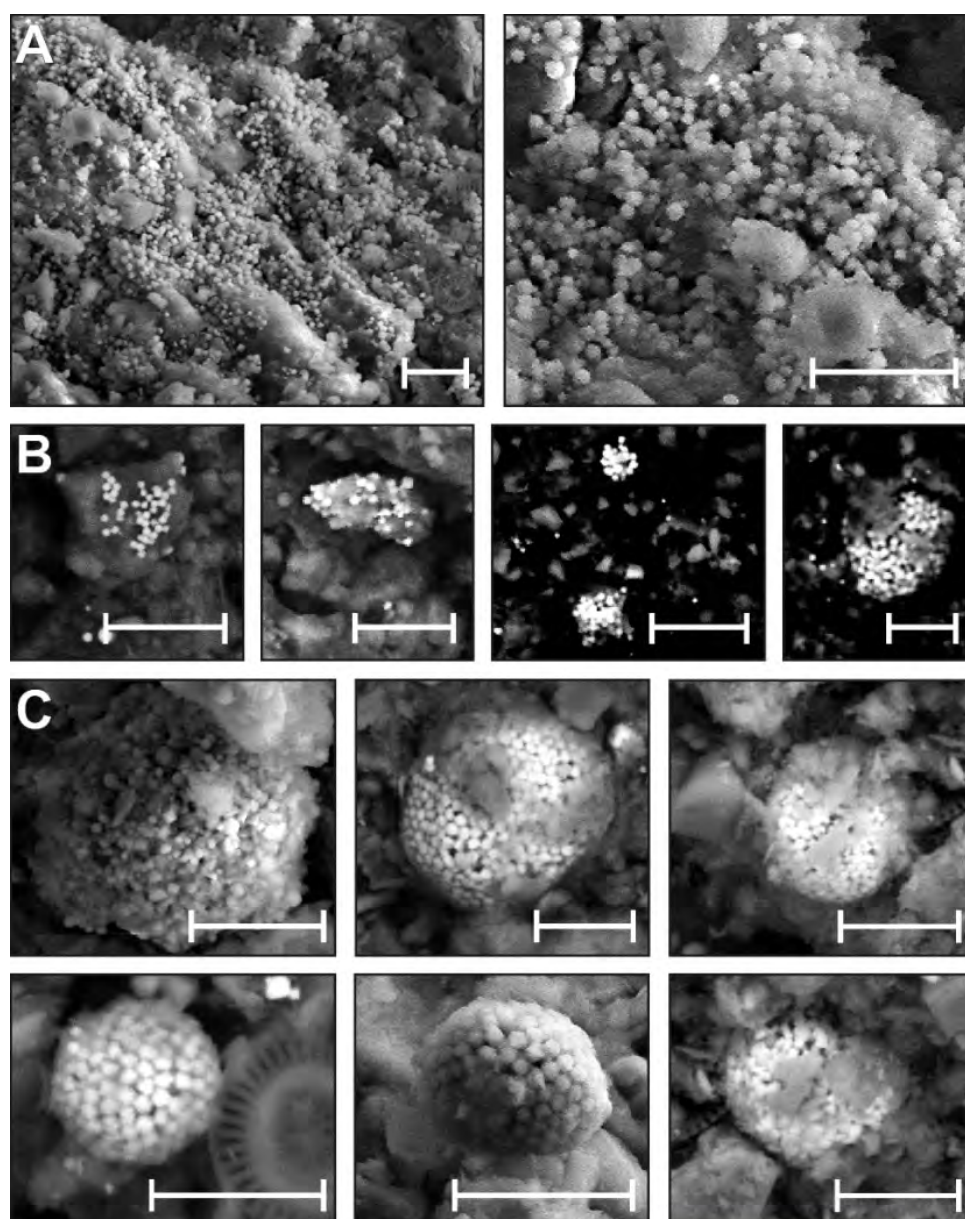


Figure 5 SEM microphotographs of framboid precursors and framboids **(A)** Disseminated single cubic crystals of iron sulfides (scales = 10 μm) **(B)** BSE images of partially aggregated iron sulfide crystals, possibly showing the stepwise process of framboid aggregation (scales = 10 μm) **(C)** Fully formed framboids (scales = 10 μm)

3.7 EDS elemental analyses

EDS analyses were performed on phosphates (Fig. 6A₁₋₄), framboids (Fig. 6B₁₋₄), matrices and specific foliated clays (Fig. 6C₁₋₂) and some accessory minerals (Annex A-5). Iron oxides such as hematite (Fe_2O_3), magnetite (Fe_3O_4), or ilmenite (FeTiO_3) were not investigated because they are very common in the catchment, and thus mostly from detrital origin. BSE images were used to distinguish phosphates within matrices. Vivianite ($\text{Fe}_3(\text{PO}_4)_2 \cdot 8 \text{H}_2\text{O}$) was the dominant identified mineral, although anapaite ($\text{Ca}_2\text{Fe}(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$), another authigenic phosphate, was also identified once. Analyses that did not match the stoichiometric ratio for vivianite perfectly were considered alteration byproducts of this mineral that most often turn to strengite ($\text{Fe}^{2+}\text{PO}_4 \cdot 2\text{H}_2\text{O}$). EDS measurements on framboids (Fig. 6B) reveal a range of compositions corresponding mainly to greigite and pyrite.

The lines on the plots shown in Figs. 6A and 6B (right side) correspond to stoichiometric compositions of specific phosphorus- and sulfur-containing minerals, respectively. Carbon and iron contents of matrices were also plotted (Fig. 6C, right side) to assess the possible sorption of organic elements onto oxides. Additional analyses were carried out on coated samples that revealed specific clays habitus (Fig. 6 C₁₋₃) and EPS remnants (Fig. 6 C₄). These results show a positive correlation between carbon and iron contents, leading to an inference for coupled iron and OM reduction in the presence of microbes.

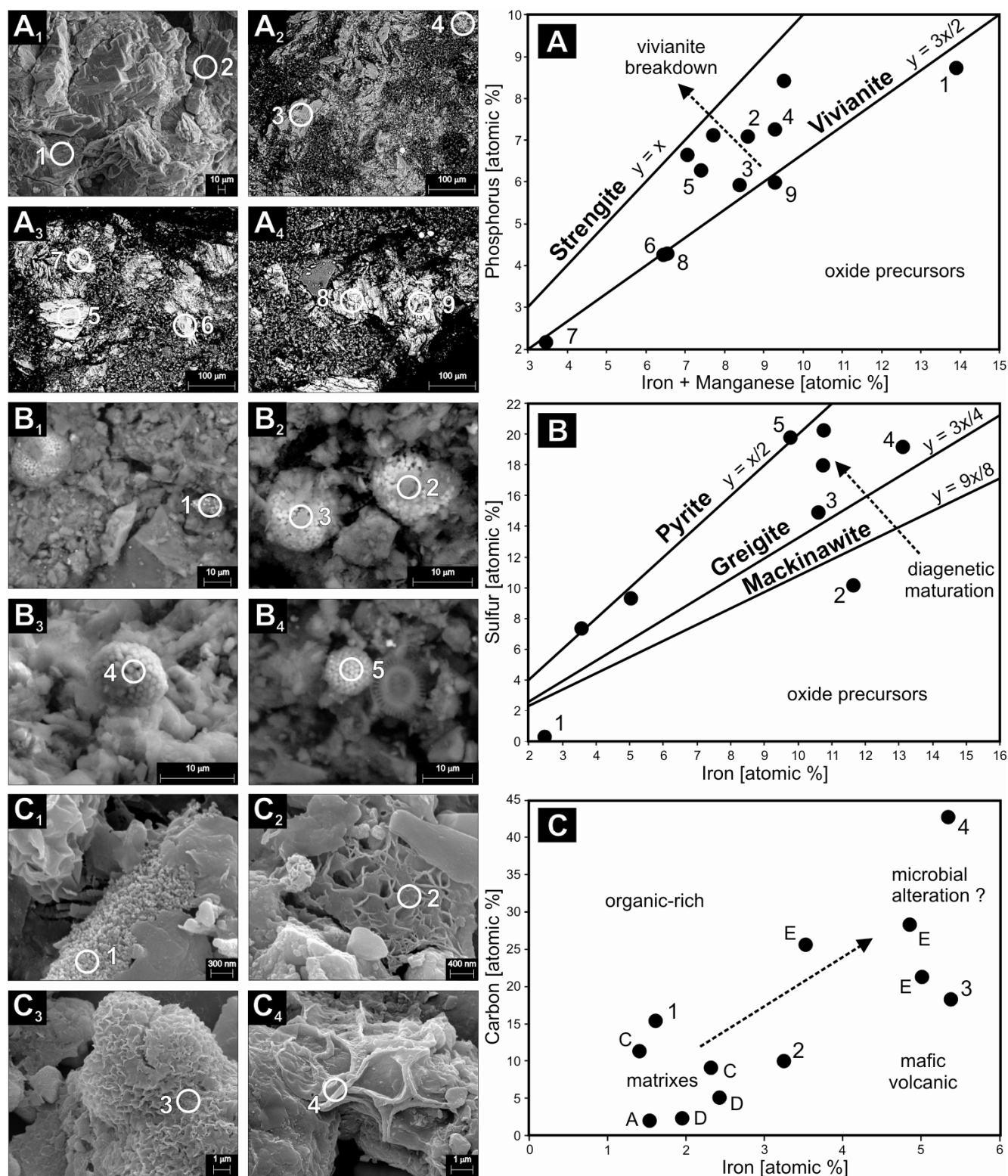


Figure 6 SEM images showing the position of points analyzed by EDS and their results are plotted on the right. Pictures C₁₋₄ were taken on coated samples. **(A)** Phosphates **(B)** Framboids **(C)** Matrices and foliated minerals interpreted as altered smectites. C₄ represents remnants of microbial EPS. C₁₋₂ and C₃₋₄ depths are 0.75 m and 60 m, respectively.

4. Discussion

4.1 Conditions for authigenic mineral formation

4.1.1 *Vivianite*

In Laguna Potrok Aike the influence of mafic volcanics on sediment geochemistry (Kliem et al. 2013) seems to have played a major role in the formation of the studied concretions. Reworking of mafic scoria and ashes from the catchment (Fig. 1) could have provided large amounts of the necessary phosphate, iron and sulfur (Park et al. 2005) whereas associated oxides (Manning et al. 1999) and smectites (Stamatakis and Koukoulas 2001) would have been ideal phosphorus sinks. Smectites are alteration products of soils and volcanic ashes and are known to occur within the Potrok Aike sediment record, where they represent up to 50 % of the clay fraction (Nuttin et al. 2013). Overall, different forms of phosphorus throughout the core reflect variations in the sedimentation regime. Sedimentary sequences (Fig. 2) of basaltic sands, overlain by thin clayey horizons, suggest sporadic inputs of AP followed by sedimentation of adsorbed NAIP (Fig. 3A). AP is insoluble and generally of detrital origin (Zhou et al. 2001). Authigenic apatite is known to form only in marine environments (Compton et al. 2007) and thus is not part of this lacustrine record. AP variations reflect changes in allochthonous input into the Potrok Aike closed basin corresponding with gravity events during the glacial interval (Kliem et al. 2013). NAIP is a bioavailable form of phosphorus that turns into an authigenic form when adsorbed onto metal oxides within the sediment, thereby restricting its solubility in sediments to reducing conditions (Anderson et al. 2001). The coupled AP and NAIP behavior can be explained by external input of phosphorus with AP, its insoluble form, which settles directly to the sediment. At the same time, NAIP is dissolved in the water column and precipitates within fine particles when adsorbed onto metal oxides (Hupfer et al. 1998). Vivianite precursors such as HFO, green rust and hydroxyphosphates (Nriagu and Dell 1974; Fredrickson et al. 1998), are known to form by the adsorption of phosphorus onto iron oxides (Fagel et al. 2005). These accumulations are transformed into vivianite during methanic diagenesis (Berner 1981). In lacustrine systems, this process has often been reported in surficial sediments (Emerson 1976; Berner 1981; Sapota et al. 2006), with frequent occurrences in anoxic, low-sulfide sedimentary environments (Manning et al. 1999) and cold, dry climate zones (Sapota et al. 2006). The low TOC content in Laguna Potrok Aike glacial sediments (Fig. 3A) is an additional factor that could have favored P fixation in the sediment (Wang et al. 2007) and limited bacterial P release from sediments (Gächter et al. 1988). Additionally, the frequent gravity events during the glacial period (annex A-4) reflect higher sedimentation rates that disrupted the microbial degradation chain that would otherwise favor quick OM mineralization and thereby prevented P diffusion to the water column.

Holocene sediments of Laguna Potrok Aike illustrate the opposite situation, with a pelagic to hemi-pelagic sedimentation (Kliem et al. 2013). Their high pore-water phosphate concentrations (Fig. 3B) were probably caused by OM degradation and associated high microbial activity (Fig. 3C) along with low sulfate concentrations (Fig. 3B). Subsequent mineralization of OP degraded by microbes may trigger the formation of authigenic forms of phosphate (Gächter et al. 1988), as indicated by the gradual increase of NAIP in bulk sediment down to 20 m depth. Such inverse correlation between OP and NAIP was only observed in relatively young sediments as the preservation potential of organic phosphorus forms in older sediments is very low and does not allow discrimination between sorption processes and mineralization. Remobilization of authigenic forms of NAIP can occur under reducing

conditions with solubilization back to the water column (Wang et al. 2007) or to interstitial waters (Zhou et al. 2001; Compton et al. 2007). Moreover, small concentrations of H_2S from sulfate reduction destabilize vivianite, possibly remobilizing its iron to sulfides and dissolving small amounts of phosphate from the concretions in deep glacial sediments (Fig. 3B). Thus, this ability of vivianite to shift from a P sink to P source within the sediment highlights the fact that caution must be used if vivianite is to be utilized as a paleoindicator of prevailing environmental conditions during its formation.

4.1.2 *Framboids*

The iron sulfide framboids (Fig. 5C) display rather small and homogenous sizes without overgrowth (Schieber 2002). Such features are probably formed within sediment porewaters under anoxic conditions (Wilkin et al. 1996; Suits and Wilkin 1998; Böttcher and Lepland 2000), rather than in the water column (Park et al. 2005). The different composition of the framboids (Fig. 6B) may result from the variety of reactive iron available (Fig. 3B) and variable redox conditions (Berner 1981; Wilkin et al. 1996). Indeed, slightly sulfided iron oxides were detected in partial aggregates (Fig. 5B), mackinawite (Fe_9S_8) and greigite (Fe_3S_4), sometimes as fully formed framboids (Fig. 6B). Greigite as a ferrimagnetic precursor to framboidal pyrite, has been documented in other lacustrine settings (Ariztegui and Dobson 1996) and implies gradual diagenetic maturation. The sulfide iron oxides have been interpreted as precursors of pyrite (FeS_2), which is the dominant framboidal mineral (Wilkin and Barnes 1997) formed from interstitial anoxic waters (Suits and Wilkin 1998; Böttcher and Lepland 2000) and probably mediated by microbial activity (Astafieva et al. 2005). The presence of dissolved iron above the framboid-containing interval (Fig. 3B) argues for iron reduction coupled with sulfate reduction, to account for precipitation of iron (Fe^{2+}) sulfides below 40 m depth. Development of framboids is linked to microcrystal aggregation by magnetic attraction (Wilkin and Barnes 1997). This plausible path of framboid formation is shown in Fig. 5B. Because availability of reactive iron is the dominant control on pyrite formation (Berner 1981), production of H_2S via sulfate reduction is sufficient to transform sulfide precursors such as mackinawite and greigite into pyrite (Holmer and Storkholm 2001). Concomitant measurement of *in situ* ATP and DAPI cell count at ~50 and ~40 m depth (Fig. 3C) is evidence for the sustainability of such microbial processes.

4.1.3 *Carbonate and other minerals*

There are abundant carbonate crystals in the two uppermost lithostratigraphic units of the Laguna Potrok Aike record (Kliem et al. 2013; Nuttin et al. 2013), whereas they are completely absent in late glacial sediments, aside from some reworked gastropod shells. Oehlerich et al. (2013) found that carbonate precipitates in modern Laguna Potrok Aike as mm-sized ikaite ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$), which rapidly disintegrates into μm -sized calcite crystals. These fine calcite crystals are morphologically identical to the calcite in the rest of the sediment record, which supports the idea that these carbonates were originally precipitated as ikaite in the water column and transformed to calcite soon thereafter. Although ikaite is normally metastable, it precipitates when calcite and aragonite nucleation is inhibited by low temperatures and high phosphate concentrations (Oehlerich et al. 2013). Carbonate precipitation today is favored by the salinity increase during lake-level lowstands of the presently closed-basin waterbody (Ohlendorf et al. 2013). Chloride and calcium profiles (Fig. 3B) reflect a salinity increase within the upper 20 m, with an increase in chloride and a decrease in calcium, the latter a consequence of carbonate precipitation.

Some accessory minerals were also encountered within the studied concretions (Annex A-5). Ilmenite, magnetite and plagioclase ($\text{NaAlSi}_3\text{O}_8$ - $\text{CaAl}_2\text{Si}_2\text{O}_8$) were also identified, reflecting detrital input from the surrounding igneous rocks.

4.2 Early diagenesis and timing of authigenic mineral formation

4.2.1 Microbial mediation

In situ ATP production and high microbial population densities (Fig. 3C) provide evidence for substantial microbial reduction processes within the topmost 10 m of sediment (Konhauser 2007). Simultaneous presence of framboids and vivianite is explained by a series of anaerobic processes related to stratified microbial communities (Nealson and Stahl 1997). Iron reduction is the first step, followed by sulfate reduction, whereby the produced H_2S reacts with dissolved iron and precipitates as iron sulfides. Methanogenesis generally starts only after sulfate depletion (Hoehler et al. 2001) and reduces vivianite precursors. High sulfate concentrations in some sediment horizons of the glacial record (Fig. 3B), however, would greatly limit methane production (Schubert et al. 2011), as sulfate reducers are known to outcompete methanogens (Schink 2002). The decrease in methane content from 2 to 8 m depth (Fig. 3C) could be explained by redeposition events, as suggested by geophysical data in the lake (Anselmetti et al. 2009), or preferential bacterial use of the substrate (Hoehler et al. 2001; Schink 2002). The high methane content at ~10 m depth (Fig. 3C) is associated with a sudden shift of $\delta^{13}\text{C}_{\text{CH}_4}$ towards lower values (~-68 ‰), indicating methane production based on ^{13}C -depleted compounds, as would be the case with advanced CO_2 reduction (Whiticar 1999). High variability of methane below 20 m depth is interpreted as the result of low TOC content within late glacial sediments, along with frequent gravity events during this time interval (Fig. 3C). These gravity event deposits, composed of mafic volcanics, supplied iron and sulfate to the basin, causing microbial iron and sulfate reduction in such mafic horizons. Simultaneously, associated high sediment loads disrupted methanogenesis in underlying organic clays and triggered methane escapes to the overlying sediments (Fig. 3C and Annex A-4). At the same time, NAIP was adsorbed on volcanic clays and iron oxides in the water column and precipitated as fine sediments. A succession of discrete mafic sand and organic clay horizons could lead in turn to competing processes, such as sulfate reduction and methanogenesis. Indeed, the shift of $\delta^{13}\text{C}_{\text{CH}_4}$ toward higher values at ~40 m depth (Fig. 3C) demonstrates that methane was used as a source of carbon, with residual methane enriched in ^{13}C (Valentine 2002; Schubert et al. 2011). A common byproduct of sulfate reduction and anaerobic oxidation of methane (AOM) is concurrent precipitation of pyrite (Schink 2002). It is still unclear, however, whether these diagenetic products were formed at shallow depths or after a longer burial time.

4.2.2 Depth of formation

Nuttin et al. (2013) demonstrated that the vivianite concretions in Potrok Aike experienced diffusion of elements to and from the surrounding sediments through time. U-Th dating gave ages much younger than the model deposition age (Kliem et al. 2013) and were thus attributed to late diagenetic precipitation under open system conditions.

In the uppermost 10 m of sediment, the NAIP content (Fig. 3A) remains low, whereas a large fraction of the phosphorus is found as OP and phosphate in pore waters (Figs. 3A and 3B). Because microbes are abundant and active throughout the Holocene record (Fig. 3C), P remineralization rates during early diagenetic stages render uncertain the formation depth of

vivianite. Furthermore, microbial activity in Potrok Aike sediments is sustained as deep as 40 m (Fig. 3C) and authigenesis can thus be considered a slowly evolving process. At the same time, high sedimentation rates related to gravity events favored the adsorption and mineralization of phosphorus by disrupting the methanogenic degradation chain, while inputs of sulfur into the system further promoted sulfate reduction over methanogenesis. *In situ* ATP detections at 48 and 34 m depth (Fig. 3C) imply that there are still substantial ongoing microbial processes at these depths, with possible sites of reduced activity at 70 and 80 m depth, associated with relatively high TOC and OP contents for the glacial record. AOM and microbially mediated pyrite formation are good examples of deep survival strategies of sulfate reducers (Schink 2002). Moreover, microbial iron reduction is known to provoke alteration of different clay minerals, especially in the presence of sulfate (Li et al. 2004), leading to significant structural changes in clay lattices (Stucki and Kostka, 2006). Besides, SRB have the ability to reduce iron-containing minerals to support their growth (Li et al. 2004; Zhang et al., 2012). Smectites, which are the most abundant clay group in Potrok Aike sediments, may originate from the weathering of mafic rocks, but from authigenic processes as well. Nuttin et al. (2013) reported a gradual decrease in their 2 θ angle peak with depth, which is commonly observed with microbial alteration (Dong et al. 2009). During microbial reduction of Fe(III) from smectites, the mineral structure has to be stabilized by the addition of interlayer cations, the habitus becoming foliated during the process (Dong et al. 2009), and as they lose their sorption capacity, altered smectites tend to release adsorbed nutrients (Kostka et al. 1999; Stucki and Kostka 2006). Figures 6C₁₋₃ show organic matter interbedded with clays with different degrees of foliation. Their respective depths, along with EPS remnants (Fig. 6C₄), indicate clay-microbe interactions, with microbial metabolism possibly increasing clay alteration over time (Zhang et al. 2012). Anoxic releases of Fe and P (Fig. 3B) from metal oxides and sediments could also result from the activity of the microbial consortium (Gächter et al. 1988; Li et al. 2004), making the elements available to the bacteria, with methane as a complementary source of carbon. Further consumption of released phosphate in the presence of reduced iron leads to vivianite precipitation (Dong et al. 2009). Although culture studies on bacterial iron reduction have shown that vivianite is a stable end product (Glasauer et al. 2003), some levels of phosphate content (Fig. 3B) seem to be associated with vivianite dissolution (Wilson et al. 2010). Vivianite could be an alternative source of phosphorus that sustains microbial communities (Smith and Prairie 2004). AOM, smectite alteration and the associated release of adsorbed organics are critical to maintain microbial activity in deep environments, thus continuing to support diagenetic processes. The microbial signal does, however, become difficult to track below 40 m sediment depth in our record, where it begins to fade.

4.3 Paleo-indicator or biosignature

Comparison of TOC, TN and NAIP (Fig. 3A) suggests that iron and phosphorus in the concretions were derived from both volcanic sources and OM mineralization. Evidence for these two different sources is found in the material incorporated in the concretions. Pumice found in concretions C, D and E (Fig. 4) illustrates the role of mafic inputs, whereas the framboids attest to microbial reduction processes. In parallel, diatom concentration peaks (Fig. 3C) that coincide with TP and NAIP argue for gravity events and associated loads of P and Fe as a trigger of blooms in the water column (Recasens et al. 2012). Moreover, methane escapes, along with high calcium, sulfate and AP values (Figs. 3C, 3B and 3A), show sudden contributions of substantial mafic material reworked into the basin (Annex A-4, sample D40).

Concretions B to D (Fig. 4), however, possess low diatom counts (Fig. 3C), demonstrating the buffering effect on primary productivity of P adsorption and sequestration in vivianite (Nriagu 1972). The high numbers of diatom frustules in sediments (Fig. 3C) and concretion A (Fig. 4A) provide evidence for the recovery of primary production. The concretions reflect both nutrient enrichment of the lake basin and P sequestration from the water column as a result of sporadic inputs of catchment material, and thus offer potential insights into paleoproductivity during the glacial period (Fagel et al. 2005). The same applies to smectites as they could be derived from weathered volcanic ashes and soils associated with greater precipitation or aeolian transport (Nuttin et al. 2013). Because vivianite can be altered easily, it remains an unreliable paleoindicator.

The use of the studied concretions as biosignatures depends upon the respective diagenetic role of chemical adsorption and microbial remineralization in their formation (Hupfer et al. 1998). Although large concretions (Fig. 2B) result from precipitation via oxide precursors reduced during methanogenic diagenesis (Berner 1981; Fagel et al. 2005), nuggets of vivianite scattered in the sediments (Fig. 2A and 2C) reflect precipitation from pore waters after OM degradation and P remineralization by microorganisms (Gächter et al. 1988; Manning et al. 1999). Culture experiments have shown that vivianite formation only occurs in the presence of sufficient Fe^{2+} and inorganic phosphorus, along with microbial respiration (Glasauer et al. 2003). Other indirect signs of microbially mediated vivianite formation include clay alteration, methane production, and possibly framboids. Aside from the fact that methanogens are capable of dissolving clay minerals and further triggering vivianite aggregates, they are easily inhibited by DIRB and outcompeted by SRB under adequate Fe(II) and SO_4 interstitial concentrations (Zhang et al. 2012). Methanogenesis limitation could be inferred from moderate $\delta^{13}\text{C}_{\text{CH}_4}$ values (-48.55 to 57.24 ‰) (Fig. 3C), which might result from relatively low isotopic fractionation during methane formation.

Whether framboids can be considered biosignatures is still a matter of debate because natural crystals are hardly distinguishable from their synthetic abiogenic counterparts. Previous studies in sedimentary environments found fossilized microbial features associated with framboids (Schieber 2002; MacLean et al. 2008). On the other hand, the conditions required to nucleate such pyrites abiotically (Ohfuji and Rickard 2005) drastically diminish the possibility of such a pathway in lacustrine sediments. Although microbial features were not unequivocally detected on SEM microphotographs, some signs indicate the microbial origin of these framboids (Fig. 5C). They include low ATP (Fig. 3C), $\delta^{13}\text{C}_{\text{CH}_4}$ values (Fig. 3C) linked to AOM and sulfate reduction (Schink 2002), gradual maturation (Fig. 6B) and related concentration shifts in pore waters (Fig. 3B), P release from vivianite destabilized by production of H_2S (Fig. 3B), along with possible smectite alteration (Fig. 6C₁₋₂) caused by structural iron reduction (Kostka et al. 1999; Li et al. 2004).

5. Conclusions

We conclude that, 50 to 45 ka ago, deposition of reworked volcanic material into Potrok Aike maar, related to gravity events, played a dominant role in forming authigenic concretions in the sediments. Large amounts of organic matter were adsorbed onto oxides and smectites, partially controlling the lake trophic status. The low OM content of glacial sediments favored P retention, in authigenic form, during burial. Early diagenetic processes linked to stratified microbial communities may explain the concomitant formation of vivianite and iron sulfides. Methanogenesis appeared initially as the dominant process during early diagenesis, but was

often disrupted by sporadic gravity events as a consequence of lake level declines during the glacial. Mafic volcanics reworked from the catchment to the lake basin acted as the main supply of iron, sulfur and phosphorus, thus influencing primary productivity and generating additional microbial metabolism. In fine organic sediments, methane production reduced inorganic phosphorus complexed to volcanic clays and iron oxides to form vivianite concretions, whereas iron and sulfate reduction started replacing methanogenesis in mafic horizons. Microbial iron and sulfate reduction were sustained throughout diagenesis and led to the formation of framboids. Mackinawite and greigite evolved towards pyrite, implying diagenetic maturation through the sediment record. Additional evidence of prolonged microbial influence during diagenesis includes *in situ* ATP detection below 30 m depth, AOM process as indicated by $\delta^{13}\text{C}_{\text{CH}_4}$, and possible microbial alteration of smectites. In the meantime, methane and phosphorus consumption by microbes likely caused the nucleation of vivianite from sediment interstitial waters.

In summary, results from this study emphasize the successive influence of volcanic materials on microbial metabolism, leading to the formation of mineral concretions. Furthermore, sustained microbial activity observed within sediments shows that processes such as mineral authigenesis and diagenesis can be under their prolonged influence. Although authigenic minerals *per se* do not constitute unequivocal biosignatures, the multiple lines of evidence used to investigate concretions in Laguna Potrok Aike indicate diagenetic processes, mediated by microbial activity, during their formation. These features can be used to reconstruct authigenic and/or diagenetic processes in similar lake basins at different geographic and temporal scales.

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CHAPTER 6

Methanogenic populations in Holocene sediments

A modified version of this chapter is under review as

Vuillemin et al. in Geomicrobiology Journal

Influence of methanogenic populations in Holocene lacustrine sediments revealed by clone libraries and fatty acid biogeochemistry

Abstract

Methanogenic populations were investigated in subsaline Laguna Potrok Aike sediments, southern Argentina. Microbial density and activity were assessed via cell count and *in situ* ATP detection for the last ~11K years. Methanogen phylogenetics highlighted species stratification throughout depth, whereas CO₂ reduction was the major pathway leading to methane production. Organic substrates, characterized using pore water analysis, bulk organic fractions and saturated fatty acids, showed a clear link between sediment colonization and initial organic sources. Concentrations and $\delta^{13}\text{C}$ compositions of methane and fatty acids provided final evidence of a microbial imprint on Holocene organic proxies in the most colonized intervals.

1. Introduction

Methane and carbon dioxide (CO₂) in anoxic lake sediments are final products of organic matter (OM) degradation. Biogenic production of these end members depends mainly on OM lability and metabolic pathways used by microbial consortia. During early diagenesis, fermentation processes gradually degrade original organic mixtures into simple molecules, sometimes leading to a deviation from the initial signatures of the bulk fraction (Freudenthal et al. 2001; Lehmann et al. 2002). In anaerobic habitats, degradation of complex organic compounds is a stepwise process taking place via hydrolysis, acidogenesis, acetogenesis and methanogenesis (Haider and Schäffer 2009; Wüst et al. 2009), during which competitive and syntrophic relationships can occur between microbes with a clear dependence on local physico-chemical conditions (McInerney et al. 2008). For example, in environments with low nitrate and sulfate concentrations, methanogens can associate with chemoheterotrophic bacteria producing methane at various stages. In freshwater environments methyl fermentation is favored and proceeds unrestricted by sulfate reduction (Whiticar et al. 1986), whereas in cold environments acetogenesis appears to dominate (Kotsyurbenko 2005; Nozhevnikova et al. 2007). This affects rates of methanogenesis (Lay et al. 1998) and the associated carbon isotope fractionation (Whiticar 1999; Conrad 2005). Thus, the produced amount of methane in sediments derives from variable organic substrata, bacterial activity and metabolic pathways (Conrad et al. 2010). Furthermore, microbes develop the ability to use refractory compounds (McInerney et al. 2009), while evolving toward energy conservation with depth (Schink 1997).

Currently, geomicrobiological investigations of methanogenesis in lakes remain limited to most surficial sediments. In autumn 2008, the ICDP-sponsored Potrok Aike Sediment Archive Drilling prOject (PASADO) dedicated a full core to geomicrobiological studies (Vuillemin et al. 2010) and allowed the investigation of microbial populations in deep lacustrine sediments for the first time. Climatic fluctuations, like those recorded during the Holocene (Recasens et al. 2012), may promote or restrain sediment colonization by microbes. In this context, bulk OM proxies were interpreted as reflecting initial OM sources and associated environmental changes, dismissing diagenetic effects within the uppermost 3 m of sediment (Mayr et al.

2009). More recently, geomicrobiological investigations along the uppermost meter have shown the impact of paleoenvironmental conditions on microbial communities (Vuillemin et al. 2013). On the contrary, paleoenvironmental studies rarely inspect the influence of microbes on OM proxies. The role of microbes as agents of early diagenesis can, however, manifest via degradation processes, leading to organic sources reworking and substrate fractionation. Moreover, the *in situ* biomass production associated with microbial activity is often neglected.

This study investigates methanogenic populations, combining fatty acids (FAs) and methane concentrations, with their corresponding $\delta^{13}\text{C}$ compositions, in the entire Holocene record of Laguna Potrok Aike. We focus on microbial species distribution throughout depth, linking methanogenic pathways to OM degradation and substrate evolution. We inspect local sediment conditions, such as pore water chemistry and organic sources, and their influence on microbial development inferred from adenosine-5'-triphosphate (ATP) detections and cell counts. We define the influence of microbial biomass in sediments using branched-chain and unsaturated FA concentrations and their $\delta^{13}\text{C}$ signatures. Finally, we discuss a potential imprint of microbial diagenesis on paleoenvironmental proxies.

2. Methods

2.1. Study area and sampling

Laguna Potrok Aike is an endorheic maar lake in the Pali Aike volcanic field of southern Patagonia, Argentina (Zolitschka et al. 2006). The maar presents a very steep geometry with a water column of a maximal depth of 100 m (Gebhardt et al. 2011). Climatic parameters exert substantial control on the lake level (Ohlendorf et al. 2013) and can lead to important changes in organic sources and geochemical conditions recorded in the sediment (Recasens et al. 2012; Hahn et al. 2013). Presently, the lake is polymictic due to the persistent overturn caused by the Westerlies (average wind speed: 7.4 m/s) with subsaline conditions and water temperatures ranging from 4°C to 10°C throughout the year. Oxic conditions prevail in the water column (Zolitschka et al. 2006; Mayr et al. 2007). However, reducing conditions at the water/sediment interface have occurred over time (Haberzettl et al. 2007) and the oxygen penetration within surface sediment appears to be restricted (Vuillemin et al. 2013).

The investigations presented here were carried out in two hydraulic piston cores 5022-1A and 5022-1D of 65 mm diameter, and 87 and 97 m long, respectively (Ohlendorf et al. 2011), retrieved from the centre of the maar at 100 m water depth in autumn 2008. The cores were sampled at a resolution of one meter, avoiding felsic tephra layers and organic macroremains. A detailed description of the field sampling strategy and sample handling can be found in Vuillemin et al. (2010). Complete procedures for *in situ* ATP detection assessment, 4', 6-diamidino-2-phenylindole (DAPI) cell counts, and denaturing gradient gel electrophoresis (DGGE) analyses of bacterial and archaeal diversities are described in Vuillemin et al. (2013).

2.2. Organic matter characterization

Stable isotope compositions of the homogenized bulk organic fraction were analyzed on decalcified ($\delta^{13}\text{C}_{\text{org}}$) and untreated ($\delta^{15}\text{N}$) samples, using an elemental analyzer (EuroVector®, Euro EA®) linked in continuous flow to an isotope-ratio mass spectrometer (IRMS; Micromass, IsoPrime®). Isotope ratios are reported in δ -notation in per mil according to the following equation: $\delta = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$, where R is the measured ratio of $^{13}\text{C}/^{12}\text{C}$ of the

sample and of the Vienna PeeDee Belemnite standard (V-PDB). Analytical precision of the isotope analysis is better than 0.1‰. Total organic carbon (TOC) and total nitrogen (TN) were calculated from the yield of CO₂ and N₂ measured after sample combustion in the elemental analyzer. Analytical precision was $\pm 3\%$ (1 σ) for C_{org} and $\pm 2\%$ (1 σ) 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 C_{org}/N ratios.

2.3. Methane headspace and methane carbon isotopic composition

The vials sampled in the field were first sonicated to homogenize sediments within the solution prior to methane headspace determinations. The gas fraction was sampled using a HP7694 chromatograph with a Headspace Sampler and separated by molecular weight on a GC column into methane, N₂O, and CO₂ and transported differentially with argon as the carrying gas to a coupled mass spectrometer (Agilent 6850 Series GC System). Methane peaks were detected in a flame ionization detector (FID) and appeared on the chromatogram at 2 min 11 sec. Methane spikes were then used to calibrate and transform detection intensities (pA) into volumes and millimolar by applying the ideal gas law $PV=nRT$ at standard conditions. Traces of nitrous and sulfidic gases were detected in the samples, but not measured any further.

The carbon isotope composition of methane ($\delta^{13}\text{C}_{\text{CH}_4}$) was determined on the same samples as for headspace chromatography, but only for three samples. Duplicate measurements were processed with an IRMS (Isoprime[®]) connected to a trace gas preconcentrator. The trace gas preconcentrator works at a helium flow of 17mL/min. H₂O is eliminated with a magnesium perchlorate trap and CO₂ in a Carbosorb[®] trap. Methane of the cleaned gas flow is oxidized to CO₂ at 960°C with copper oxide. The evolved CO₂ is trapped and concentrated in a cryogenic trap. It is cleaned and separated in a porabond GC column before entering the IRMS. Results are given in the standard δ -notation relative to V-PDB and the precision for a duplicate $\delta^{13}\text{C}_{\text{CH}_4}$ analyses is 2-3 ‰ V-PDB.

2.4. Interstitial water chemistry

Pore water samples were obtained from core 5022-1A using soil moisture samplers (Rhizon SMS, Eijkelkamp) introduced into the sediment through small holes drilled in the core liners. Fluids were extracted using syringes screwed to the microrhizons and maintained under low pressure. After sampling, recovered waters were split for cation and anion analyses and flushed with helium gas immediately thereafter to avoid any shift in water chemistry. The transfer of pore water samples into sealed vials was performed under a N₂ protective atmosphere in small chamber. Samples for cation analyses were acidified with 100 μL HNO₃ (65%). Cations were measured by ICP-MS whereas anions were analyzed by ion-chromatography.

2.5. Fatty acid extraction

FA extractions, targeting compounds from 12 to 35 carbons, were carried out on fresh sediments preserved under anoxic conditions in hermetically sealed bags (Vuillemin et al. 2013). The extraction method was previously described by Naeher et al. (2012). Sediments (2 cm³) were ultrasonically extracted (40 min) with 2 mL methanol (MeOH) and 6 mL dichloromethane (DCM), and heated (2 min at 330 W and 6 min at 500 W) in teflon tubes using a high performance microwave (Milestone[®]). Liquid and sediment fractions were

separated by centrifugation. Sediments were retrieved, dried and weighed. Liquid extracts were combined in a separatory funnel with 20 mL milli-Q water DCM extracted NaCl solution (5%). The aqueous phase was extracted with 10 mL DCM twice, and the collected DCM fraction reduced by rotary evaporation (Rotavapor® R-210, Buchi). Samples were dissolved in DCM, dried with Na₂SO₄, blown down with N₂ at 30°C, redissolved in DCM, passed through a copper column to eliminate sulfur traces and blown down again. 3 mL KOH 6% was added and saponification carried out for 3 hours at 80°C. 1 mL of DCM extracted H₂O was added to stop the reaction. Neutral lipids were extracted with 1 mL n-hexane (4×), blown down, dissolved in DCM, dried with Na₂SO₄ and kept in the freezer. After acidification with HCl 6M (~0.85 mL), FAs were extracted with 1 mL n-hexane (4×), blown down, dissolved in 1 mL BF₃/CH₃OH 10% (Sigma Aldrich) to produce the methyl esters (FAMES). Derivatization was carried out for 30 min at 60°C, and the reaction stopped with 2 mL of DCM extracted H₂O. FAs were extracted with 1 mL n-hexane (4×), blown down, dissolved in DCM, dried with Na₂SO₄, blown down once more, and dissolved in 100 µL n-hexane. Aliquots (20 µL) were used for measurements. The precision for double injection is better than 0.5% and better than 8% for the whole method including extraction.

2.6. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

The resulting fractions were measured using a GC System 6890 Series Plus (Agilent®) with a MultiPurposeSampler MPS2 (Gerstel®) and flame ionisation detection (GC-FID, Agilent 6890, with a 60 m VF-5 column x 0.25 mm inner diameter x 0.25 µm film thickness). Peaks quantification was achieved by using spikes of an external standard (C₃₆). The second run of analyses was performed on a GCMS-QP 2010 Ultra (Shimadzu®) with an autoinjector AOC-201 (Shimadzu®). The retention times of FAs detected in samples were compared with retention times of known compounds (FAME and BAME, Supelco®) on the GCMSSolution® version 2.70 software (Shimadzu®). Corroboration of FA identification was by comparison of mass spectra to those in the NIST 08 Mass Spectral Library. A third run of analyses was performed on a Network GC System 6890N (Agilent®) coupled to a Micromass IRMS (Isoprime®) to measure $\delta^{13}\text{C}$ composition of FAs. An nC_{25:0} alkane standard ($\delta^{13}\text{C} = -31.7\text{‰}$) was regularly measured to check for instrumental deviation. The precision of samples measured in triplicate is better than 0.5 ‰. The linearity of the IRMS is consistent from 0.5 to 2.0 nA. Samples were concentrated to reach at least 5 pA for a reliable measurement.

2.7. DNA extraction and PCR amplification

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 µmol/L of each of the primers, 200 µmol/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. Bacterial 16S rRNA gene amplifications were performed using the bacterial universal primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). Reaction mixtures were held at 95°C for 2 min followed by 30 cycles of 94°C for 30 s, 52°C for

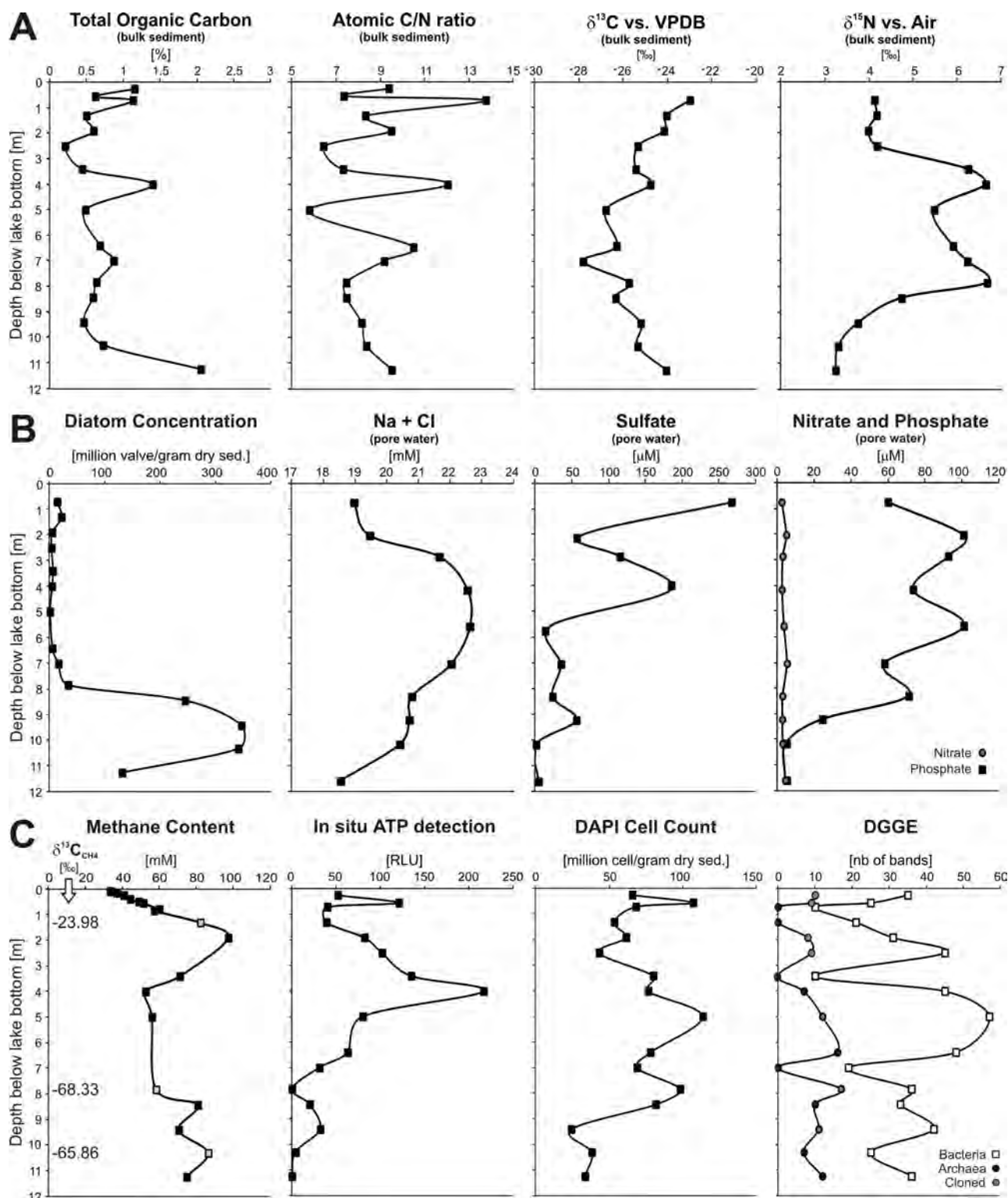


Figure 1 Multiproxy dataset displaying bulk OM characteristics, pore water concentrations and microbial indexes. **Top row (A) left to right:** TOC [% dry weight]; atomic $\text{C}_{\text{org}}/\text{N}$ ratio; $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ of the bulk fraction [‰]. **Medium row (B) left to right:** Diatom concentration [million valve/gram dry sediment]; pore water Na + Cl concentrations [mM]; pore water sulfate concentrations [μM]; pore water nitrate and phosphate concentrations [μM]. **Bottom row (C) left to right:** Methane content [mM] and some of its respective $\delta^{13}\text{C}$ values [‰]; *in situ* ATP detections [RLU]; DAPI cell counts [million cell/gram dry sediment]; number of DGGE bands for Bacteria (white squares) and Archaea (black dots with grey ones indicating cloned samples).

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 archaeal gene amplifications, a nested PCR approach with overlapping primers was selected to avoid an enrichment step by cultures (Vissers et al. 2009). 4F (5'-TCY GGT TGA TCC TGC CRG-3') with Univ1492R (5'-CGG TTA CCT TGT TAC

GAC TT-3') were used as the initial primer pair (Dong et al. 2006). Archaeal 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 used in the second PCR round with the overlapping forward primer 3F (5'-TTC CGG TTG ATC CTG CCG GA-3') associated with 9R (5'-CCC GCC AAT TCC TTT AAG TTT C-3') as the reverse primer.

2.8. Cloning procedure and phylogenetic analyses

PCR products were purified using the High Pure[®] PCR Product Purification Kit (Roche Diagnostics SA), measured with a Nanodrop[®] ND-1000 Spectrophotometer (Witec AG), and diluted to a concentration of 10 ng/µL. 2 µL PCR template were ligated to the pCR[®]4-TOPO[®] vector (Invitrogen[™] by life technologies[™]) and cloned into competent *Escherichia coli* cells. Cloning procedure was performed using the TOPO[®] TA Cloning[®] Kit (Invitrogen[™] by life technologies[™]) following the manufacturer's recommendations. Transformed cells were incubated at 37°C for 20 hours on a LB medium containing 1g/L NaCl, 1 g/L Bactotryptone, 0.5 g/L Bactoyeast, 1.5 g/L Bactoagar and 2 mL/L ampicillin. Archaeal clones were selected from sediment samples at 0.3, 0.6, 1.9, 2.5, 5.0, 7.8, 9.4 m depth to constitute the libraries. Sequencing cycles were performed using the BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied BioSystems) with vector primers D4 and R5. Sequencing was performed on an ABIPRISM[®] 3130xl Genetic Analyzer (Applied BioSystems, Hitachi).

Sequences were assembled with CodonCode Aligner[®] v.3.7.1 (CodonCode Corporation), aligned on Seaview[®] v.4.3.0 (Gouy et al. 2010) using the clustalW2 algorithm, and primers selectively cut off with VecScreen (www.ncbi.nlm.nih.gov/VecScreen). Chimeras were detected using the online program Bellerophon[®] (Huber et al. 2004) (<http://comp-bio.anu.edu.au/bellerophon/>). 16S ribosomal DNA gene sequences were identified using the megx[®] Geographic-BLAST (<http://www.megx.net>) and SILVA[®] comprehensive ribosomal RNA databases (Pruesse et al. 2007). Rarefaction curves (see annex A-6) were established for 5 and 10% divergence, using the Mothur[®] v. 1.25.1 software (Schloss et al. 2009). Sequences related to methanogens were selected for phylogenetic analyses. References were obtained from the SILVA[®] browser (<http://www.arb-silva.de>) and a phylogenetic tree produced on the Ribosomal Database Project (<http://rdp.cme.msu.edu>) using the Weighbor Joining method (Bruno et al. 2000), including results from a bootstrap test using 100 replicates.

The sequences generated in this study have been deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>) under accession numbers JX272064 to JX272122.

3. Results

3.1. Bulk organic matter proxies and organic remains (diatoms)

TOC contents (Fig. 1A) average 0.6%, with values over 1% at 0.3, 0.7, 6.6 m depth and a maximum (2%) at 11.3 m depth. Atomic C_{org}/N ratios (Fig. 1A) display variations with depth, with maxima at 0.7, 4.0 and 10.4 m depth and minima at 0.6, 2.5 and 5.0 m depth. Organic carbon and nitrogen isotope compositions of the bulk fraction show opposite trends. $\delta^{13}\text{C}_{\text{org}}$ decreases gradually from -23.0‰ at the surface to -27.8‰ at 6.2 m depth (Fig. 1A) and increases to -24.1‰ at 11.3 m depth. Conversely, the $\delta^{15}\text{N}$ values are 4.0‰ in the uppermost sediments, increase to 6.6‰ at 3.0 m depth (Fig. 1A), decrease to 5.5‰ at 5.0 m depth,

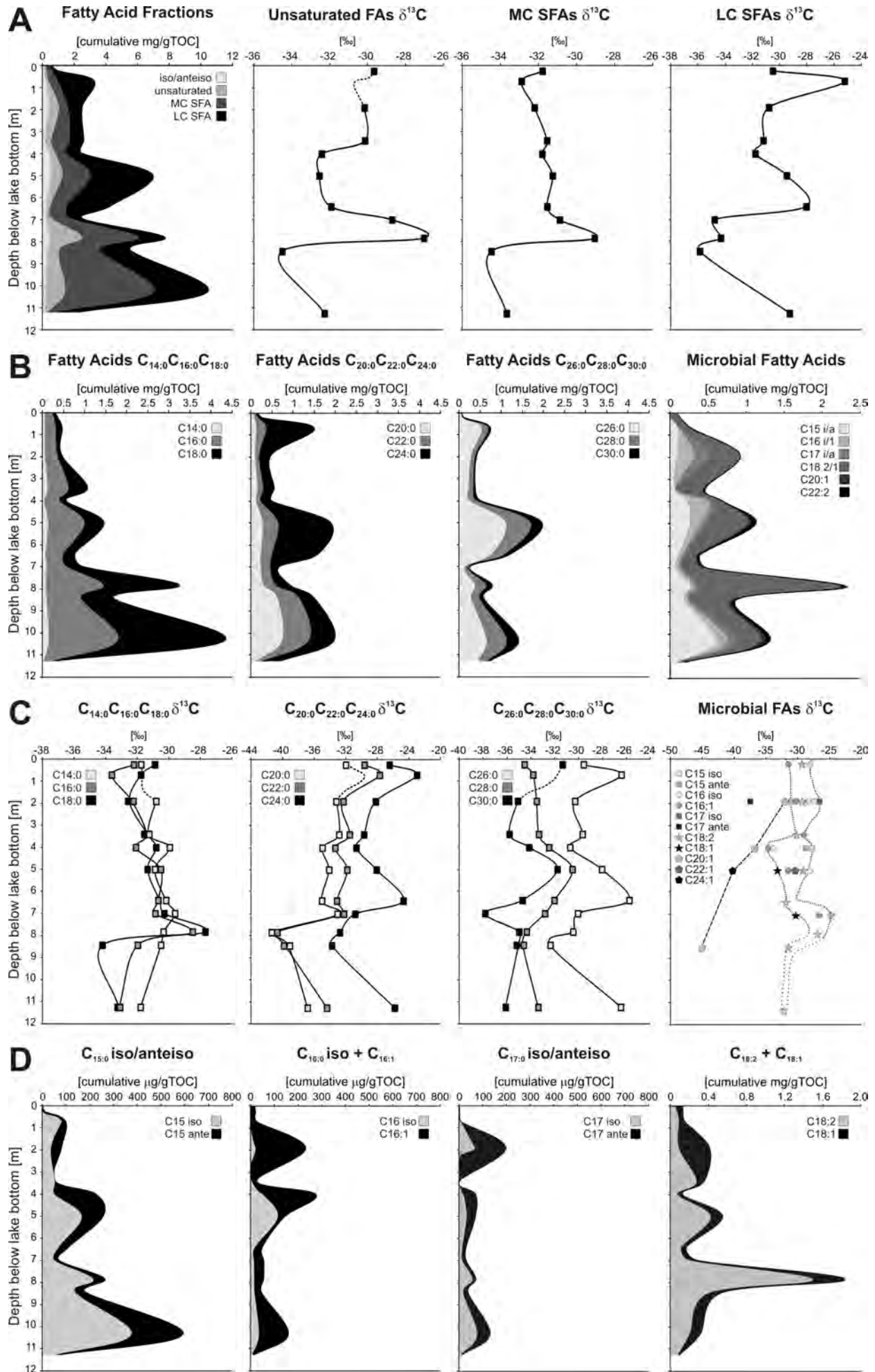


Figure 2 Multiproxy dataset displaying FA concentrations and their respective carbon isotope signatures. **First row (A) left to right:** Cumulative total concentrations of FAs displayed as specific fractions (iso/anteiso, unsaturated, MC SFAs, LC SFAs) [mg/gTOC]; $\delta^{13}\text{C}$ of branched and unsaturated FAs [‰]; $\delta^{13}\text{C}$ of MC SFAs [‰]; $\delta^{13}\text{C}$ of LC SFAs [‰]. **Second row (B) left to right:** Concentrations of FAs $\text{C}_{14:0}$, $\text{C}_{16:0}$ and $\text{C}_{18:0}$ [mg/gram TOC]; concentrations of FAs $\text{C}_{20:0}$, $\text{C}_{22:0}$ and $\text{C}_{24:0}$ [mg/gram TOC]; concentrations of FAs $\text{C}_{26:0}$, $\text{C}_{28:0}$ and $\text{C}_{30:0}$ [mg/gram TOC]; cumulative FAs concentrations indicative of microbial biomass [mg/gram TOC]. **Third row (C) left to right:** $\delta^{13}\text{C}$ of FAs $\text{C}_{14:0}$, $\text{C}_{16:0}$ and $\text{C}_{18:0}$ [‰]; $\delta^{13}\text{C}$ of FAs $\text{C}_{20:0}$, $\text{C}_{22:0}$ and $\text{C}_{24:0}$ [‰]; $\delta^{13}\text{C}$ of FAs $\text{C}_{26:0}$, $\text{C}_{28:0}$ and $\text{C}_{30:0}$; $\delta^{13}\text{C}$ of various microbial fatty acids [‰]. **Fourth row (D) left to right:** $\text{C}_{15:0}$ iso/anteiso concentrations [$\mu\text{g}/\text{gram TOC}$]; $\text{C}_{16:0}$ iso and $\text{C}_{16:1}$ concentrations [$\mu\text{g}/\text{gram TOC}$]; $\text{C}_{17:0}$ iso/anteiso concentrations [$\mu\text{g}/\text{gram TOC}$]; $\text{C}_{18:2}$ and $\text{C}_{18:1}$ concentrations [mg/gram TOC].

increasing to 6.6‰ at 7.8 m depth and decreasing to 3.2‰ at 11.3 m depth. A sharp peak in diatom concentration, an indicator of algal paleoproductivity, is observed between 9.4 and 11.3 m depth (Fig. 1B; Recasens et al. 2012).

3.2. Pore water chemistry

Salinity concentrations (i.e. $\text{Na}^+ + \text{Cl}^-$) range between 22.62 (5.6 m depth) and 18.53 mM (11.6 m depth), reflecting subsaline conditions (Fig.1B). Sulfate concentrations (Fig. 1B) are maximal (267 μM) in surficial sediments, decrease to 63 μM at 2.0 m depth, increase to 184 μM at 4.2 m depth, with sulfate depletion (ca. 20 μM) occurring below 5.0 m depth. Nitrate concentrations are all below 5 μM , while phosphate oscillates between 60 and 100 μM in the uppermost 8 m of sediment and is depleted (ca. 10 μM) at 9.2 m depth (Fig. 1B).

3.2. Total methane content and its carbon isotopic composition ($\delta^{13}\text{C}_{\text{CH}_4}$)

Methane content (Fig. 1C) displays a constant increase from the sediment surface to 1.9 m depth, reaching 100 mM. It decreases subsequently to 50 mM at 4.0 m and fluctuates between 60 and 85 mM thereafter. The carbon isotope compositions of methane ($\delta^{13}\text{C}_{\text{CH}_4}$) were measured on selected samples at 1.3, 7.8 and 10.3 m depth. $\delta^{13}\text{C}_{\text{CH}_4}$ values correspond to -24.0‰ in shallow sediments, decreasing to -68.3‰ and -65.9‰ in bottom sediments.

3.4. Microbial indexes

ATP is only produced by living organisms and its measurements are presently used as an indicator of ongoing microbial activity. Background readings measured on milli-Q water varied between 25 and 30 relative luminescence units (RLU). 30 RLUs were, thus, systematically removed from every reading. Average values increase from 50 at 0.3 m depth to 217 RLUs at 4.0 m depth (Fig. 1C), with sharp peaks occurring at 0.6 and 4.0 m depth. ATP values then decrease to a minimum at 7.8 m depth, with a minor final increase at 9.4 m depth. DAPI cell counts (Fig. 1C) varies throughout depth, displaying three maxima at 0.6, 5.0 and 7.8 m depth, local minima at 2.5 and 7.0 m depth and a minimum in the lowermost sediment. The number of DGGE bands indicates the dominance of Bacteria over Archaea (Fig. 1C), but both trends are similar, with maxima and minima at identical depths. Low band numbers are found at 0.71, 3.41, and 7.0 m depth and maximal band numbers at 0.3, 2.5, 5.0, and 9.4 m depth.

3.5. Fatty acid concentrations and their $\delta^{13}\text{C}$ isotopic signatures

For each sample, we identified 27 compounds from $\text{C}_{14:0}$ to $\text{C}_{30:0}$ (see annex A-7). Concentrations are expressed in % of dry sediment normalized to TOC. $\delta^{13}\text{C}$ compositions of unsaturated FAs, medium-chain saturated fatty acids (MC SFAs) and long-chain saturated

fatty acids (LCFAs) represent mean values normalized by each compound concentration (Fig. 2A). Total FAs reach 1% of the TOC content at 5.0, 7.8 and 10.3 m depth (Fig. 2A), and display an overall increase with depth. Unsaturated and iso/anteiso FA concentrations show minor maxima at 2.5, 5.0 and 7.8 m depth. Their corresponding $\delta^{13}\text{C}$ signatures (Fig. 2A) display values between -34 and -32‰ from 4.0 to 7.0 m depth, a maximum (-27.0‰) at 7.8 m depth, followed by a minimum (-34.5‰) at 9.4 m depth. MC SFAs represent a substantial proportion of FAs from 4.0 to 7.0 m depth, but clearly dominate in lowermost sediments (7.0 to 11.3 m depth). Their $\delta^{13}\text{C}$ values gather around -32‰ with few variations (Fig. 2A), with a maximum (-29.1‰) followed by a minimum (-34.5‰) in the lowermost record. LC SFAs are substantial at 1.3 and 5.0 m depth and display an opposite $\delta^{13}\text{C}$ trend compared to unsaturated FAs and MC SFAs (Fig. 2A), with maximal values (-25.2 and 28.0‰) at 0.7 and 6.4 m depth and minimal values (-34.8 to 35.9‰) from 7.0 to 9.4 m depth.

Concentrations for $\text{C}_{14:0}$, $\text{C}_{16:0}$ and $\text{C}_{18:0}$ (Fig. 2B) are high in lowermost sediments, indicating the substantial presence of labile OM. Their $\delta^{13}\text{C}$ signatures are identical (Fig. 2C), showing an increase with depth from a minimum of -33.6‰ to a maximum of -27.7‰ at 7.8 m depth. In contrast, $\delta^{13}\text{C}$ profiles for $\text{C}_{20:0}$ and $\text{C}_{22:0}$ values (Fig. 2C) slightly decrease from -27.7‰ to -36.7‰ with depth, reaching a minimum (-41.3‰) at 7.8 m depth. Refractory OM is represented by $\text{C}_{24:0}$ to $\text{C}_{30:0}$. $\text{C}_{24:0}$ and $\text{C}_{26:0}$ concentrations (Fig. 2B) are substantial at 1 m and from 4.0 to 7.0 m depth, with respective $\delta^{13}\text{C}$ values ranging from -23.0 to -33.8‰ (Fig. 2C), whereas $\text{C}_{28:0}$ and $\text{C}_{30:0}$ constitute a minor fraction, displaying low $\delta^{13}\text{C}$ values (-30.5 to -37.8‰).

Bacterial FAs constitute 2 to 23% of total FAs, and 8 to 37% including all unsaturated FAs. Generally, microbial FA concentrations (Fig. 2B) are maximal at 1.9, 5.0, 7.8 and 10.3 m depth. Peaks associated with specific bacterial compounds (Fig. 2D) occur at: 5.0, 7.8 and 10.3 m depth for $\text{C}_{15:0}$ iso and $\text{C}_{15:0}$ anteiso; 1.9 and 4.0 m depth for $\text{C}_{16:0}$ iso and $\text{C}_{16:1}$; 1.9 m depth for $\text{C}_{17:0}$ iso and $\text{C}_{17:0}$ anteiso; 1.9, 5.0 and 7.8 m depth for $\text{C}_{18:2}$ and $\text{C}_{18:1}$. Because detection was not achieved for compounds of concentrations below 100 $\mu\text{g/gTOC}$ (Fig. 2C), these $\delta^{13}\text{C}$ composition results are partial (Fig. 2D). A first trend referring to bacterial chains from $\text{C}_{15:0}$ iso to $\text{C}_{18:1}$ was detected, with values included in a 5‰ interval (Fig. 2C, dotted lines) with decreasing tendencies at 1.9, 4.0 and 5.0 m depth and a maximum value (-24.9‰) at 7.0 m depth. At 1.9 m depth, $\text{C}_{17:0}$ anteiso displays a very low $\delta^{13}\text{C}$ value (-37.5‰). Bacterial chains over twenty carbons show a linear decrease with depth from -31.6‰ to -45.0‰ (Fig. 2C, dashed line), indicating important fractionation processes linked to methane production.

3.6. Archaeal phylogenetics

Archaeal clone libraries were established from samples at 0.25, 0.55, 1.91, 2.51, 5.0, 7.84 and 9.4 m depth. Sequences in uppermost sediments are mainly related to the Marine Group 1 and Marine Benthic Groups B and D (Fig. 3) with few methanogens, whereas methanogens and the Miscellaneous Crenarchaeotic Group (MCG) dominate in samples from 1.9 to 9.4 m depth. South African Gold Mine Group (SAGMEG) sequences appear below 7.0 m depth.

A phylogenetic tree was built with 58 methanogen sequences (Fig. 4) from Laguna Potrok Aike sediments, evidencing a majority of *Methanomicrobiales* and *Methanosarcinales* with an overall low diversity of species. The defined clusters highlight a stratification of methanogenic populations in Holocene sediments. Most sequences at 0.3, 0.6 and 2.5 m depth are identified

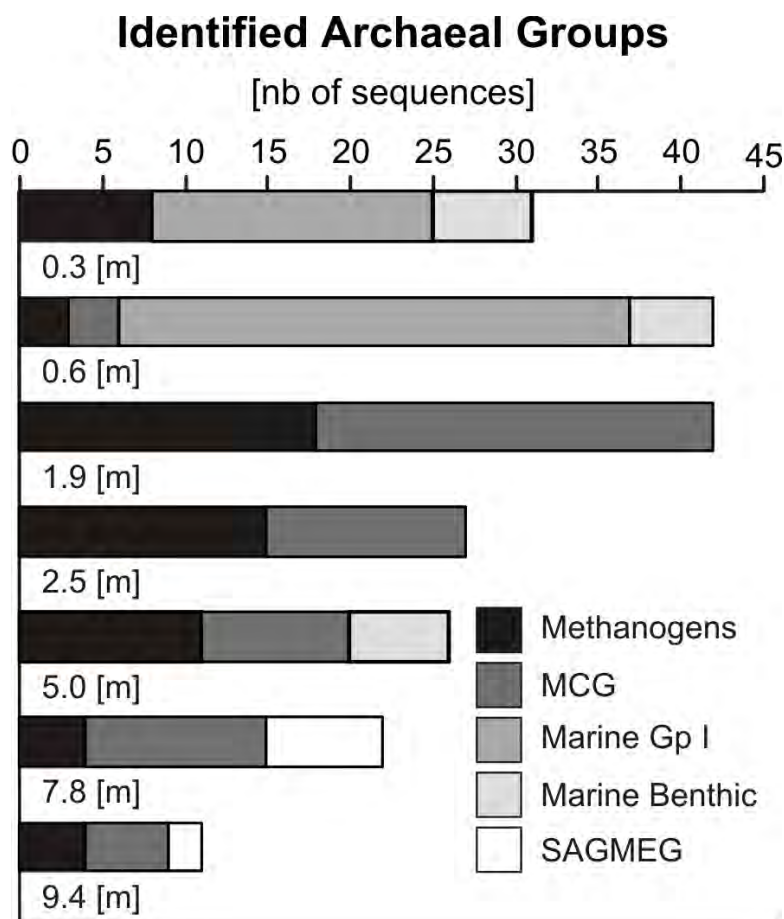


Figure 3 Histogram of all retrieved archaeal sequences and their respective identifications.

as *Methanolinea* sp., whereas sequences at 1.9 m depth relate to *Methanosarcinales* Gulf of Mexico Archaea 1 (GOM Arc 1). Sequences at 5.0 and 7.8 m depth comprise *Methanoregula* sp. exclusively. The few sequences at 9.4 m depth are identified as *uncultured Methanomicrobiaceae* branching in between *Methanosphaerula palustris* and *Methanolinea tarda*.

These results include two bacterial sequences retrieved at 5.0 m depth, because they relate to *Syntrophus aciditrophicus* (Table 1), which is known to establish syntrophic relationships with methanogens (Jackson et al. 1999).

4. Discussion

4.1. Bulk organic matter characteristics and microbial development

OM sources in bulk sediments are often traced using C_{org}/N ratio, $\delta^{13}C_{org}$ and $\delta^{15}N$. Although these parameters generally allow the distinction between terrestrial and aquatic contributions, they can hardly discriminate individual sources (Das et al. 2008). As algal OM is preferentially degraded during sinking and sedimentation, source interpretations are often biased towards macrophytes and land plants (Meyers 1994). Diagenesis generates the preferential preservation of refractory OM with a preferential removal of nitrogen over carbon (Giani et al. 2010). Moreover, ammonium adsorption in clay lattices often leads to a decrease in C_{org}/N ratios with depth and over time (Freudenthal et al. 2001). Still, high C_{org}/N ratios can indicate sporadic terrestrial inputs into Laguna Potrok Aike sediments (Fig. 1A), and high diatom concentrations attest of high algal productivity (Fig. 1B).

However, microbial processes can extend deep into the sediments, as shown by the ATP values at 4.0 m depth (Fig. 1C). DAPI cell counts and DGGE (Fig. 1C) also indicate that microbial populations have preferentially colonized and proliferated in those horizons with low atomic C_{org}/N ratios (Fig. 1A). Additionally, microbial development appears to be strongly correlated with phosphate concentrations in pore water (Fig. 1B), which are directly dependent on OM degradation. Microbial colonies, with regard to their alteration potential (Lehmann et al. 2002) and biomass production (Freudenthal et al. 2001), influence and become part of the labile organic sources. Beside the fact that bacterial growth is higher when associated with such labile OM (Amon and Benner 1996), the conversion of refractory components into methane can be carried out via trophic links between fermenting microbes and methanogens (Wüst et al. 2009). However, methanogenesis is considered a late stage of degradation as it normally occurs after depletion of available oxidants by layered microbial communities (Nealson 1997; Konhauser 2007). In Laguna Potrok Aike sediments, methanogenic activity should start around 0.7 m depth to correspond with the transition to CO₂ as the main oxidant (Vuillemin et al. 2013). Coexistence can nevertheless be achieved via mutualistic interactions and syntrophy (Wang et al. 2010), which are essential to the complete conversion of natural polymers (McInerney et al. 2009). In lacustrine anoxic sediments, syntrophic populations are widespread and present a strong potential for carbon fractionation (Borrel et al. 2011).

Table 1 Closest matches of bacterial 16S rRNA gene sequences, retrieved at 4.97 m sediment depth, to cultured and environmental syntrophic organisms.

Clone No.	Length (bp)	Maximum score in GenBank database	Query coverage	Maximum identity	Accession N°
POTROK_AIKE_Bact D7/Clone 03	1425	<i>Syntrophus aciditrophicus</i> (GU993263) <i>Syntrophaceae uncultured</i> (FJ484515)	99 % SILVA	87 % SILVA	JX272064
POTROK_AIKE_Bact D7/Clone 39	1437	<i>Syntrophus aciditrophicus</i> (GU993263) <i>Syntrophaceae uncultured</i> (AM086113)	99 % SILVA	94 % SILVA	JX272065

4.2. Depth distribution of methanogenic species.

Archaeal distribution with depth (Fig. 3) shows that methanogens become dominant from 1.9 m depth, coinciding with the highest methane concentration (Fig. 1C). The phylogenetic tree (Fig. 4) emphasizes four depth-dependent clusters, revealing the stratification of methanogenic populations in the sediment. The first cluster (ca. 0.3-2.5 m depth downward) is related to *Methanolinea tarda* (Fig. 4), a species that tolerates a 1.5% salinity and grows using C1 compounds exclusively (e.g. formate, H₂/CO₂), thus referring to the methyl fermentation and CO₂ reduction pathways (Imachi et al. 2008). A syntrophic relationship with sulfate-reducing bacteria (SRB) is often observed under sufficient sulfate concentrations (Fig. 1B; Imachi et al. 2006 and 2008). The second cluster (ca. 1.9 m depth) is composed of *GOM Arc 1 Methanosarcinales*. These methanogens have been documented in relation to the anaerobic oxidation of methane (AOM) and are referred to as AOM- associated Archaea (AAA; Borrel et al. 2011; Schubert et al. 2011). Growth and methane production are based on fermentative products, such as methanol, methylamines, H₂/CO₂ and possibly acetate (Simankova et al. 2001). Methanogenesis derived from methylamines leads to the production of ammonium (Hedderich and Whitman 2006), which can be critical for microbial development under nitrogen-limiting pore water conditions (Fig. 1B). Substrate competition does not occur with *Methanolinea* or SRB (Borrel et al. 2011). The third cluster (ca. 5.0 to 7.8 m depth) is composed of *Methanoregula sp.* (Fig. 4), a halotolerant but sulfide sensitive species producing methane from H₂/CO₂ exclusively (Bräuer et al. 2011), thus indicating the CO₂ reduction pathway. The presence at the same depth of *Syntrophus*-related sequences (Table 1) provide

evidence for a syntrophic relationship with *Methanoregula* (Jackson et al. 1999; Garcia et al. 2000), thus potentially enabling an advanced metabolism of refractory polymers by microbes (McInerney et al. 2009; Wüst et al. 2009). The fourth cluster (ca. 10.3 m depth) corresponds to *uncultured Methanomicrobiaceae*, which have been previously reported in syntrophic acetate oxidation processes (Schnürer et al. 1994; Nüsslein et al. 2001). Nearest related species use mainly formate and H_2/CO_2 during methanogenesis, but require acetate for growth (Asakawa and Nagaoka 2003; Cadillo-Quiroz et al. 2009).

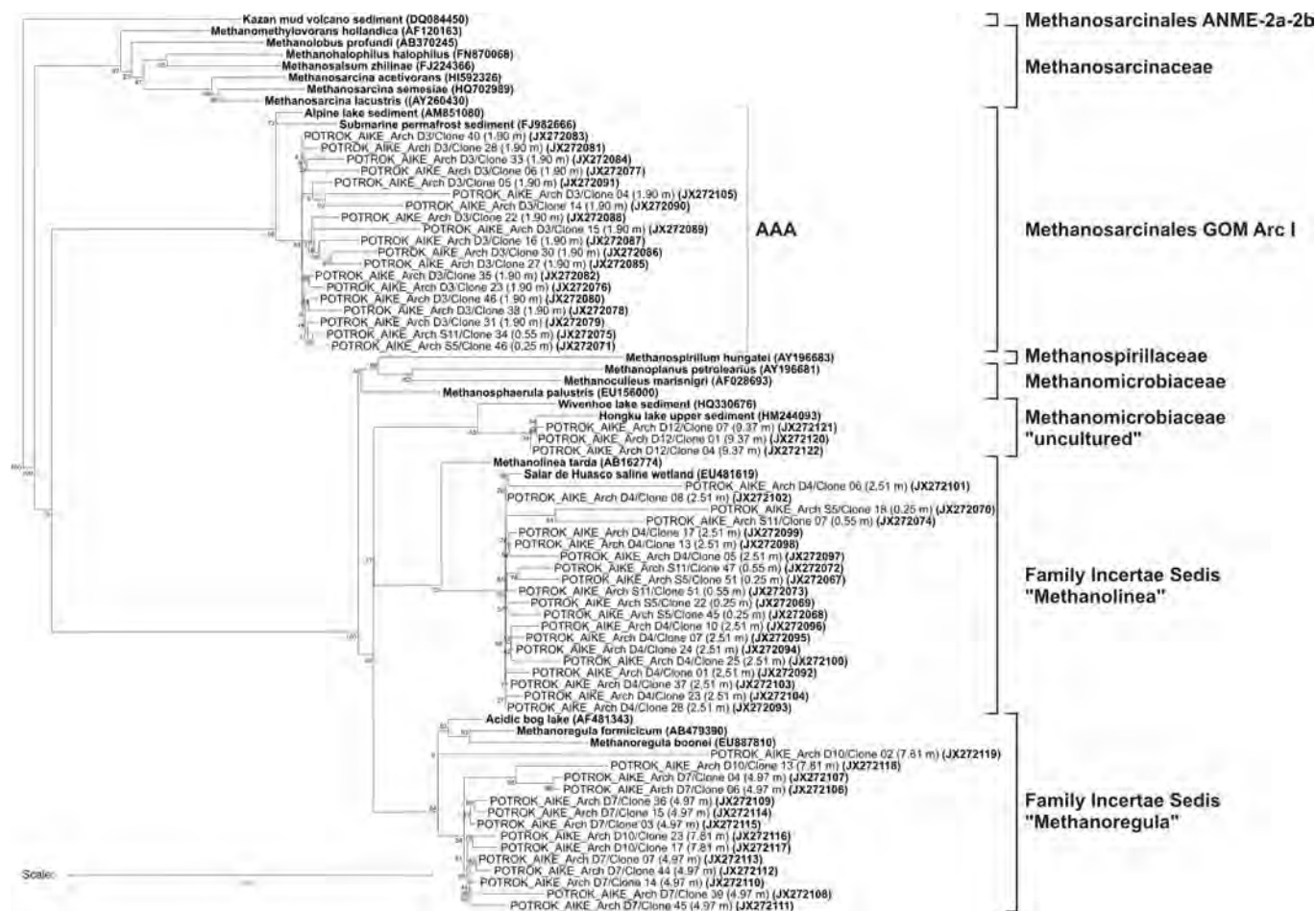


Figure 4 Phylogenetic tree (Weighted Neighbor Joining) of partial 16S rRNA gene sequences (900 bp) related to methanogenic species. Sequences were retrieved at various depths from Laguna Potrok Aike anoxic sediments. *Methanosarcinales* present in Laguna Potrok Aike sediments are related to the AAA lineage (i.e. AOM-associated Archaea).

Methanogenic populations at Laguna Potrok Aike are dominated by hydrogenotrophs, with possible syntrophic partnerships involving *Methanoregula* and *Methanolinea*, which is in agreement with many documented freshwater environments of low trophic levels (Borrel et al. 2011). Conditions in Laguna Potrok Aike water column and sediment (Zolitschka et al. 2006) also suggest that these species are halo and psychrotolerant (Simankova et al. 2001; Simankova et al. 2003). The stratification of methanogenic species with depth may derive from the gradual degradation of OM which provides successive substrates to each species (Fig. 5). Methanogenesis starts with the methyl fermentation pathway and shifts to CO_2 reduction at 2.5 m depth (Fig. 5). The transition depth is colonized by *Methanosarcinales*, perhaps due to their ability to use variable fermentative products as substrate. In lowermost sediments, we propose that the *uncultured Methanomicrobiaceae* perform syntrophic oxidation of acetate as a late stage of methanogenesis (Fig. 5).

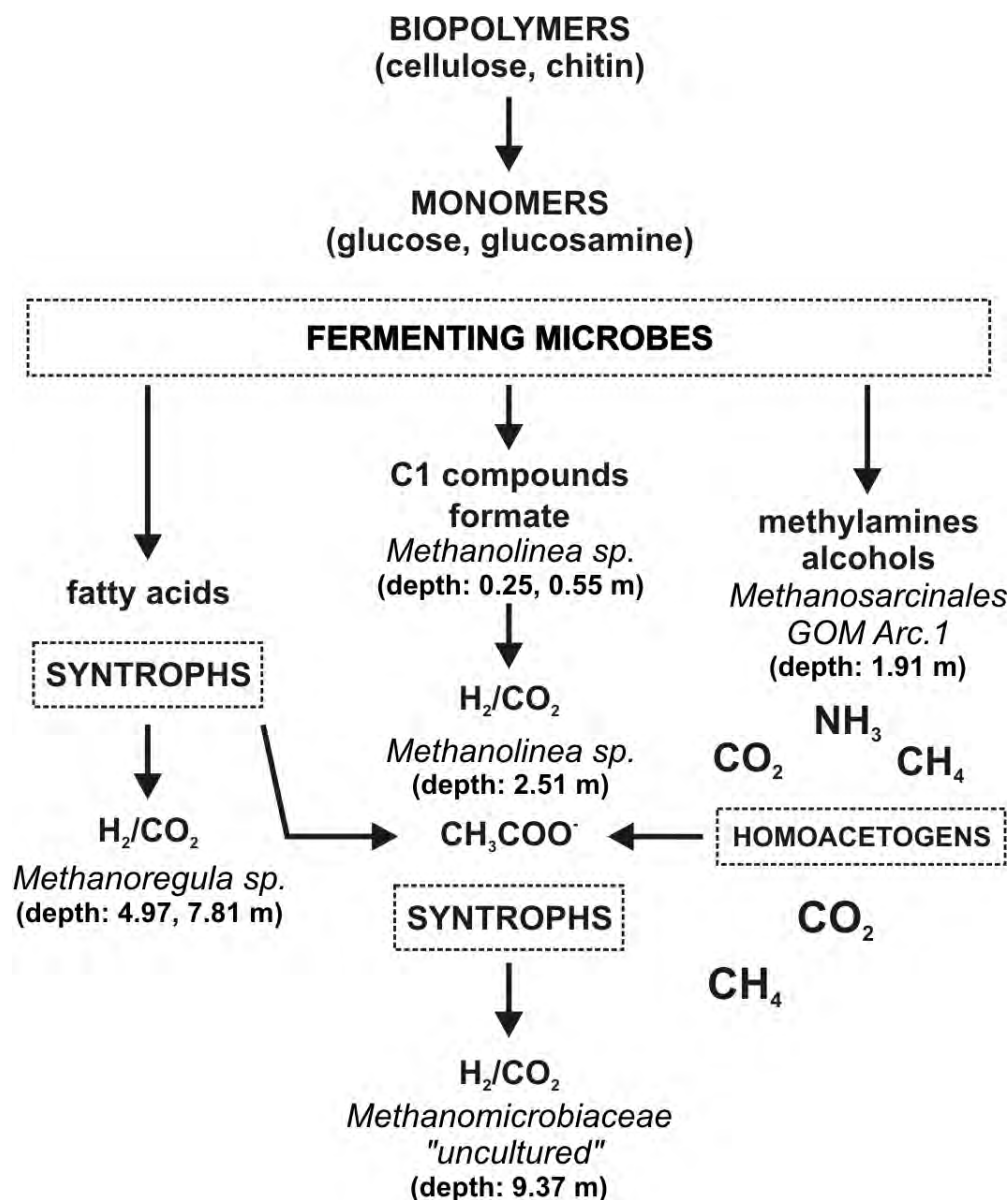


Figure 5 Schematic summary showing the stepwise OM degradation. Successive steps are hydrolysis, acidogenesis (or fermentation), acetogenesis and methanogenesis. Methanogens from the present study are placed with respect to their substrates and depths. It shows that methanogenesis takes place during different degradation stages. Final products can reenter the cycle by being reduced (CO_2 reduction) or oxidized (CH_4 anaerobic oxidation).

4.3. Methane $\delta^{13}C$ signature and organic matter degradation

Methane production is mainly based on methyl fermentation in freshwater shallow sediments, in which CO_2 reduction plays a minor role (Whiticar 1999). In Laguna Potrok Aike, methane content and microbial activity do not correlate (Fig. 1C), mainly because methanogenesis depends on non-competitive substrates (Whiticar 1999; Conrad et al. 2010), whereas microbial assemblages are dominated by Bacteria (Fig. 1C). The linear increase of methane content in shallow sediments may reflect gas diffusion toward the surface from a zone of maximal production around 2 m depth. The $\delta^{13}C_{CH_4}$ composition at 1.3 m depth is relatively high (-24.0‰), corresponding with the bulk $\delta^{13}C_{org}$. AOM processes are known to occur in freshwater sediments in link to nitrate, iron, manganese and sulfate reduction processes (Deutzmann and Schink 2011; Schubert et al. 2011). During this process, the uptake of the light carbon isotope by methane oxidizing microbes results in more positive $\delta^{13}C_{CH_4}$ values of the residual methane

fraction (Holler et al. 2009). Nitrate in pore water is presently depleted (Fig. 1B) while iron and manganese were below detection limits (not shown). Conversely, sulfate concentration in pore water is sufficient to allow AOM processes (Boetius et al. 2000). In the absence of anaerobic methane-oxidizing Archaea (ANME), this $\delta^{13}\text{C}_{\text{CH}_4}$ value can be interpreted as an early stage of methyl fermentation performed by *Methanolinea* or as due to some microaerobic oxidation of methane. Still, the potential uptake of ^{12}C by SRB in association with AAA methanogens during AOM processes has to be considered (Borrel et al. 2011).

These *Methanosarcinales* can use additional substrates (Fig. 4), leading to maximal methane concentration at 1.9 m depth (Fig. 1C). Thereafter, methane concentrations decrease sharply, which may reflect the shift of methanogenic pathway to CO_2 reduction (Whiticar 1999). Indeed, methanogenic populations are essentially composed by *Methanoregula* sp. (Fig. 4) which grows on H_2/CO_2 exclusively (Bräuer et al. 2011; Fig. 5). Syntrophy with *Methanoregula* can explain the current sustainability of microbial populations (Fig. 1C) because it allows an efficient conversion of polymers into methanogenic substrates (McInerney et al. 2009; Fig. 5). The very negative $\delta^{13}\text{C}_{\text{CH}_4}$ value at 7.8 m depth (-68.3‰) can correspond to an advanced stage of CO_2 reduction (Whiticar et al. 1986) derived from the use of refractory OM by syntrophic consortia (Penning et al. 2005). Deeper in the sediment (ca. 9.4-11.3 m depth), methanogenesis relies possibly on H_2/CO_2 produced during anaerobic oxidation of acetate (Fig. 5), which tends to accumulate during acetogenesis and degradation of FAs (Schink 1997). This is only partially inferred from the $\delta^{13}\text{C}_{\text{CH}_4}$ value (-65.9‰) and presence of *uncultured Methanomicrobiacea*. These methanogens have been reported from deep lacustrine sediments in which they outcompete acetoclastic methanogenesis via syntrophic acetate oxidation (Nüsslein et al. 2001; Hattori 2008).

4.4. Fatty acid sources

According to Mayr et al. (2009), the $\delta^{13}\text{C}_{\text{org}}$ composition of the bulk organic fraction measured in sediment traps of Laguna Potrok Aike deep waters varies between -24.2 and -25.5‰. Several OM sources were identified in the OM fraction, whose relative proportions explained the $\delta^{13}\text{C}_{\text{org}}$ bulk values: Diatom oozes (mean: -25.6‰), cyanobacteria (mean: -21.8‰), terrestrial vegetation (mean: -26.0‰), aquatic mosses (mean: -32.2‰), macrophytes (mean: -13.0‰) and soils (mean: -25.0‰).

Terrestrial and planktonic sources are normally traced using LC SFAs and MC SFAs, respectively (Naraoka and Ishiwatari 2000; Niggemann and Schubert 2006). High concentrations of $\text{C}_{16:0}$ to $\text{C}_{20:0}$ FAs along with diatom valves, observed at 10.3 m depth (Figs. 1B and 2B), provide evidence for a substantial contribution of algal sources. Such preservation of labile OM can be attributed to an increased sedimentation rate associated with redeposition events during the Early mid-Holocene (Anselmetti et al. 2009). On the contrary, concentrations of LC SFAs do not directly correlate with high $\text{C}_{\text{org}}/\text{N}$ ratios (Figs. 1A and 2B). The comparison of ^{13}C compositions between LC SFAs and initial organic sources shows that $\text{C}_{24:0}$ and $\text{C}_{26:0}$ (Fig. 2C) reflect terrestrial sources values coming from the Patagonian steppe, whereas $\text{C}_{26:0}$ and $\text{C}_{30:0}$ correspond to aquatic mosses values presently growing on the lake shores. This shows that the ^{13}C signature is best preserved in refractory compounds (Naraoka and Ishiwatari 2000). Their $\delta^{13}\text{C}$ shift towards more positive values (up to 6‰) in between 4.0 and 7.0 m depth coincides with the maximal density, activity and diversity of microbial populations (Fig. 1C) and likely indicates a ^{12}C uptake by microbes and partial reworking of the

bulk OM sources. At 1.3 m depth, similar features are observed for the $C_{20:0}$ to $C_{26:0}$ profiles (Fig. 2C), emphasizing the necessity of syntrophy partnership in the degradation of long carbon chains (McInerney et al. 2009).

Additionally, $\delta^{13}C$ values for $C_{14:0}$ to $C_{18:0}$ are too ^{13}C -depleted (-27.7 to -34.2‰) as regards possible algal sources (Sun et al. 2004), with even more depleted $\delta^{13}C$ values for $C_{20:0}$ and $C_{22:0}$ in lowermost sediments (from -36.9 to -41.2‰). The contrasting $\delta^{13}C$ trends displayed by unsaturated FAs and LC SFAs (Fig. 2A) further imply that some FAs get ^{13}C -depleted and some ^{13}C -enriched during diagenesis (Gong and Hollander 1997; Freudenthal et al. 2001), with a significantly higher turnover of labile compounds (Amon and Benner 1996). As microbes discriminate against the heavy isotope, FAs produced by microbes tend to be depleted in ^{13}C in comparison to the initial substrate, while residual FAs are normally enriched in ^{13}C (Niggemann and Schubert 2006).

The relative contribution of bacterial biomass to sedimentary OM can be estimated by branched chain and unsaturated FA concentrations (Fig. 2D; Bechtel and Schubert 2009; Volkman et al. 2008), although the use of these sole compounds sometimes results in an underestimation (Teece et al. 1999). Firstly, bacterial FAs appear more abundant in horizons of low C_{org}/N ratios (Figs. 1A and 2B), implying that labile OM derived from algal sources promotes a better development of heterotrophic bacteria. Secondly, depth distribution of bacterial compounds can account for differences in microbial populations (Boschker and Middelburg 2002). Peaks in $C_{16:1}$ and $C_{17:0}$ anteiso concentrations (ca. 1.9 and 4.0 m depth) coincide with higher sulfate concentrations (Fig. 1B), possibly indicating the production of these compounds by SRB (Volkman et al. 2008; Bechtel and Schubert 2009). From 9.4 to 11.3 m depth, high concentrations of $C_{15:0}$ iso/anteiso, MC SFAs and diatoms (Figs. 1B, 2B and 2D) show that algal OM is the main substrate in the microbial production of these compounds (Niggemann and Schubert 2006). Moreover, the $C_{18:2}$ peak at 7.8 m depth, which is associated with the decrease in diatom concentration, shows little difference with the $\delta^{13}C$ signature of diatomaceous oozes (Figs. 1B and 2D). In addition, main bacterial FAs display $\delta^{13}C$ values ranging from -24.9 to -34.3‰ (Fig. 2C, dotted lines), which possibly correspond with a gradual fractionation of algal matter. Although most ^{13}C signatures of FAs produced and/or reworked by microbes average ~ -31 ‰, fractionation varies for each compound and can be influenced by different metabolic types and substrates. SRB growing heterotrophically produce certain compounds that are sometimes depleted in ^{13}C by 12‰ compared to their substrates (Londry et al. 2004). Moreover, methane can presently act as an additional source of carbon, leading to highly ^{13}C -depleted FAs when oxidized (Yang et al. 2011). Thus, $\delta^{13}C$ values of the $C_{17:0}$ anteiso (-37.5‰) and methane (-24.0‰) at 1.9 m depth may indicate the production of this compound by SRB along with some microaerobic oxidation of methane (Deutmann and Schink 2011).

However, AOM processes related to AAA methanogens or other oxidants cannot be excluded (Schubert et al. 2011). On the contrary, unsaturated compounds of more than 20 carbons were previously linked to methanic environments and possibly to methanotrophs (Stefanova and Disnar 2000; Pancost et al. 2000). Such microbial compounds that are methane-derived show even more ^{13}C -depleted (Pancost et al. 2000). Thus, the linear $\delta^{13}C$ decrease of $C_{20:1}$ and $C_{24:1}$ with depth may be connected with methanogenic activity (Yang et al. 2011) as this trend is parallel to the evolution of the methane ^{13}C composition (Figs. 1C and 2C).

Bacterial FAs represent a small fraction of the sedimentary TOC, but can constitute more than 30% of FA sources within sediments. Moreover, these results do not even include long chain FAs constituting methanogen membranes (Boschker and Middleburg 2002) as their extraction requires another protocol. Such ^{13}C -depleted archaeal compounds have been reported to accumulate in horizons showing prolonged methanogenic activity (Noble and Henk 1998; Pancost et al. 2000). Microbial synthesis under anaerobic conditions also produces saturated FAs, sometimes falsely attributed to terrestrial sources as they can be depleted by up to 12‰ relatively to the substrate (Teece et al. 1999; Niggemann and Schubert 2006).

4.5. Imprints on bulk organic proxies

From 3.4 to 7.0 m depth, methanogenic populations are highly active, as emphasized by the ATP results (Fig. 1C) and involve syntrophic partnerships (Table 1). Cell counts and microbial FA concentrations (Figs. 1C and 2D) denote a significant microbial biomass relatively enriched in ^{12}C (Fig. 2C), which is often not taken into account when using organic proxies for paleoenvironmental reconstructions. Excursions in $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ seen in the same interval (Fig. 1A) are difficult to explain using organic sources modeling (Mayr et al. 2009). Conversely, methanogenic populations, as the ones identified in Laguna Potrok Aike, can potentially fractionate nitrogen from the substrate (Lojen et al. 1997). Because *Methanosarcinales* drive OM ammonification (Hedderich and Whitman 2006), microbial biomass could get enriched in ^{15}N via the assimilation of ammonium (Macko and Estep 1984) leading to more positive $\delta^{15}\text{N}$ values (Lojen et al. 1997). The capacity to grow solely on CO_2 and ammonium has been documented for several methylotrophs and hydrogenotrophs (Kenealy et al. 1982; Raemakers-Franken et al. 1991). As syntrophic OM degradation goes on, the recycling of byproducts into microbial biomass can turn into an additional factor of isotope fractionation (Freudenthal et al. 2001).

At 7.8 m depth, microbial activity, diversity and density start decreasing, while $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ compositions converge showing a positive correlation again (Figs. 1A and 1C). Although possible early diagenetic imprints on organic fractions were inspected down to 3 m depth, alteration and production of successive substrates by microbes (McInerney et al. 2009) are factual means of their prolonged activity (Fig. 1C). Hence, we propose that microbial reworking of bulk OM initial signatures is occurring within specific sedimentary layers that are sheltering dense and active methanogenic populations.

5. Conclusions

Our combined bulk OM, pore water, fatty acids, isotopic and phylogenetic study of Laguna Potrok Aike sediments show that methanogenic species stratify over depth depending on the substrate. Syntrophic interactions along with a stepwise degradation of OM allow microbial populations to sustain their activity in deep sediments, leading to the partial reworking of refractory compounds. The succession of methanogenic pathways with depth gradually increases carbon fractionation, resulting in very negative $\delta^{13}\text{C}$ values of methane and specific FAs. The capacity of methanogens to grow on fermentation byproducts solely makes them an additional source of biomass enriched in ^{12}C and ^{15}N within sediments. Microbial biomass sometimes accounted for a third of FA sources within sediments. As specific sedimentary horizons have been exposed to the prolonged influence of these methanogenic populations, the isotope compositions of the bulk OM fraction can be considered partially overprinted by

microbial diagenesis. Thus, these results demonstrate that diagenesis of labile organic fractions mediated by microbes should be considered a potential bias of paleoenvironmental reconstructions using bulk OM proxies.

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CHAPTER 7

Microbial interactions with depth and lithology

Article in preparation to be submitted as

Vuillemin et al. to an international journal

Microbes staying alive until 35 ka in deep lacustrine sediments

Abstract

Lacustrine sediments represent excellent archives of past climatic conditions, but provide also a wide range of ecological niches for microbes. Currently, microbiological studies in this realm are confined to surficial sediments, with a poor characterization of the evolution and survival capacities of the hosted populations. In addition, the recycling of nutrients and by-products of fermentation represents metabolic strategies, potentially inducing diagenetic changes on the original environmental signal. Although microbes show a clear dependency to initial geological and geochemical conditions in marine environments, the role of species and metabolisms acting in deep lacustrine settings remain elusive. A unique dataset from a Patagonian maar lake indicates that microbial consortia remain active in the sediment as deep as 40 m depth, and even below. Clone libraries reflect at first the associated lithologies, while displaying a diversity of species corresponding to a reduction in trophic level. Parallel to the depletion of organic substrates, bacterial populations develop syntrophic relationships and evolve towards lithotrophy. In Holocene sediments, organic-rich clays reveal a microbial community adapted to subsaline conditions, the presence of organotrophs capable of degrading refractory organic matter, and syntrophic partnerships with methanogens. Volcanic-rich sediments from the Last Glacial period show a substantial number of acidophilic Archaea associated with a syntrophic consortium of sulphate-reducing Bacteria performing anaerobic oxidation of methane. Thus, such mutualistic microbial communities gradually reduce their energy requirement and sustain an activity in 35 ka old sediments under nutrient-depleted conditions.

1. Study site

The Potrok Aike maar lake is a closed basin located within the Pali Aike volcanic field (Zolitschka et al. 2013). Its sedimentary sequence contains a climate record of southernmost South America that is mostly paced by changes in the Westerly winds and ice caps fluctuations (Mayr et al. 2007). Presently, the water column is unstratified with a maximum depth of 100 m and annual temperatures ranging between 4 and 10 °C. Climatic-induced changes in sedimentation are reflected by horizons of distinctive lithologies, salinities and organic sources (Recasens et al. 2012). Redeposition events associated with lake level oscillations of the Potrok Aike maar were frequent throughout the glacial record (Fig. 1; Zolitschka et al. 2013; Recasens et al. 2012), whereas conditions became subsaline during the Younger Dryas due to an important drop in lake level (Fig. 1; Vuillemin et al. 2013 a; Zolitschka et al. 2013). Higher nutrient concentrations in the sediment could be associated with lake level oscillations (Zolitschka et al. 2013) and correlated with positive temperature excursions in the southern hemisphere (Fig. 1; Jouzel et al. 2007). Nutrient inflows to the lake and warmer temperatures favor primary productivity and lead to preferential colonization of these sedimentary layers by microbes. Thus, two intervals with sustained microbial activities (Fig. 1) were clearly identified after the first geomicrobiological investigations carried out during the deep drilling of Laguna Potrok Aike sedimentary infilling (Vuillemin et al. 2013 a). Clone libraries in samples from these two intervals were established in order to define the evolution of microbial phylogenetic diversity with respect to sediment depth, dominant lithology (Inagaki et al. 2003) and prevailing paleoenvironmental conditions.

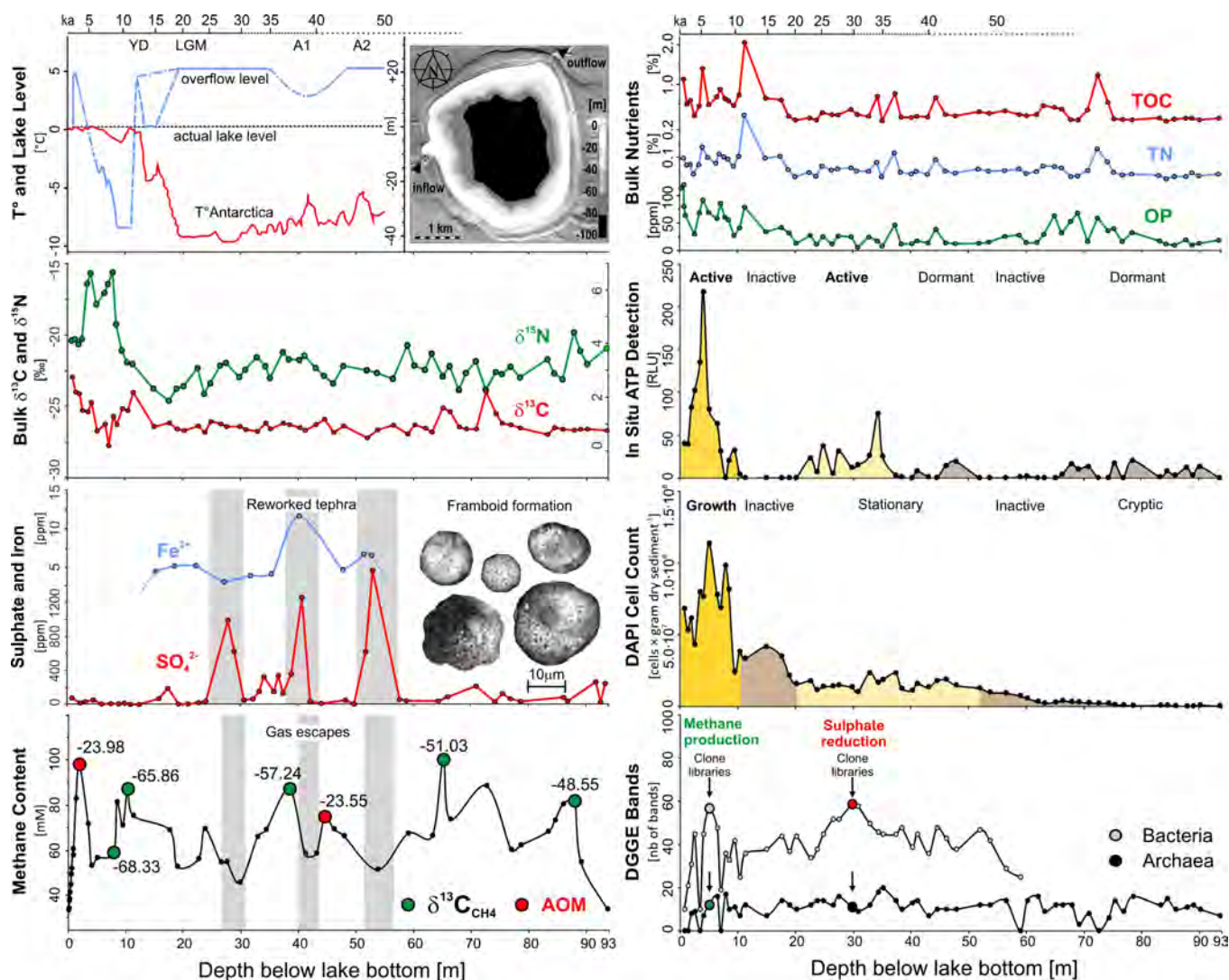


Figure 1 Results for multiple analyses on the bulk fraction and pore water **From top to bottom and left to right:** Curves of lake level fluctuations (after Zolitschka et al. 2013) and Antarctica surface temperatures (after Jouzel et al. 2007), with a bathymetric map of the lake as insert; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bulk sediment; sulphate and iron contents in pore water, with SEM pictures of framboidal sulphides as insert; methane content and its relative carbon isotope values ($\delta^{13}\text{C}_{\text{CH}_4}$). Grey shadings indicate gravity events composed of mafic tephra and associated methane escapes from the sediment; total organic carbon, total nitrogen and organic phosphorus of bulk sediment; adenosine triphosphate (ATP) detection used as an index of in situ microbial activity; 4',6-diamidino-2-phenylindole (DAPI) cell counts; number of denaturing gradient gel electrophoresis (DGGE) bands, with colour dots highlighting methanogenesis and sulphate reduction processes.

2. Holocene horizon

The first horizon at 5 m depth, with a corresponding age of ~6 Ka, consisted of methane bearing clays and subsaline pore waters (Vuillemin et al. 2013) and displayed the maximum microbial population activity and density of the entire record (Fig. 1). 16S rDNA sequences provided evidence for the presence of alkalotolerant species, such as Dehalococcoidetes (Fig. 2; Moe et al. 2009), some uncultured Clostridia (Nakagawa et al. 2006) along with Archaea belonging to marine benthic groups B and D (Fig. 3; Takai and Horikoshi, 1999; Jiang, H. et al. 2008) and confirmed the control exerted by salinity gradients on the distribution of microbial species. These organotrophs ferment mainly labile compounds including alkanes, acids and alcohols (Wüst et al. 2009). As early diagenetic processes progress, the turnover rate of labile organic matter increases, gradually orienting organotrophs toward refractory compounds, which tend to accumulate within sediments. Actinobacteria and Bacteroidetes (Fig. 2) were

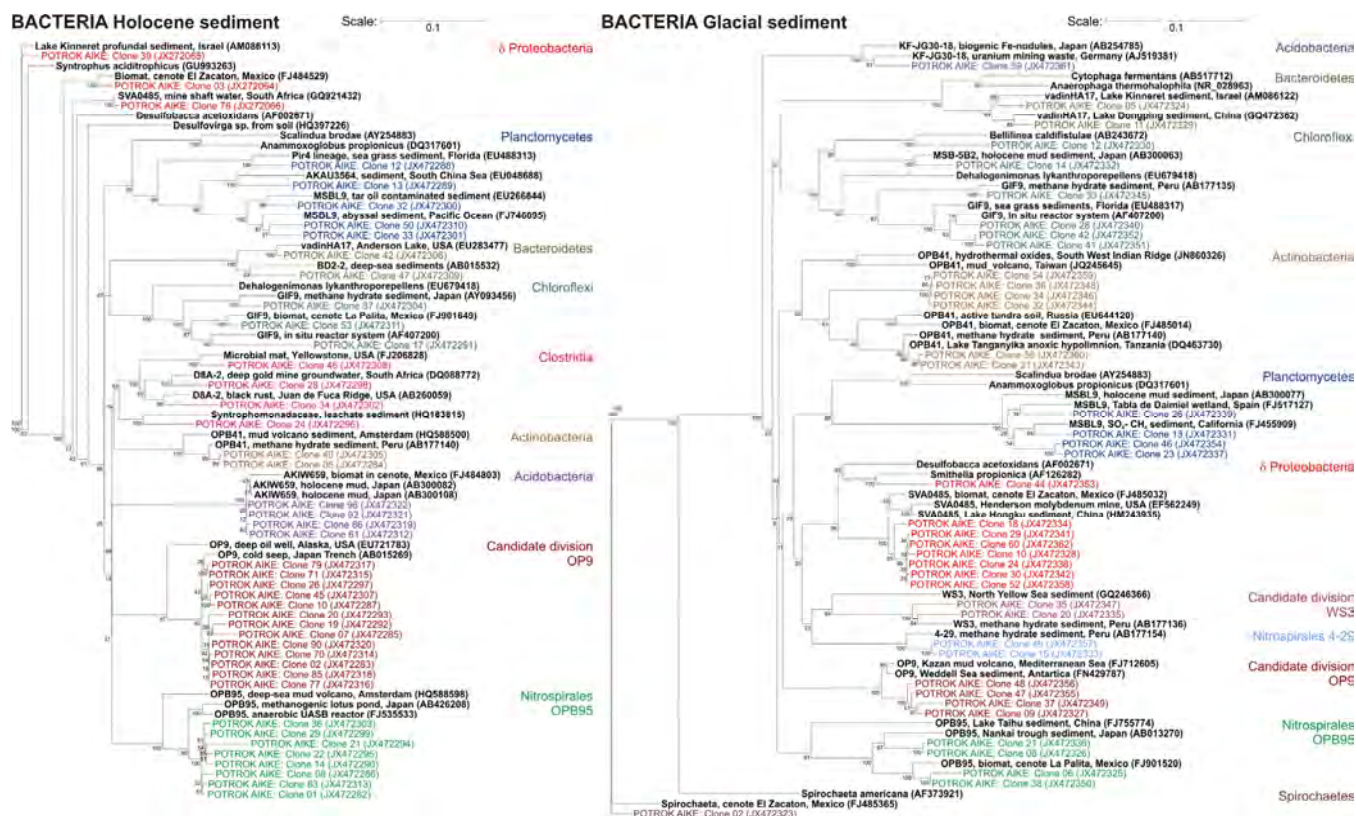


Figure 2 Bacterial phylogenetic trees **Left:** Bacterial diversity in Laguna Potrok Aike sediment at 5 m sediment depth - Phylogenetic relationships of bacterial 16S rRNA gene sequences of representative clones (1400 bp) emphasize a dominant colonization of the substrate by OP9 and OPB95-related Bacteria. Organic matter degradation is an important ongoing process in this horizon. Boldface types signify databases references with their sequence accession numbers in parentheses. **Right:** Bacterial diversity in Laguna Potrok Aike sediment at 29.7 m sediment depth - Phylogenetic relationships of bacterial 16S rRNA gene sequences of representative clones (1400 bp) displaying various divisions among which sulphate reducers are dominant. The alteration of basaltic tephra provides inorganic nutrients, and although the microbial activity is low, it appears to be sustained by complementary interactions.

among identified anaerobic Bacteria, holding the capacity to degrade cellulose, chitin and lignins (Pachiadaki et al. 2011). Meanwhile, fermentation reactions generate H_2 and CO_2 , which serve as substrates for methane production by Methanomicrobiales (Wüst et al. 2009)¹⁴, of which only *Methanoregula* (Bräuer et al. 2011) was identified at that depth (Fig. 3). Concurrent *Syntrophus* sequences (Fig. 2) attested syntrophic interactions among microbes and the metabolic capacity to degrade fatty acids (Wüst et al. 2009; Jackson et al. 1999). However, methane content decreased simultaneously to a net increase in microbial activity and density (Fig. 1), which possibly implied homoacetogenesis as a concomitant process, competing with methanogenesis (Wüst et al. 2009). Candidate division OP9, which was first documented in hot springs (Hugenholtz et al. 1998) and reported thereafter in subseafloor sediments (Inagaki et al. 2003), dominated among retrieved sequences (Fig. 2). Their phylogenetic proximity to homoacetogens (Hugenholtz et al. 1998)³ suggests a lithotrophic growth on H_2 and CO_2 issued from fermentation processes and designate them as possible competitors of methanogens (Wüst et al. 2009). Similarly, Nitrospirales OPB95 (Fig. 2), former division OP8 (Dojka et al. 1998), were documented in relation to nitrogen turnover in anoxic sediments (Freitag and Posser, 2003), possibly implying nitrification processes coupled with organic matter ammonification under present methanogenic conditions (Hugenholtz et al. 1998). However, it remains uncertain whether these organisms have the capacity to growth under such conditions or only survive during this period (Pachiadaki et al. 2011; Freitag and

Prosser 2003). Conversely, Planctomycetes sequences (Fig. 2) related to *Pirellula* (Glöckner et al. 2003) were found preserved in the sediment, probably due to the resistance of their cell membrane.

In brief, the microbial consortium at 5 m depth in these anoxic organic-rich clays (Fig. 1) reflects initial geochemical conditions, with an adaptation to salinity and different capacities of organic matter degradation (Wüst et al. 2009; Pachiadaki et al. 2001). It displays complementarities in the degradation chain via the establishment of syntrophic relationships with hydrogenotrophs (Wüst et al. 2009; Dojka et al. 1998) and geochemical cycling of degradation products, in which candidate divisions seems to regulate the cycling of fermentation endproducts in order to sustain microbial consortium activity. The diagenetic influence of such processes on the initial paleoenvironmental signal remains to be clearly established as the bulk isotope excursion observed in the Holocene record (Fig. 1) can result from global microbial processes associated with the thawing of permafrost and peatlands.

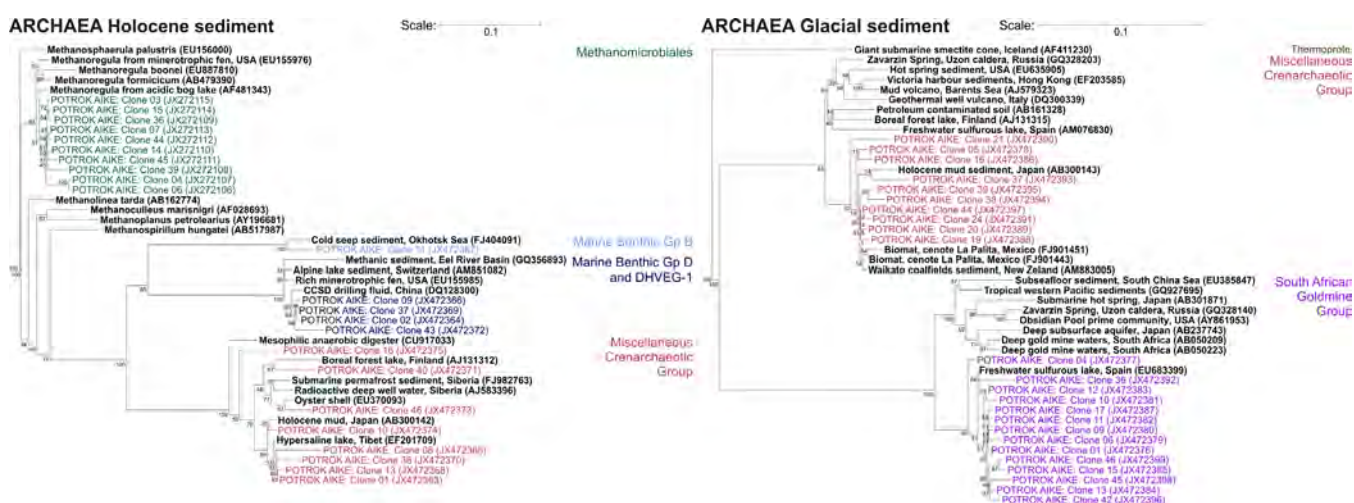


Figure 3 Archaeal phylogenetic trees **Left:** Archaeal diversity in Laguna Potrok Aike sediment at 5 m sediment depth - Phylogenetic relationships of archaeal 16S rRNA gene sequences of representative clones (900 bp) are dominated by methanogens along with MCG Archaea. The identified sequences previously allocated only to marine benthic groups denote the importance of local subsaline conditions ruling the assemblage. **Right:** Archaeal diversity in Laguna Potrok Aike sediment at 29.7 m sediment depth - Phylogenetic relationships of archaeal 16S rRNA gene sequences of representative clones (900 bp) showing a restricted diversity limited to SAGMEG and MCG divisions.

3. Glacial horizon

The second horizon at 30 m depth consisted of organic-poor silts, overlain by basaltic fine sands with a high sulphate content in pore water (Vuillemin et al. 2013 a), and corresponded to an age of ~30 Ka. Microbial populations in this horizon showed sustained activity but low population density (Fig. 1; Vuillemin et al. 2013 a). Several discrete layers along the same interval contain high sulphate concentrations (Fig. 1), derived from basaltic tephra reworked from the catchment into the basin. These abrupt loadings triggered methane escapes (Fig. 1; Vuillemin et al. 2013 a), while changes in lithology resulted in a diversification of the microbial population characteristics (Inagaki et al. 2003). The mafic composition provided iron and sulphur (Fig. 1), thus engendering metabolisms based on these oxidants (Nakagawa et al. 2006). Indeed, numerous δ Proteobacteria sequences (Fig. 2) were identified as sulphate-reducing Bacteria along with one syntroph related to *Smithella propionica* (Liu et al. 1999). Iron sulphides found as framboids were observed at similar depths (Vuillemin et al. 2013) in

association with an important increase in the $\delta^{13}\text{C}_{\text{CH}_4}$ composition (Fig. 1). These features provided evidence for processes of anaerobic oxidation of methane, mostly coupled to sulphate reduction (Boetius et al. 2000; Inagaki et al. 2006). Additionally, the significant presence of SAGMEG Archaea (Takai et al. 2001)⁷ (Fig. 3) suggested the use of inorganic nutrients derived from mineral sources (Johnson 1998)²³, while still reflecting the volcanic nature of these sediments. Sequences related to Acidobacteria (Liesack et al. 1994), *Spirochaeta* (Hoover et al. 2003), Actinobacteria (Pachiadaki et al. 2011) and Bacteroidetes (Fig. 2) were interpreted as the consortium metabolic capacity to degrade cellulose and chitin, resulting in the production of labile substrates, which could be used by the Chloroflexi and δ Proteobacteria thereafter. On the contrary, the presence of WS3, Nitrospirales 4-29 (Fig. 2) and MCG Archaea (Fig. 3) seemed to stem rather from the low trophic state of the sediment (Pachiadaki et al. 2011; Dojka et al. 1998). OP9 (Inagaki et al. 2003), Nitrospirales OPB95 (Hugenholtz et al. 1998) and Dehalococcoidetes (Moe et al. 2009) were still among retrieved sequences (Fig. 2), which could indicate either their capacity to grow continuously on fermentation derivatives or their preservation in a dormant state. The presence of Phycisphaerae (Glöckner et al. 2003) could be attributed with certainty to preservation since these organisms come from the water column.

The multiproxy dataset (Fig. 1) and microorganisms identified in this second horizon (Figs. 2 and 3) provide evidence for ongoing processes of sulphate reduction and anaerobic oxidation of methane. The produced H_2S promotes the formation of iron sulphides, found as framboids, and supplies molecular hydrogen to lithotrophic species. At the same time, the alteration of mafic minerals represents an important source of iron in pore water. The degradation of refractory compounds (Wüst et al. 2009) appears essential for sustaining of microbial activity, since it provides labile substrates to microorganisms with low metabolic capacities and, thus, accounts for a mutualistic partnership. Moreover, microbial communities are stratified within the sedimentary record (Inagaki et al. 2006), as methanogenic species tend to be replaced by additional candidate divisions with depth. The evolution of microbial assemblages can actually result from the gradual depletion of organic substrates, while still reflecting the geochemical characteristics of the sediment (Inagaki et al. 2003).

4. Conclusions

In terms of diversity, Bacteria dominated the microbial assemblage with a wide distribution of candidate divisions. Active metabolisms were all anaerobic with the tendency to shift from organotrophy to lithotrophy, while remaining in adequation. In parallel, the degradation of chitin, cellulose, humic acids and fatty acids was proved effective and its byproducts recyclable, potentially generating the isotope shifts observed in Holocene bulk sediments. Competition in the use of H_2/CO_2 could be evidenced between methanogens and other hydrogenotrophs. Yet, methanogenesis was a dominant process in Holocene sediments, whereas the volcanic material sedimented during the glacial period promoted important iron and sulphate reduction in discrete volcanic-rich horizons. The results of this study indicate that prevailing conditions in the catchment exert an initial control on the nature of the sedimented material, thus defining sedimentary horizons that will be preferentially colonized. Lithology and initial salinity conditions are then fundamental in determining the microbial assemblages developing in the sediment. The understanding of microbial interactions between active species of the consortium is also necessary to resolve successive diagenetic impacts on both the sedimentary organic matter and/or the mineral fraction. Our data further indicate a

structural stratification of microbial communities associated with the partial degradation of organic substrates, their exhaustion driving microbes towards metabolic simplicity. Metabolic survival strategies include reciprocity in the degradation of organic matter, syntrophic partnership with methanogens in the use of H_2/CO_2 , and mutualism in the use of molecular hydrogen supplied from sulphate reduction. Whereas methanogenesis and acetogenesis make use of H_2/CO_2 derived from fermentation processes, candidate divisions in depth appear to rely on inorganic substrates, possibly of mineral origin, in a similar way to the early life on Earth. Thus, deep lacustrine sediments constitute ecological niches of substantial long-term survival sheltering high phylogenetic diversities.

5. Methods summary

5.1 Clone libraries

All procedures used in the establishment of the multiproxy have been published elsewhere (Vuillemin et al. 2013 b). DNA extraction and PCR amplifications are described in detail elsewhere (Vuillemin et al. 2013 b). PCR products were purified using the High Pure[®] PCR Product Purification Kit (Roche Diagnostics SA), measured with a Nanodrop[®] ND-1000 Spectrophotometer (Witec AG), and diluted to a concentration of 10 ng/ μ l. 2 μ l PCR product were ligated to the pCR[®]4-TOPO[®] vector (Invitrogen[™] by life technologies[™]) and cloned into competent *Escherichia coli* cells. Cloning procedure was performed using the TOPO[®] TA Cloning[®] Kit (Invitrogen[™] by life technologies[™]) following the manufacturer's recommendations. Transformed cells were incubated at 37°C for 20 hours on a LB medium containing 1g L⁻¹NaCl, 1 g L⁻¹ Bactotryptone, 0.5 L⁻¹ Bactoyeast, 1.5 g L⁻¹Bactoagar and 2 ml L⁻¹ ampicillin. 102 and 62 bacterial clones plus 31 and 39 archaeal clones were selected from samples at 5 m and 30 m depth, respectively, to constitute the libraries. Sequencing cycles were performed using the BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied BioSystems) with primers 27F and 1492R for Bacteria and vector primers D4 and R5 for Archaea. Sequencing was performed on an ABIPRISM[®] 3130xl Genetic Analyzer (Applied BioSystems, Hitachi).

5.2 Phylogenetic analyses

Sequences were assembled with CodonCode Aligner[®] v.3.7.1 (CodonCode Corporation), aligned on Seaview v.4.3.0 (Gouy et al. 2010) with clustalW2. Primers were selectively cut off. Chimeras were detected using the online program Bellerophon (Huber et al. 2004) (<http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl>). 16S rDNA sequences were identified using the megx[®] Geographic-BLAST (<http://www.megx.net>) and SILVA[®] comprehensive ribosomal RNA databases (Pruesse et al. 2007). Phylogenetic references were downloaded from the SILVA[®] browser (<http://www.arb-silva.de>) and NCBI genbank[®] (<http://www.ncbi.nlm.nih.gov/genbank/>). Clone and reference sequences were uploaded on the Ribosomal Database Project (<http://rdp.cme.msu.edu>) and phylogenetic trees produced with the Weighor Joining method (Bruno et al. 2000), including results from a bootstrap test using 100 replicates.

Present sequences are deposited in the GenBank database under accession numbers JX272064 to JX272122 and JX472282 to JX472399.

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CHAPTER 8

Conclusions

Conclusions

The first goal of this dissertation was to characterize the microbial role shaping the organic matter fraction preserved in lacustrine sediments. The main conclusion is that lacustrine organic matter can provide an excellent record of paleoenvironmental changes as shown by the comparison of the Laguna Potrok Aike organic record with other independent, non-organic proxies. However, the results of these geomicrobiological investigations - carried out for the first time in the framework of an ICDP project – have sounded a note of caution when interpreting some widely used bulk organic matter proxies alone. These results show that the often-neglected microbial impact on the sedimentary organic matter can be very important changing the original paleoenvironmental signal.

In the following paragraphs the main conclusions of the six research chapters are briefly recapitulated.

Chapter 2: Sampling

Geomicrobiological investigations at Laguna Potrok Aike provided a first dataset to start fulfilling the existing lack of geomicrobiological studies in deep lacustrine sediments. A specific protocol was applied in the field allowing the aseptic recovery and conditioning of sediment samples on a 1 m long gravity core and a 93 m long hydraulic piston core. ATP measurements obtained in the field provided good assessments of *in situ* microbial activity as shown by their comparison with results of further laboratory analyses.

Chapter 3: Surficial sediments

The study of surficial sediments showed a very good correlation between paleoenvironmental conditions of deposition and the different living microbial communities. Microbiological proxies are reflecting distinctive climatic intervals, with low and high degrees of sediment colonization during dry conditions (Medieval Climate Anomaly) and wet conditions (Little Ice Age), respectively. Thus, a clear dependence was shown between predominant environmental conditions of deposition and microbial settlement, development and organization.

Chapter 4: Sustainability of microbial activity

Geochemical and microbiological results from the 93 m long core showed that microbial communities sustain their activity within deep sediments. Dissolved phosphate derived from OM degradation was a main factor for microbial development, whereas sediments adsorbing P were poorly colonized. Comparison with paleoclimatic reconstructions indicated that intervals colonized by active microbes corresponded with climatic periods of positive temperature excursions. The sedimentary regime also exerted significant control on microbial development, as frequent re-depositional events, associated with lake-level oscillations, disrupted the settlement of microbial populations along the Glacial record. Nevertheless, microbial activity was maintained down in 30 ka old sediments. In parallel, the presence and influence of microbial communities turned out to be high in Holocene organic rich sediments, in which the recycling of the excreted ammonium by microbes led to increased $\delta^{15}\text{N}$ values. Overall results point to the influence of paleoconditions on microbial development, the persistence of living microbes in deep sediments and their relative impact on bulk OM.

Chapter 5: Searching for biosignatures

Vivianite concretions were found in deep glacial sediments of Laguna Potrok Aike raising the question of a possible microbial mediation in their formation. SEM images and EDS allowed the identification of vivianite along with iron sulfides found as framboids within the concretions. Methanogenesis acted as the initial microbial process reducing phosphorus-iron oxides to vivianite in fine organic sediments. Since microbial populations were disrupted by sporadic gravity events, mafic volcanics reworked from the catchment also acted as main supplies of iron, sulfur and phosphorus arousing additional microbial metabolisms, such as iron and sulfate reduction. These metabolisms appeared to be sustained throughout diagenesis leading to the formation of iron sulfides. Single cubic sulfides aggregated into framboids of mackinawite and greigite that further evolved towards pyrite, implying diagenetic maturation. ATP measurements and $\delta^{13}\text{C}_{\text{CH}_4}$ values indicated ongoing AOM processes below 30 m depth, while dissolved iron in pore water and the foliated aspect of certain clay minerals also suggested microbial iron reduction.

Chapter 6: Methanogenic populations in Holocene sediments

The bulk OM isotopic signatures of Laguna Potrok Aike record were first interpreted as only reflecting initial OM sources. We have shown that microbial populations are dense and active in Holocene sediments. Thus, initial OM sources, microbial reworking and the production of a secondary biomass by microbes have been traced using methane and fatty acid quantification along with their isotopic compositions in order to characterize the relative microbial impact in the bulk OM proxies. Methanogenic species were inspected via clone libraries, appearing stratified within the sediments, with pathways shifting from methyl fermentation to CO_2 reduction, and to syntrophic acetate oxidation throughout depth. $\delta^{13}\text{C}_{\text{CH}_4}$ compositions showed that carbon fractionation during methane production increased with depth. The $\delta^{13}\text{C}$ compositions of saturated fatty acids revealed a partial reworking of refractory sources due to the microbial uptake of the light isotope, while the resynthesis of labile OM sources by microbes depleted $\delta^{13}\text{C}$ compositions. Branched and unsaturated fatty acid $\delta^{13}\text{C}$ compositions evidenced algal sources as the main substrate used by microbes as well as the role of methanogens in producing a ^{12}C -enriched biomass.

Chapter 7: Species interactions

Bacterial and archaeal clone libraries were established in Holocene and Glacial horizons displaying ongoing microbial activity. These results evidenced a direct relationship between microbial species and the sediment mineralogy as well as mutualistic interactions associated with the depletion of trophic conditions. In terms of diversity, Bacteria dominated the microbial assemblage. Active metabolisms were all anaerobic with species able to degrade chitin, cellulose, humic acids and fatty acids. Competitive and syntrophic interactions in the use of H_2/CO_2 could both be evidenced. Methanogenesis dominated in Holocene clayey sediments, whereas volcanites reworked from the surroundings into the basin during the Glacial period promoted important iron and sulfate reduction. Identified candidate divisions in depth seemed to rely on substrates of mineral origin similarly to the Earth early life. These results clearly indicate that the lithology and initial salinity conditions determine prevalent microbial assemblages in the sediments. They also show that deep lacustrine sediments constitute a niche for substantial long-term survival of microbial life.

Outlook

This pioneer study has also raised more research questions. In this section, I propose some strategies to try to answer some of them. For every research chapter, these consist of two parts: i) how to identify the microbial impact on bulk organic matter proxies; and ii) how to use these results in future deep lacustrine drilling projects.

Chapter 2

In order to optimize future field sampling and methodological approaches, some of the problems encountered with the used protocol could be improved as follows: i) pore water extraction using microrhizons should be carried out by drilling a small hole in the core liner prior to window opening in order to avoid the loss of vacuum within sediments; ii) the rims of headspace vials for methane determinations have to be cleaned before crimping, and stored upward to avoid leaking; iii) sediments fixed with formaldehyde have to be rinsed on the field if molecular probes are planned; iv) ATP measurements can be inhibited by high salinity and this factor should be taken into account; v) sampling windows have to be sealed hermetically to avoid sediment oxidation. In addition, spikes sold by the manufacturer can be used to calibrate the ATP relative luminescence results into quantitative units. It is recommendable to consider the transport of sensitive samples specialized companies that offer this service at reasonable costs in order to secure a better preservation of them.

Chapter 3

It is always dubious to interpret microbiological proxies in term of processes as long as additional evidences are not provided. The presence of layered microbial communities can be supported by pore water analyses and/or fluorescence in situ hybridization (FISH) cell counting. Additionally, the degree of OM preservation in the water column as well as events of stratification could be documented via pigment analyses.

Chapter 4

One major issue in the accurate estimation of microbial diversity with DGGE resides in the preservation of fossil DNA within the sediments. Extracting intracellular and extracellular DNA separately allows the distinction between living microbes and molecular fossils. Similarly, the accumulation of dead cells in the sediment can be checked with live/dead viability assays. These results can be further used to assess turnover rates of microbial biomass. Fluxes from the sediment to pore water in the form of compounds excreted by microbes (e.g. DIC, CO₂, CH₄, NH₃, H₂S) can be quantified via chromatography, with their respective isotopic compositions providing essential information on microbial fractionation potentials.

Chapter 5

The microbe-mineral interface needs to be documented precisely to define unequivocal biosignatures. The role of EPS, for example, can be investigated using confocal and/or cryogenic microscopy. The determination of precursors and diagenetic phases requires high accuracy measurements at a very small scale that can be achieved with a microprobe or nanoSIMS. The formation of framboids, microbial metal and sulfate reduction processes can be traced by Mn, Fe and S speciation.

Chapter 6

The δD compositions of methane give valuable information on the fractionation processes occurring during methane formation, while dissolved CO_2 gas samples can complete the methane dataset. Archaeal compounds can be specifically inspected via hopanoids and glycerol dialkyl glycerol tetraethers (GDGTs).

Chapter 7

Identifications based on the 16S rDNA do not provide any indication on whether species are active or dormant. An approach targeting messenger ribonucleic acids (mRNA) evidences active species and can provide helpful information on the role of candidate divisions. However, this technique is extremely sensitive to contaminations. In addition, specific pore water analyses can be achieved in order to discriminate the use of organic and/or inorganic nutrients by microbes and define organotrophic/lithotrophic interactions.

ANNEXES

Supplementary materials

Chapter 4 (3 annexes)

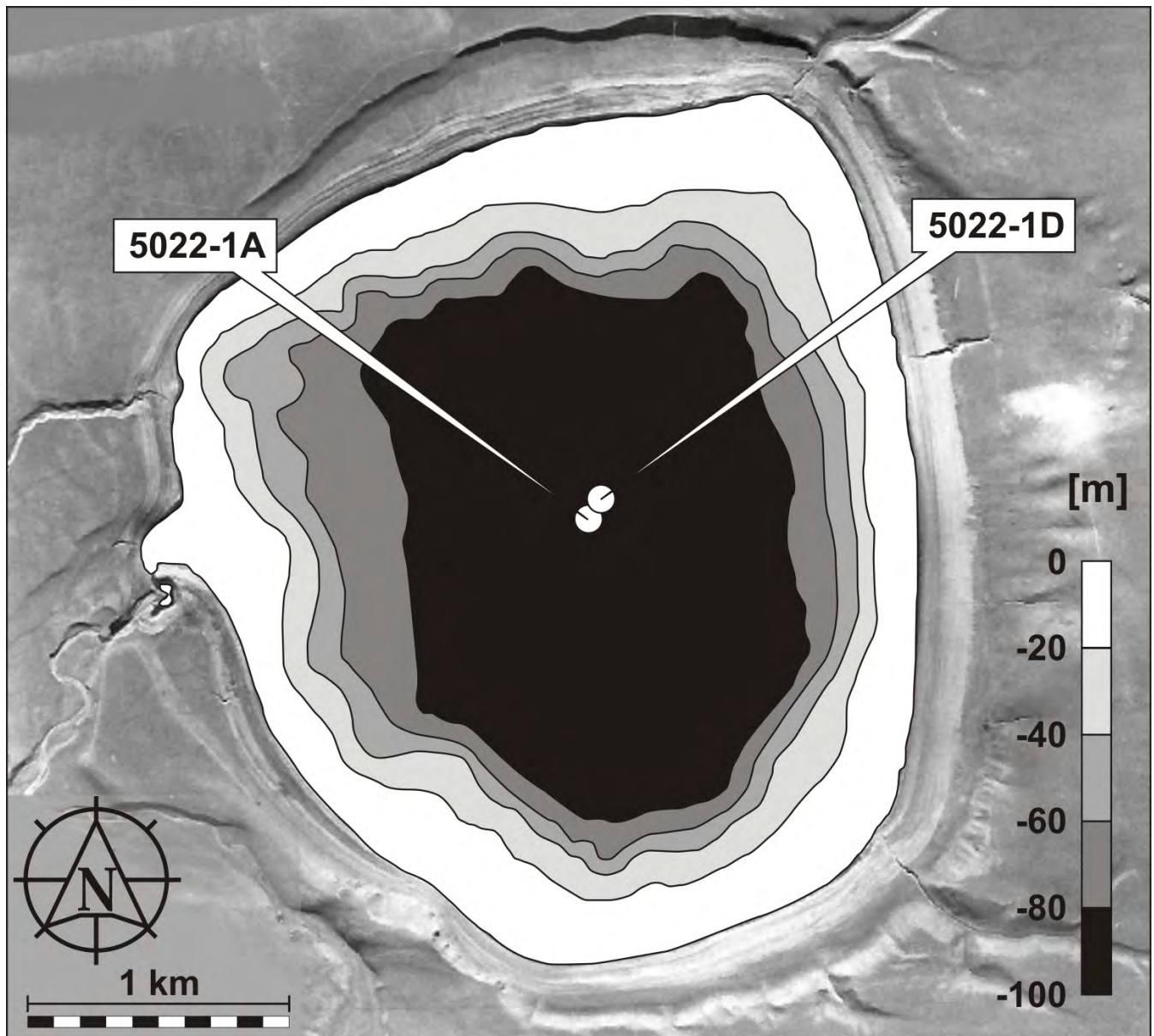
Chapter 5 (2 annexes)

Chapter 6 (2 annexes)

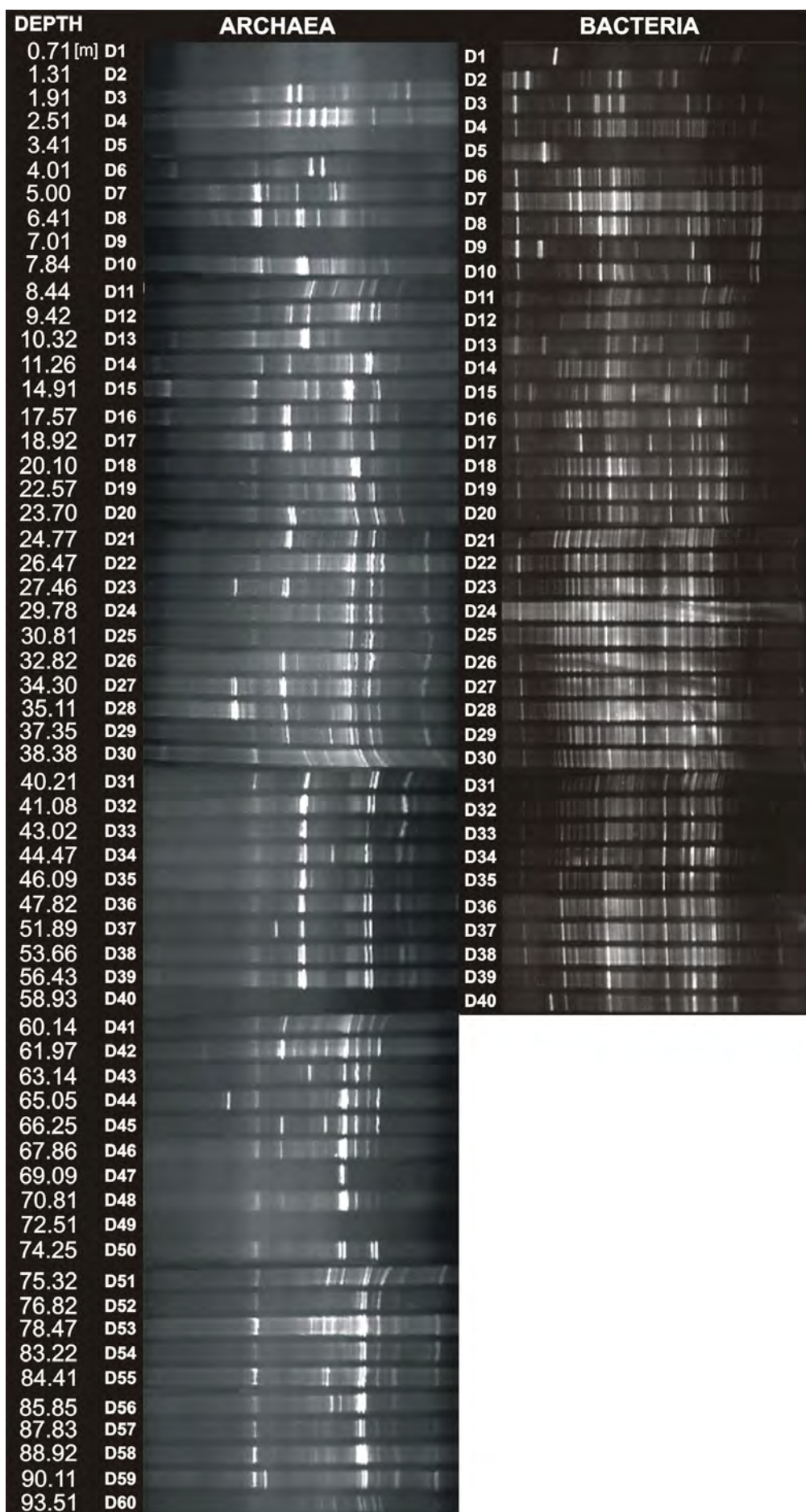
Chapter 7 (1 annexe)

Annexes

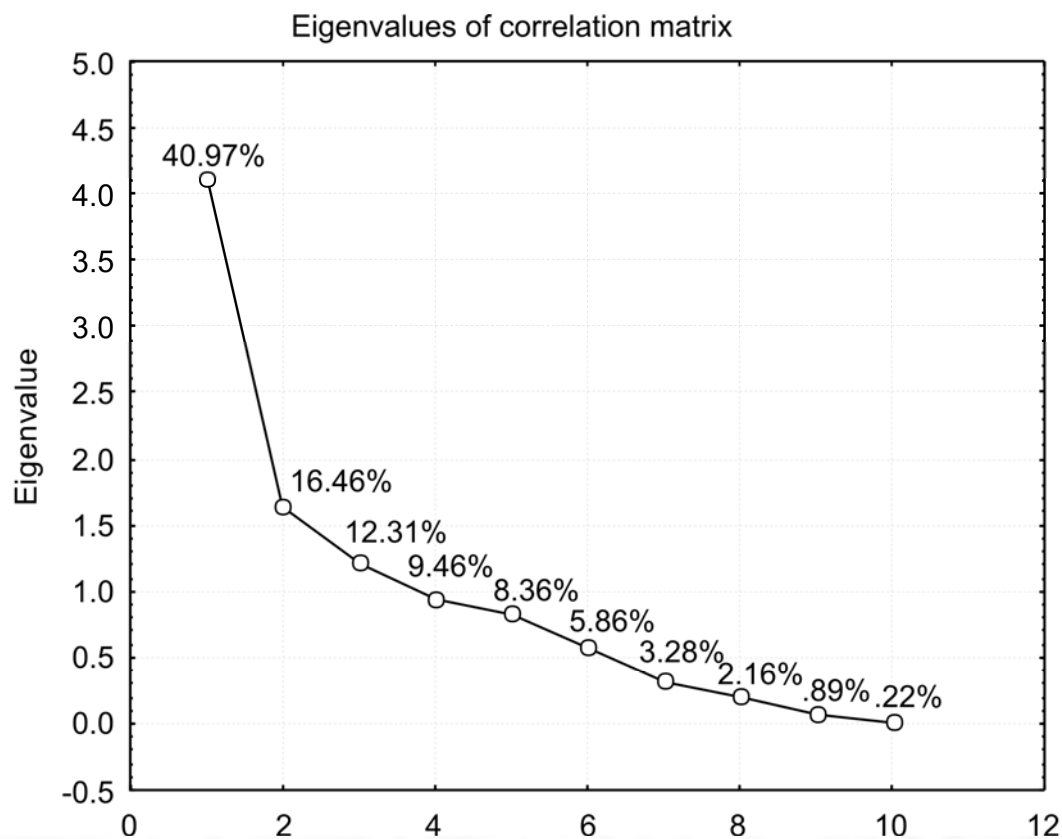
Chapter 4



Annex A-1 Bathymetric map of Laguna Potrok Aike (modified after Zolitschka et al. 2006) showing the positions of the two hydraulic cores studied in this paper.



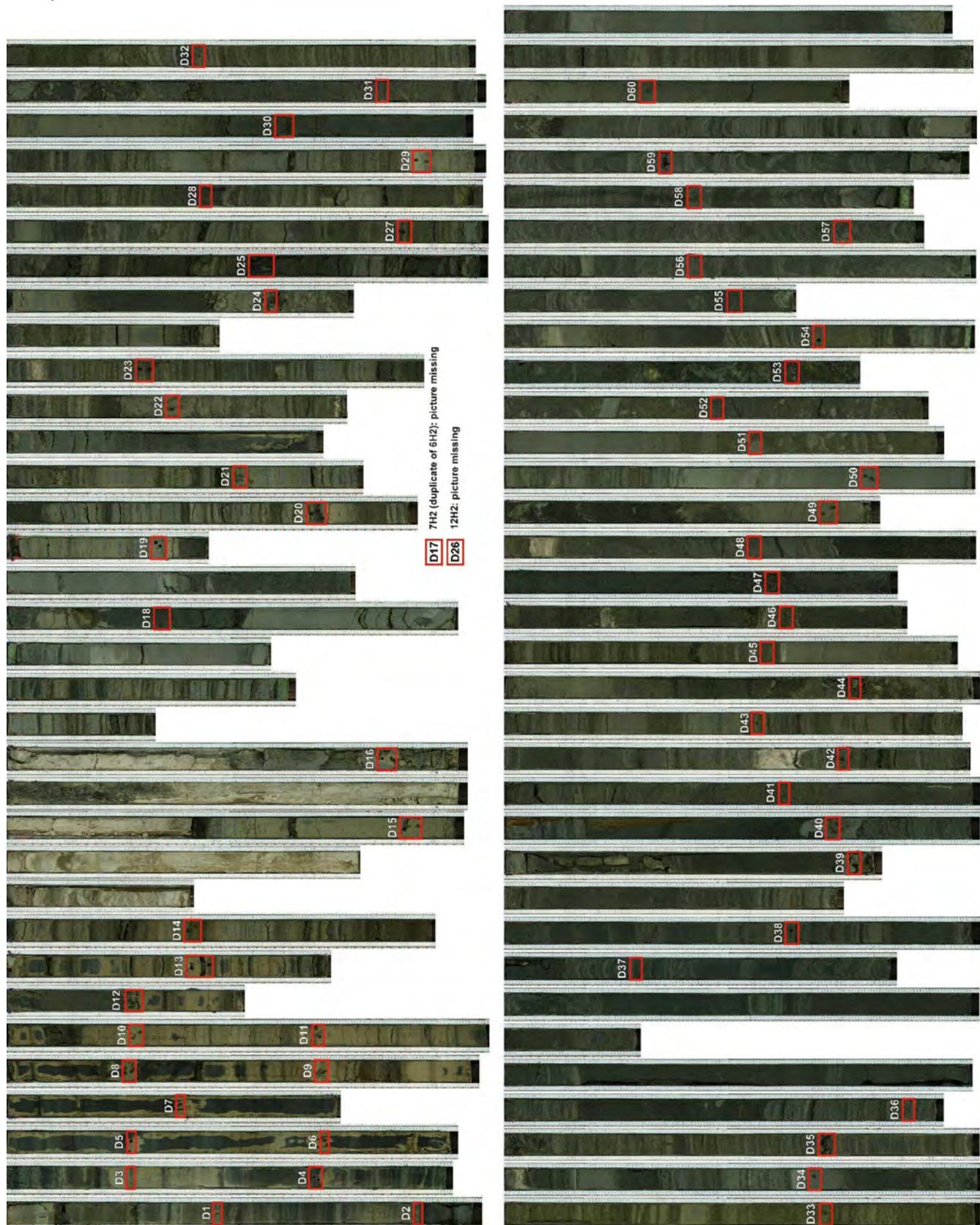
Annex A-2 DGGE gels pictures with gradient from 30% (left) to 70 % (right). The depth of each sample is signified on the left.



SAMPLE	Clays	TN	TOC	OP	NAIP	d13C	d15N	ATP	DAPI	DGGE	SAMPLE	Clays	TN	TOC	OP	NAIP	d13C	d15N	ATP	DAPI	DGGE
D1	12.89	0.096	1.13	122.61	88.22	-22.99	4.11	40	6.81E+07	10	D32	6.73	0.048	0.2	21.37	104	-26.73	3.54	8	1.58E+07	59
D2	11.81	0.069	0.5	67.7	117.11	-24.05	4.15	39	5.33E+07	21	D33	18.51	0.037	0.17	17.46	325.96	-26.32	3.09	1	1.34E+07	43
D4	13.69	0.038	0.21	33.63	52.15	-25.34	4.16	102	4.31E+07	54	D34	8.73	0.09	0.66	42.09	159.3	-25.96	2.8	0	1.78E+07	58
D5	10.99	0.071	0.45	71.56	91.83	-25.43	6.22	135	7.98E+07	10	D35	21.22	0.048	0.23	17.4	98.39	-26.88	2.51	14	1.88E+07	53
D6	17.29	0.136	1.4	95.03	129.15	-24.78	6.61	217	7.64E+07	52	D36	10.87	0.041	0.2	16.14	69.79	-26.42	3.16	19	1.48E+07	48
D7	17.55	0.098	0.49	72.58	112.43	-26.78	5.45	80	1.13E+08	69	D37	7.77	0.055	0.3	16.77	93.75	-27.26	3	0	1.27E+07	59
D8	13.26	0.077	0.68	61.59	86.48	-26.29	5.87	63	7.79E+07	64	D38	8.29	0.048	0.27	18.62	172.39	-26.71	2.9	0	9.82E+06	54
D9	11.24	0.111	0.87	89.08	120.46	-27.81	6.2	31	6.88E+07	19	D39	9.45	0.05	0.32	29.41	434.94	-26.37	2.69	0	9.34E+06	43
D10	16.15	0.1	0.64	63.89	77.89	-25.74	6.64	0	9.81E+07	53	D40	18.11	0.056	0.24	31.68	169.65	-26.99	3.93	1	7.38E+06	25
D11	19.84	0.092	0.59	59.34	103.59	-26.33	4.72	20	8.16E+07	43	D41	19.39	0.04	0.2	18.06	97.37	-26.35	3.18	2	5.67E+06	14
D12	21.01	0.066	0.46	31.28	104.24	-25.21	3.74	32	2.42E+07	53	D42	8.66	0.046	0.24	18.24	95.77	-26.58	3.02	0	3.49E+06	16
D13	13.57	0.101	0.73	44.83	70.87	-25.34	3.29	4	3.82E+07	32	D43	10.12	0.067	0.46	33.04	147.38	-26.85	3.61	0	2.24E+06	9
D14	16.75	0.252	2.05	82.4	136.15	-24.06	3.23	0	3.35E+07	48	D44	16.62	0.065	0.41	66.97	233.56	-25.15	2.77	0	2.86E+06	12
D15	16.91	0.095	0.65	38.2	205.49	-26.46	2.32	0	4.16E+07	45	D45	17.62	0.059	0.37	35.61	309.27	-25.43	3.16	4	3.96E+06	12
D16	18.59	0.102	0.6	45.55	256.83	-26.21	1.87	0	3.49E+07	58	D46	13.24	0.029	0.11	56.14	69.43	-26.5	2.27	16	3.23E+06	12
D17	23.59	0.053	0.19	35.15	100.65	-26.64	2.31	0	2.15E+07	49	D47	2.72	0.031	0.1	72	121.65	-26.63	2.9	10	3.03E+06	3
D18	22.66	0.028	0.09	16.84	156.31	-26.75	2.41	0	1.57E+07	54	D48	1.66	0.05	0.35	19.85	249.52	-26.64	3.34	13	2.57E+06	8
D19	5.1	0.043	0.15	31.4	82.45	-26.42	3.09	23	1.77E+07	46	D49	11.58	0.129	1.23	62.2	239.51	-24.02	2.27	0	2.21E+06	0
D20	15.61	0.03	0.09	15.08	60.83	-26.87	2.13	7	1.16E+07	52	D50	20.93	0.082	0.54	39.82	231.51	-25.55	2.93	0	2.21E+06	6
D21	5.11	0.066	0.28	28.97	492.37	-26.11	2.52	37	1.39E+07	59	D51	21.66	0.044	0.13	43.44	982.08	-26.23	2.86	17	1.19E+06	14
D22	13.71	0.052	0.23	15.46	141.36	-26.28	3.17	5	1.42E+07	64	D52	21.61	0.034	0.11	20.14	179.31	-26.34	3.13	0	9.52E+05	9
D23	8.55	0.057	0.22	29.74	89.92	-26.47	3.28	31	1.49E+07	68	D53	6.08	0.038	0.11	36.05	124.07	-26.57	2.73	20	5.86E+05	16
D24	9.46	0.06	0.36	28.73	103.58	-26.46	2.75	12	1.35E+07	70	D54	15.37	0.032	0.15	21.38	107.52	-27.02	3.4	1	7.49E+05	12
D25	18.62	0.049	0.24	9.58	40.1	-26.68	3.02	15	1.01E+07	67	D55	8.75	0.021	0.06	16.03	70.82	-26.55	2.89	8	8.13E+05	15
D26	1.93	0.047	0.19	27.94	166.04	-26.79	3.48	26	2.32E+07	62	D56	2.1	0.029	0.12	13.19	54.02	-26.65	2.66	3	3.95E+05	10
D27	7.95	0.081	0.69	21.51	146.57	-26.22	3.16	75	1.66E+07	64	D57	4.77	0.028	0.14	22.95	95.91	-26.72	4.41	12	1.36E+05	11
D28	14.82	0.028	0.08	11.47	61.68	-26.63	2.71	25	1.86E+07	65	D58	6.57	0.042	0.14	14.13	69.97	-26.65	3.72	3	1.33E+05	12
D29	2	0.114	0.76	50.41	225.62	-26.23	3.68	3	2.32E+07	59	D59	7.88	0.031	0.11	17.21	72.85	-26.64	3.23	13	8.01E+05	10
D30	54.18	0.044	0.17	15.71	97.36	-26.33	3.4	1	1.25E+07	58	D60	4.07	0.038	0.14	22.35	90.57	-26.72	3.83	0	3.33E+05	7
D31	6.83	0.037	0.15	16.33	161.97	-26.54	3.38	0	1.12E+07	52											

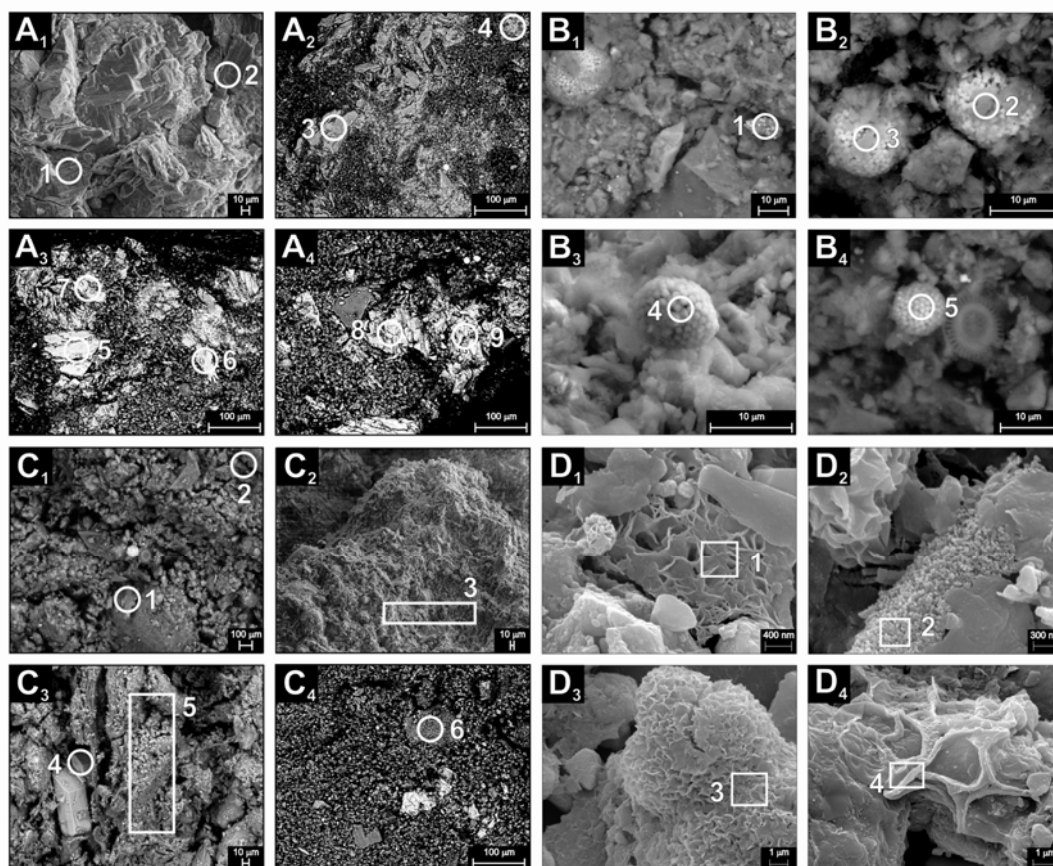
Annex A-3 Principal component analysis (PCA) with loading factors and complete datasets of variables used in the PCA.

Chapter 5



Annex A-4 Complete stratigraphic record of the 5022-1D core with sample positions

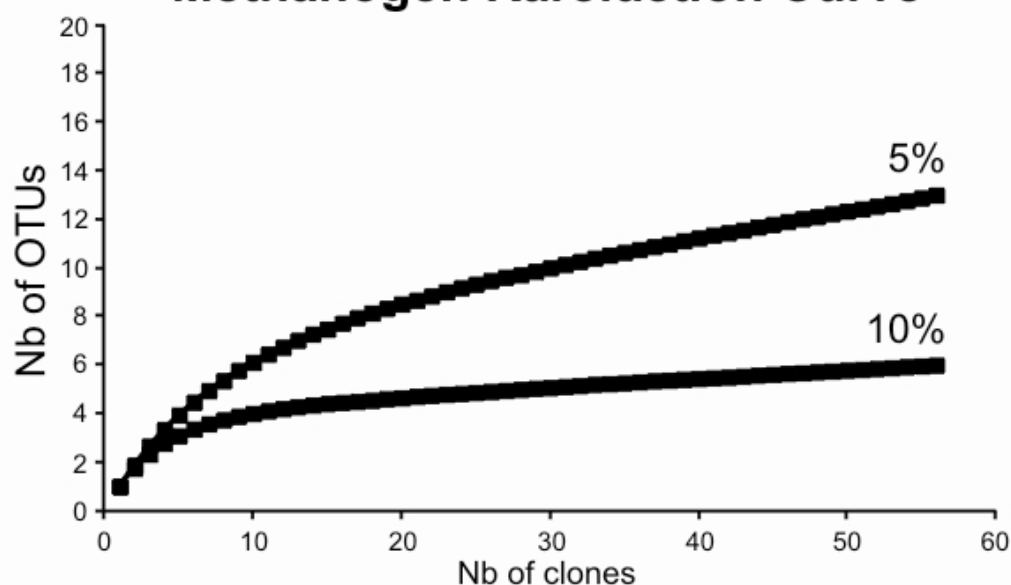
Elements Atomic Percentages [%]																	
A	Phosphates	C	O	Na	Mg	Al	Si	P	S	K	Ca	Ti	Mn	Fe	Total	Fe+Mn/P	Type
	1	7.00	66.92	--	--	0.71	1.91	8.74	--	--	0.14	--	0.69	13.89	100	1.59	Vivianite
	2	11.60	68.55	0.35	0.32	0.79	1.92	7.10	--	--	0.09	--	0.70	8.58	100	1.31	Oxidized V.
	3	19.45	58.63	0.40	0.40	1.53	4.31	5.94	--	0.26	0.13	--	0.58	8.37	100	1.51	Vivianite
	4	12.37	64.42	0.36	0.44	1.23	3.97	7.27	--	--	0.19	--	0.48	9.27	100	1.34	Oxidized
	5	17.51	62.67	0.31	0.28	1.15	3.40	6.29	--	0.14	0.33	--	0.53	7.39	100	1.26	Oxidized
	6	22.26	59.49	0.30	0.33	1.53	4.74	4.27	--	0.19	0.21	--	0.25	6.43	100	1.51	Vivianite
	7	4.68	70.67	0.70	0.80	3.75	12.79	2.18	--	0.38	0.43	0.08	0.10	3.44	100	1.58	Vivianite
	8	7.22	62.98	0.40	0.58	2.22	14.11	4.30	--	0.32	0.98	0.13	0.23	6.53	100	1.52	Vivianite
	9	17.48	61.34	0.37	0.35	1.13	3.34	6.00	--	0.13	--	--	0.59	9.27	100	1.55	Vivianite
B	Framboids	C	O	Na	Mg	Al	Si	P	S	K	Ca	Ti	Mn	Fe	Total	Fe/S	Type
	1	30.90	52.03	0.73	0.41	2.48	9.17	0.47	0.26	0.33	0.33	0.11	0.14	2.64	100	10.15	Iron precursor
	2	30.24	38.12	0.35	0.31	1.77	6.53	0.38	10.18	0.25	0.24	--	--	11.63	100	1.14	Mackinawite
	3	24.59	41.61	0.29	0.54	1.70	5.02	0.27	14.93	0.19	0.27	--	--	10.59	100	0.71	Greigite
	4	--	55.50	0.69	0.57	2.37	7.96	--	19.20	0.23	0.38	--	--	13.10	100	0.68	Greigite
	5	16.60	44.89	0.74	--	1.79	5.49	0.46	19.81	0.25	0.21	--	--	9.76	100	0.49	Pyrite
	6	19.11	53.78	0.23	0.79	2.89	7.41	0.33	9.33	0.45	0.66	--	--	5.02	100	0.54	Pyrite
	7	19.21	58.84	--	0.22	1.04	9.06	0.51	7.37	0.10	0.10	--	--	3.55	100	0.48	Pyrite
	8	20.61	41.54	0.35	0.41	1.68	4.04	0.23	20.28	0.11	--	--	--	10.75	100	0.53	Pyrite
	9	26.89	39.90	--	--	0.90	2.36	1.00	18.00	0.12	0.10	--	0.16	10.57	100	0.59	Pyrite
C	Matrices	C	O	Na	Mg	Al	Si	P	S	K	Ca	Ti	Mn	Fe	Total	Alk/Si	Type
	1	11.39	65.42	1.16	0.70	4.04	14.85	0.39	--	0.39	0.25	--	--	1.41	100	0.54	----
	2	9.16	65.45	0.84	0.92	5.02	14.53	0.71	--	0.80	0.24	--	--	2.33	100	0.70	----
	3	2.37	68.98	1.07	0.80	4.74	18.56	--	--	0.80	0.56	0.16	--	1.96	100	0.54	----
	4	28.39	47.87	0.31	7.65	1.24	8.90	0.28	--	0.14	0.33	--	--	4.89	100	1.64	Organic rich
	5	25.69	53.08	0.69	0.43	3.13	11.28	0.55	0.21	0.55	0.60	0.24	--	3.55	100	0.79	Organic rich
	6	2.05	69.24	1.48	0.82	5.75	18.05	--	--	0.56	0.35	0.16	--	1.54	100	0.58	----
D	Foliated clays	C	O	Na	Mg	Al	Si	P	S	K	Ca	Ti	Mn	Fe	Total	Alk/Si	Type
	1	10.06	45.07	2.93	1.42	6.08	29.94	--	--	1.23	--	--	--	3.27	100	0.50	----
	2	15.21	46.79	3.04	1.15	5.59	24.95	--	--	--	1.34	--	--	1.58	100	0.51	----
	3	18.35	42.25	1.99	2.42	7.72	20.90	--	--	--	0.95	--	--	5.42	100	0.88	----
	4	43.15	28.55	1.23	0.75	3.34	11.22	--	1.05	0.90	1.36	1.52	1.20	5.67	100	0.88	Sheath ?
	Others	C	O	Na	Mg	Al	Si	P	S	K	Ca	Ti	Mn	Fe	Total	Ratio	Type
	1	2.69	72.11	0.37	0.79	2.70	8.18	--	--	0.23	0.16	6.26	0.19	6.32	100	Fe/Ti	Ilmenite
	2	13.37	59.23	2.27	--	7.29	14.98	--	--	0.16	2.26	--	--	0.44	100	Na/Ca	Plagioclase
	3	14.86	59.35	2.04	0.17	6.75	13.37	0.43	--	0.15	1.98	--	--	0.90	100	Na/Ca	Plagioclase
	4	24.10	57.42	0.31	0.32	1.27	3.51	4.92	0.34	0.13	5.90	0.09	0.35	1.34	100	Ca/Fe	Anapaite



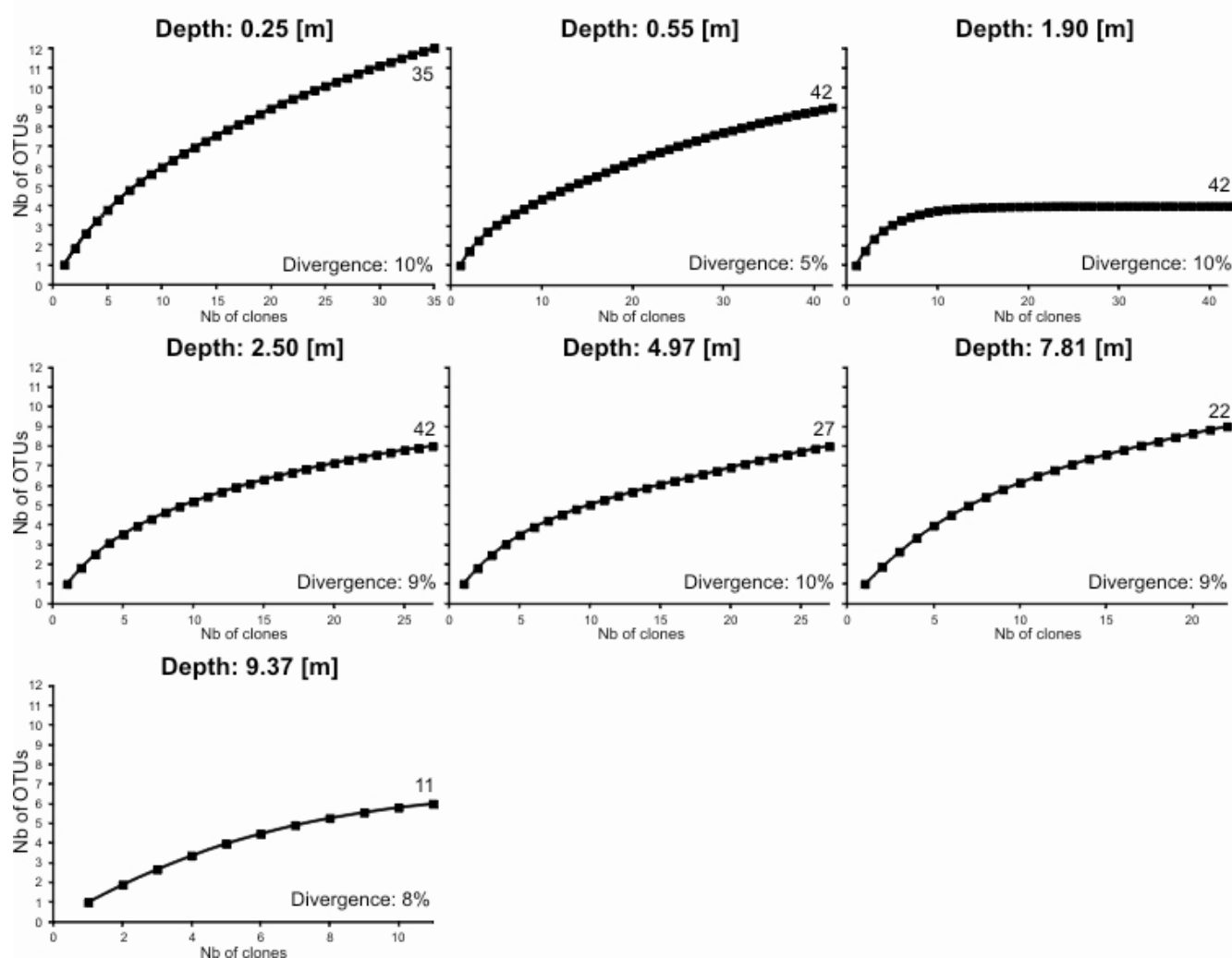
Annex A-5 Detailed EDS analyses for phosphates (A₁₋₄), frambooids (B₁₋₄), matrices (C₁₋₄) and specific foliated clays (D₁₋₃), EPS remnants (D₄) and some accessory minerals

Chapter 6

Methanogen Rarefaction Curve

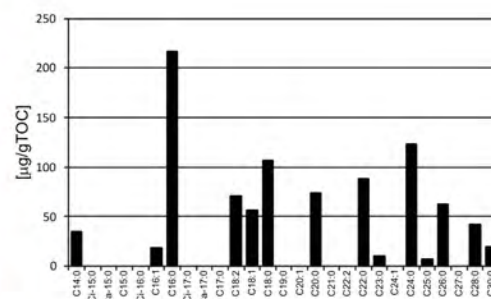
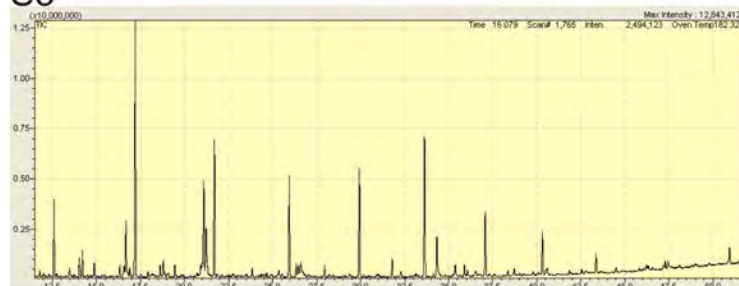


Archaea Rarefaction Curves

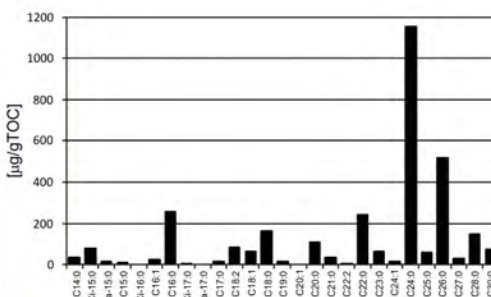
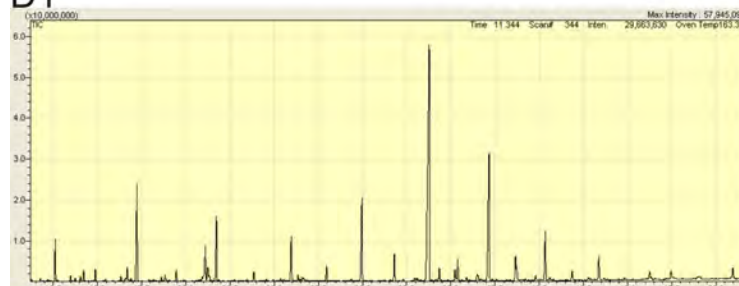
Annex A-6 Rarefaction curves were established using the Mothur[®] v. 1.25.1 software

Spectra used for identification

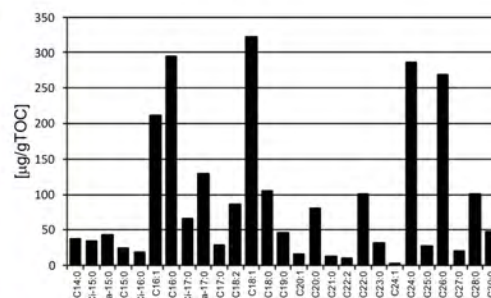
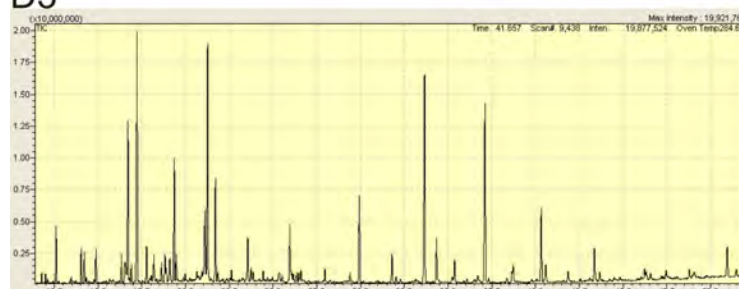
S5



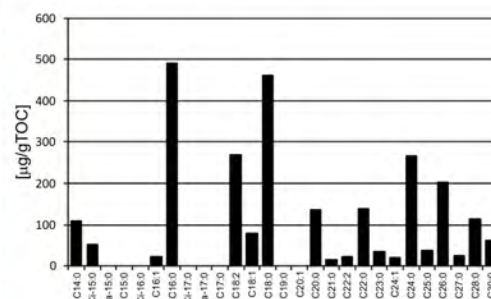
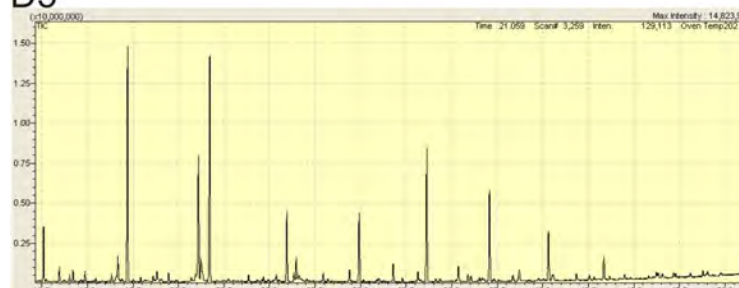
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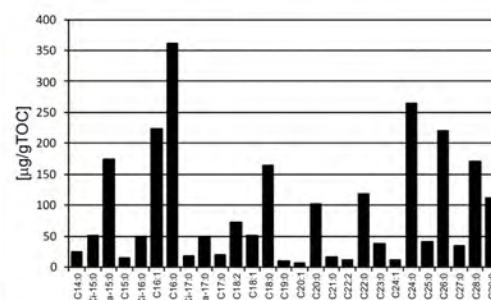
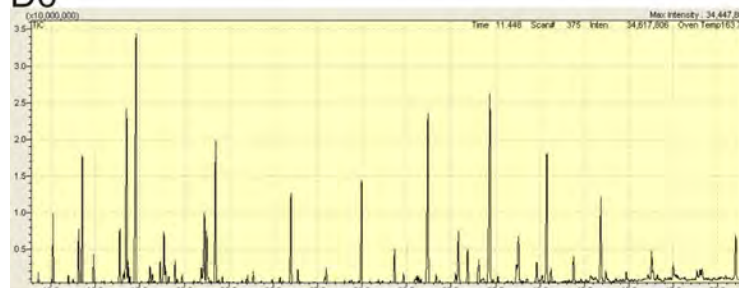
D3



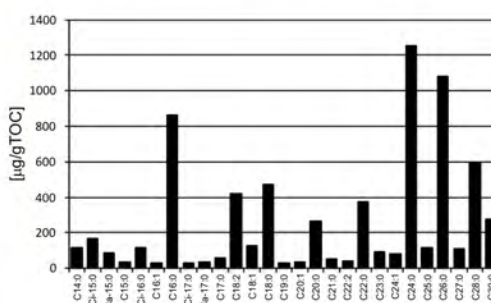
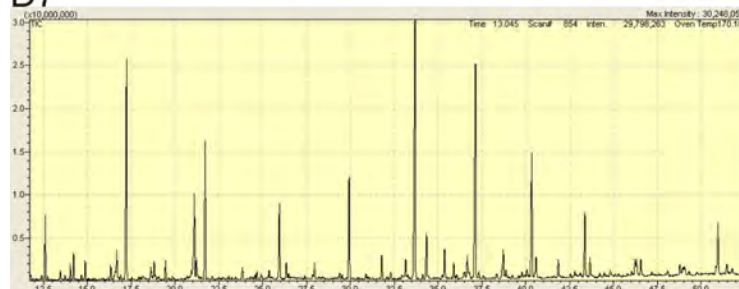
D5



D6

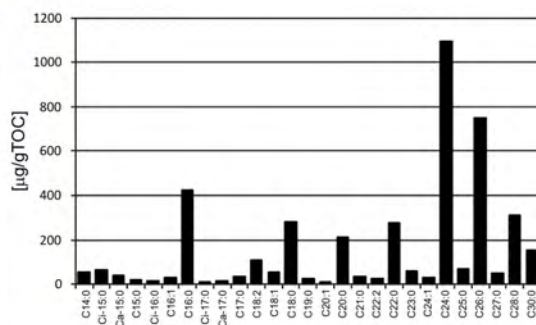
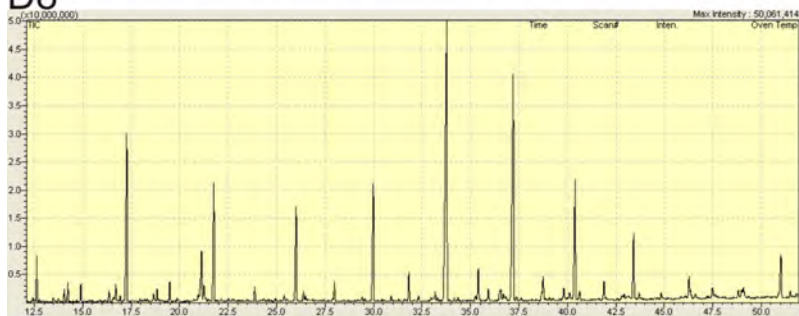


D7

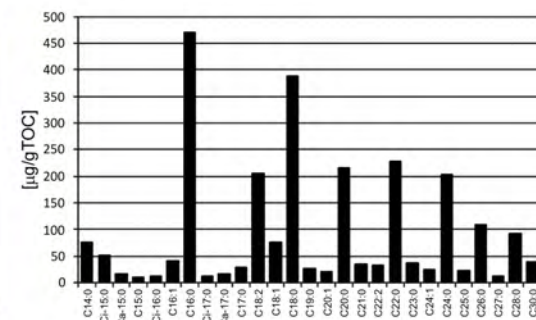


Spectra used for identification

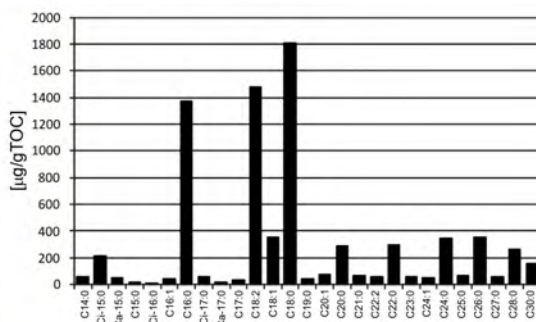
D8



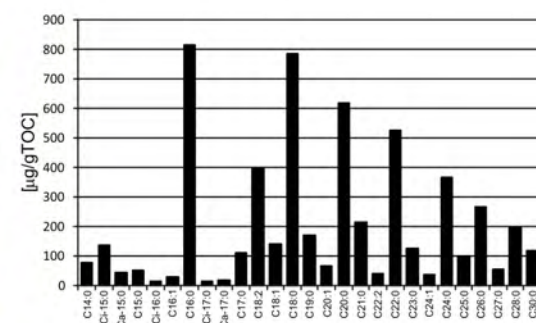
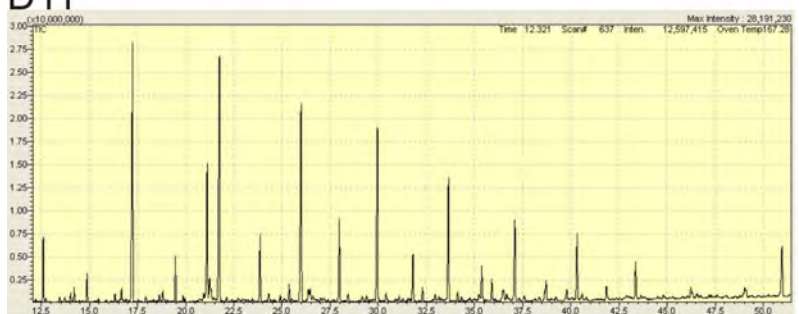
D9



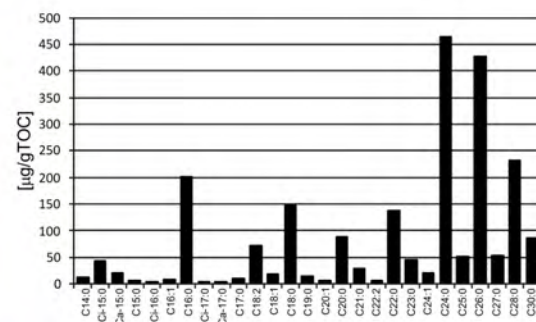
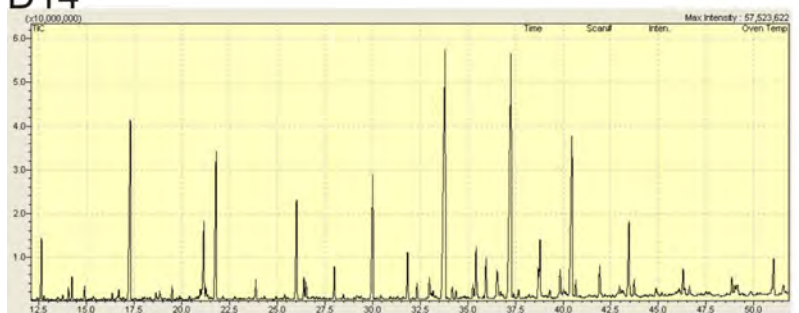
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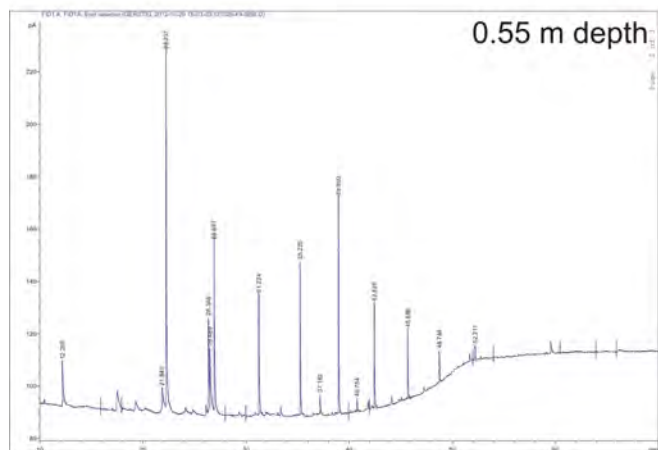
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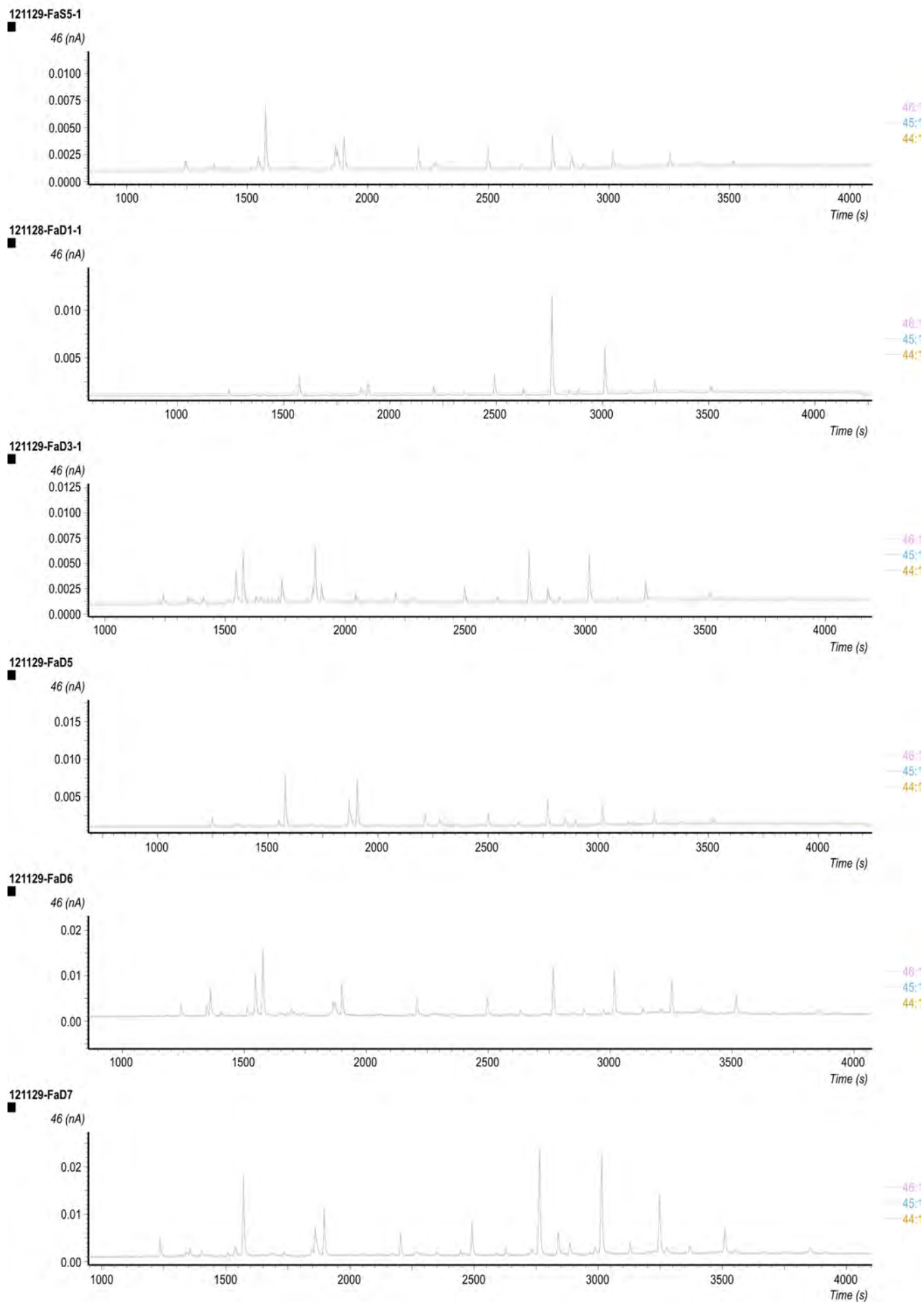
D14



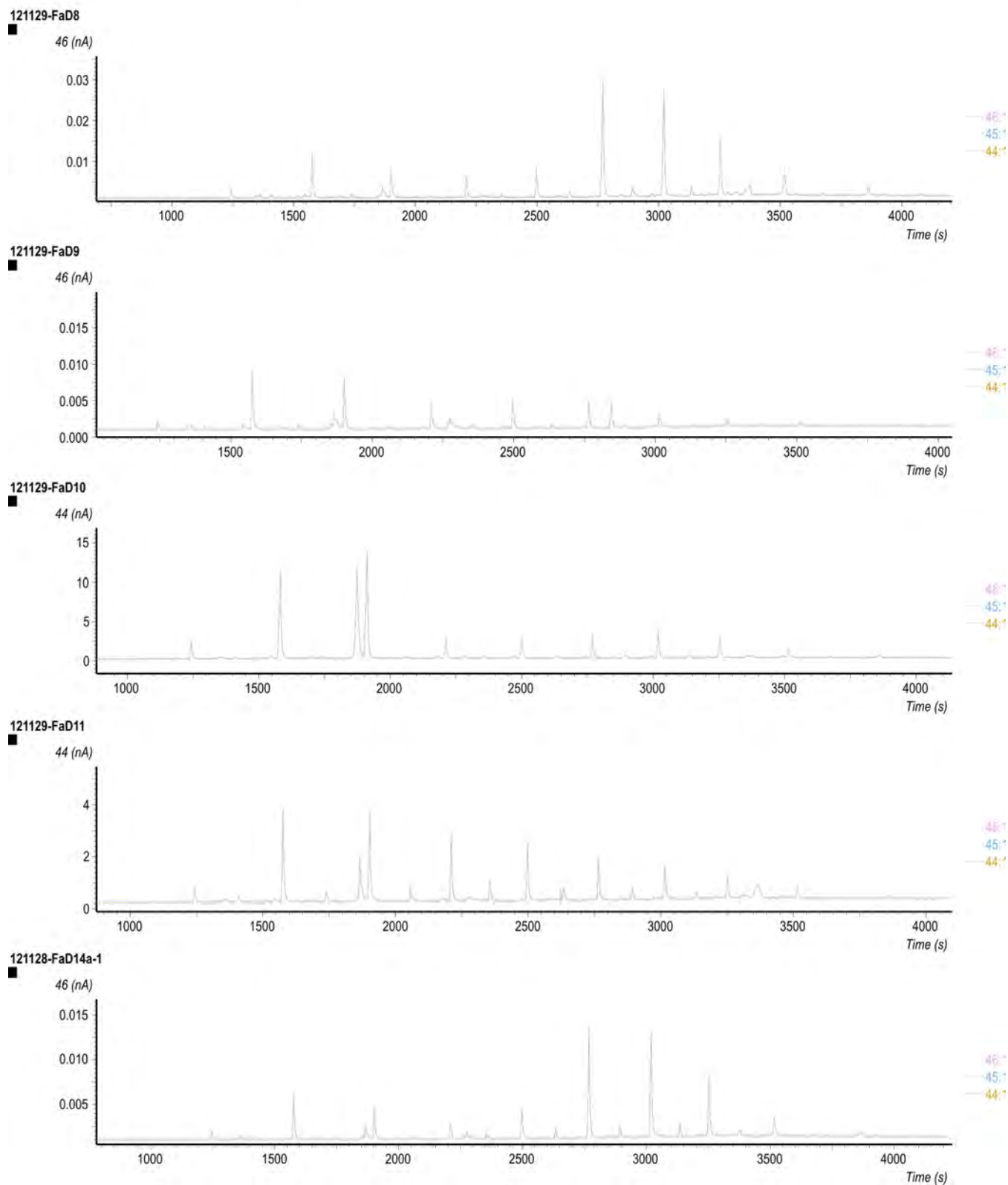
Spectra used for quantification



Spectra used for isotopic compositions



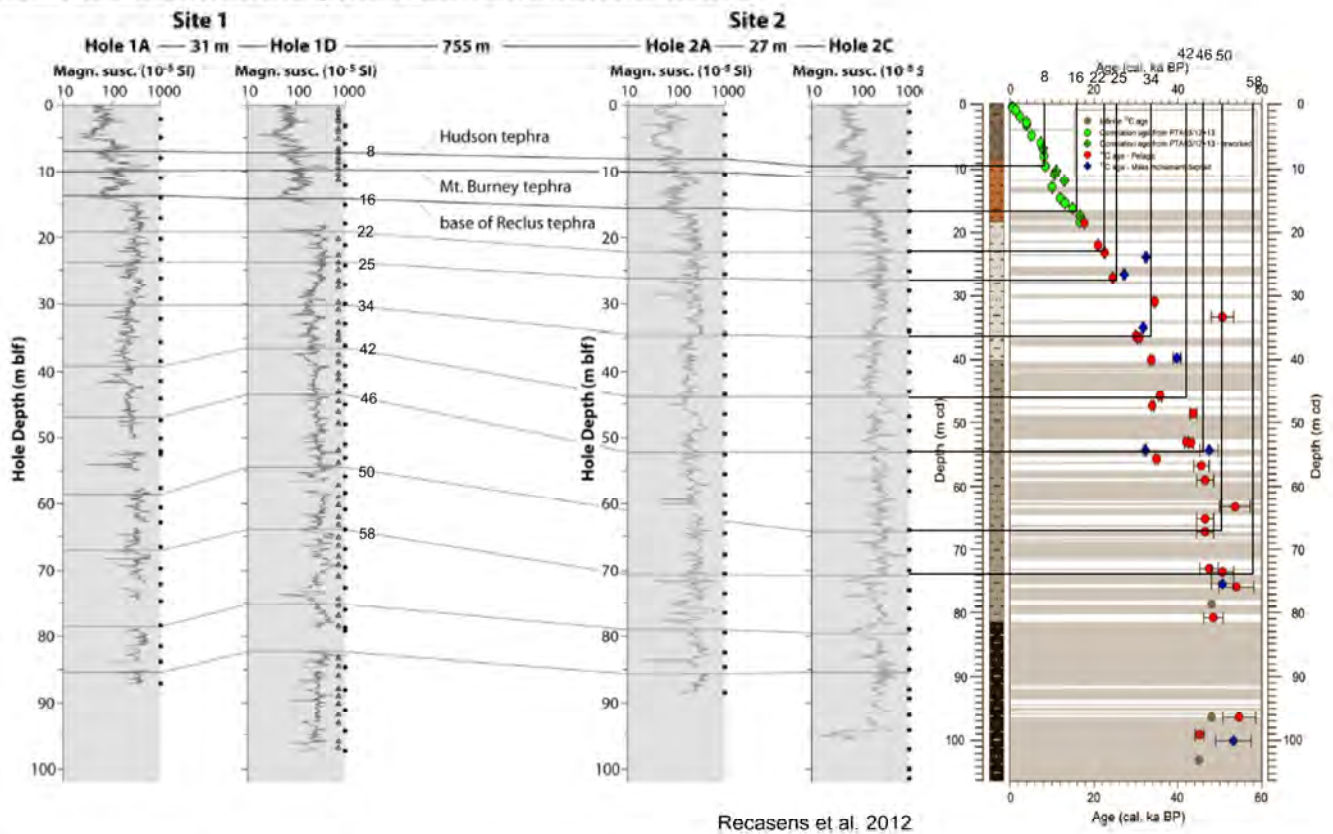
Spectra used for isotopic compositions



Annex A-7 FAs concentrations based on the identification of 27 compounds identified from $C_{14:0}$ to $C_{30:0}$

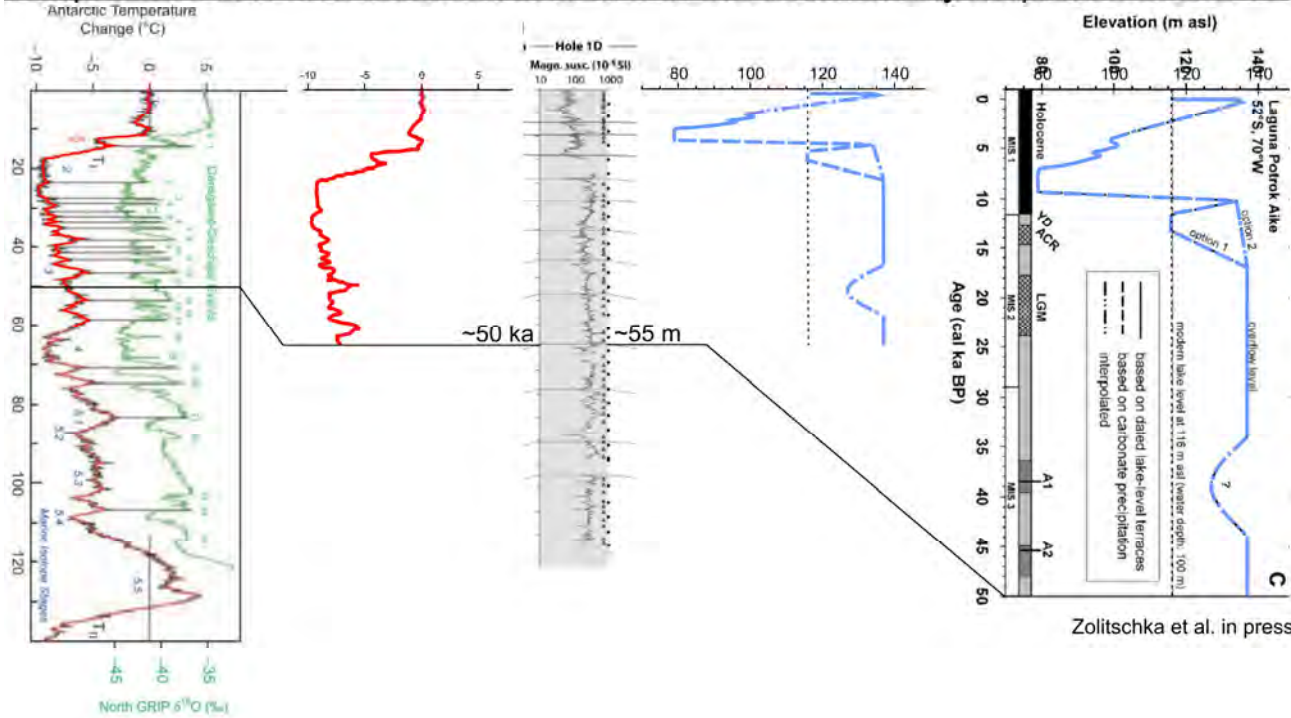
Chapter 7

Depth and chronology correlation between sites



Kliem et al. in press

Temperature and lake level correlation with the sedimentary sequence of site 1D



Jouzel et al. 2007

Annex A-8 Depth, age, temperature and lake level correlations established for the sedimentary sequence of site 1D