

# Ancient DNA: A window to paleo-environmental changes in microbial ecosystems as illustrated by an Antarctic lake and a fjord

Marco J.L. Coolen\* and Jaap S. Sinninghe Damsté

Marine or lake sediments frequently serve as archives of past aquatic microbial communities and, consequently, as recorders of environmental and climate change. Usually, these archives are read using specific chemical fossils, called biomarkers. We have shown that the study of fossil genetic material (DNA) can add a lot of information on the composition of past microbial communities to the biomarker approach. We studied changes in the microbial ecosystems of two Antarctic Lakes at an unprecedented species-level in sediments of over 10,000 years old.

Preservation conditions for ancient DNA were expected to be highest in cold and anoxic settings such as prevail in the Holocene sediment records of Antarctic lakes and fjords of the Vestfold Hills, eastern Antarctica. Our two study sites were the Small Meromictic (i.e. permanently stratified) Basin (SMB) in Ellis Fjord and the nearby, also permanently stratified, saline, Ace Lake (Fig. 1). Both basins are characterized by sulfide-rich anoxic bottom waters and sediments. Whereas SMB was continuously connected to the surrounding ocean during the Holocene, Ace Lake was initially a freshwater filled melt-water lake but became connected to the ocean 9,400 years ago as a result of post-glacial sea-level rise and then became permanently stratified and anoxic.

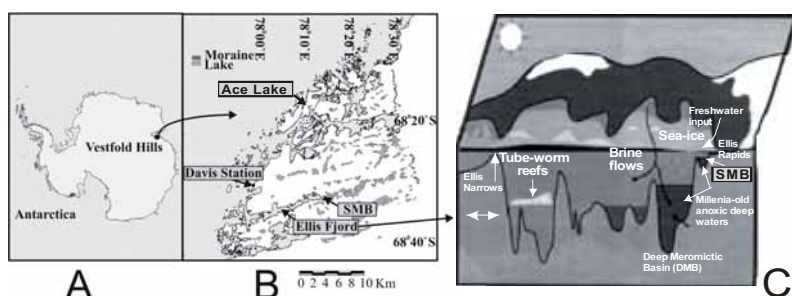


Fig. 1. (A) Location of the Vestfold Hills in Eastern Antarctica and (B) the two sampling sites in the Vestfold Hills: the Small Meromictic Basin (SMB) and Ace Lake and the nearby Australian Davis Station. (C) Location of the permanently stratified SMB with millenia-old anoxic deep waters in Ellis Fjord.

Using gravity cores, we reconstructed up to 10,000-year-old (Holocene) records of phytoplankton and bacterioplankton members, indicative for environmental change, based on their fossil genetic signatures. In order to ground-truth these ancient DNA or "paleogenomical" findings, we performed a parallel analysis of

chemical fossils and their isotopic signatures as an indication for the biochemical pathways used to biosynthesize them.

Previous work had shown that the Holocene sediments of the SMB in Ellis Fjord, contained numerous diatom skeletons and indeed we found high contents of a specific biomarker for certain species of diatoms belonging to the genera *Navicula*, *Haslea*, *Pleurosigma* or *Rhizosoleni*; i.e. the highly branched isoprenoid (HBI)  $C_{25:2}$  alkadiene. In order to test the suitability of fossil DNA as a novel paleoecology proxy, we focused our search on the algae which might have produced this specific HBI biomarker based on preserved genetic markers of diatoms. We used the sensitive Polymerase Chain Reaction (PCR) technique for this purpose. Despite the finding that the HBI biomarker had reacted with sulfide within 500 years after their deposition, the genetic material of the diatoms was relatively well preserved for



Gravity coring at Ace Lake, Vestfold Hills, Antarctica. The sediment core was 150 cm long, spanning 10,450 calendar years of deposition.

\*Corresponding author: mcoolen@whoi.edu

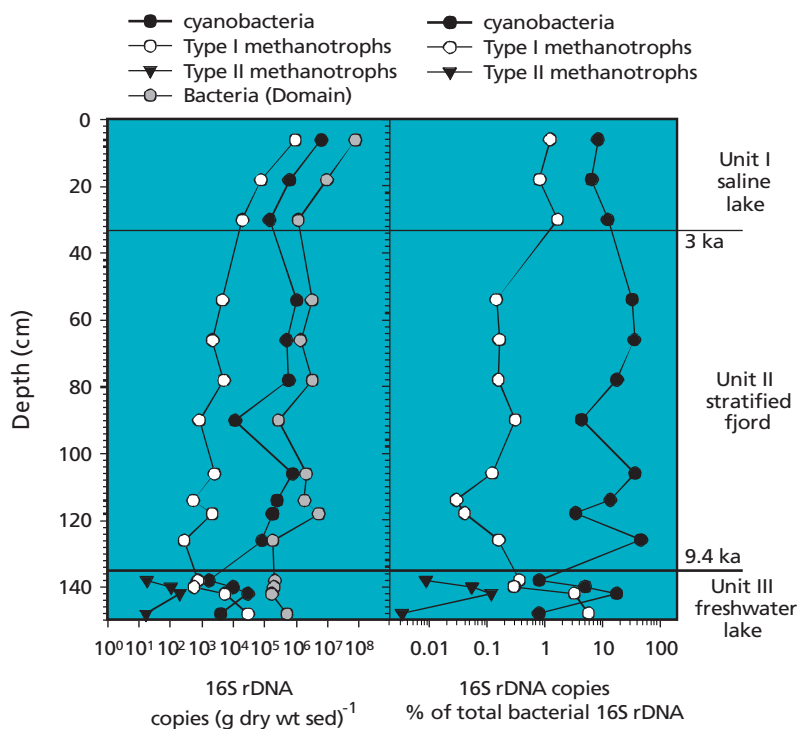


Fig. 2. Quantitative distribution of sedimentary 16S rDNA of bacteria (general), cyanobacteria, and aerobic methanotrophic bacteria (Type I and II) as revealed by quantitative polymerase chain reaction (PCR). (A); Quantity expressed as copies per gram of dry weight sediment or (B); as the percentage of total bacterial copy numbers. Unit III: freshwater sediments deposited >9,400 calendar years ago. Unit II: Post-glacial input of marine waters at 9,400 calendar years ago caused water column stratification and bottom water anoxia. Unit I: Closed stratified saline lake with anoxic bottom waters.

surface water and the anoxic, sulfidic high-salinity bottom waters when Ace Lake had become a stratified lake as a result of post-glacial input of marine waters. In addition, aerobic methane consumption could have taken place in the top few cm of oxygenated sediment when Ace Lake was still vertically well-mixed freshwater lake more than 9400 years ago. Since oxygen is absent from the sediment record, these buried bacteria could not have been active any more, which proves that their DNA in the fossil record is ancient.

Using our fossil DNA approach, we could indeed identify several methanotrophic bacteria and some species were unique for the old freshwater sediments (>9,400 years ago). We could also identify new species of methanotrophic bacteria from the high salinity period as the sources of the isotopically very light BHPs. Furthermore, the distribution of the methanotrophic species as well as additional sources of BHPs (freshwater cyanobacteria) enabled us to refine the environmental history of Ace Lake (Fig. 2).

at least 2,500 years and we were able to identify different strains of the genera *Haslea* and *Navicula* as the Holocene sources of the HBI biomarkers.

Another aspect of the project was to study Holocene biological sources of the biomarker bacteriohopanoids in Ace Lake. Bacteriohopanoids are widespread chemical fossils in the geosphere, but because many aerobic and anaerobic bacteria have these structures in their cell walls, they are not very specific as indicators for ecological and environmental changes. Therefore, we searched for more specific genetic markers to identify the Holocene bacterial species which were the sources of these biomarker compounds, the bacteriohopanepolyols, (BHPs). A suite of intact BHPs were identified with a variety of molecular structures throughout the sediment core from Ace Lake, reflecting changes in bacteria populations caused by large changes in salinity. These large and sudden changes in salinity is caused by the fact that Ace

Lake was initially an isolated, wind-mixed and non-stratified fresh water lake until about 9,400 years ago. When the sea-level rose after the last ice-age, water from the Southern Ocean could flow over a dam into the lake, immediately sinking to the bottom because of its high salinity. Some BHPs showed an isotopically very light carbon signal ( $\delta^{13}C$ ), which is indicative for bacteria incorporating carbon from methane into their cells. This aerobic consumption of methane must have taken place by bacteria which lived in the interface between the oxygenated fresh

This project showed that preserved genetic markers can reveal a large subset of Holocene planktonic organisms varying from algae such as diatoms, to cyanobacteria and methanotrophic bacteria which carry information about past environmental conditions. In addition, the identification of species based on these preserved genetic markers can help to identify fossil sources of lipid biomarkers. As such, the combination of both methods yielded much more paleo-environmental information than expected from the individual analysis.

