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RESEARCH

Temporal succession of bacterial and archaeal communities in a Mediterranean high-mountain lake over the last 430 years using sedimentary DNA

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Abstract

Despite the known influence of climate change on high-altitude ecosystems, the long-term response of prokaryotic communities in Mediterranean high-mountain lakes remains poorly understood. Here, we investigate the temporal dynamics of prokaryotic communities over the past ~ 430 years in a Mediterranean high-mountain lake, utilizing sedimentary ancient DNA (sedDNA). By examining a sediment core from Borreguil Lake in the Sierra Nevada (Spain), we evaluated bacterial and archaeal abundance, diversity, and community composition (β-diversity) in relation to paleoenvironmental and climate data. Our findings revealed a significant restructuring of prokaryotic communities, particularly since ca. 1960. A Random Forest model identified dissolved organic carbon, organic nitrogen, Saharan atmospheric dust inputs, and temperature as key drivers of the abundance, diversity, and composition of prokaryotic communities, particularly in the modern era. Notably, the abundance and diversity of bacterial communities increased in response to increased dissolved organic carbon, elevated temperatures, and dust deposition, while archaea demonstrated a more nuanced response linked to organic nitrogen availability and dust inputs. The temporal shifts in microbial community composition point to broader ecological changes within the lake, shaped by climate-driven environmental variations. For example, the increased relative abundance of Cyanobacteria and other taxa linked to higher nutrient availability indicates ongoing eutrophication processes, likely intensified by climate warming. This study highlights the importance of high-mountain lakes as indicators of climate change, contributing valuable insights into microbial ecology's response to long-term environmental change. Our findings offer a foundational understanding for predicting microbial responses in sensitive ecosystems under future climate scenarios.

Keywords Prokaryotic communities · Sedimentary ancient DNA · High-mountain lake · Microbial diversity · Climate change · Saharan dust

Introduction

High-mountain areas play a crucial role as sensors of anthropogenic climate change and global warming due to their sensitivity to environmental shifts. These mountain ecosystems are particularly responsive to temperature variations since the rate of warming is often amplified with elevation [1, 2]. Within global high-mountain regions, the Mediterranean area has been identified as a significant "climate change hot-spot" [3]. Mediterranean high-mountain

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freshwater ecosystems are particularly susceptible to the impacts of global warming, as evidenced by the observed gradual increase in mean air temperatures and a decline in annual precipitation over recent decades [3–6]. Improving our knowledge of how Mediterranean high-mountain freshwater ecosystems respond to past and recent environmental changes can improve our ability to predict and mitigate the potential impacts of ongoing climate change.

The Sierra Nevada Mountain range in southern Spain extends approximately 80 km in an east–west direction and includes the highest peaks on the Iberian Peninsula, reaching elevations above 3400 m a.s.l. [7]. This region serves as a key unique biodiversity refuge in southern Europe [8]. The high-altitude lakes of the Sierra Nevada, most of them situated at elevations exceeding 2800 m a.s.l., are recognized as critical indicators of global change due to their specific geographic location, isolation, altitude, high exposure to ultraviolet radiation (UVR), and the significant diversity of water bodies within this compact mountain range [9]. Sigró et al. [6] analyzed the temperature and precipitation trends in Sierra Nevada and found a warming of 0.13 °C/decade from 1930 to 2020, especially in spring and summer temperatures. They also observed a significant negative trend in summer precipitation, which intensified in recent decades to – 13.4%/decade for 1975–2020. These climate changes and climate-driven limnological changes have been shown to have significant effects on the abundance and composition of primary and secondary producers in the lakes [10, 11

In addition, Sierra Nevada's high-mountain lakes are subjected to atmospheric inputs of Saharan dust [10, 12], which introduce organic matter, nutrients (mainly phosphorus and calcium), and microorganisms to the lakes, ultimately affecting primary production and species community composition [10–13]. Paleoecological studies on Sierra Nevada lakes also indicate significant changes in climate and Saharan dust deposition during the Holocene [14, 15]. Therefore, the Sierra Nevada Mountain area is an excellent site to study long-term climate change effects due to its sensitivity to environmental changes and limited human impacts [16].

Prokaryotic communities play a critical role in the ecosystems of the oligo-mesotrophic high-mountain lakes in the Sierra Nevada [17]. For example, bacterial and archaeal communities play key roles in biogeochemical processes including carbon (C) [18] and nitrogen (N) cycling in these ecosystems [17, 19], among other processes. Yet, little is known about the influence of climate warming and other climate-driven limnological changes (e.g., changes in nutrient availability, water temperature, water lake volume, etc.) on the abundance, diversity, and composition of prokaryotic communities on long time scales in Sierra Nevada lakes and in freshwater ecosystems in general. For instance, a recent study showed that strong Saharan dust deposition events can increase the abundance and diversity and alter the composition of bacterial communities in sediments of high-mountain lakes in Sierra Nevada [17]. Other studies on alpine lakes in the European Alps and the Rocky Mountains (USA) have documented increases in microbial abundance and diversity in response to post-1950 warming and associated environmental shifts [20–22].

The use of sedimentary ancient DNA (sedDNA) has received increased attention as a suitable approach to reconstruct the history of aquatic ecosystems over geological scales [21, 23]. DNA obtained from the sediment core can serve as a proxy for the microbial communities present at the time of sediment deposition. The stratigraphic distribution of sedDNA in dated sediment cores enables the reconstruction of temporal dynamics in these microbial communities [21, 23]. This information, together with climatic and paleoecological data, can allow researchers to explore how the prokaryotic sediment microbiomes have responded to changing environmental conditions over extended timescales [23], and in lakes in different lake regions [21, 24]. In addition, the identification of responsive taxa to changes in environmental factors, as well as the strength of environmental drivers controlling these changes, can also be studied [25].

Previous paleoecological studies in alpine high-mountain lakes of Sierra Nevada mountains have contributed to our understanding of changes in algal biomass, cladocerans, diatoms, sedimentary algal pigments as well as changes in paleoenvironmental conditions over the past ~ 180 years [11, 14–16, 26–28]. Although this information has advanced our understanding of past environmental changes and helped constrain future environmental scenarios, we still lack similar paleoecological studies on the long-term changes in prokaryotic communities of Sierra Nevada high-mountain lakes. Therefore, our overall goal was to explore the temporal dynamics of prokaryotic communities (using a sedDNA approach) in a Mediterranean high-mountain lake by studying variations in the abundance, diversity, and composition of bacterial and archaeal communities in response to changing environmental conditions over the past ~ 430 years using a sedDNA approach. Through the integration of microbial analyses, paleoenvironmental variables, and climate data, we aim to uncover patterns of community abundance, diversity, and composition across time, and identify key environmental drivers of these changes and the key responsive taxa. We hypothesized that the diversity and composition of prokaryotic communities may be affected by variations in past environmental conditions, and that recent warming will increase microbial richness and diversity and promote variations in the relative abundance of specific taxa of the sediment microbiome.

Materials and Methods

Study Site and Field Sampling

The Borreguil Lake (37° 03′ 09.53″ N, 3° 17′ 59.03″ W) is a small oligo-mesotrophic lake located above the tree line at an elevation of 3020 m a.s.l. on the south face of Sierra Nevada Mountain (South of Spain) (Fig. 1). It has a maximum depth of 2.5 m and a surface area of 0.18 ha. In Sierra Nevada, the ice-free period usually extends from the middle of June to the end of October although there is considerable interannual variability [29, 30]. The catchment bedrock is siliceous, largely composed of mica schist with graphite and/or feldspar, and the soil is poorly developed, on which agriculture and forestry are not possible [31]. The catchment vegetation consists mainly of scarce xerophytic shrubland [32] and alpine meadows mainly consisting of Cyperaceae and Poaceae. Borreguil Lake is surrounded by alpine meadows with an overall meadow area of 0.55 ha. The lake has a permanent inflow and outflow, which currently flows throughout the entire ice-free period. Additional chemical and biological details on the site are available elsewhere [10, 11].

A slide-hammer gravity corer (Aquatic Research Instruments, Hope, ID, USA) with an inner core-tube diameter of 6.8 cm was used to collect a 26 cm sediment core from a sampling point at ~ 2-m depth of the Borreguil Lake (Fig. 1) in September 2021. The sediment core is hereafter referred to as SSBG-21. The sediment core was sectioned using sterile material on-site at 0.25-cm intervals for the first 9.25 cm and at 0.5-cm intervals for the remaining sediment. For each

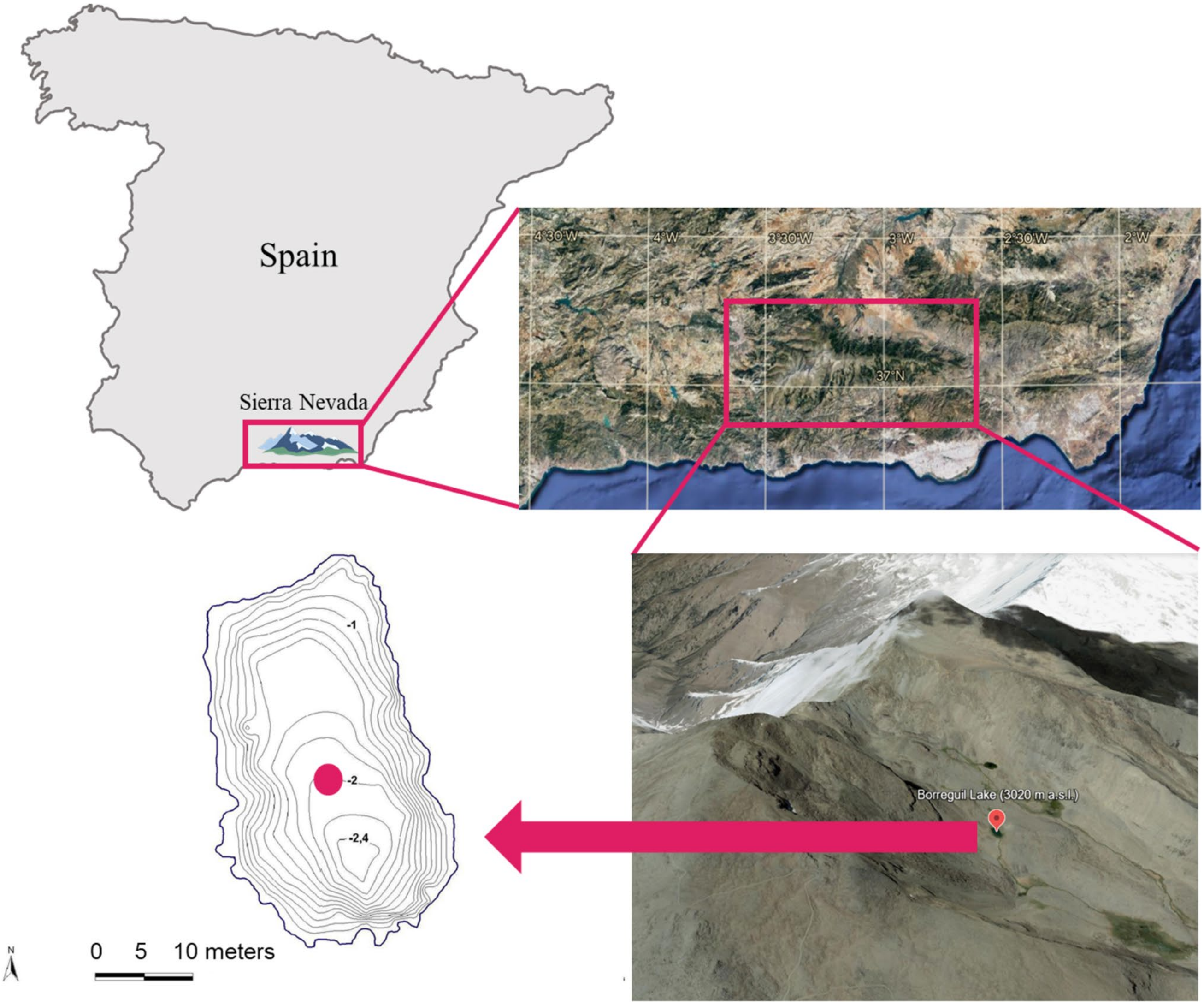


Fig. 1 Location of the study lake in Sierra Nevada and bathymetry of Borreguil Lake (digitized map of the bathymetry report from Egmasa S.A.). The orange dot represents the core location

interval, a fraction of sediment was taken through single-use sterile spatulas and stored in sterile vials for DNA analysis. These samples for DNA were placed into dark bags in a cooler until they were transported to our laboratory where they were frozen at − 80 °C until analysis. The remaining sample of each interval was placed into a plastic zip bag and transported in the dark to the laboratory for a later freeze-dry process. Freeze-dried sediment was stored at 4 °C for later analysis.

Dating, Age Models, and Paleoenvironmental Variable Analyses

Dating and Age Models

Freeze-dried subsamples were analyzed for ^{210}Pb, ^{226}Ra, and ^{137}Cs by direct gamma assay in the Liverpool University Environmental Radioactivity Laboratory. A selection of 18 sedimentary intervals was analyzed for ^{210}Pb activity following the technique outlined in Schelske et al. [33]. Sediment ages were estimated from unsupported ^{210}Pb activities using the constant rate of supply (CRS) and constant initial concentration (CIC) models [34]. ^{137}Cs is an artificial fallout nucleotide emitted during thermonuclear bomb testing (~1950–1980 with its peak in 1963) and nuclear accidents such as the Chernobyl accident in 1986 or the Fukushima accident in 2011. The analysis of ^{137}Cs in the sediment core was used to identify these dates [35].

Paleoenvironmental Variable Analyses

Freeze-dried sediment samples were analyzed for sedimentary chlorophyll-*a* (Chl-*a*) using the methodology and log transformed data from Michelutti et al. [36] with the equation: chl a + derivatives = EXP (0.83784 * LN (peak area 650–700 nm) + (− 2.48861)). Importantly, this approach incorporates the isomers and the main diagenetic products of chlorophyll a. Sediment-inferred lake-water dissolved organic carbon (DOC$_w$) was reconstructed by employing a transfer function between VNIR spectra of lake sediment following the methodology described in Meyer-Jacob et al. [37]. Both Chl-*a* and DOC$_w$ were analyzed at the Paleoecological Environmental Assessment and Research Laboratory (PEARL), Queen's University (Canada). In addition, a sediment fraction of ~ 1 g of wet weight (WW) sediment that has been previously freeze-dried was treated with 1 M hydrochloric acid overnight at 50 °C to remove carbonates. This sediment was used to determine the sediment total organic carbon (TOC$_s$) and total organic N (expressed as percentage of N in the organic matter—organic N %) using a Thermo Scientific Flash 2000 coupled to a mass spectrometer at the Scientific Instrumentation Center of the University of Granada. The C/N ratio of the organic matter was calculated from the mass data and expressed as an atomic ratio. Further analyses of stable isotopes were performed using the remaining carbonate-free sediment samples with an elemental analyzer (Flash HT Plus coupled to IRMS DELTA V Advantage at the Scientific Instrumentation Center of the University of Granada). Carbon isotope ($\delta^{13}C_{org}$) and nitrogen isotope ($\delta^{15}N$) composition was determined from the bulk sediment organic matter and reported in the conventional delta (δ) notation versus Vienna PeeDee Belemnite (V-PDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

Climate Data

The climate data used in the statistical analysis to examine relationships with the prokaryotic communities was sourced from Sigró et al. [6], who developed a dedicated climate database for the Sierra Nevada Mountains (http://www.c3.urv.cat/climadata.php). We used the mean annual temperature anomaly (MATA) and annual precipitation anomaly (APA). The annual climate series were available for the period ~ 1894–2020 year CE.

Previous studies have linked the intensity of Saharan dust emission and transport to the winter North Atlantic Oscillation (wNAO; [38]) and to drought conditions in the Sahel region [39]. Furthermore, Jiménez et al. [10] demonstrated that the Sahel Precipitation Index (SPI) and the wNAO index can be effectively used as predictors of Saharan dust transport and intensity in Sierra Nevada. Consequently, both indices were utilized for this purpose in the present study. The wNAO index (DJFM) is calculated based on the difference in normalized sea level pressure between the Azores High (Lisbon, Portugal) and the Iceland Low (Stykkisholmur, Iceland) stations [10]. Hurrell's dataset for the wNAO index provides monthly mean values dating back to 1864 (https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-station-based). The Sahel Precipitation Index (SPI), extending back to 1900, is available from the University of Washington and the Joint Institute for the Study of the Atmosphere and Ocean (http://research.jisao.washington.edu/data/sahel/), offering a standardized measure of rainfall for the Sahel region in northern Africa. The climate and dust data, resolved on an annual scale, were averaged over the accumulation period corresponding to each dated interval, effectively combining instrumental records with paleolimnological data [10, 11, 27]. Final weighted data are only available for the period 1905–2020.

DNA Extraction and Quantification

Total DNA was isolated from 250 mg of sediment samples utilizing the PowerSoil® DNA Isolation Kit (Qiagen, Germantown, MD, USA) following the protocol provided by the manufacturer. The concentration of the extracted DNA was determined using a Qubit 4™ Fluorometer in conjunction with the dsDNA HS Assay Kit™ (Thermo Fisher Scientific, Wilmington, DE, USA). The DNA samples were subsequently stored at – 80 °C until further analysis.

Quantification of Total Bacterial and Archaeal Communities

The absolute abundance of bacterial (16SB) and archaeal (16SA) communities was measured through quantitative PCR (qPCR), employing the 16S rRNA gene as a molecular marker. Amplifications were performed following the PCR conditions and primer sets detailed by Castellano-Hinojosa et al. [19] on a QuantStudio 3 Real-Time PCR system (Thermo Fisher, USA) (Supplementary Table S1). Calibration curves exhibited correlation coefficients exceeding 0.997 across all assays, and the PCR amplification efficiency ranged from 89.2% to 97.6%.

Sequencing and Microbial Community Analysis

The DNA was sequenced at Novogene Europe (Cambridge, UK) utilizing an Illumina MiSeq Sequencer. The bacterial and archaeal communities were targeted using the primers 341 F and 806R and Arch519F and Arch915R, respectively. The raw sequencing reads were processed and analyzed with QIIME2 version 2023.7, following the procedures outlined previously [40]. In summary, sequence reads were assembled and dereplicated into representative amplicon sequence variants (ASVs) using the DADA2 pipeline [41]. The ASVs were taxonomically classified against the SILVA 138.2 database in QIIME2 using a naïve Bayes classifier [42]. On average, 53,866 and 41,266 high-quality sequences per sample were obtained for the bacterial and archaeal communities, respectively. The raw sequence data have been deposited in the National Center for Biotechnology Information (NCBI) under BioProject PRJNA1275809. Alpha diversity metrics, including the number of amplicon sequence variants (ASVs) and Shannon index values, were calculated using the "Phyloseq" package in R, as described by Castellano-Hinojosa et al. [40]. Changes in microbial compositional turnover in microbial communities along the sediment core were explored using Detrended Correspondence Analysis (DCA), performed with the "vegan" package in R. We interpreted the scores of samples along the first DCA axis (DCA1) as a proxy for temporal variation in microbial community structure, following similar approaches in paleoecological studies [43]. To provide a complementary and quantitative measure of beta diversity, we conducted Non-metric Multidimensional Scaling (NMDS) based on Bray–Curtis dissimilarities (Supplementary Fig. S1), which provided a distance-based visualization of microbial compositional turnover.

Statistical Analyses

Random Forest (RF) classification was used to assess the influence of the paleo-environmental variables (Chl-*a*, $\delta^{13}C_{org}$, $\delta^{15}N$, TOC_s, organic N %, atomic C/N ratio, and DOC_w) and climate data (APA, MATA, SPI, and wNAO index) on the absolute abundance (number of copies/g sediment), and alpha (number of ASVs and values of the Shannon index) and beta diversity (first axis of the NMDS analysis) of archaeal and bacterial communities. RF is an ensemble learning method well-suited for both classification and regression tasks. It operates by constructing a multitude of decision trees during training, with predictions made by averaging (for regression) or taking a majority vote (for classification) across these trees. This method is particularly advantageous in paleoecological studies due to its robustness against overfitting and its ability to handle complex interactions among predictor variables, as demonstrated in previous research [44, 45]. In this study, the RF model was built using 1000 trees. The importance of each factor was ranked, with paleo-environmental variables and climate data serving as predictor variables, while the abundance, alpha, and beta diversity of archaeal and bacterial communities were treated as dependent variables. For data preprocessing, a Z-score transformation was applied to each predictor variable (mean-centered and scaled to unit variance) to ensure all variables contributed on a comparable scale. This standardization step mitigates any bias from variable magnitude differences, facilitating a balanced importance ranking of the paleo-environmental and climate predictors. The RF analysis was run using two main periods: from 1594 to 1905 and from 1905 to 2020 because climate data were not available for Sierra Nevada summits until 1905. The importance of each predictor variable was quantified and ranked, enabling the identification of key factors influencing microbial community structure over time. This analysis was conducted using the "ggRandomForest" package in R statistical software (version 4.2.2, http://www.rproject.org/).

Results

Core Chronology and Dating

The total ^{210}Pb activity profile exhibited an exponential decline with sediment depth (Fig. 2A). Total ^{210}Pb activity reaches values close to equilibrium with the supporting ^{226}Ra at a depth of between 6 and 7 cm. The ^{137}Cs concentrations have a well-defined peak between 1.5 and 1.75 cm (Fig. 2A) that most probably recorded the 1986 radioisotopic fallout from the Chernobyl accident. Both CIC and CRS models suggested that the sedimentation rate was relatively constant throughout the core with a small increase in recent

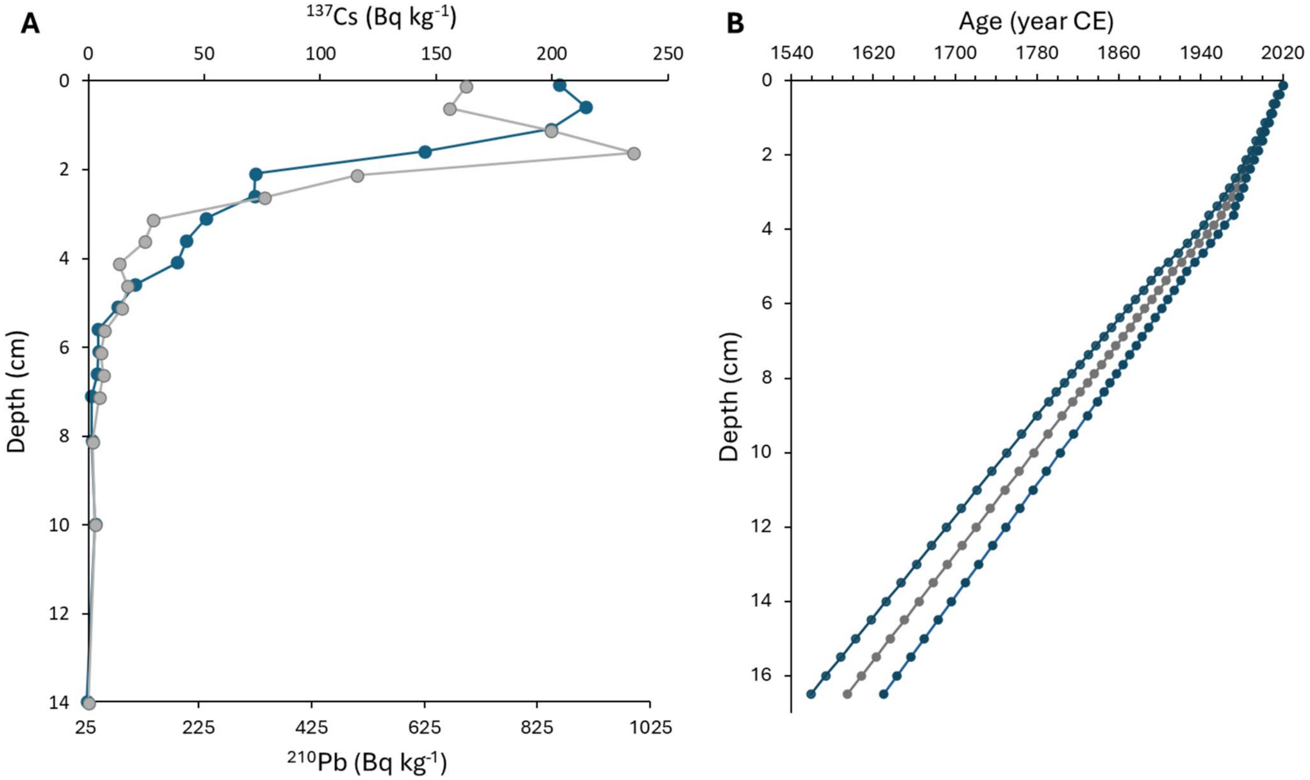


Fig. 2 **A** Radiometric chronology showing ^{210}Pb (blue circle) and ^{137}Cs (gray circle) activity (Bq kg^{-1} dried sediment) for SSBG-21 sediment core. **B**.^{210}Pb-estimated age versus core depth (black line) and the associated errors (gray lines)

years (uppermost 1 cm of the core) (Fig. 2B). Excluding the uppermost 1 cm, the mean sedimentation rate was 0.0098 ± 0.008 g cm^{-2} year^{-1} (or 0.041 cm year^{-1}) (Fig. 2B). The temporal resolution of the sediment cores ranged between 3 and 1 years per interval for the past ~ 150 years (at 0.25 cm intervals).

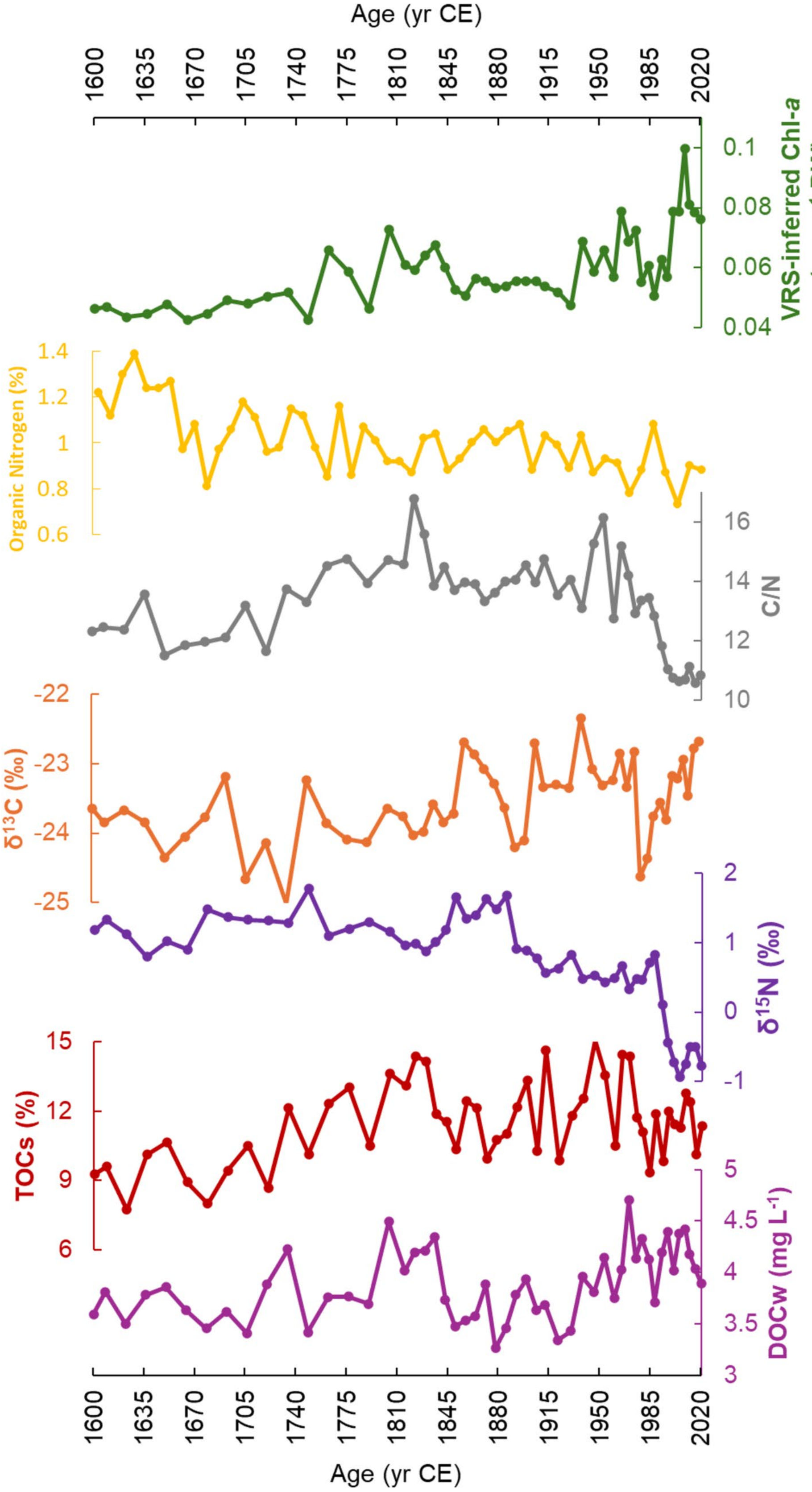
Paleoenvironmental Variable Analyses and Climate Data

Although our dating framework covers the last ~ 170 years, we also evaluated changes that occurred in earlier periods, recognizing that these extrapolated dates should be interpreted with caution.

Variations in the C/N ratio values (10.6–16.8) and δ^{13}Corg values (– 25.02 to – 22.34‰) were observed throughout the sediment core (Fig. 3). From 1594 to the early 1700 s, the sedimentary record shows relatively minor fluctuations in Chl-*a* and δ^{13}Corg, accompanied by a gradual increase in DOCw and TOCs (Fig. 3). The atomic C/N ratio remained relatively stable during this period. Between the late 1700 s and ca. 1850, Chl-*a* and TOCs exhibited notable fluctuations, while the C/N ratio increased, reaching a peak around 1850. In this same interval, δ^{13}Corg values declined from the early 1700 s to the ca. 1770 s, followed by recovery and stabilization towards ca. 1850 (Fig. 3). Between ca. 1850 and 1905, sedimentary values of Chl-*a*, DOCw, δ^{15}N, TOCs, organic N %, and the C/N ratio remained relatively stable, with only minor changes (Fig. 3).

From the early twentieth century onward, instrumental climate data show an increasing trend in MATA and a decreasing trend in APA (Fig. 4). These shifts coincide with a progressive increase in Chl-*a* and organic N %, and a steady decline in the C/N ratio, which reached its lowest values in the last ~ 40 years (Fig. 3). δ^{15}N values also declined after ca. 1905, with a more pronounced decrease after ca. 1970. The SPI showed a shift towards more negative values beginning around the mid-twentieth century, particularly during the 1960s–1990s (Fig. 4). The wNAO index also showed a gradual upward trend, especially from the ca. 1970 s onward, with an increase in the frequency of positive phases (Fig. 4). Altogether, the paleoenvironmental and climatic proxies in Borreguil Lake identified two distinct temporal phases: an early period up to the ca. 1920 s characterized by colder and wetter conditions, and a second period from the ca. 1920 s to the present marked by increasingly warmer and drier conditions, particularly after ca. 1960 (Figs. 3–4).

Fig. 3 Profiles of sedimentary chlorophyll-*a* (Chl *a*), $\delta^{13}C_{org}$, $\delta^{15}N$, TOCs, DOCw, atomic C/N ratio, and organic N in the sediment core. The annual precipitation anomaly (APA) and air temperature anomaly (MATA) are shown from ca. 1905 to 2020. DOCw sediment-inferred lake-water dissolved organic carbon, TOCs sediment total organic carbon



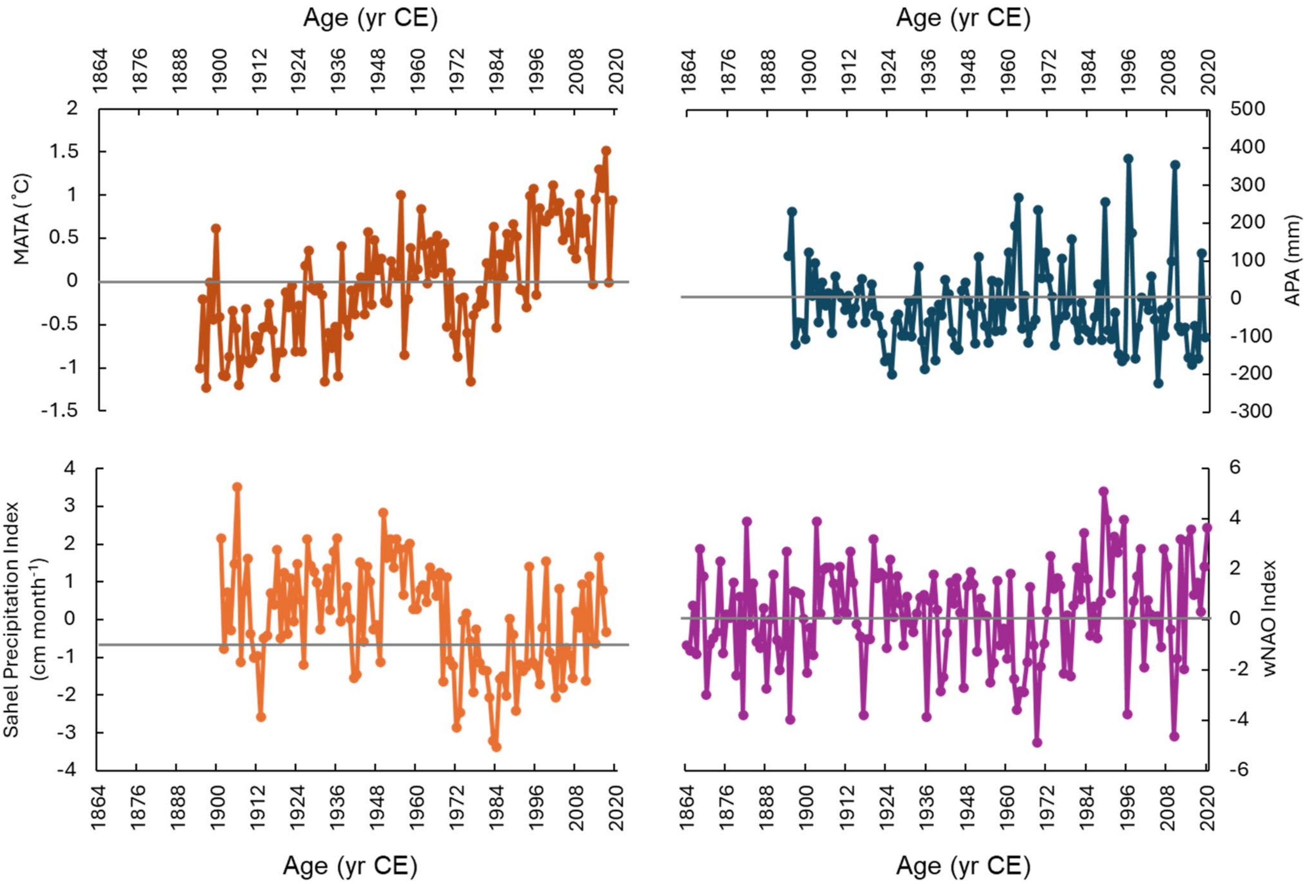


Fig. 4 Variations in the annual precipitation anomaly (APA), the air temperature anomaly (MATA), SPI and the wNAO. wNAO winter North Atlantic Oscillation, SPI Sahel Precipitation Index

Temporal Changes in the Absolute Abundance, Alpha and Beta Diversity, and Structure of Prokaryotic Communities

The ~ 430-year sedimentary record revealed gradual increases in the absolute abundance of both archaeal and bacterial communities, with a pronounced rise after 1960 (Fig. 5). Slight increases in the number of observed archaeal ASVs (~ 300–400) were observed over the ~ 430-year record, while increases in Shannon diversity of the archaea community (ranging from 2.6 to 5.3) were more marked, particularly over the past ~ 60 years (Fig. 5). Similarly, bacterial communities showed gradual increases in ASV numbers (310–953) and Shannon diversity (3.8–6.1), reflecting a parallel trend in community diversification (Fig. 5). The DCA analysis further highlights the gradual increases in community structure for archaeal and bacterial communities throughout the sedimentary records, with more pronounced shifts occurring from ca. 1950–1960 to 2020 (Fig. 5). In addition, the NMDS analysis based on Bray–Curtis dissimilarities (Supplementary Fig. S1) revealed substantial temporal variation in beta diversity, further confirming the restructuring of microbial community composition across the studied period.

Temporal Changes in the Composition of Archaeal Communities

An overview of the composition of the archaeal community at the phylum and genus taxonomic levels is provided in Fig. 6A, C, respectively. The archaeal community was dominated by the phylum Crenarchaeota from ca. 1594 to 1843, with values of relative abundance in the range of 67.5–88.8%, respectively. Thereafter, the relative abundance of this phylum gradually decreased to less than 20% by ca. 1979. From ca. 1843 to 1970, there were gradual increases in the relative abundance of the phyla Thermoplasmadota (from 7.3% to 18.7%), Micrarchaeota (from 4.7% to 27.8%), and Nanoarchaeota (from 6.3% to 14.5%). From ca. 1970 to 2020, the archaeal community was mainly formed by the Nanoarchaeota, Thermoplasmadota, and Crenarchaeota phyla, with average relative abundances of 27.5%, 18.6%, and 14.4%, respectively.

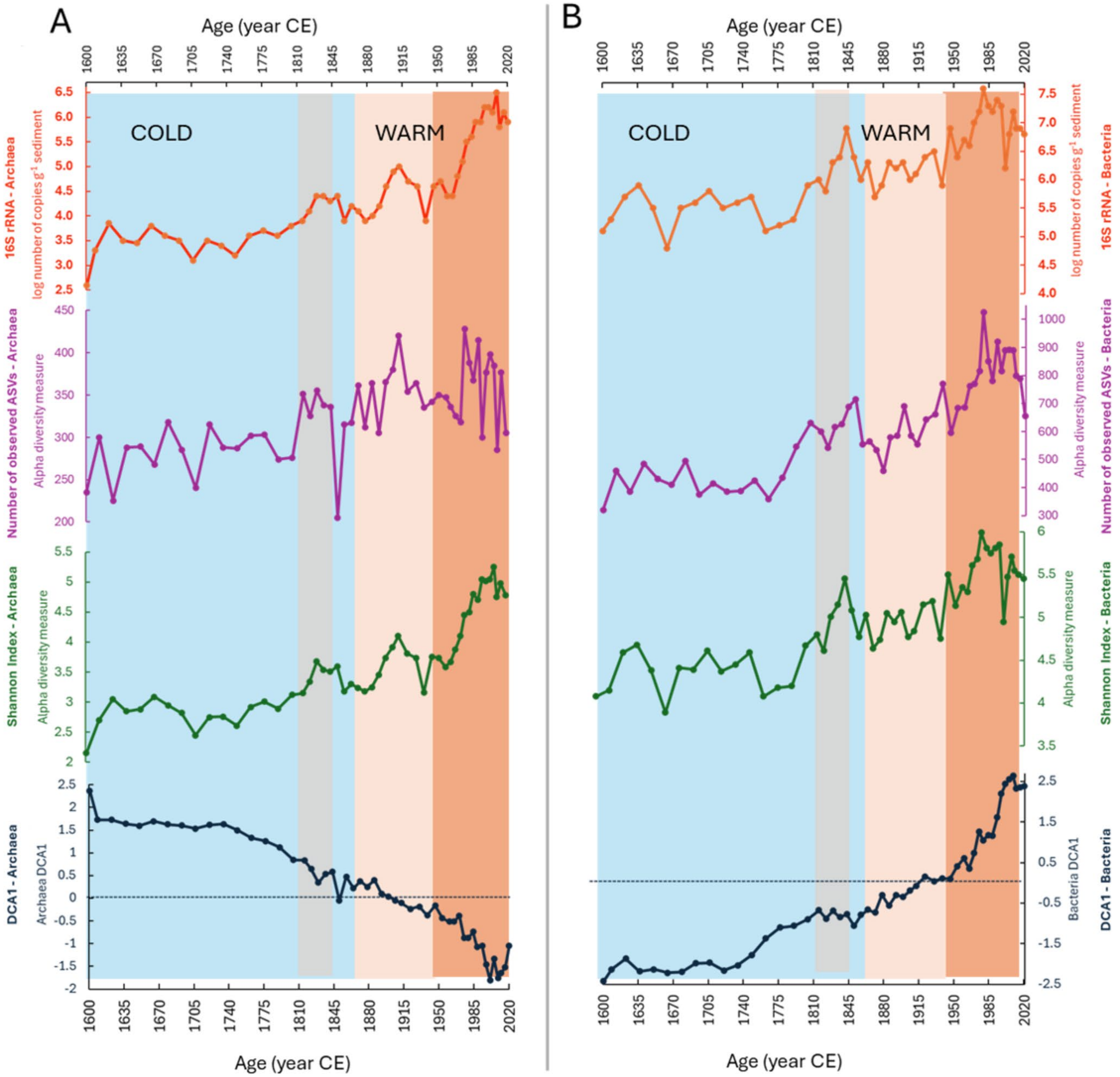


Fig. 5 Absolute abundances, number of observed amplicon sequence variants (ASVs), values of the Shannon diversity index, and DCA axis 1 for the archaeal (**A**) and bacterial (**B**) communities recovered from the sediment core

From ca. 1594 to 1748, the archaeal community was mainly dominated by the genus *Bathyarchaeia* (Crenarchaeota phylum) with values of relative abundance in the range of 67.3–88.6%, respectively. From ca. 1748 to 1970, there were gradual increases in the relative abundance of ten different genera, including *Marine_Benthich_Group_D_and_DHVEG-1* (from 7.1% to 18.5%; the Thermoplasmadota phylum), GW2011_GWC1_47_15 (from 2.2% to 17.5%; Thermoplasmadota phylum), and *CG1-02–32-21* (from 2.2% to 17.5%; Micrarchaeota phylum). From ca. 1970 to 2020, the archaeal community was dominated by the order Woesearchaeles (Nanoarchaeota phylum), *Marine_Benthich_Group_D_and_DHVEG-1*, GW2011_GWC1_47_15, and *Bathyarchaeia* with average relative abundances of 24.4%, 14.2%, 13.2%, and 12.4% respectively. Of note, methanogenic genera belonging to the families Methanomassiliicoccaceae (Thermoplasmadota phylum), Methanoregulaceae (Halobacterota phylum), Methanosaetaceae (Halobacterota phylum), and Methanosarcinaceae (Halobacterota phylum) were only detected during the period ca. 1892–2020, with

Fig. 6 Relative abundances of archaeal (**A, C**) and bacterial (**B, D**) amplicon sequence variants (ASVs) at the phylum and genus taxonomic levels in the sediment core. Phyla and genera with at least 1% of relative abundance are shown

values of relative abundance in the range of 0.9–1.8%, suggesting that recent changes in environmental conditions favored these groups.

Temporal Changes in the Composition of Bacterial Communities

An overview of the composition of the bacterial community at the phylum and genus taxonomic levels is provided in Fig. 6B, D, respectively. The bacterial community composition showed significant shifts over the ~430-year period, reflecting broader ecological changes. From ca. 1594 to 1970, the bacterial community was dominated by the phyla Chloroflexi, Bacteroidota, and Acidobacteriota. During this period, the relative abundance of the phyla Chloroflexi and Acidobacteriota gradually decreased from 64.5% to 24.2% and from 23.1% to 3.1%, respectively, whereas that of Bacteroidota remains stable at around 20%. From ca. 1970 to 2020, there were gradual increases in the relative abundance of the phyla Cyanobacteria (from 4.4% to 16.1%) and Proteobacteria (from 1.9% to 13.8%). Gradual increases in the relative abundances of the phylum Verricomicrobiota were observed from ca. 1594 (0.8%) to 2005 (14.2%) and thereafter decreased to about 10% of relative abundance towards the present.

Gradual decreases in the relative abundance of the genera SCGC-AB-539-J10 and Sh765B-TzT-20 belonging to the phylum Chloroflexi were observed from ca. 1594 (39.1% and 7.8%, respectively) to 1762 (5.1% and 0.8%, respectively), but then were no longer recorded. The genus *MSBL5* (Chloroflexi phylum) gradually increased in relative abundance from ca. 1594 (1.8%) to 1762 (35.8%) and afterwards gradually decreased to less than 0.7% of relative abundance by ca. 1997. From ca. 1970 to 2020, there were gradual increases in the relative abundance of *Cyanobium_PCC-6307* (from 3.5% to 22.4%; phylum Cyanobacteria), *BSV13* (from 1.3% to 4.5%; phylum Bacteroidota), *ADurb.Bin063-1* (from 1.2% to 5.2%; phylum Verrucomicrobiota), and genera belonging to the family Lentimicrobiaceae (from 0.7% to 3.1%; phylum Bacteriodota). Taxa belonging to the order Aminicenantales (Acidobacteriota phylum) and the genus *GIF9* (Chloroflexi phylum) gradually decreased in relative abundance over the ~430-year record from values of about 5–6% to less than 1.5%.

Contributions of Paleoenvironmental Drivers to Changes in the Archaeal and Bacterial Communities.

An RF analysis was used to assess the influence of the available paleo-environmental variables and climate data on the absolute abundances, as well as the alpha diversity, and community structure of archaeal and bacterial communities over the ~ 430-year period. The performance of the Random Forest models was generally high, with coefficient of determination (R^2) values ranging from 0.55 to 0.84 and root mean square error (RMSE) values between 0.08 and 0.20, depending on the microbial parameter and time period (Supplementary Table S2). These results indicate that the selected paleo-environmental and climate variables explained a substantial proportion of the variation in microbial community abundance, diversity, and composition. From ca. 1594 to 1905, before significant anthropogenic influences, the C/N ratio and organic N % emerged as the dominant drivers influencing both the abundance and alpha diversity of archaeal communities (Fig. 7). Similarly, for bacterial communities, organic N % was the primary determinant of changes in both abundance and alpha diversity. Variations in the composition of archaeal and bacterial communities (β-diversity) were influenced by most paleo-environmental variables from ca. 1594 to 1905 (Fig. 7).

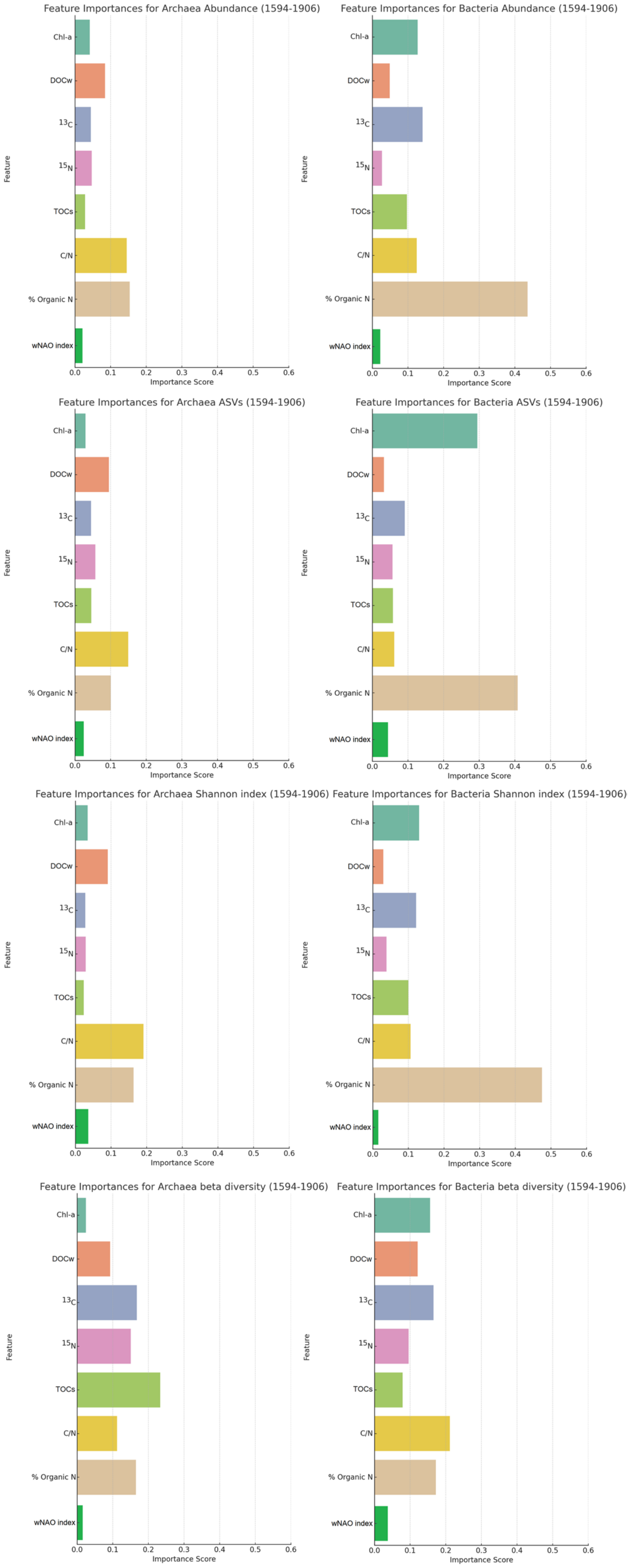
From ca. 1905 to 2020, the inclusion of climate variables (APA, MATA, and SPI) introduced additional drivers of microbial community characteristics (Fig. 8). The DOC_w ratio and the SPI were identified as the main paleoenvironmental and climate drivers influencing bacterial abundance and alpha diversity from ca. 1905 to 2020 (Fig. 8). As for the period from ca. 1594 to 1905, variations in the β-diversity of the archaeal and bacterial community were influenced by most paleo-environmental variables from ca. 1905 to 2020, but especially by the SPI (Fig. 8). The organic N%, MATA, and SPI were important for predicting changes in the abundance and Shannon diversity values of archaeal communities from ca. 1905 to 2020, respectively.

Discussion

Our analyses provide a comprehensive assessment of the long-term dynamics of sedimentary prokaryotic communities in a Mediterranean high-mountain lake over the past ~ 430 years, integrating sedimentary DNA (sedDNA) data with paleoenvironmental and climatic proxies. Our findings reveal clear evidence of how microbial diversity and composition have responded to historical and recent environmental changes, with particularly strong restructuring since the mid-twentieth century due to regional warming and associated alterations in nutrient and dust deposition regimes.

The paleoenvironmental proxies examined in this study provide critical insights into the organic matter dynamics and climatic forcing that shaped the sedimentary record of Borreguil Lake. The variations in the C/N ratio and δ^{13}Corg values suggest a persistent mixture of algal-derived and terrestrial organic matter inputs, which is consistent with the lake's small surface area, relatively large catchment, and partial coverage by alpine meadow vegetation. These values fall within the ranges reported for other Sierra Nevada lakes [10, 11, 27, 46, 47], supporting the idea of sustained mixed-source inputs. From ca. 1594 to the early 1700 s, minor fluctuations in Chl-*a* and δ^{13}Corg, combined with a decreasing trend in C/N and increasing DOCw and TOCs, suggest relatively stable, oligotrophic conditions dominated by autochthonous organic matter production. During the late eighteenth and early nineteenth centuries, increasing Chl-*a* and DOCw values and a peak in C/N around 1850 point to enhanced terrestrial inputs and a temporary boost in productivity, likely driven by the warming phase following the Little Ice Age, which are consistent with other records from the region [47, 48]. This is further supported by δ^{13}Corg trends, which show a decline until the 1770 s followed by stabilization, indicating a shift in the balance between sources of organic matter. Between ca. 1850 and 1905, the geochemical signals (Chl-*a*, DOCw, δ^{15}N, TOCs, organic N %, and C/N) remained stable with minor fluctuations, suggesting relatively unchanging productivity under intermediate environmental conditions.

A marked transition began in the early twentieth century, as warming (increasing MATA) and drying (declining APA) trends intensified across the Sierra Nevada [6]. These changes were accompanied by a rise in Chl-*a* and organic N %, indicating enhanced primary productivity, while the progressive decline in C/N reached minimum values over the past ~ 40 years, indicating an increasing dominance of algal-derived organic matter [49]. These changes were likely associated with longer growing seasons and higher water temperatures with the onset of the twentieth century rise in air temperature and the decrease in precipitation in Sierra Nevada [11, 16]. Increased anthropogenic nutrient inputs and climate change in the modern period have been related to higher primary productivity in remote lakes [50, 51]. Warming trends could have led to a longer ice-free period in the Borreguil Lake after ca. 1905, increasing both light availability and average water temperature. This change can result in longer water residence times due to decreased inflow and increased evaporation, along with more snowmelt and weathering, which boosts solute input into the lakes [20]. These factors may promote biological production and allow for greater annual biomass accumulation in the Borreguil lake. Additionally, the deposition of P-rich Saharan dust over the past ~ 50 years may have contributed to the observed increases in sedimentary Chl-*a*



Feature Importances for Archaea Abundance (1594-1906)

Feature Importances for Bacteria Abundance (1594-1906)

Feature Importances for Archaea ASVs (1594-1906)

Feature Importances for Bacteria ASVs (1594-1906)

Feature Importances for Archaea Shannon index (1594-1906)

Feature Importances for Bacteria Shannon index (1594-1906)

Feature Importances for Archaea beta diversity (1594-1906)

Feature Importances for Bacteria beta diversity (1594-1906)

Fig. 7 Random Forest (RF) model of the influence of the paleo-environmental variables (Chl-*a*, $\delta^{13}C_{org}$, $\delta^{15}N$, TOCs, DOCw, organic N %, and atomic C/N ratio) on the absolute abundance (number of copies/g sediment), and alpha (number of ASVs and values of the Shannon index) and beta (first axis of the NMDS analysis) diversity of archaeal and bacterial communities for the period between ca. 1594 and 1905. DOCw sediment-inferred lake-water dissolved organic carbon, TOCs sediment total organic carbon, wNAO winter North Atlantic Oscillation, SPI Sahel Precipitation Index

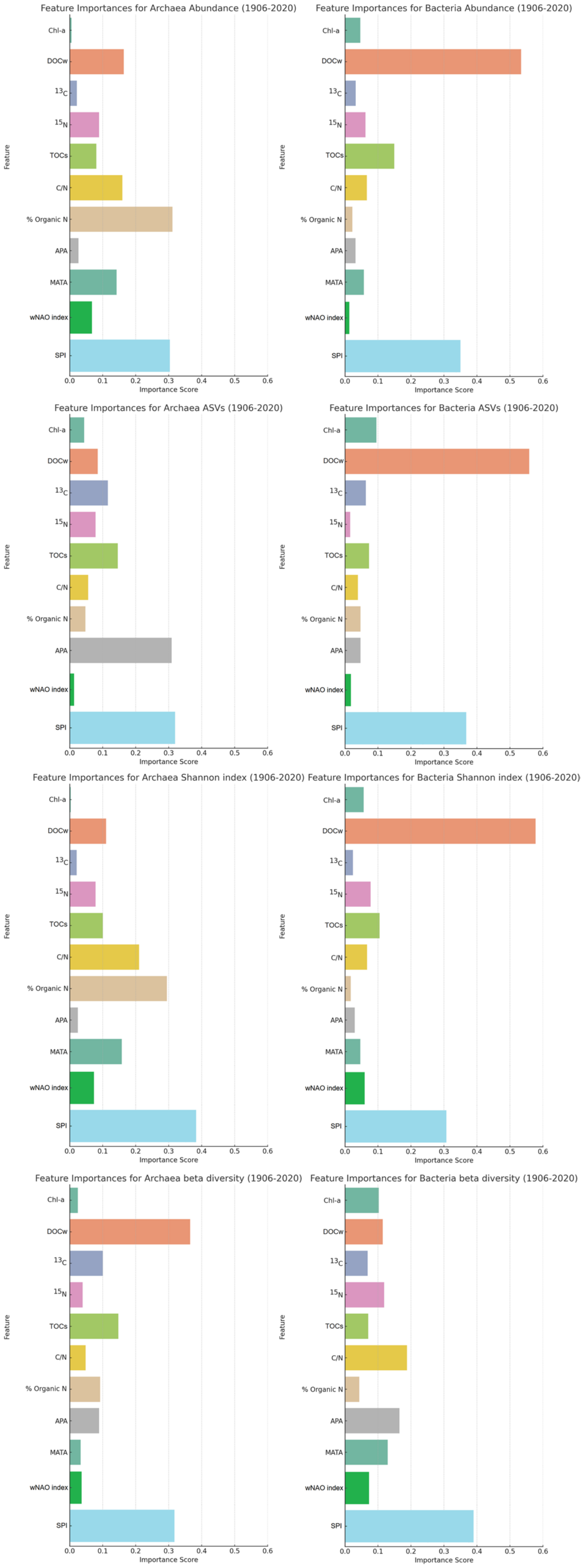


Fig. 8 Random Forest (RF) model of the influence of the paleo-environmental variables (Chl-*a*, $\delta^{13}C_{org}$, $\delta^{15}N$, TOCs, DOCw, organic N %, and atomic C/N ratio) and climate data (APA and MATA, SPI, and wNAO) on the absolute abundance (number of copies/g sediment), and alpha (number of ASVs and values of the Shannon index) and beta (first axis of the NMDS analysis) diversity of archaeal and bacterial communities for the period between ca. 1905 and 2020. DOCw sediment-inferred lake-water dissolved organic carbon, TOCs sediment total organic carbon, wNAO winter North Atlantic Oscillation, SPI Sahel Precipitation Index ▶

concentrations in the Borreguil Lake, similar to findings in other Sierra Nevada lakes [10, 31]. Therefore, the combination of warmer temperatures, extended growing seasons, and increased P deposition likely led to significant rises in Chl-*a* in the Borreguil Lake [28]. The declining C/N ratio and $\delta^{15}N$ post-1905 and especially post-1970 further support the notion of enhanced autochthonous productivity likely due to warming and biological N_2 fixation, potentially linked to increased nutrient cycling under warmer conditions and enhanced Saharan P deposition [28].

The mid-twentieth century onwards was characterized by more negative SPI values, particularly between the ca. 1960 s and 1990 s, which we linked to Sahel droughts and increased Saharan dust emissions, and a shift towards more frequent positive phases of the wNAO, reinforcing arid conditions and long-range dust transport toward southern Europe [10, 12]. Such dust deposition can deliver substantial amounts of bioavailable phosphorus and micronutrients, stimulating primary production in oligotrophic systems [12, 13]. Positive wNAO phases are typically associated with higher pressure over the Azores and lower pressure over Iceland, strengthening the westerlies and thus displacing storm tracks northward. This pattern can lead to drier conditions over the western Mediterranean, including Sierra Nevada. These broader atmospheric patterns, likely contributed to the observed rise in primary production in Borreguil Lake over the past century.

The Saharan dust input interacts with local warming in Sierra Nevada which determines two periods since the Little Ice Age (LIA). Altogether, the environmental trends inferred from our organic proxies align well with the existence of these two periods in the long-term late Holocene changes observed in nearby alpine wetlands [10, 27, 52]. A first period, spanning up to the ca. 1920 s, characterized by colder and wetter conditions, likely reflects the environmental response to warming after the LIA. A second period, from the ca. 1920 s to the present, marked by drier and warmer conditions and intensified after ca. 1960, captures the response of the Borreguil Lake to recent and ongoing climate change. The identification of these drier and warmer environmental periods since ca. 1960 is consistent with an abrupt environmental shift recorded in other alpine peatlands in the region around the 1920 s and 1960–1970 [10, 16, 27, 52]. Collectively, these results indicate a regional-scale response to climate change.

The long-term record from Borreguil Lake reveals clear temporal shifts in microbial abundance and diversity, particularly after the mid-twentieth century, in parallel with intensifying climate change. Both archaeal and bacterial communities showed gradual increases in absolute abundance over the ~ 430-year period, with a marked rise after ca. 1960. This increase was more pronounced in bacterial communities, which consistently exhibited higher absolute abundance levels than archaeal communities. This observation may reflect the broader ecological niches and metabolic plasticity commonly attributed to bacterial taxa, which can enable them to persist and expand under changing environmental conditions [17, 19–23, 50]. While such traits are often associated with higher adaptability and growth potential, we recognize that diversity and abundance metrics alone do not directly quantify physiological adaptability or proliferation rates. Thus, this interpretation should be viewed as a plausible ecological inference supported by consistent patterns observed in other high-altitude lakes undergoing climate-driven change [50, 53, 54]. Patterns of alpha diversity also changed through time: while the number of archaeal ASVs remained relatively stable, Shannon diversity increased notably in recent decades, suggesting greater evenness in community structure. Bacterial communities followed a similar trend, with gradual increases in ASV richness and Shannon diversity. In parallel, DCA and NMDS analyses indicated progressive changes in community structure and beta diversity for both domains, with the most substantial shifts occurring between ca. 1950–1960 and the present. These changes align with documented responses in other alpine lakes in the European Alps and the Rocky Mountains (USA), where post-1950 warming and associated alterations in nutrient inputs and hydrology have driven increases in microbial abundance, diversity, and community turnover [17, 19–23, 50]. The post-1950 period, characterized by accelerated warming and nutrient enrichment, has led to the restructuring of microbial communities in high-mountain lakes globally [50, 53, 54]. The significant community restructuring observed in Borreguil Lake during this period reflects this broader pattern of ecological change driven by global climate dynamics, further emphasizing the role of high-altitude lakes as sensitive indicators of environmental change.

Clear changes in the composition of the archaeal community were observed over time. These temporal shifts can be attributed to the ecological characteristics and adaptive strategies of the dominant archaeal phyla and genera identified in the sediment core. Members of the Micrarchaeota and Nanoarchaeota phyla are typically found in symbiotic or parasitic relationships with other microorganisms in sediments (particularly other archaea) and are known to participate in organic matter decomposition [55, 56]. The increase in their relative abundances may indicate changes in the microbial community structure that created opportunities for these phyla to thrive, possibly due to increases in organic matter availability. The increase in Thermoplasmadota may be linked to their metabolic versatility and ability to thrive under anoxic and carbon-rich conditions. Members of this phylum are known to participate in the degradation of complex organic matter and may be favored under enhanced redox gradients and increased organic inputs, as indicated by the observed rise in Chl-*a* and organic N % in sediments [57].

From ca. 1594 to 1748, the archaeal community was mainly dominated by the genus *Bathyarchaeia.* These microorganisms are often associated with oxygen-limited environments where they play significant roles in organic matter degradation and sulfur reduction [58]. The dominance of *Bathyarchaeia* during this period suggests stable and low-energy conditions. Although primary productivity was not particularly high during this study, our data point to *Bathyarchaeia* species being the main drivers of organic matter deposition. From ca. 1748 to 1970, there were gradual increases in the relative abundances of ten different taxa including Marine_Benthic_Group_D_and_DHVEG-1. This group is often found in environments with fluctuating redox conditions and is known for its ability to degrade complex organic compounds under anaerobic conditions [59]. The gradual increase in their relative abundance may reflect the onset of more variable environmental conditions, possibly due to slight warming after the LIA and/or nutrient enrichment (e.g., increased deposition of terrestrial organic matter and DOCw), which could create more diverse ecological niches and favor specific archaeal genera. Of note, methanogenic genera were only detected during the period 1892–2020, suggesting that recent changes in environmental conditions favored these groups. Methanogens are sensitive to changes in temperature and organic matter input, both of which can influence methane production [60]. The observed increases in these methanogenic archaea could therefore be a response to rising temperatures and enhanced nutrient input, which promote conditions favorable for methanogenesis.

The bacterial community composition also changed substantially over time. From the ca. 1970 s onward, groups such as Cyanobacteria and Proteobacteria increased in relative abundance, consistent with eutrophication, higher algal biomass, and warmer water temperatures, which are known to favor these groups [61, 62]. These changes are consistent with the observed rise in primary productivity indicators (e.g., Chl-*a*) and increased nutrient inputs, which have been documented in other lake studies as key drivers of bacterial community shifts [23, 45]. The increase in Cyanobacteria, particularly genera such as *Cyanobium_PCC-6307*, highlights not only the influence of eutrophication and climate warming on microbial dynamics [51], but also supports recent findings that challenge the traditional view of mountain lakes as cyanobacteria-free due to their oligotrophic nature [23, 45, 63]. Despite their generally low contribution, cyanobacteria appear to be more widespread in ultraoligotrophic systems than previously recognized [63]. *Cyanobium* is a photosynthetic genus contributing to primary production in aquatic ecosystems. However, unlike some other cyanobacterial genera, *Cyanobium* does not fix atmospheric nitrogen (N_2) due to the lack of specialized nitrogen-fixing cells, such as heterocysts. This limitation makes *Cyanobium* reliant on external sources of nitrogen, which are often more available in eutrophic conditions.

We acknowledge that universal primers were used in this study, which may have limited the detection of specific cyanobacterial groups. Therefore, future studies should consider the use of cyanobacteria-specific primers to achieve a more comprehensive characterization of the cyanobacterial community. Decreases in the relative abundances of oligotrophic taxa (e.g., order Aminicenantales and genus *GIF9*) further support the idea of a transition to more nutrient-rich conditions, which may have disadvantaged these groups that are better adapted to low-nutrient environments [64]. In contrast, oligotrophic-associated taxa such as certain Chloroflexi and Acidobacteriota genera declined, reflecting a transition toward more productive, nutrient-rich conditions. Chloroflexi bacteria, particularly genera such as *SCGC-AB-539-J10* and *Sh765B-TzT-20*, are often associated with organic-rich, anaerobic environments, where they contribute to the degradation of complex organic compounds [65]. Their decline from the mid-1700s onwards may be linked to changes in the source of organic matter in the lake, as a greater contribution of lake autochthonous organic matter input with respect to terrestrial inputs was observed during this period. Algae-derived organic matter is less complex than plant-derived organic matter [45]. The linkage between Chloroflexi and organic matter origin has been previously observed in other lakes [24]. Gradual increases in the relative abundance of the phylum Verricomicrobiota were also observed from ca. 1594 to 2005 and afterward decreased to about 10% of relative abundance towards the present. The increases in Verrucomicrobiota, organisms that are known for their polysaccharide-degrading capabilities, may reflect a higher input of complex organic matter associated with primary productivity during this period [66].

The RF analysis showed that procaryotic community shifts were tightly coupled with paleoenvironmental proxies and climatic drivers. From 1594 to 1905, microbial diversity and abundance were primarily driven by nutrient proxies (e.g., organic N %, C/N ratio), indicating that endogenous lake productivity and organic matter inputs shaped the sedimentary microbiota. Nevertheless, the incorporation of climatic variables subsequent to,ca. 1905 in the analyses results in the identification of these variables as predominant controllers. The significant influence of climate variables during this period aligns with previous studies showing the impact of recent climate warming on algal biomass, diatoms, and cladoceran in Sierra Nevada high-mountain lakes during this period [10, 11]. Notably, DOCw and SPI were the strongest predictors of bacterial diversity and abundance, suggesting that Saharan dust deposition and carbon availability have become major drivers of bacterial ecology in recent decades. Morales-Baquero et al. [31] and Reche et al. [12] reported similar results in short-term studies in Sierra Nevada. DOC_w in the water column provides a foundational energy source for microbial metabolism, fueling growth and promoting diversity by creating ecological niches for bacteria with varied metabolic capabilities. Increased DOC_w often correlates with greater organic matter deposition, which fosters bacterial abundance and diversification in sediments [67]. This carbon influx not only sustains bacterial communities but also alters sediment redox conditions, favoring anaerobic bacteria and expanding microbial diversity by enabling taxa that thrive in oxygen-depleted layers. Consequently, DOC_w dynamics reflect both historical lake productivity and present-day ecological conditions, reinforcing DOC_w's role as a key driver of bacterial community structure over time under a warming climate. Saharan dust deposition events are known to deliver bioavailable phosphorus and other nutrients that can stimulate microbial activity and primary production in oligotrophic high-mountain lakes, and ultimately alter the diversity and composition of bacterial communities in Mediterranean high-mountain lakes of Sierra Nevada [12, 17]. Consequently, the observed association between SPI and bacterial alpha diversity suggests that enhanced dust deposition may have contributed to increased nutrient availability in Borreguil Lake, thereby promoting more complex and productive microbial assemblages in surface sediments. This interpretation aligns with previous evidence showing Saharan dust as a significant external driver of lake productivity in Sierra Nevada during recent decades [10, 13, 31]. This underscores the influence of regional climatic dynamics—not only as modulators of hydrological balance, but also as key vectors of nutrient deposition through dust transport. The link between SPI and community turnover suggests that variability in external dust inputs shaped sedimentary microbial communities by modifying both nutrient inputs and geochemical microenvironments over decadal timescales in line with recent studies in high-mountain lakes of Sierra Nevada, Spain [17]. The influence of SPI extended beyond alpha diversity. For both archaea and bacteria, SPI was a key predictor of beta diversity from 1905 to 2020.

Notably, organic N %, MATA, and SPI were strong predictors of archaeal abundance and Shannon diversity from ca. 1905 to 2020. Although the role of temperature increases in driving microbial diversification is known, we show a greater influence of MATA on archaea than bacteria in Borreguil Lake during this period. This differential response could be attributed to several factors. For instance, archaea are known for their ability to adapt to changes in temperature in many different ecosystems, including lake sediments [68]. Additionally, the unique metabolic pathways of archaea, such as those involved in methane production and consumption, could be more directly influenced by temperature shifts, leading to a greater impact of MATA on their abundance and diversity. The influence of the SPI on archaeal communities likely reflects the cascading effects of regional atmospheric conditions on sediment chemistry and microbial habitat structure. On the other hand, bacteria, with their broader ecological niches and metabolic diversity, might be more influenced by other factors such as nutrient availability or organic carbon dynamics, which could buffer the effects of temperature changes.

Interestingly, MATA and APA significantly influenced bacterial beta diversity, yet they did not exert a strong effect on bacterial abundance or alpha diversity. This differential response suggests that while climatic variables such as temperature and precipitation can shape the β-diversity of bacterial communities—likely by selecting for different taxa adapted to specific environmental conditions—they do not necessarily affect the overall richness or evenness of those communities, nor the total abundance of bacterial cells in sediments.

Overall, the results from the RF analysis highlight the differential responses of archaeal and bacterial communities to long-term environmental changes, with climate variables becoming increasingly influential in the modern era. These findings are consistent with broader ecological trends observed in other high-altitude lakes, where recent warming and associated nutrient changes (e.g., organic matter and N) have led to significant restructuring of microbial communities [21, 54]. Our findings also emphasize the sensitivity of prokaryotic communities to long-term environmental changes and recent climate warming. The identified shifts in microbial composition and diversity are reflective of broader ecological changes within the lake ecosystem, driven by climate-driven variables.

While our study provides valuable insights into the long-term dynamics of microbial communities, we acknowledge certain limitations. The potential for DNA degradation with increasing sediment age could bias community reconstructions, though we minimized this by employing rigorous laboratory procedures and bioinformatics analyses. Importantly, the temporal patterns of microbial diversity and abundance closely align with independent geochemical and climate proxies, suggesting that these biological trends likely reflect ecological responses to environmental change rather than being solely artifacts of DNA degradation. Although we interpret the observed microbial shifts primarily as temporal responses to environmental change, we acknowledge that sediment depth can also correlate with geochemical gradients, particularly redox conditions, which may influence microbial community structure. While direct redox measurements were not available, trends in organic matter-related proxies such as TOC, organic N %, and DOCw—which have been associated with redox-sensitive microbial processes [57, 59, 67]—suggest that changes in sediment chemistry may have contributed to microbial turnover. Similar depth-associated microbial stratification has been reported in other sedDNA studies from lake sediments [22, 24]. However, the strong correspondence between microbial changes and paleoenvironmental trends (e.g., Chl-a, δ^{13}Corg, C/N ratio, SPI; [15, 27]), supports the interpretation of these shifts as temporal ecological responses rather than solely depth-related artifacts. This distinction is especially relevant in sedimentary DNA studies, where DNA is generally considered to reflect the community present at the time of deposition, particularly in high-mountain lakes with low sedimentation rates, where bioturbation and post-depositional microbial migration are minimal and preservation conditions are more stable [21, 23]. Future studies could benefit from integrating additional proxies, such as sedimentary pigments and isotopic analyses, to corroborate DNA-based reconstructions and provide a more holistic view of historical community dynamics. Moreover, while we accounted for major environmental drivers, the complex interplay between multiple factors (e.g., nutrient loading, hydrodynamics, and climate change) necessitates further investigation. Advanced modeling approaches and sequencing methods (e.g., metagenomics) could help disentangle the relative contributions of these factors and predict future changes in microbial communities under different climate scenarios.

Conclusions

We provide a comprehensive analysis of the temporal dynamics of prokaryotic communities in a Mediterranean high-mountain lake, focusing on how these communities have responded to changing environmental conditions over the past ~ 430 years. By integrating microbial data with paleoenvironmental variables and climate data, we identified significant shifts in the abundance, diversity, and composition of archaeal and bacterial communities, particularly in response to recent warming trends, atmospheric dust deposition events, and associated environmental changes. Our findings reveal that both archaeal and bacterial communities have undergone substantial restructuring, especially in the period since ca. 1960, corresponding with the onset of significant anthropogenic climate change. The observed increases in microbial abundance and diversity during this time underscore the sensitivity of these communities to changes in temperature, nutrient dynamics, and other environmental factors in freshwater high-mountain ecosystems. Notably, this study highlights the importance of dissolved organic carbon, organic nitrogen, Saharan atmospheric dust inputs, and temperature as key drivers of microbial community changes, particularly in the modern era. The temporal shifts in the composition of these microbial communities reflect broader ecological changes within the lake, driven by a combination of climate-driven variables. For instance, the rise in the relative abundance of Chl-*a* and Cyanobacteria and other taxa associated with increased nutrient availability points to ongoing eutrophication processes, exacerbated by climate warming. These shifts have significant implications for the lake's biogeochemical cycles and overall ecosystem health, reinforcing the role of high-altitude lakes as sensitive indicators of environmental change. Overall, this research contributes valuable insights into the long-term ecological dynamics of high-mountain freshwater ecosystems and underscores the need for continued monitoring and research. Given the fragility and importance of these ecosystems, further studies are necessary to better understand the complex interplay between climate change, nutrient loading, and microbial community dynamics. Such efforts will be crucial for informing conservation strategies and policy decisions aimed at preserving the ecological integrity of high-mountain lakes in the face of ongoing global change.

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Author Contribution Antonio Castellano-Hinojosa: Writing—Original Draft, Conceptualization, Investigation—Data collection, Visualization; Joana Llodrà-Llabrés: Review & Editing, Conceptualization, Investigation—Data collection, formal analysis, Visualization; Eloísa Ramos-Rodríguez: Review & Editing, Data collection; John P. Smol: Review & Editing, Data collection; Carsten Meyer-Jacob: Review & Editing, Data collection; Javier Sigro: Review & Editing, Data collection; Carmen Pérez-Martínez: Review & Editing, Conceptualization, Investigation—Data collection, formal analysis; Supervision, financial.

Author contribution A.C-H: Writing—Original Draft, Conceptualization, Investigation—Data collection, Visualization; J.L-L: Review & Editing, Conceptualization, Investigation—Data collection, formal analysis, Visualization; E.R.R: Review & Editing, Data collection; J.P.S: Review & Editing, Data collection; C.M-J: Review & Editing, Data collection; J.S: Review & Editing, Data collection; C.P-M: Review & Editing, Conceptualization, Investigation—Data collection, formal analysis; Supervision, financial.

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Data Availability Raw sequence data were deposited into the National Library of Medicine (NCBI) under BioProject PRJNA1275809.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest The authors declare no competing interests

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