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Sedimentary DNA reveals the link between microbial community dynamics and climate during the late last glaciation in the offshore region of the Zambezi River, southwest Indian Ocean

Haoyu Li[a], Hucai Zhang[a,*], Huayong Li[b], Lizeng Duan[a], Xiaonan Zhang[a], Youhong Gao[a], Ian R. Hall[c], Sidney R. Hemming[d], Leah J. LeVay[e] & the Expedition 361 Scientists†

[a] *Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, 650500 Kunming, China*

[b] *School of Resource Environment and Tourism, Anyang Normal University, Anyang 455000, China.*

[c] *Department of Earth Sciences, Cardiff University, Main College, Park Place, PO Box 914, Cardiff Wales CF10 3AT, United Kingdom.*

[d] *Lamont-Doherty Earth Observatory, Columbia University, 61 Route 9W, Palisades NY 10964, USA.*

[e] *International Ocean Discovery Program, Texas A&M University, College Station TX 77845, USA.*

* Corresponding author at: Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming, China.

E-mail addresses: zhanghc@ynu.edu.cn

ABSTRACT

Reconstructing the relationship between microbial communities and past abrupt climate change is of great importance for understanding current biodiversity patterns and predicting changes under future climate scenarios. However, little is currently known about how microbial communities respond to changes in key environmental stages due to a lack of research in this area. Here, we examine the variability in the communities of bacteria, archaea, and fungi from sediments deposited offshore of the Zambezi River between 21.7 and 9.6 thousand years ago (ka) (covering the last glacial maximum, or LGM, and the early Holocene) using DNA metabarcoding approach via high-throughput sequencing. The results showed that (1) microbial assemblages differed across three key time intervals, with the last deglaciation having the most homogeneous prokaryotic assemblages, while for fungal communities in the LGM, and the early Holocene and LGM differing the most; (2) the warm early Holocene showed the highest diversity, whereas the lowest diversity was found in the LGM; and (3) the selected indicator species better reflected the climatic characteristics of different environmental stages. These results highlight the power of ancient sedimentary DNA to refine our understanding of microbial dynamics in marine sedimentary systems near large rivers, thus providing a basis for better modeling ecological processes in further research.

***Keywords**:* Sedimentary DNA; Metabarcoding; Microbial dynamics; Abrupt climate change; Offshore Zambezi River

1. Introduction

Marine microbes account for ≥90% of the total marine biomass (Suttle, 2007), and contribute ~50% of global primary productivity (Arrigo, 2005). The global diversity and distribution patterns of microorganisms in the marine realm are strongly related to climate-driven variability in primary production (Lozupone and Knight, 2007; Hoshino et al., 2020), with strictly anaerobic organic-rich sediments found along the continental margins (Lipp et al., 2008). As important engines of global changes, marine microbes not only affect climate change, but also play vitally important roles in the origin and evolution of life on Earth and biogeochemical cycles (Kasting and Siefert, 2002; Falkowski et al., 2008). In addition, these microbes are highly sensitive to climate change, and their community variations can serve as sentinels to examine the long-term changes in ecosystems (Yao et al., 2006; Mitchell et al., 2009). Therefore, understanding the composition and dominant groups of microbial communities, especially in sedimentary environments, is essential for elucidating a number of urgent ecological issues, including microbial succession, microbial interactions and climate feedback.

The diversity of biological dynamics contained within DNA signatures can be examined using DNA metabarcoding via high-throughput sequencing. More importantly, sedimentary DNA (sedDNA) enables the identification of a wide range of organisms that leave few or no traces in the fossil record, including prokaryotes and eukaryotes, thus allowing for the exploration of ecological impacts on a finer scale far beyond traditional morphological observations using microscopes (Pawlowski et al., 2018; Cabrera et al., 2019). The study of past biodiversity using sedDNA has been extensively reviewed (Li et al., 2023; Capo et al., 2021; Armbrecht et al., 2019; Ruppert et al., 2019; Thomsen and Willerslev, 2015). It is worth noting that most of these studies have suggested that microorganisms are an excellent target for sedDNA-based research and are particularly interrelated in climate–environment–ecosystem reconstructions.

The abrupt climate change events since the Last Glacial Maximum (LGM) have had profound effects on the global climate and environmental evolution (Lambeck et al., 2014; Cotton et al., 2016 Hahn et al., 2021), and are among the key topics in the field of global change research. Microbial dynamics in subseafloor sediments have been a research focus for decades (Coolen et al., 2013; More et al., 2018; Armbrecht et al., 2021), whereas limited research

has been conducted in shallow marine sediments influenced primarily by river discharge. Considering the critical roles of microorganisms in marine ecosystems, more attention should be paid to their responses to climate change. Taken together, the primary objectives of this study were as follows: (1) to elucidate the microbial dynamics in sediments supplied by the offshore Zambezi River using the metabarcoding approach; (2) to identify potential indicator species among the microbial communities related to the specific environmental conditions; and (3) to reveal the climatic signatures that could affect microbial communities.

2. Materials and methods

2.1. Study area and sample collection

Site U1477, from the International Ocean Discovery Program (IODP) Expedition 361, is located in the western Mozambique Channel on the upper continental slope, ~65 n mile east of the Zambezi River Delta, at a water depth of 429.2 m below sea level, and receives fluvial material exported from the Zambezi River Catchment (Hall et al., 2017). The Zambezi River, originating in northern Zambia, the longest river in southern Africa (over 2500 km in length). The mean volume of the runoff is 3000 m^3/s, with an annual sediment load of ~51 Mm^3, making it the largest single source of suspended sediment supply to the Mozambique margin (Walford et al., 2005). The Zambezi River Catchment, with a total of area of ~1.385 million km^2, is situated in the southernmost reach of the African monsoonal precipitation belt referred to as the Intertropical Convergence Zone (ITCZ) and the Congo Air Boundary (CAB). It has mean annual rainfall of 95 cm, and an average annual discharge of 103 km^3 (MacDonald, 2008). Peak precipitation occurs during the austral summer (December, January, and February), when the ITCZ is located at its most southerly position (Hall et al., 2017). In the Mozambique Channel, there is a dominant southward surface flow accompanied by multiple quasi-stationary counterclockwise eddies (Fig. 1). The discharge of the Zambezi River sediments into this system is reflected by the high sedimentation rates, up to 1 m/ka, recorded at Site U1477 (Hall et al., 2017), providing a potential sedimentary archive that records regional hydroclimate changes in the Zambezi River Catchment at an unprecedented temporal resolution. The samples were collected from Hole U1477B (19°21.2822′S, 36°54.8958′E) of IODP Expedition 361 during the RV "JOIDES Resolution" cruise on March 14, 2016.

2.2. Sedimentary DNA extraction and analysis

Sedimentary DNA was extracted from a total of 38 sediment samples (triplicate for each sample) that spanned the core interval of 5.666–17.495 m below the sea floor using the MOBIO PowerSoil® DNA Isolation Kit (MOBIO

Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA concentrations were quantified using the Thermo NanoDrop One (Thermo Fisher Scientific, MA, USA), and the extracted DNA was stored at −80°C until amplification. For prokaryotes, the V4 region of the 16S rRNA gene was amplified using the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Zhou et al., 2016). This targeted gene region (about 300 bp) could provide enough resolution for the accurate taxonomic classification of prokaryote (bacteria and archaea) sequences. For eukaryotes, the primer set 528F (5′-GCGGTAATTCCAGCTCCAA-3′) and 706R (5′-AATCCRAGAATTTCACCTCT-3′) was used to amplify the V4 region of the 18S rRNA gene (Lutz et al., 2016), with a targeted gene region of about 350–390 bp. Fifty-microliter polymerase chain reaction (PCR) reaction systems were prepared in duplicate, and each contained 50 ng of DNA template, 25 μl 2× Premix Taq DNA polymerase (Takara Biotechnology, Dalian Co., Ltd., China), 1 μl of each primer (10 μm), and 20 μl nuclease-free water. The PCR thermal regime consisted of an initial denaturation of 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. PCR was replicated in triplicate and the products of each sample were pooled and purified. The pooled PCR products were visualized on 1% purified agarose gel and purified using the E.Z.N.A.® Gel Extraction Kit (Omega, USA). The purified products were finally quantified with the Quant-iT™ Broad-Range DNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Library construction was performed using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). Sequencing was conducted on an Illumina Nova 6000 platform for PE250 (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China).

2.3. Bioinformatics analysis

The raw sequencing data were initially processed using fastp (an ultra-fast all-in-one FASTQ Preprocessor, v0.14.1) (https://github.com/OpenGene/fastp) with a sliding window to cut the two-terminal raw reads. Furthermore, cutadapt (v1.14) (https://github.com/marcelm/cutadapt/) was used to remove the primer to obtain the paired-end clean reads after quality filtering. Paired-end clean reads that overlapped more than 16 bp were merged into a single sequence, and sequences containing ambiguous bases were removed to obtain raw tags using usearch-fastq_mergepairs (v10.0.240) (http://www.drive5.com/usearch/). The raw tags were processed using fastp with a sliding window to obtain clean tags. Furthermore, after the chimeras and singletons were discarded, the generated high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the UPARSE workflow (Edgar, 2013). The representative sequences were selected from each OTU and aligned against the SILVA

(v138) database at a bootstrap cutoff of 80% using usearch-sintax (v10.0.240). In this study, for the prokaryotic dataset, both bacteria and archaea were considered for further analysis. For the eukaryotic dataset, OTUs that were not classified into fungi were filtered for further analysis. Finally, because uneven distribution in sample sequences might affect statistical analysis, the data were resampled based on the lowest sequence number across all samples (72,152 and 23,850 for prokaryotes (Table S1) and micro-eukaryotes (Table S2), respectively) for downstream analysis. Sequencing data were analyzed on the Mgigene Cloud Platform (http://cloud.magigene.com/). The raw sequences have been uploaded and are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) Database (Accession Nos: PRJNA1021399 for prokaryotic microbes and PRJNA1021574 for eukaryotic microbes).

2.4. Statistical analysis

Samples were generally grouped based on time windows representing specific climate intervals, where the interval transitions corresponded to major changes in microbial community structure. The alpha diversity of the microbial communities (the Richness, Shannon, and Simpson indices) were estimated with the "vegan" package in R (Oksanen et al., 2022) based on grouped samples. On this basis, one-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference (HSD) tests were employed using the R package "multcomp" (Hothorn et al., 2008) to determine the differences among environmental intervals. For beta diversity, to identify the grouping patterns of microbial communities that were significantly different from each other at the OTU level, non-metric multidimensional scaling (NMDS) analysis based on the Bray–Curtis distance with 999 permutations was performed using the "vegan" package in R (Oksanen et al., 2022). Accordingly, to determine the significance of the community differences between two or more classes of objects, similarity analysis (ANOSIM) was performed using the "vegan" package in R (Oksanen et al., 2022). ANOSIM was carried out using 999 permutations and the Bray–Curtis distance. Moreover, to determine whether there would be differences between different stages, we conducted analyses of the temporal beta diversity index (TBI; Legendre, 2019), which measures temporal beta differentiation. The TBI was calculated using the R package "adspatial" with the "aka Bray-Curtis" method and 999 permutations (Dray et al., 2023). Then, we decomposed the TBI into beta diversity explained by either species loss or gain over time. These components describe the different contributions of species loss and gain to the overall differences, thus giving us an opportunity to understand of the relative importance of these effects (Legendre, 2019). In the analyses mentioned above, including NMDS, ANOMIS, and TBI, we performed all procedures in each time window or between the

different windows for the all species and indicator species (which were the OTUs), respectively.

In addition, to detect differences in OTU abundance among grouped samples, a negative binomial generalized linear model was implemented with the R package "edgeR" (Robinson et al., 2010) using the trimmed means of M (TMM) method, and the normalized counts were expressed as the relative abundance counts per million (CPM). All *p* values were calibrated using the false discovery rate (FDR). After screening, the significantly changed OTUs were displayed in a heatmap using the R package "pheatmap" (Kolde, 2019).

Finally, to investigate the differences in the occurrence or abundance of a small set of OTUs in the selected time periods, correlation based on indicator species analysis (ISA) was conducted using the R package "indicspecies" (Cáceres and Legendre, 2009). The analysis for individual time windows only (rather than combinations of time windows) was conducted with 999 permutations and considered significant at $p < 0.05$. This analysis generated two indicator values (i.e., A and B), where an A-value of 1 indicates that the species in question occurs in only one indicator group, while an optimal B-value of 1 means that this species occurs in all samples in that category. In this study, we chose to use the A value to measure the contribution of the indicator species. We then further identified the best environmental indicator species with reference to the approach of Coolen et al. (2013). All statistical analyses were conducted in R v4.2.2 (R Core Team, 2022).

3. Results and discussion

3.1. Chronology of the core

Six mixed planktonic foraminifera samples were collected for ^{14}C dating using accelerator mass spectrometry (AMS) at Beta Analytic, USA (Table 1). The ^{14}C dates were calibrated using the Marine20 calibration curve (Reimer et al., 2013). The age-depth model was produced using Bacon v2.2, with a Bayesian modeling of accumulation rates (Blaauw and Christen, 2011). Based on these models, the calibrated dates and their 95% confidence intervals were calculated for the boundaries of the stratigraphic sections, and then linear interpolation was used to determine the ages for each individual sample.

3.2. Microbial community composition

After bioinformatics processing, taxonomic filtering, rarefaction, and normalization, a total of 8,225,328 high-quality prokaryotic 16S rRNA gene (Table S1) and 2,718,900 micro-eukaryotic 18S rRNA gene (Table S2) sequence counts were assigned across all samples. The subsampled sequences numbered 72,152 and 23,850 for the prokaryotic

and fungal communities, respectively. The Venn diagram showed that 471 OTUs were shared by bacterial and archaeal communities, while there were 79 shared OTUs for fungal communities across the late last glaciation (Fig. S1). We defined the three time windows based on our dataset, namely the LGM (21.7–19.1 cal ka BP; 17.495–14.2 m depth), last deglaciation (18.7–12.2 cal ka BP; 13.8–6.966 m depth), and early Holocene (11.5–9.6 cal ka BP; 6.666–5.666 m depth). Among them, for the bacterial and archaeal communities, there were 236 shared OTUs for the LGM and the last deglaciation, 223 shared OTUs for the last deglaciation and the early Holocene, and 6 shared OTUs for the LGM and the early Holocene (Fig. S1a); for the fungal communities, there were 50 shared OTUs for the LGM and the last deglaciation, 28 shared OTUs for the last deglaciation and the early Holocene, and 2 shared OTUs for the LGM and the early Holocene (Fig. S1b). Overall, microbial diversity increased from the LGM to the early Holocene (Tables S1 and S2).

For the prokaryotic dataset, these sequences were clustered into 1,609 OTUs (including bacteria of 1320 OTUs in 82%, archaea of 289 OTUs in 18% were assigned) at the cutoff of 97% sequence identity, ranging from 148 to 333 OTUs per sample across all samples. Of these, 826 OTUs could be identified to the family level, excluding the "uncultured" and "Unknown_Family" (51%), eliminating the "uncultured" for 543 OTUs to the genus level (34%), and 137 OTUs to the species level (9%) after removing the "uncultured." The most abundant taxa were Proteobacteria and Bacteroidota at the phylum level, while *Pseudomonas* was the most abundant at the genus level, and a large proportion was "unassigned" (Fig. 2). Bacterial and archaeal communities were mainly composed of members from the phyla Proteobacteria and Bacteroidetes, and from Crenarchaeota and Asgardarchaeota, respectively. In the bacterial and archaeal communities, the phylum Proteobacteria, of which Gammaproteobacteria and Alphaproteobacteria are the most widely distributed and abundant classes in marine sediments (Ambati and Kumar, 2022), which is consistent with the results of the present study.

For the fungal dataset, a total of 279 OTUs were assigned to fungi at the phylum level at a similarity level of 97%, ranging from 14 to 64 OTUs per sample across all samples. Of these, 119 OTUs could be identified to the family level (42%), 50 OTUs to the genus level excluding from the "uncultured" (18%), and 10 OTUs to the species level (4%) removing the "uncultured." Ascomycota (75%) and Basidiomycota (16%) are the most dominant taxa at the phylum level (Table S2), which was consistent with the results of Tisthammer et al. (2016) and Hoshino et al. (2020), and are slightly more abundant than in the Pearl River estuary of southern China (Cheung et al., 2018). In addition, most abundant taxa are Eurotiomycetes and Sodariomycetes at the class level, while at the genus level "unassigned" is the most dominant, followed by *Exophiala* (Fig. 3).

For the alpha diversity, the prokaryotic communities from the last deglaciation were similar, while other stages were dissimilar, with the exception of the early Holocene, for the Shannon and Simpson indices (Fig. 4a–c). In particular, the Simpson index was the most similar between different stages. The fungal communities from the same stage were significantly more similar than those from other stages, as verified by multiple dissimilarity tests (Fig. 4d–f). In particular, the Shannon and Simpson indices were more consistent between different stages. Overall, the alpha diversity was highest in bacteria and archaea, while it was lowest in fungi.

Ordination using NMDS reveals a less pronounced grouping of samples in three different time windows across the records. The samples displayed concentric distribution between time windows, accounting for their similar OTU compositions. The prokaryotic communities were more divergent (Fig. 5a) than the fungal communities (Fig. 5b). Furthermore, ANOSIM revealed that the overall prokaryotic communities differed slightly (Fig. 5c), while there were no evident changes in fungal communities (Fig. 5d). Specifically, only minor differences were observed in both the prokaryotic and fungal communities between environmental stages (Fig. S5), suggesting the existence of some similar general conditions in the environmental habitats between different stages. In particular, for the prokaryotic community, the last deglaciation showed the greatest differences in the three time windows (Fig. S7a), with species loss being dominant (Fig. S8b), indicating that the loss of new species contributed the most to the overall differences at this stage. However, for fungal communities, species loss was dominant in the LGM and had the largest dissimilarities (Fig. S7b and Fig. S8f).

Differential OTU abundance analysis identified 699 enriched OTUs in prokaryotic communities and 99 enriched OTUs in fungal communities (Table S3 and Table S4). Taxonomy at the phylum level showed few differences, with Proteobacteria and Crenarchaeota contributing the greatest differences in both periods for the prokaryotic communities (Fig. S2), while the Ascomycota exhibited the greatest differences in the fungal communities (Fig. S3).

3.3. Microbial indicator taxa

For the prokaryotic communities, ISA identified 338 significant ($p < 0.05$) indicator OTUs from prokaryotic communities (Table S5) and 47 for the fungal communities during the selected study period (Table S6). Proteobacteria (phylum) in bacteria, with 120 OTUs, constituted the dominant group of indicator species, followed by Crenarchaeota (phylum) in archaea, with 52 OTUs. For the fungal communities, the majority of these indicator species were Ascomycota (phylum) with 40 OTUs. For both prokaryotic and fungal communities, indicator OTUs were the most diverse in the early Holocene, followed by the last deglaciation, and finally the LGM. Specifically, at

the phylum level for bacteria, ISA identified 179 significant indicator taxa for the early Holocene (mainly Proteobacteria and Actinobacteriota), 86 for the last deglaciation (mainly Proteobacteria), and 87 for the LGM (mainly Proteobacteria). For archaea at the phylum level, ISA identified 62 significant indicator taxa for the early Holocene (49 Crenarchaeota, 7 Asgardarchaeota, 3 Thermoplasmatota, 2 Nanoarchaeota, and 1 Aenigmarchaeota), 21 for the last deglaciation (14 Crenarchaeota, 4 Asgardarchaeota, 2 Thermoplasmatota, and 1 Nanoarchaeota), 6 for the LGM (3 Crenarchaeota, 2 Halobacterota, and 1 Thermoplasmatota). For fungi at the phylum level, ISA identified 34 significant indicator taxa for the early Holocene (33 Ascomycota and 1 Basidiomycota), 21 for the last deglaciation (18 Ascomycota and 3 Basidiomycota), and 8 for the LGM (5 Ascomycota, 2 Basidiomycota, and 1 Chytridiomycota).

This study then investigated the effects of the three environmental stages, and the transitions between these stages, on the microbial community structure using NMDS. NMDS using only these indicator species revealed stronger clustering between environmental stages (Fig. S4a–b). The shifts in community composition were clearly displayed by the NMDS, where the samples in the LGM were distinctly separated from the samples in the last deglaciation and the early Holocene. The ANOSIM results suggested that the prokaryotic communities differed significantly among the three intervals ($R = 0.429$, $P = 0.001$), while the differences in fungal communities were not significant (Fig. S4c–d). In particular, for the prokaryotic communities, the LGM and early Holocene showed strong differences ($R = 0.657$, $P = 0.001$) (Fig. S6b), which may be due to the extreme cold (LGM) and warm (early Holocene) climates.

Additionally, the NMDS plot suggests a strong reduction of temporal beta diversity, as indicated by a decline in sample dispersion and the size of the confidence ellipse between the LGM and the early Holocene. Specifically, for the prokaryotic communities, the last deglaciation showed the greatest differences in the time windows (Fig. S7c), with species loss being dominant (Fig. S9b); this suggests that the loss of new species contributed the most to the total dissimilarity at this stage. However, for fungal communities, species loss was dominant in the LGM, resulting in the largest differences (Fig. S7d and Fig. S9f).

Furthermore, to identify the indicator species that exhibited the strongest relationship with environmental conditions, we assessed the species that best reflected the biotic or abiotic state of the environment. For prokaryotic communities, at the phylum level, Proteobacteria and Halobacterota were the best indicator taxa for the LGM; Proteobacteria, Actinobacteriota, and Thermoplasmatota were the best indicators for the last deglaciation; and Myxococcota and Crenarchaeota were the best indicators for the early Holocene (Fig. 6).

Similarly, for fungal communities, at the phylum level, Basidiomycota was the best indicator taxa for both the LGM and the last deglaciation, while Ascomycota was the best indicator taxa for the early Holocene (Fig. 7).

3.4. Relationships between indicator species and climate

Abrupt climate change during the large transitions from the LGM to the early Holocene contributed to changes in sea level, monsoon dynamics, precipitation patterns across the southeast Africa, and associated runoff of the Zambesi River that strongly affected the microbial ecosystem in Site U1477 sediments (Fig. 8). The LGM was followed by a rapid climate warming and deglaciation event initiated at 19 ka cal BP (Clark et al., 2009), with the corresponding sea level rise leading to current interglacial conditions. This transition was of crucial importance due to the occurrence of drastic climate oscillations over a short period. This was of great interest because the climate in the southern Hemisphere has undergone several distinct changes, such as the LGM, the last deglaciation, and the early Holocene with cold and warm phases. The three time windows are represented by the LGM, with the coldest climate and the lowest sea levels; the last deglaciation period, with moderate climate and sea levels; and the early Holocene, with a warm climate and high sea levels. However, these climate events were not linear, and included some very rapid climatic oscillations. Generally, Northern Hemisphere ice sheet boosting can initiate the southward displacement of the ITCZ, thereby contributing to increased precipitation in southeast Africa (Schefuß et al., 2011) and generating the maximum rainfall and Zambezi runoff (Moore et al., 2008). Consequently, after the early Holocene the record revealed stronger monsoon precipitation (Fig. 8a, c). Hence, the abrupt switch between different environmental stages may partially be attributed to a southward displacement of the ITCZ.

During the LGM, the increased runoff from the Zambesi River with higher precipitation, resulting in an increase in nutrients, may have contributed to the growth of Proteobacteria. However, Proteobacteria were absent or less abundant during the transition from the LGM to the last deglaciation; this was because of weak monsoons, which resulted in reduced mixing, and thus fewer nutrients. In contrast, at the onset of the LGM, Halobacterota and Basidiomycota were highly abundant, suggesting less nutrient availability with weak monsoons. To be precise, the genera *Oceanicaulis* and *Pseudomonas* (phylum Proteobacteria), the phylum Halobacterota, and the genus *Malassezia* (phylum Basidiomycota) are the best environmental indicator taxa (Fig. 8e–h). *Oceanicaulis* colonized marine environments (WoRMS, 2023a), while *Pseudomonas* inhabited diverse environments (marine, brackish, freshwater, and terrestrial environments) (WoRMS, 2023b) in oxic sediments (Hoshino et al., 2020). In particular, the wide distribution of *Pseudomonas* suggests a significant degree of physiological and genetic adaptability (Spiers et al., 2000). Although Halobacterota mainly inhabit high-salinity environments (Oren, 1994), the results showed that Halobacterota can be found under low-salinity conditions with high rainfall, which was consistent with the results of Kulp et al. (2007) and Genderjahn et al. (2018).

During the last deglaciation, Actinobacteriota and Thermoplasmatota were identified as potential indicators for nutrient-limited conditions, which may be related to weak monsoons leading to weak mixing due to intensified upper-ocean stratification, despite the increased terrestrial input. Specifically, the genus *Promicromonospora* (phylum Actinobacteriota), the class Alphaproteobacteria (phylum Proteobacteria), the class Thermoplasmata (phylum Thermoplasmatota), and the genus *Exobasidium* (phylum Basidiomycota) are the best environmental indicator taxa (Fig. 8e–h). Both *Promicromonospora* (WoRMS, 2023c) and Thermoplasmata (WoRMS, 2023d) colonized marine conditions with high terrestrial-derived organic matter contents (Fig. 8d, e and g), indicating a coastal distribution pattern. Exobasidium settled in terrestrial conditions (WoRMS, 2023e), followed by a lower sea level (Fig. 8b and h). The Alphaproteobacteria inhabited multiple environments (marine, brackish, freshwater, and terrestrial environments) (WoRMS, 2023f), possibly due to an adaptation to the rapid rise of sea levels. In addition, Promicromonospora and Thermoplasmata were found to be remarkable indicator taxa for the occurrence of increased runoff and high terrestrial-sourced organic matter contents.

In the early Holocene, the climate responded rapidly to the warmer conditions with a sea-level highstand, as seen in the increased abundance of Myxococcota, Crenarchaeota, and Ascomycota. During this period, the relatively weak monsoon was accompanied by less precipitation and the runoff decreased, resulting in a decrease in terrestrial-derived organic matter (Fig. 8a, c and d). The best environmental indicator species are the family Myxococcaceae (phylum Myxococcota), the class Bathyarchaeia (phylum Crenarchaeota), and the family Herpotrichiellaceae (phylum Ascomycota) (Fig. 8e, g and h). Myxococcaceae colonized the marine environment (WoRMS, 2023g) and Bathyarchaeia was prevalent within the anaerobic sediments with high marine-derived organic matter content (Hoshino et al., 2020; Romano et al., 2021), indicating anoxic conditions along the coasts in the early Holocene. Herpotrichiellaceae inhabited both marine and terrestrial environments (Index Fungorum Partnership, 2023), suggesting a marine–terrestrial transition with a higher sea level (Fig. 8b). Furthermore, Bathyarchaeia and Herpotrichiellaceae are found to be prominent indicator species for the occurrence of less runoff and high marine-sourced organic matter contents.

4. Conclusion

This investigation on the temporal patterns of prokaryotic and fungal communities from the sediments at the offshore region of the Zambezi River in the southwest Indian Ocean reveals that the phyla Proteobacteria and Bacteroidota were the most abundant taxa for bacterial communities, while the phyla Crenarchaeota and

Asgardarchaeota were the most abundant taxa for archaeal communities during the transition from late glaciation to the Holocene. With respect to fungi, the phyla Ascomycota and Basidiomycota dominated in abundance across the core. The findings of this study suggested that there were distinct temporal distribution patterns of prokaryotic and fungal indicator taxa, which were mainly impacted by climatic transitions. These findings add to the growing consensus that the regional oceanic conditions were highly sensitive to the monsoon. In particular, nutrient availability plays an important role in regulating the temporal variations of prokaryotic and fungal communities.

Although there are limitations in chronology, this study provides baseline data on microbial dynamics and may benefit the further reconstruction and prediction of microbes in southeast Africa in the framework of climate change. Future investigations will need to consider modern samples and more comprehensive methods (such as metagenomics, metatranscriptome, and metaproteome techniques) to confirm the present findings. Such integrated research will provide new insights on the temporal evolution of prokaryotic and eukaryotic communities from marine sediments and their responses to climate change in depth.

CRediT authorship contribution statement

Hucai Zhang: Conceptualization, Methodology, Funding Acquisition, Supervision, Validation, Writing - review & editing; **Haoyu Li:** Funding Acquisition, Software, Data acquisition, Data curation, Visualization, Writing - original draft; **Huayong Li, Lizeng Duan, Xiaonan Zhang:** Data acquisition; **Ian R. Hall, Sidney R. Hemming, Leah J. LeVay:** Review & editing.

Declaration of competing interests

None declared.

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Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article.

† The Expedition 361 Scientists (a complete list):

I.R. Hall[1], S.R. Hemming[2], L.J. LeVay[3], S. Barker[4], M.A. Berke[5], L. Brentegani[6], T. Caley[7], A. Cartagena-Sierra[8], C.D. Charles[9], J.J. Coenen[10], J.G. Crespin[11], A.M. Franzese[12], J. Gruetzner[13], X.B. Han[14], S.K.V. Hines[15], F.J. Jimenez Espejo[16], J. Just[17], A. Koutsodendris[18], K. Kubota[19], N. Lathika[20], R.D. Norris[21], T. Periera dos Santos[22], R. Robinson[23], J.M. Rolinson[24], M.H. Simon[25], D. Tangunan[26], J.J.L. van der Lubbe[27], M. Yamane[28], and H. C. Zhang[29].

[1]Department of Earth Sciences, Cardiff University, Main College, Park Place, PO Box 914, Cardiff Wales CF10 3AT, United Kingdom. [2]Lamont-Doherty Earth Observatory, Columbia University, 61 Route 9W, Palisades NY 10964, USA. [3]International Ocean Discovery Program, Texas A&M University, College Station, TX, USA. [4]School of Earth and Ocean Sciences, Cardiff University, Cardiff, UK. [5]Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, USA. [6]Earth and Environmental Sciences, University of Technology Queensland, Brisbane, Australia. [7]EPOC, UMR CNRS 5805, University of Bordeaux, Pessac, France. [8]Department of Earth and Environmental Sciences, Lamont-Doherty, Earth Observatory of Columbia University, Palisades, NY, USA. [9]Scripps Institution of Oceanography, University of California, San Diego, La Jolla, USA. [10]Department of Geology, Northern Illinois University, DeKalb, IL 60115, USA. [11]EPOC, UMR CNRS 5805, University of Bordeaux, Pessac, France. [12]Natural Sciences Department, School of Earth and Environmental Sciences, Hostos Community College (C.U.N.Y.), Bronx NY, USA. [13]Alfred-Wegener-Institute, Helmholtz-Zentrum für Polar- und Meeresforschung, Am Alten Hafen 26, D- 27568 Bremerhaven, Germany. [14]Second Institute of Oceanography (SOA), Key Laboratory of Submarine Science, Hangzhou City, P.R. China. [15]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, USA. [16]Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Natsushima-cho 2-15 Yokosuka 237-0061, Japan. [17]Geologisches Institute, Universität Köln, Germany. [18]Institute of Earth Sciences, Heidelberg University, Heidelberg, Germany. [19]Atmosphere and Ocean Research Institute, University of Tokyo, Kashiwashi Chiba, Japan. [20]Ice Core Laboratory, National Centre for Antarctic and Ocean Research (NCAOR), Head Land Sada, Vasco da Gama Goa,403804, India. [21]Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, USA. [22]Programa de Geociências (Geoquímica), Universidade Federal Fluminense, Niterói, Brazil. [23]Graduate School of Oceanography, University of Rhode Island, Narragansett, USA. [24]Chemistry Department, University of Otago, Dunedin, New Zealand. [25]NORCE Norwegian Research Centre, Bjerknes Centre for Climate Research, Bergen, Norway. [26]Department of Geosciences, University of Bremen, Bremen, Germany. [27]Department of Earth Sciences, Cluster Geochemistry & Geology, Vrije Universiteit VU, Amsterdam, the Netherlands. [28]Department of Biogeochemistry, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Japan. [29]Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming 650500, China.

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Fig. 1. Location map of Site U1477 (adapted from Hall et al., 2017). The main surface currents in the southwest Indian Ocean are indicated by yellow and orange arrows. Black double-headed arrows and dashed lines indicate the main pathways of atmospheric circulation over southern Africa during austral summer (December, January, and February), alongside the approximate position of the ITCZ and CAB. Purple shading indicates the Zambezi Catchment and green shading the Limpopo Catchment. ITCZ, Intertropical Convergence Zone; CAB, Congo Air Boundary; EACC, East Africa Coastal Current; SEC, South Equatorial Current; NEMC, Northeast Madagascar Current; SEMC, Southeast Madagascar Current; MC, Mozambique Channel; AC, Agulhas Current.

Fig. 2. Temporal trends of the top 15 phyla and genera based on the relative abundance (⩾0.01%) of the prokaryotic community composition (bacteria and archaea) at the phylum (**a**) and genus (**b**) levels. The data were averaged across three replications for each sample. LGM, last glacial maximum.

Fig. 3. Temporal trends of the relative abundance (⩾0.01%) of fungal community composition at the class (**a**) and genus (**b**) level. The data were averaged across three replications for each sample. LGM, last glacial maximum.

Fig. 4. Variation patterns of microbial diversity at the OTU level for all samples. Prokaryotic (**a–c**) and fungal (**d–f**) communities. ES, environmental stage. Different letters in (a–c) indicate significant differences ($p < 0.05$). LGM, last glacial maximum.

Fig. 5. Temporal variations in microbial communities across the three time windows. (**a**) Non-metric multidimensional scaling (NMDS) using all 1,609 OTUs across all samples for prokaryotic communities (bacteria and archaea). (**b**) NMDS using all 282 OTUs across all samples for micro-eukaryotic communities (fungi). Box plots showing the dissimilarity of prokaryotic communities (**c**) and fungal communities (**d**) estimated using analysis of similarities (ANOSIM). Samples are color-coded according to three time windows based on our dataset. Ellipses show the variability of each time window with 95% confidence intervals. Between represents the difference among the compositions of the three period samples. LGM, last glacial maximum.

Fig. 6. Relationships between the weighted average scores of species (the operational taxonomic units, OTUs) for ordination configuration from the nonmetric multidimensional scaling (NMDS) analysis and indicator values (IVs) of component "A" from the indicator species analysis (ISA). Bacteria (**a–b**) and archaea (**c–d**) are shown. OTUs with the highest scores and IVs (the best environmental indicator species) are labeled in the panels. LGM, last glacial maximum.

Fig. 7. Relationships between the weighted average scores of species (the operational taxonomic units, OTUs) for ordination configuration from the nonmetric multidimensional scaling (NMDS) analysis and indicator values (IVs) of component "A" from the indicator species analysis (ISA). Fungi are shown (**a**–**b**). OTUs with the highest scores and IVs (the best environmental indicator species) are labeled in the panels. LGM, last glacial maximum.

Fig. 8. Comparison of paleo-environmental and thermal changes in southeast Africa. (**a**) $\delta^{18}O_{sw}$ record used as a proxy for monsoon changes over southern Africa (Ma et al., 2021). (**b**) Red Sea relative sea level (RSL) (black line) and global RSL (red line) records from Grant et al. (2014) and Waelbroeck et al. (2002), respectively. (**c**) Ba/Ca as a proxy for the strength of the Zambezi runoff (Weldeab et al., 2014), and rainfall data from Khon et al. (2014). (**d**) BIT index used as a proxy for indicating the riverine transport of soil organic matter (Kasper et al., 2015). (**e**–**h**) Normalized relative abundance of selected highly relevant environmental indicator species for the three environmental stages. Cyan shaded areas indicate the peaks in abundance of selected environmental indicator species. LGM, last glacial maximum.

Table 1. Radiocarbon ages from core 1477B.