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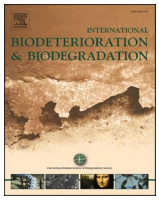

Investigation of the microbiota of historical parchment artifacts: Bacterial and fungal communities and their potential for biodegradation

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ABSTRACT

Written artifacts made from parchment constitute a significant category of the world's documentary heritage. The deterioration of parchment's collagen structure presents a challenge for the conservation of these artifacts. In addition to environmental conditions, the influence of microorganisms is one of the main causes of this deterioration. To better counteract microbial decay, it is necessary to gain an understanding of the microbiota of parchment. The present study was conducted to characterize the microbiota of parchment with a focus on microorganisms that may be involved in decay. Therefore, 12 parchment manuscript fragments showing signs of decay were analyzed by amplicon sequencing. The five most abundant fungal genera were *Aspergillus*, *Alternaria*, *Plectosphaerella*, *Wallemia*, and *Fusarium*, while the five most abundant bacterial genera were *Bacillus*, *Skermanella*, *Streptococcus*, *Metabacillus*, and *Cytobacillus*. The key organisms that can contribute to the decay of written artifacts are those that can form collagenases. The main genera in this context, *Bacillus* and *Clostridium*, were both found within the microbiota of the parchment samples analyzed, with *Bacillus* being the most prevalent genus. This study underlines the importance of a comprehensive understanding of the microbiota of parchment in order to counteract microbial decay.

1. Introduction

For centuries, human knowledge has been recorded in written artifacts made from organic materials of animal or plant origin (Creydt and Fischer, 2023). Since the 2nd century BCE, parchment, which is produced from skins of animals such as calves, sheep, goats, and lambs (Piñar et al., 2022; Wang et al., 2025), has been used as a writing support and covering material for books. To make parchment, untanned animal skins are cured in salt, then dehaired (often after being limed), and dried under stretching while the unwanted layers of derma are scraped off. Additional finishing treatments, such as surface polishing, pouncing or dyeing, were applied upon need. The main component of parchment, the protein collagen, consists of the three amino acids glycine, proline, and hydroxyproline, which form the primary structure. The secondary structure comprises an α-helix, and three of these helical chains form a triple helix structure. These triple helices are covalently bound together to form microfibrils, creating collagen fibers (Haulica et al., 2020; Piñar et al., 2022).

Since the organic material of parchment can degrade under certain conditions such as extremes of humidity and temperature, or the activity of microorganisms, the conservation of this cultural heritage poses a major challenge (Wang et al., 2025). The structural decay of collagen fibers and heat-induced changes in gelatin structures are important causes of parchment deterioration. Long exposure to high temperatures destroys the internal hydrogen bonds of collagen and opens the twisted collagen chains, resulting in a disordered, random coil configuration (Wang et al., 2025). The biological decomposition of parchment is due to the activity of microorganisms capable of secreting extracellular enzymes that enable them to metabolically break down proteins. Organisms with these abilities are able to degrade the collagen fibers of parchment through hydrolysis with collagenases (Zhang et al., 2022). The organisms most commonly associated with this activity are

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members of the genera *Bacillus*, *Clostridium*, and *Pseudomonas* (Gao et al., 2024).

Mold contamination is also a significant risk to parchment artifacts. Fungal colonization on parchment often manifests as colored spotting, and members of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Chaetomium*, *Chrysosporium*, *Paecilomyces*, *Penicillium*, *Phoma*, and *Stachybotrys* have all been associated with this sign of deterioration. Purple spots, for example, have been linked to *Aspergillus* species (Kwaśna et al., 2020; Creydt and Fischer, 2023); however, Piñar et al. (2022) reported similar staining caused by members of the Actinobacteria, mainly *Saccharopolyspora* spp.

The autochthonous microbiota describes the microorganisms that occur naturally in a habitat. Owing to its inherent material properties, the methods used in its production, and human handling, parchment has a very diverse autochthonous microbiota (Piñar et al., 2022). The most common bacteria within the microbiota of parchment include those that are common in saline habitat zones, such as *Halobacterium* spp. and *Halococcus* spp., and those that are typical for the human microbiota, such as *Staphylococcus* spp. and *Streptococcus* spp. (Piñar et al., 2022). Halophilic bacteria are known to be linked to the biological degradation of parchment. Migliore et al. (2017) proposed a succession model for the degradation in which *Halobacterium* spp. is suspected to be the initial colonizer. On the other hand, Cappitelli and Sorlini (2005) found, based on literature references, that the most common genera on parchment were *Streptomyces*, *Cladosporium*, *Scopulariopsis*, *Fusarium*, *Sporendonema*, *Ophiostoma*, *Aspergillus*, *Mucor*, *Penicillium*, *Nocardia*, *Alternaria*, *Trichoderma*, *Botryotrichum*, *Micrococcus*, *Bacillus*, and *Serratia*. Farag et al. (2018) examined 40 parchment artifacts from various libraries and identified *Aspergillus niger*, *A. flavus*, *Fycomycetes*, *Penicillium funiculosum*, and *Trichoderma viride*. *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Virgibacillus*, and *Micromonospora*, on the other hand, were isolated from damaged parchments stored in the Slovak National Library (Kraková et al., 2012). Sterflinger (2010) concluded from other studies that the most common fungal species on parchment were *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Phlebiopsis gigantea*, *Penicillium chrysogenum*, and *Thanatephorus cucumeris*. Although Piñar et al. (2022) attributed these different results to distinct stages in a succession model of decay, they may also reflect differences in analytical methods or actual variations in microbiota composition.

The present study aims to characterize the autochthonous microbiota of parchment and to improve understanding of the microorganisms potentially involved in its biodegradation. For this purpose, 12 parchment manuscript fragments dating from the ninth to 11th centuries CE - originally part of the library of the Great Mosque of Kairouan and now housed at the National Laboratory for the Preservation and Conservation of Parchment and Manuscripts in Raqqada, Kairouan, Tunisia, a division of the National Heritage Institute - were analyzed. The elemental and morphological characterization of the same fragments will be discussed elsewhere (publication in preparation by members of the Centre for the Study of Manuscript Cultures' Mobile Lab).

In November 2024, a four-person delegation from Tunisia travelled to Hamburg with 15 parchment manuscript fragments for a period of two weeks. As mentioned, 12 of these were analyzed in this study. Together, the Tunisian delegation and Centre for the Study of Manuscript Cultures' researchers, including external Centre-affiliated specialists, examined the objects in the Centre's' Artefact Labs. The visit and analysis were coordinated by, and conducted as part of, the Kairouan Manuscript Project, a Centre-based international cooperation project dedicated to facilitating and advancing the care, management, study, and promotion of the National Laboratory for the Preservation and Conservation of Parchment and Manuscripts' collection.

The core of this collection consists of manuscripts from the historical library of the Great Mosque of Kairouan, with additional manuscripts coming from other mosques and religious institutions across the city. According to Brockopp (2017), it is the oldest near-intact historical collection of Islamic manuscripts, 'the most important repository of early Islamic manuscripts in the world', and 'the most extraordinary collection of early Arabic manuscripts'. It contains 23 of the 30 known surviving non-Christian, non-Qur'anic manuscripts dating to approximately 900 CE or earlier, in addition to several early Qur'anic manuscripts. Al-Rammāh (1996), former director of the National Laboratory, asserts that its holdings constitute the largest collection of Islamic parchment manuscripts in the world, with current laboratory staff estimating more than 42,500 disbound leaves.

2. Materials and methods

2.1. Parchment samples, sample processing and DNA isolation

The 12 parchment manuscript fragments analyzed show signs of degradation including gelatinization, shrinkage, cockling, brittleness, tears, losses, moisture staining, and brown spots, with the latter possibly due to bacterial or fungal activity. To understand the causes of degradation, the custodian of the artifacts, the National Laboratory for the Preservation and Conservation of Parchment and Manuscripts in Raqqada, Kairouan, Tunisia, and its parent organization, the National Heritage Institute, requested the assistance of the Centre for the Study of Manuscript Cultures at the University of Hamburg, which has significant expertise in developing and applying non- or minimally-invasive and non-destructive analytical methods to study the materiality of written artifacts.

For the sampling, the surface of the parchment manuscript fragments was swabbed with dry sterile cotton swabs (80.625, Sarstedt, Nümbrecht, Germany) in one to three locations per fragment. Sampling locations were chosen to include areas with and without visible signs of degradation on the same page. For locations with visible signs of degradation, cockling and brown spots were selected, as microbial activity is primarily associated with these features. Only areas without writing were sampled. During sampling, areas showing signs of degradation were swabbed over their entire surface. Areas showing no signs of degradation were sampled in a square measuring approximately four by 4 cm. Swabbing was carried out first horizontally across the entire surface, then vertically across the entire surface of an area. This swabbing method was used for all sampled areas. An overview of the sampling areas is summarized in Table 1. Fig. 1 shows the three sampling locations on one of the parchment manuscript fragments examined (shelf mark R49, folio 195b).

For the gDNA isolation, the tip of the sterile cotton swabs was cut off with a sterile scalpel and transferred directly to the PowerBead® Tube of the DNeasy® PowerSoil Pro Kit (Qiagen, Hilden, Germany), which was used to isolate the gDNA from the microorganisms adhering to the sterile swabs. The kit was used according to the manufacturer's instructions. The concentration of gDNA was checked with the NanoPhotometer® NP80 (Implen, Munich, Germany). gDNA was stored at −20°C.

2.2. Library preparation and illumina sequencing

2.2.1. Partial amplification of bacterial 16S rRNA genes

Illumina library preparation was based on amplicons of the hypervariable regions V3 and V4 of the 16S rRNA gene. Therefore, a nested PCR approach was used to increase amplification success from low-biomass samples with potentially degraded DNA typical of historical parchment. The primers 616V (5′-AGA GTT TGA TYM TGG CTC A-3′), and 630R (5′-AAG GAG GTG ATC CAR CC-3′, both Eurofins Genomics, Ebersberg, Germany) according to Chenoll et al. (2011) were used to obtain amplicons of the hypervariable regions V1 to V9. The expected amplicon length is approximately 1500 bp. The amplicons of the hypervariable regions V3 and V4 could then be obtained from these amplicons using the primers 341FSeq containing the adapter sequence (underlined) (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′), and 805RSeq containing the adapter sequence (underlined) (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA

Table 1
Overview of the investigated parchment samples concerning parchment code (shelf mark and folio), sample code, sample number, total OTUs and total reads of the bacterial and fungal community.

Parchment code (shelf mark and folio)	Sample code	Signs of degradation[a]	Sample number	Bacterial community		Fungal community	
				Total OTUs	Total Reads	Total OTUs	Total Reads
R3/826_f002b	R3/826_f002b_top	n	1	300	76,512	-	-
	R3/826_f002b_centre	b, c	2	115	89,647	28	107,572
	R3/826_f002b_right side	c	3	-	-	26	103,939
R11_f208b	R11_f208b_spot	b, c	5	435	81,346	29	102,923
R11_f205a	R11_f205a_right side	n	6	500	74,134	44	86,663
	R11_f205a_centre	b, c	7	702	70,403	15	98,767
R11_f172a	R11_f172a_top left side	b, c	8	632	71,509	24	109,430
	R11_f172a_bottom left side	b	9	383	70,260	37	107,704
	R11_f172a_bottom right side	n	10	-	-	32	101,253
R11_f175b	R11_f175b_top right side	b	11	766	61,045	32	92,690
	R11_f175b_centre	b	12	636	35,969	22	94,536
	R11_f175b_bottom left side	c	13	670	39,340	31	96,300
R24_f021b	R24_f021b	n	16	-	-	30	82,942
R24_f024a	R24_f024a	c	17	699	77,479	9	104,651
	R24_f024a_piece	b, c	36	-	-	12	104,284
R25_f066	R25_f066	b, c	18	261	75,969	10	106,646
R49_f137b	R49_f137b_top right side	b	19	520	61,580	13	96,561
	R49_f137b_left side	n	20	641	45,925	28	93,288
R49_f138a	R49_f138a_fold	c	21	546	63,717	27	91,127
R49_f195b	R49_f195b_right side	b	22	533	73,566	17	88,181
	R49_f195b_left side	n	23	674	65,529	31	91,632
	R49_f195b_fold	c	24	564	67,940	11	99,127
R49_f027	R49_f027	b, c	25	-	-	19	96,719
R59_f017b	R59_f017b_top	b	26	-	-	26	43,396
	R59_f017b_bottom	n	27	327	85,911	23	71,359
R59_f026b	R59_f026b_left side	b, c	28	125	88,997	17	88,005
	R59_f026b_centre	b	29	-	-	13	94,607
	R59_f026b_right side	n	30	233	88,376	27	82,544
R115_f133b	R115_f133b_bottom left side	n	31	210	97,863	17	98,924
	R115_f133b_bottom spot	b	32	180	69,892	27	94,629
R115_f074–075	R115_f074-075_piece	b, c	33	545	72,589	19	102,678
	R115_f074-075_little piece	b, c	34	-	-	9	40,722

[a] n = none; b = brown spots; c = cockling.

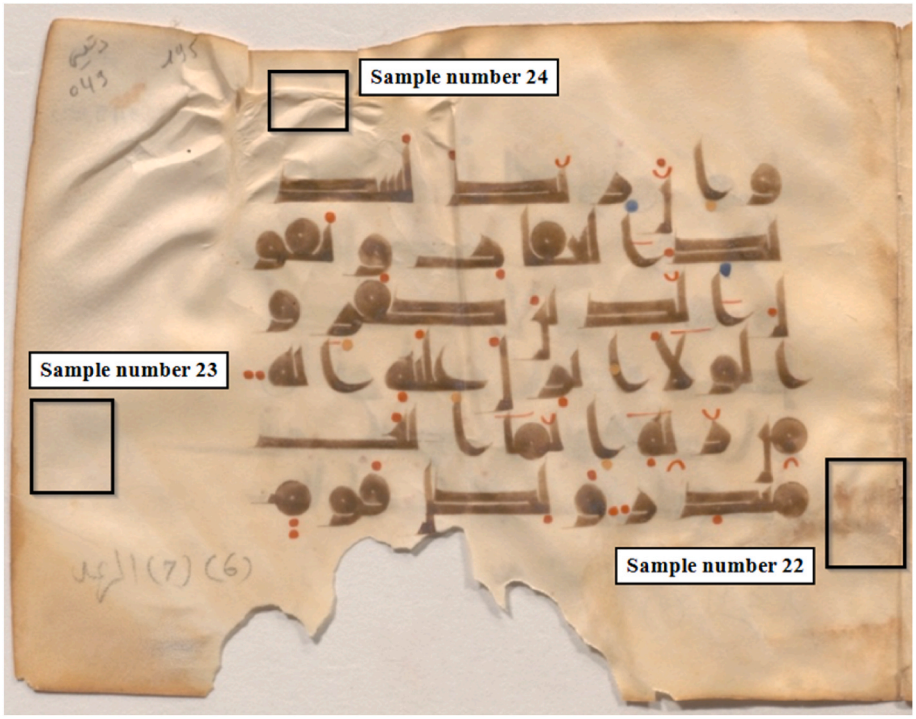

Fig. 1. Parchment R49_f195b. The three sampling locations are marked with black boxes. Sample number 22 showed signs of degradation in the form of brown spots, sample number 23 showed no signs of degradation, and sample number 24 showed signs of degradation in the form of cockling. Manuscript image courtesy of the National Heritage Institute, reproduced with permission. Annotations © the authors. Further reproduction of the manuscript image requires permission from the National Heritage Institute. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′, both Eurofins Genomics, Ebersberg, Germany). The primer sequences according to Klindworth et al. (2013) and the adapter sequences were provided by Microsynth AG (Balgach, Switzerland), who performed the sequencing. The expected amplicon length is approximately 500 bp.

All PCR reactions were performed on the Mastercycler® nexus GX2 (Eppendorf, Hamburg, Germany). Each PCR reaction was performed with a no template control. For the first 20 μL PCR reaction, the PCR mixture contained 10 μL of 2 × Phusion Flash PCR MasterMix (F-548, Thermo Fisher Scientific, Waltham, MA, USA), 0.5 μL (10 μM) of each primer (616V and 630R), 6 μL of the gDNA extract, and 3 μL of PCR grade water. The PCR program using primers 616V and 630R included an initial denaturation (95°C for 300 s), 35 cycles (95°C for 40 s; 58°C for 60 s; 72°C for 90 s), followed by a final elongation at 72°C for 300 s, and cooling down to 4°C.

For the second 20 μL PCR reaction, the PCR mixture contained 10 μL of 2 × Phusion Flash PCR MasterMix (F-548, Thermo Fisher Scientific, Waltham, MA, USA), 0.5 μL (10 μM) of each primer (341FSeq and 805RSeq), and 9 μL of the 616V/630R amplicons from the first PCR reaction. The PCR program using primers 341FSeq and 805RSeq included an initial denaturation (95°C for 300 s), 25 cycles (95°C for 30 s; 52°C for 30 s; 72°C for 90 s), followed by a final elongation at 72°C for 300 s, and cooling down to 4°C.

2.2.2. Partial amplification of fungal ITS2 regions

Illumina library preparation was based on amplicons of the ITS2 region. Therefore, a nested PCR approach was used. This approach included amplification of regions ITS1 and ITS2 using the primers ITS1F_KYO2 (5′-TAG AGG AAG TAA AAG TCG TAA -3′), and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′, both Eurofins Genomics, Ebersberg, Germany) according to Toju et al. (2012) and amplification of the ITS2 region using the amplicons from the first PCR reaction and the primers ITS3Seq containing the adapter sequence (underlined) (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GCA TCG ATG AAG AGA CAG GCA TCG ATG AAG AAC CGC AGC -3′), and ITS4Seq containing the adapter sequence (underlined) (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GAT GTG TAT AAG AGA GAC AGT CCT CCG CTT ATT GAT ATG C-3′, both Eurofins Genomics, Ebersberg, Germany). The primer sequences according to Toju et al. (2012) and adapter sequences were provided by Microsynth AG (Balgach, Switzerland), who performed the sequencing. The expected amplicon length when using ITS1F_KYO2 and ITS4 is approximately 500 to 750 bp. When using ITS3Seq and ITS4Seq, the expected amplicon length is 300 to 500 bp.

For the first 20 μL PCR reaction, the PCR mixture contained 10 μL of 2 × Phusion Flash PCR MasterMix (F-548, Thermo Fisher Scientific, Waltham, MA, USA), 0.5 μL (10 μM) of each primer (ITS1F_KYO2 and ITS4), 6 μL of the gDNA extract, and 3 μL of PCR grade water. The PCR program using primers ITS1F_KYO2 and ITS4 included an initial denaturation (95°C for 300 s), one cycle (98°C for 15 s; 53.0°C for 30 s; 72°C for 30 s), one cycle (98°C for 15 s; 52.5°C for 30 s; 72°C for 30 s), one cycle (98°C for 15 s; 52.0°C for 30 s; 72°C for 30 s), one cycle (98°C for 15 s; 51.5°C for 30 s; 72°C for 30 s), one cycle (98°C for 15 s; 51.0°C for 30 s; 72°C for 30 s), one cycle (98°C for 15 s; 50.5°C for 30 s; 72°C for 30 s), 25 cycles (98°C for 15 s; 50.0°C for 30 s; 72°C for 30 s), followed by a final elongation at 72°C for 300 s and cooling down to 4°C.

For the second 20 μL PCR reaction, the PCR mixture contained 10 μL of 2 × Phusion Flash PCR MasterMix (F-548, Thermo Fisher Scientific, Waltham, MA, USA), 0.5 μL (10 μM) of each primer (ITS3Seq and ITS4Seq), and 9 μL of the ITS1F_KYO2/ITS4 amplicons from the first PCR reaction. The PCR program using primers ITS3Seq and ITS4Seq included an initial denaturation (95°C for 300 s), 25 cycles (95°C for 30 s; 80.0°C for 30 s; 72°C for 90 s), followed by a final elongation at 72°C for 300 s and cooling down to 4°C. The annealing temperature of 80°C was used to minimize non-specific amplification products observed during preliminary optimization experiments.

2.3. Purification of the PCR products and sequencing

An agarose gel electrophoresis was used to confirm the success of the PCR amplifications. Purification of the PCR amplicons was performed using enzymatic PCR cleanup with Exonuclease I (M0293S, New England Biolabs, Frankfurt am Main, Germany), Exonuclease I buffer (B0293S, New England Biolabs, Frankfurt am Main, Germany), and recombinant Shrimp Alkaline Phosphatase (rSAP, M0371S, New England Biolabs, Frankfurt am Main, Germany) as described earlier (Griese et al., 2025). Purified amplicons were checked for quality and concentration using the NanoPhotometer® NP80 (Implen GmbH, München, Germany).

Sequencing of the PCR libraries was performed on an Illumina NovaSeq platform by Microsynth AG (Balgach, Switzerland). Raw sequencing data were deposited in the Short Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA1275800.

2.4. Data analysis

Produced Paired-end reads passed the Illumina chastity filter. Demultiplexing and trimming of Illumina adaptor residuals were performed with the Illumina software bcl2fastq version v2.20.0.422. The read quality was checked with FastQC version 0.11.8. Reads that had an average Q-score of less than 24 or contained uncalled bases were excluded from further analysis. The locus specific V34 primers were removed with cutadapt version v3.2 (Martin, 2011). Trimmed forward and reverse reads of each paired-end read were merged using USEARCH version 11.0.667 (Edgar, 2010). Merged reads that contained ambiguous bases or were outliers regarding the expected amplicon size distribution were also discarded. Samples that resulted in less than 5000 merged reads were discarded, to not distort the statistical analysis. Surviving reads were denoised using the UNOISE algorithm (Edgar, 2016) implemented in USEARCH to form zero-radius OTUs (zOTUs), discarding singletons and chimeras in the process. The resulting operational taxonomic unit (OTU) abundance table was then filtered for barcode bleed-in contaminations using the UNCROSS algorithm (Edgar, 2018). OTU sequences were compared to the reference sequences of the NCBI RefSeq Targeted Loci database (downloaded on 11 March 2025), provided through the NCBI database resources (Sayers et al., 2022). Taxonomic assignment and calculation of confidence were carried out using the SINTAX algorithm implemented in USEARCH without applying a fixed SINTAX confidence threshold. Data analysis described above were performed by Microsynth AG (Balgach, Switzerland).

Calculations of abundances as well as graphical representations were performed using Microsoft Excel version 2311 (Microsoft Corporation, Redmond, Washington, USA).

3. Results and discussion

This study aimed to characterize the microbiota of 12 parchment manuscript fragments dating from the 9th to 11th centuries CE that were once part of the library of the Great Mosque of Kairouan and are now housed at the National Laboratory for the Preservation and Conservation of Parchment and Manuscripts in Raqqada, Kairouan, Tunisia, a division of the National Heritage Institute. The study placed particular emphasis on microorganisms potentially involved in the biodegradation of these manuscripts. For this purpose, parchment manuscript fragments showing signs of degradation were analyzed. An Illumina-based approach for the 16S rRNA gene and the ITS region was performed as described above to investigate the microbial community of parchment.

3.1. The bacterial community of parchment

The numbers of reads and OTUs generated in the bacterial community analysis are shown in Table 1 for all samples. No 16S rRNA gene

amplicons could be generated from the isolated gDNA of samples 3, 10, 16, 25, 26, 29, 34, and 36, so no sequencing could be performed. Among these, only samples number 10 and 16 showed no signs of degradation (Table 1).

After sequencing of amplicons of the hypervariable regions V3 and V4 of the bacterial 16S rRNA genes of the remaining 24 samples, the sequences were grouped into 1450 OTUs. The OTUs were assigned to a total of 16 phyla and 221 genera. The phyla identified were Bacillota (6.83% to 99.67%), Pseudomonadota (0.01% to 93.16%), Planctomycetota (0% to 18.80%), Deinococcota (0% to 9.41%), Chloroflexota (0% to 5.38%), Cyanobacteriota (0% to 4.32%), Verrucomicrobiota (0% to 3.31%), Thermodesulfobacteriota (0% to 1.65%), Armatimonadota (0% to 1.55%), Actinomycetota (0% to 1.31%), Gemmatimonadota (0% to 1.28%), Bacteroidota (0% to 1.27%), Myxococcota (0% to 1.13%), Acidobacteriota (0% to 1.12%), Abditibacteriota (0% to 0.55%), and Fusobacteriota (0% to 0.25%). The relative abundance of bacterial phyla is shown in Fig. 2.

The 20 most abundant genera in all 24 samples were *Bacillus* (1.26% to 51.16%, in 24 samples), *Streptococcus* (0% to 58.78%, in 15 samples), *Metabacillus* (0% to 30.19%, in 22 samples), *Cytobacillus* (0.19% to 24.44%, in 24 samples), *Skermanella* (0% to 94.96%, in 5 samples), *Staphylococcus* (0% to 37.18%, in 17 samples), *Paracoccus* (0% to 40.65%, in 16 samples), *Virgibacillus* (0.01% to 29.68%, in 24 samples), *Mesobacillus* (0% to 12.88%, in 21 samples), *Lactococcus* (0% to 23.79%, in 8 samples), *Niallia* (0% to 10.57%, in 22 samples), *Oceanobacillus* (0% to 9.46%, in 20 samples), *Acidicaldus* (0% to 45.04%, in 2 samples), *Moraxella* (0% to 15.84%, in 5 samples), *Neobacillus* (0% to 5.45%, in 21 samples), *Romboutsia* (0% to 17.33%, in 9 samples), *Lactobacillus* (0% to 36.73%, in 2 samples), *Clostridium* (0% to 7.20%, in 12 samples), *Heyndrickxia* (0.09% to 4.57%, in 24 samples), and *Azospirillum* (0% to 15.48%, in 9 samples). The distribution of the 20 most common bacterial genera is shown in Fig. 3.

According to Piñar et al. (2022), halophilic taxa are the first colonizers of parchment due to the salting process during production. However, these are replaced by halotolerant organisms as humidity increases and salt content decreases. Human handling also leads to the presence of organisms associated with the human microbiota. For the parchments analyzed in this study, Bacillota was found to be the most abundant phylum, with 75% of the most common genera classified in this phylum. The phylum Bacillota includes many salt-tolerant genera, such as *Bacillus*, *Mesobacillus*, *Metabacillus*, *Oceanobacillus*, *Virgibacillus*, and *Cytobacillus* (Gupta et al., 2020; Khalilova et al., 2021; Patel and Gupta, 2020; Yumoto et al., 2005), which were also found in the present study. It also comprises many genera associated with the human microbiota, such as *Streptococcus* and *Staphylococcus*, which are mainly found on the skin and in the oral cavity, and *Lactobacillus*, *Lactococcus*, and *Clostridium*, which are found as part of the human gut microbiota (Lugli et al., 2025; Manos, 2022) with all these genera having already been detected on historical parchment artifacts in this study and other studies (Piñar et al., 2022; Vassallo et al., 2026). Also, *Paracoccus* and *Moraxella* were already identified among the rare taxa in the study by Vassallo et al. (2026). The findings of Piñar et al. (2022) and Vassallo et al. (2026) were thus confirmed in the present study, and it could be argued that these salt-tolerant genera and those associated with the human microbiota can be regarded as part of a putative core microbiota of parchment. Interestingly, this study also identified other genera

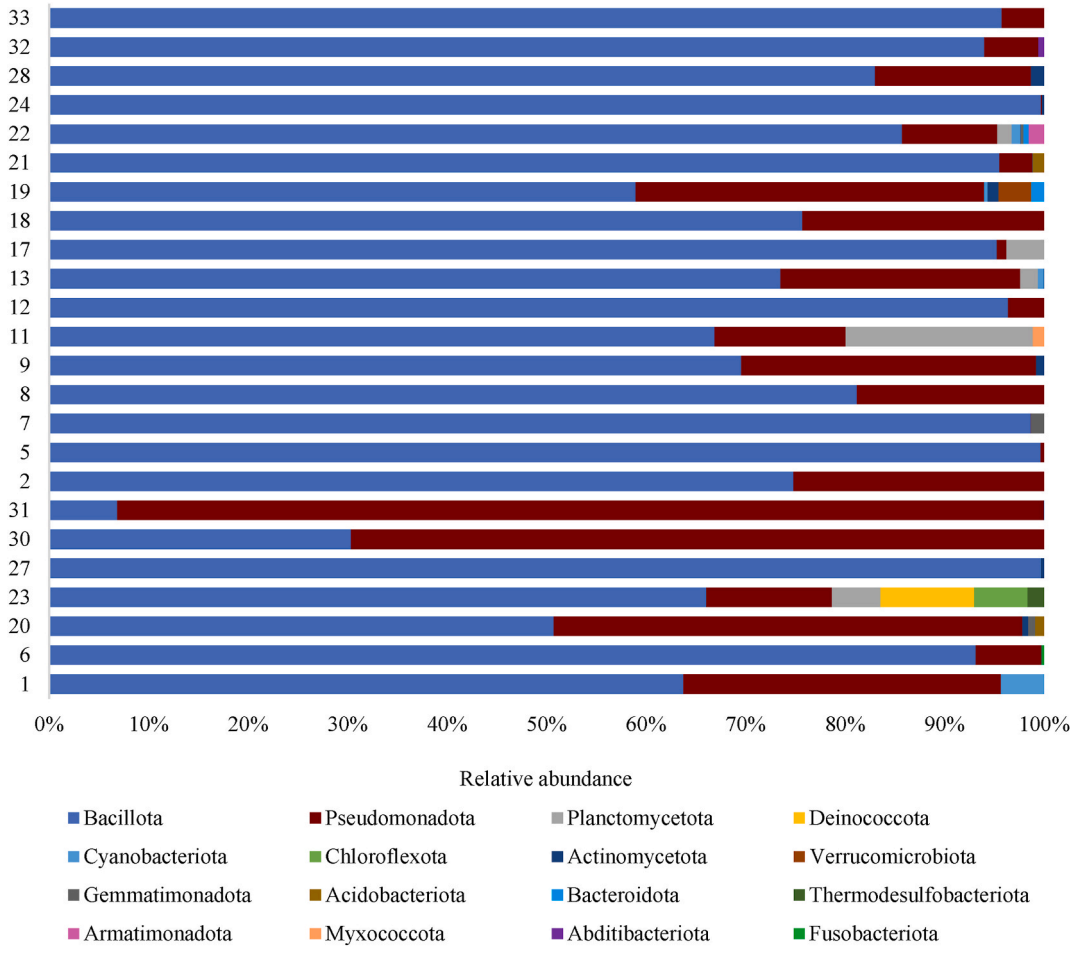

Fig. 2. Relative abundance of bacterial phyla. Sampled areas 1, 6, 20, 23, 27, 30, and 31 show no signs of degradation. Sampled areas 2, 5, 7, 8, 9, 11, 12, 13, 17, 18, 19, 21, 22, 24, 28, 32, and 33 show signs of degradation.

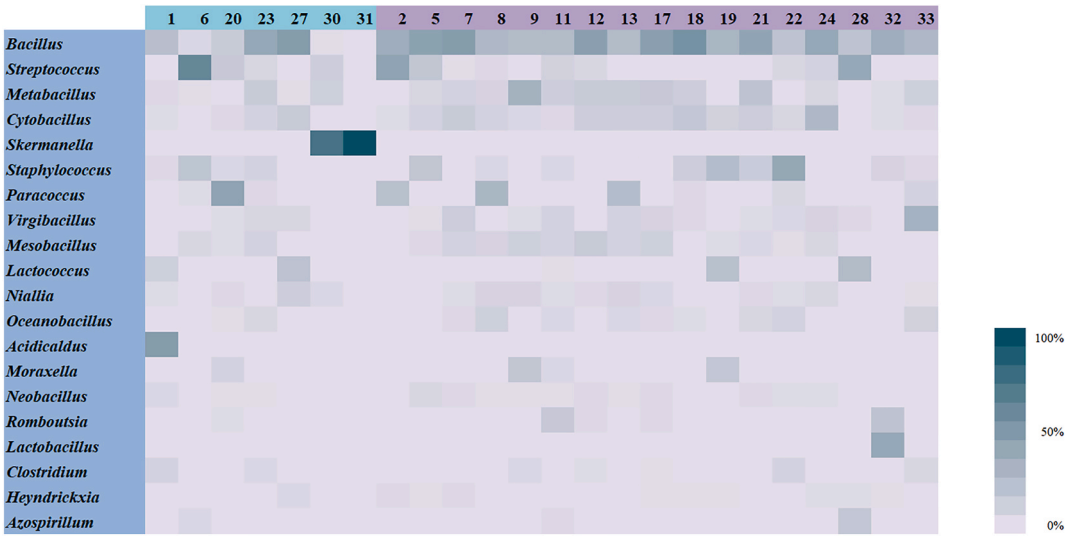

Fig. 3. Distribution of the 20 most common bacterial genera. Sampled areas 1, 6, 20, 23, 27, 30, and 31 show no signs of degradation. Sampled areas 2, 5, 7, 8, 9, 11, 12, 13, 17, 18, 19, 21, 22, 24, 28, 32, and 33 show signs of degradation.

associated with the human gut which have not yet been detected on historical parchment artifacts, such as *Romboutsia* and *Neobacillus* (Gerritsen et al., 2018; Patel and Gupta, 2020). Furthermore, the present study also found genera that have not yet been associated with parchment, such as *Skermanella*, *Azospirillum,* and *Heyndrickxia*, which have for example already been isolated from soil (Lewin et al., 2021; Lin et al., 2014). The occurrence of *Acidicaldus* in this study is also of interest, as this species has not yet been detected on parchment and has not previously been linked to the human microbiota. Species of this genus are acidophilic and moderately thermophilic, and have so far been isolated from extreme environments such as the Yellowstone National Park (Johnson et al., 2006). In particular, the finding of these rare taxa highlights that, as expected, portable historical written artifacts have highly individualized microbiota shaped by their varied histories of storage, handling, and use.

In the biodegradation of parchment, organisms that are able to break down the collagen structure of parchment using collagenases are critical (Zhang et al., 2022). Organisms that have a key role in this context belong to the genera *Bacillus*, *Clostridium*, and *Pseudomonas* (Gao et al., 2024). In the present study, *Bacillus* was found to be the most common genus on all samples. The genus *Clostridium* was also found on 12 samples, but at significantly lower abundance. *Bacillus* spp. are able to form endospores that can outlast many years and germinate again under favorable environmental conditions, such as improper storage conditions with excessive humidity (Creydt and Fischer, 2023). To confirm that *Bacillus* spp. is a potential decay agent for parchment, experiments should be conducted on contemporary parchment samples - not historical parchment artifacts - deliberately inoculated with selected strains and monitored over time.

Looking at the distribution of the most common bacteria on the sampled areas (Fig. 3), it is noticeable that the genus *Bacillus* occurs more frequently in sampling areas showing signs of degradation. This may indicate that the presence of *Bacillus* spp. is related to the degradation observed. This trend is not apparent for any of the other genera. However, it should be noted that no bacteria were detected in eight of the 32 sampling areas; among these eight, only two showed no signs of degradation. This could be related to the minimally invasive method chosen to harvest the microbiota from the parchment. It is possible that using (micro-)invasive methods by analyzing whole small pieces of parchment instead of swabbing parchment surfaces could provide a more comprehensive understanding of the autochthonous microbiota.

3.2. The fungal community of parchment

The numbers of reads and OTUs generated in the fungal community analysis are shown in Table 1 for all samples. No ITS amplicons could be generated from the isolated gDNA of sample 1, so no sequencing could be performed. This sample was taken from an area without signs of degradation (Table 1).

After sequencing of ITS2 amplicons of the remaining 31 samples, the sequences were grouped into 169 OTUs. The OTUs were assigned to a total of three phyla and 64 genera. The phyla identified were Ascomycota (19.47% to 97.96%), Basidiomycota (2.04% to 80.53%), and Mucoromycota (0% to 26.45%). The relative abundance of fungal phyla is shown in Fig. 4.

The 20 most abundant genera in all samples were *Aspergillus* (0% to 43.38%, in 25 samples), *Alternaria* (0% to 60.81%, in 18 samples), *Plectosphaerella* (0% to 42.56%, in 14 samples), *Wallemia* (0% to 72.57%, in 6 samples), *Fusarium* (0% to 22.33%, in 17 samples), *Stagonosporopsis* (0% to 30.46%, in 9 samples), *Malassezia* (0% to 52.01%, in 28 samples), *Penicillium* (0% to 25.37%, in 24 samples), *Cladosporium* (0% to 19.96%, in 20 samples), *Neopyrenopeziza* (89.13%, in 1 sample), *Vishniacozyma* (0% to 62.14%, in 6 samples), *Umbelopsis* (0% to 26.45%, in 6 samples), *Trichophaea* (38.39%, in 1 sample), *Botryotrichum* (0% to 27.16%, in 2 samples), *Aureobasidium* (0% to 13.15%, in 4 samples), *Itersonilia* (0% to 13.70%, in 7 samples), *Sarocladium* (22.21%, in 1 sample), *Geosmithia* (21.56%, in 1 sample), *Fomitopsis* (0% to 10.83%, in 4 samples), and *Clitocybe* (0% to 28.92%, in 3 samples). The distribution of the 20 most common fungal genera is shown in Fig. 5.

Previous studies have shown that *Aspergillus*, *Alternaria*, *Fusarium*, *Penicillium*, and *Cladosporium* are among the most common fungal genera found on parchment (Cappitelli and Sorlini, 2005). These genera could therefore be considered part of a putatively core mycobiota of parchment. The genus *Malassezia*, which was detected frequently in the present study, has likewise been associated with parchment (Pinheiro et al., 2022). Yeasts of this genus occur naturally on animal and human skin, making their frequent occurrence plausible. All other fungal genera identified among the 20 most abundant in this study were only detected at a few sampling locations, such as *Sarocladium* (22.21%, in 1 sample). Thus, in addition to the putatively core organisms, the fungal community, like the bacterial one, shows a highly individualized composition.

Based on the method used in this study, it is not possible to indicate whether the detected microorganisms are alive or dead, nor can any conclusions be drawn about when the microorganisms arrived on the

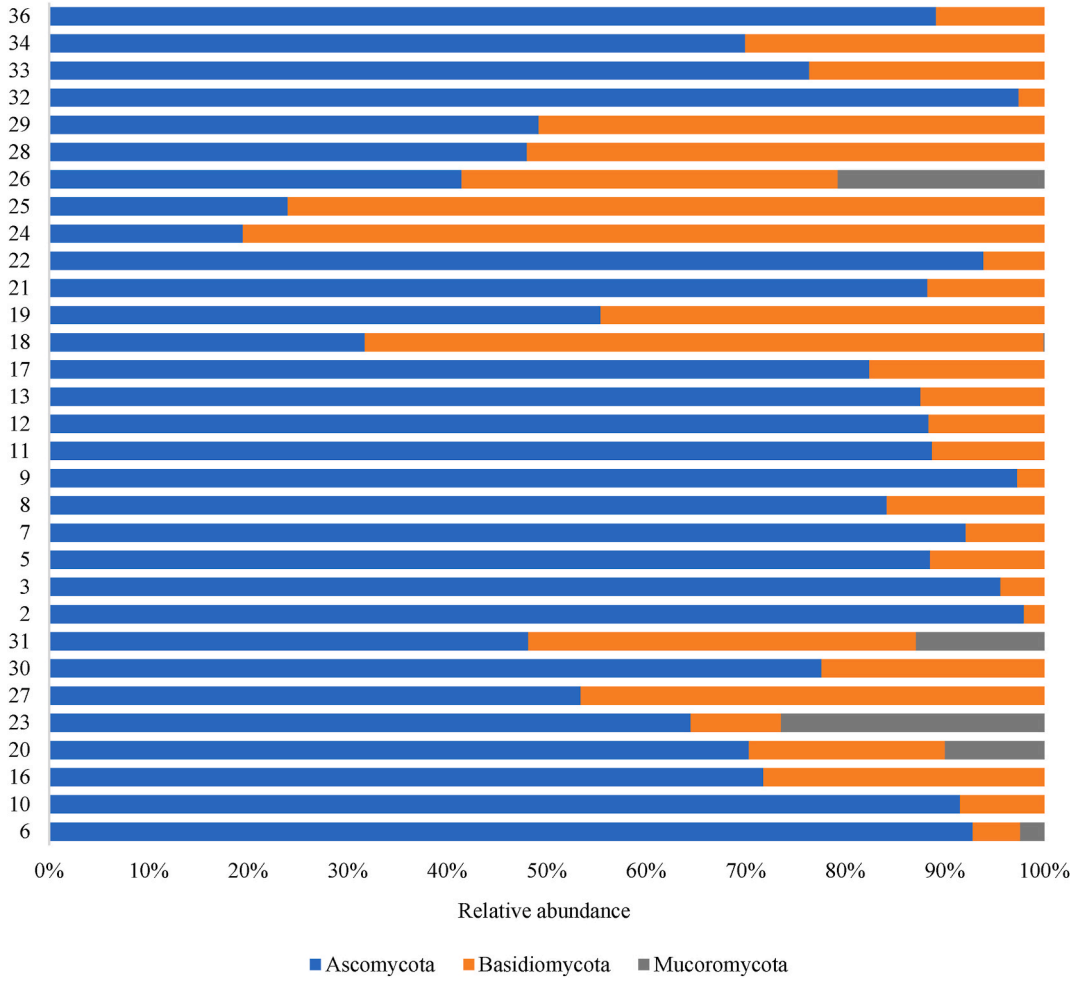

Fig. 4. Relative abundance of the fungal phyla. Sampled areas 6, 10, 16, 20, 23, 27, 30, and 31 show no signs of degradation. Sampled areas 2, 3, 5, 7, 8, 9, 11, 12, 13, 17, 18, 19, 21, 22, 24, 25, 26, 28, 29 32, 33, 34, and 36 show signs of degradation.

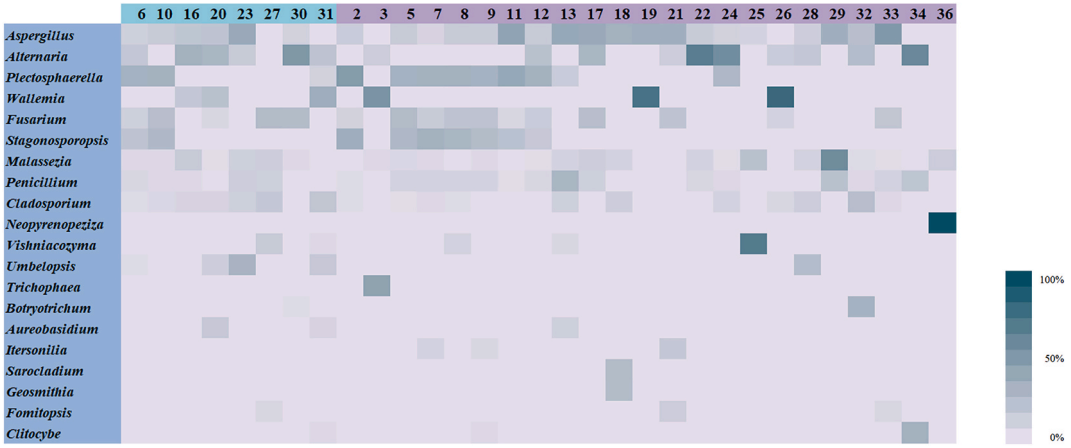

Fig. 5. Distribution of the 20 most common fungal genera. Sampled areas 6, 10, 16, 20, 23, 27, 30, and 31 show no signs of degradation. Sampled areas 2, 3, 5, 7, 8, 9, 11, 12, 13, 17, 18, 19, 21, 22, 24, 25, 26, 28, 29 32, 33, 34, and 36 show signs of degradation.

objects. It is conceivable that mold spores were already present on the analyzed parchment manuscript fragments many years before they were incorporated into the collections of the National Laboratory. Although molds prefer warm environments, they can also grow at colder temperatures and especially in high humidity.

Fungal colonization is a well-documented contributor to the biodeterioration of cultural artifacts. Fungi of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Chaetomium*, *Chrysosporium*, *Paecilomyces*, *Penicillium*, *Phoma*, and *Stachybotrys* were particularly mentioned in this context (Kwaśna et al., 2020). Like bacteria, some fungi are capable of producing collagenases that can break down the structure of parchment. In addition, fungal colonization often manifests as various forms of

discoloration (Kwaśna et al., 2020). It is therefore plausible that the fungi identified in the present study may be related to the damage observed on the parchment manuscripts.

Examining the distribution of the most common fungal genera on the sampled areas (Fig. 5), no clear distinction is apparent between areas showing signs of degradation and those that do not. This observation may indicate that the mere presence of fungal DNA does not necessarily correspond to visible signs of degradation. For example, spores that had not yet germinated and which, due to their state of survival, have not caused any detectable changes to the parchment, may be present in areas showing no visible signs of degradation. Conversely, visible signs of degradation may also indicate past microbial activity rather than ongoing colonization. As the amplicon sequencing approach used does not distinguish between viable and non-viable microorganisms, the detected fungal communities should be interpreted as an indication of presence rather than a sign of active biological degradation.

In principle, the presence of an autochthonous microbiota on parchment is not inherently problematic, and the National Laboratory's parchment holdings do not require decontamination, disinfection, or sterilization as a general, collection-wide measure. Rather, a preventive conservation program based on the maintenance of appropriate and stable storage conditions represents the most effective means of limiting the growth and/or germination of damage-causing organisms. If environmental parameters - particularly relative humidity - are not adequately controlled, bacterial and fungal spores already present on the parchment may germinate, leading to microbial activity and subsequent degradation. The storage and environmental monitoring practices currently implemented at the National Laboratory are consistent with this preventive approach and are intended to limit the risk of microbial activation.

4. Conclusions

In conclusion, this study provides important information regarding the microbiota of 12 samples of parchment manuscript fragments exhibiting signs of deterioration, especially regarding the most common bacteria and fungi and their spoilage potential. The five most abundant genera identified were *Aspergillus*, *Alternaria*, *Plectosphaerella*, *Wallemia*, and *Fusarium* for fungi, and *Bacillus*, *Skermanella*, *Streptococcus*, *Metabacillus*, and *Cytobacillus* for bacteria. The most common bacterial genera are mainly salt-tolerant bacteria and those associated with the human microbiota, such as *Bacillus*, *Metabacillus*, *Streptococcus*, and *Staphylococcus*, with representatives of the genus *Bacillus* being cited as one of the main causes of the biodegradation of parchment. Among the most common fungi, genera that can contribute to the biodegradation of parchment were also identified, such as *Aspergillus*, *Alternaria*, *Penicillium*, and *Fusarium*. It has been shown that, in addition to a number of putative core organisms, there is a highly individual microbiota on parchment. Overall, the study contributes to a better understanding of the composition and variability of parchment-associated microbial communities, particularly in historical parchment artifacts. Strategies to prevent the biodegradation of parchment should aim to limit the growth and/or germination of damage-causing organisms such as *Bacillus* spp., which may have already been present on the parchment prior to its inclusion in the collections of the National Laboratory. These findings are significant for the preservation of cultural heritage, as they support the early identification of taxa potentially involved in biodegradation, which can induce preventive conservation measures.

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CRediT authorship contribution statement

Jennifer Griese: Formal analysis, Investigation, Methodology, Writing – original draft. **Davidson MacLaren:** Writing – review & editing. **Adel Bedri:** Resources. **Samir Gdah:** Resources. **Manel Rammeh:** Resources. **Tarek Baccouche:** Project administration, Writing – review & editing. **Claudia Colini:** Writing – review & editing. **Agnes Weiss:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Raw sequencing data were deposited in the Short Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA1275800.

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