Original Research Article

Comparative 16S rRNA amplicon profiling of surface microbial communities on historic buildings across southern and northern China

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Abstract: Bacterial biofilms are often discussed as a secondary problem in the conservation of old buildings, but on damp or dust-laden surfaces they can become an active part of material decay. Here, 72 surface samples from historic buildings in Foshan, Guangzhou, and Shanxi/Taiyuan were compared to examine how much of the bacterial pattern is shared across regions and how much is shaped by individual buildings and substrates. The sample set covered a humid subtropical setting in southern China and a temperate monsoon setting in northern China, and included wood, tile, brick, wall, statue, and cave-wall surfaces. Community profiles were generated from 16S rRNA amplicon sequencing and interpreted with taxonomic summaries, rarefaction analysis, PCA, Bray-Curtis PCoA, PERMANOVA, and FAPROTAX-based functional prediction. Four phyla, Proteobacteria, Actinobacteriota, Firmicutes, and Bacteroidota, together made up more than 80% of the reads in each region, suggesting that these surfaces carry a broad but recognizable bacterial core. At genus or species level the picture was less uniform. Pseudomonas, Sphingomonas, Acinetobacter, and Bacillus appeared repeatedly, yet their abundance changed with building context and surface material. Ordination results pointed in the same direction: The separation among buildings and substrates was clearer than the separation among broad geographic regions, and PERMANOVA confirmed significant compositional differences ($p < 0.001$). Predicted functions were dominated by chemoheterotrophy and aerobic chemoheterotrophy. Nitrification-related categories were somewhat more prominent in the Shanxi/Taiyuan group, a pattern that may be linked to nitrogen deposition, bird-derived inputs, and semi-enclosed spaces. The results argue for conservation assessment at a finer scale than climate zone alone.

Keywords: historic buildings; microbial communities; cultural heritage conservation; 16S rRNA sequencing; proteobacteria; beta diversity

1. Introduction

The surfaces of historic buildings are not inert boundaries between architecture and the environment. They collect dust, salts, aerosols, fragments of plant and animal material, and moisture, sometimes for decades without deep cleaning. These deposits create small, uneven habitats on wood, brick, tile, stone, plaster, and painted or sculpted surfaces. Once microorganisms become established, they may form biofilms and alter the surface chemically or physically through organic acids, extracellular polymers, pigments, enzymes, and mineral-transforming reactions. The visible outcomes are familiar to conservators: Staining, powdering, weakened wooden elements, detaching wall layers, and local acceleration of salt- or moisture-related damage. Microorganisms rarely act alone, but they often push already vulnerable materials further toward deterioration [1-3].

For years, heritage microbiology relied on culture-based isolation, which yielded testable living strains but failed to capture most of the microbial community—Many environmental bacteria from nutrient-poor, dry or extreme surfaces do not grow on standard media. Sequencing-based approaches, especially 16S rRNA gene surveys, expanded observation scales, describing bacterial communities on various heritage surfaces (monuments, grottoes, wooden relics, etc.), with Proteobacteria, Actinobacteriota, Firmicutes, and Bacteroidota frequently reported as major groups[4].

2. Materials and methods

2.1. Study sites and sampling design

Samples came from three regional groups. Foshan contributed 32 samples, Guangzhou 15, and Shanxi/Taiyuan 25, giving a total of 72 surface samples. The two Guangdong groups represent humid subtropical monsoon conditions, whereas the Shanxi/Taiyuan group represents a temperate monsoon setting with a drier atmospheric background. Within each region, sampling covered several historic buildings and several kinds of surface. The materials included wooden components, ceramic tiles, ordinary wall surfaces, brick walls, statue surfaces, corridors, wooden columns, and stone-brick cave walls. The design was not meant to treat each building as identical replication. Instead, it was intended to preserve the real heterogeneity that conservators encounter when assessing active heritage sites.

2.2. DNA sequencing and community profiling

Bacterial composition was obtained from 16S rRNA gene amplicon sequencing. The source analysis used rarefaction curves to judge whether the sequencing depth was adequate before comparing communities. Taxonomic summaries were then examined at phylum level and, where classification was sufficiently resolved, at genus or species level. Because the three regional analyses did not always report the same fine taxonomic rank, this synthesis gives greatest weight to robust phylum-level patterns and to recurrent named genera that appeared across more than one region.

Sequencing coverage was checked with Shannon-Wiener rarefaction curves, and species accumulation information was considered where it was available. To compare community composition among samples, the analysis used PCA and Bray-Curtis PCoA. These two ordination views are complementary: PCA gives a broad view of compositional gradients, whereas Bray-Curtis PCoA is more sensitive to abundance differences among ecological communities. Group separation was evaluated with PERMANOVA, which is appropriate for multivariate community data when parametric assumptions are not realistic.

2.3. Functional prediction

Functional interpretation was performed with FAPROTAX-style assignment. This method links bacterial taxa to ecological functions that have been reported for known representatives [5]. The categories considered here included chemoheterotrophy, aerobic chemoheterotrophy, nitrification, denitrification, nitrogen fixation, fermentation, methylotrophy, and sulfide oxidation. These predictions should be read as ecological clues rather than direct measurements of gene abundance or activity, because 16S rRNA data alone cannot prove that a pathway was active on the sampled surface.

3. Results

3.1. Sequencing depth and sample coverage

The rarefaction curves for all three regions flattened after an initial rise. This pattern suggests that the sequencing effort was sufficient for the main comparisons and that additional reads would have added relatively few new abundant taxa. In the Foshan dataset, the species accumulation curve also approached a plateau when the number of samples reached roughly 30, which is consistent with the 32-sample design. Guangzhou and Shanxi/Taiyuan showed the same general tendency toward saturation. These checks do not mean that every rare organism was recovered, but they do indicate that the dominant surface communities were captured well enough for cross-sample comparison.

3.2. Phylum-level community composition

At phylum level, the three regional datasets were strikingly similar. Proteobacteria, Actinobacteriota, Firmicutes, and Bacteroidota were the main bacterial phyla across the sampled surfaces, and together they accounted for more than 80% of the community in each region. Proteobacteria usually occupied the largest share, although its dominance weakened in several wood-related samples from Foshan, Guangzhou, and Shanxi/Taiyuan. That decline is important because it suggests that wood surfaces may permit a broader redistribution of bacterial dominance than some mineral surfaces.

Actinobacteriota were common on brick, wall, and tile samples. Their presence fits well with what is known about many actinobacterial lineages: They tolerate dryness, low nutrient availability, and exposed mineral settings. Firmicutes were not evenly dominant, but they became conspicuous in particular niches, including statue surfaces, enclosed or cave-like wall spaces, and some wooden columns. The spore-forming capacity of many Firmicutes offers a plausible explanation for their success on surfaces exposed to drying, heat pulses, or intermittent disturbance.

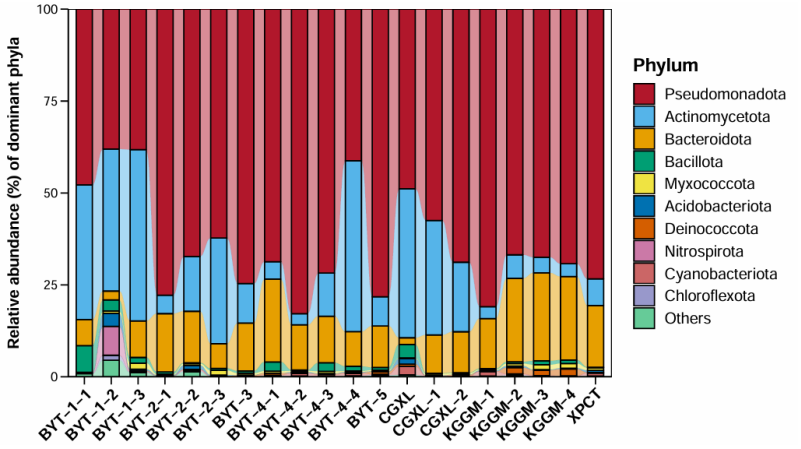

Figure 1. Dominant bacterial phyla in surface samples across regions.

3.3. Recurrent dominant genera and species

The genus-level results from Foshan and Guangzhou overlapped in several places. Pseudomonas, Sphingomonas, Acinetobacter, and Bacillus were repeatedly among the more abundant named groups. Pseudomonas appeared strongly in some wall and temple samples, which is plausible given the metabolic flexibility of the genus and its ability to use a wide range of organic compounds. Sphingomonas was more noticeable in several wooden components and column samples, where roughness, moisture retention, and limited nutrients may favor biofilm-forming oligotrophs. Acinetobacter was also detected in multiple samples, most likely reflecting its broad environmental distribution in dust, soil, water, and human-influenced settings.

Bacillus and related spore-forming taxa were especially visible in some statue, wall, cave-wall, and wood-column samples. From a conservation perspective, their presence deserves attention less because every Bacillus taxon is damaging, and more because spores allow these bacteria to persist through unfavorable periods and then respond quickly when moisture returns. In the Shanxi/Taiyuan material, many species-level assignments remained unclassified, which is itself informative: Historic building surfaces may contain lineages that are poorly represented in current reference databases.

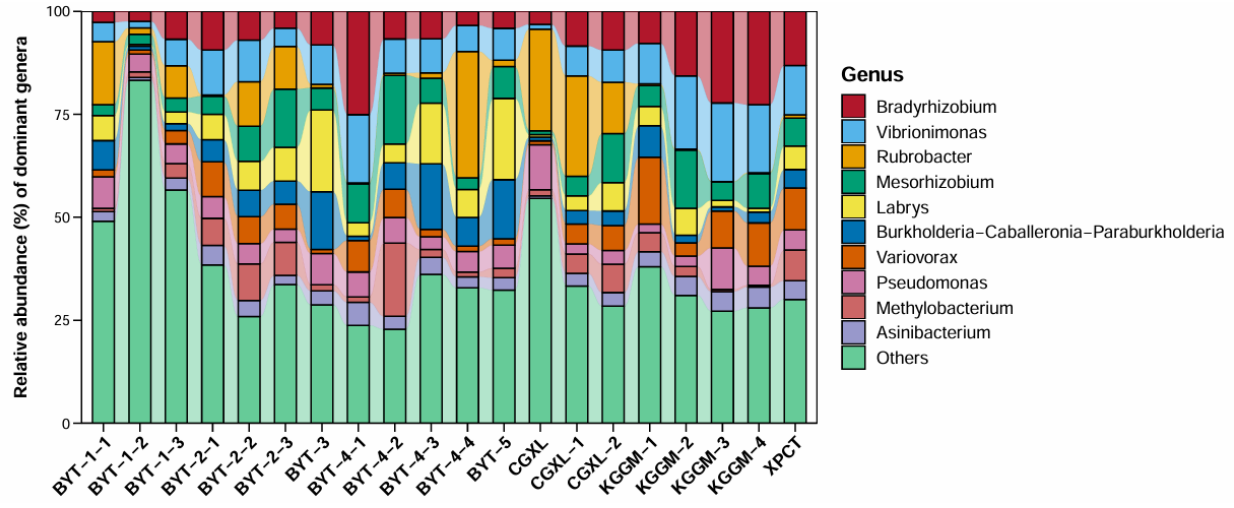

Figure 2. Relative abundance of dominant bacterial genera.

3.4. Beta diversity and community separation

In Foshan, the Bingyutang samples clustered apart from samples from Chegong Xianniang Temple and Kanggong Ancient Temple along the first principal component. In Guangzhou, samples from the Jian Clan Ancestral Hall and Zhanmei Jian Ancestral Hall separated along the main axis, while wooden columns and walls added smaller within-building differences. In Shanxi/Taiyuan, Mingxiu Temple samples were distinct from those of Chongshan Temple, Chunyang Palace, and Taiyuan Ancient County Chengguan Temple. These patterns show that the microbial community is not simply a regional climate signature; it also records the conditions of each building. Wood samples and brick or wall samples often occupied different positions even when they came from the same building. Across all three regions, the distance among buildings was generally greater than the distance expected from region alone. PERMANOVA detected significant compositional separation ($p < 0.001$), so the observed grouping cannot be dismissed as random scatter. The agreement between PCA and PCoA is useful because it shows that the main pattern does not depend on a single ordination method.

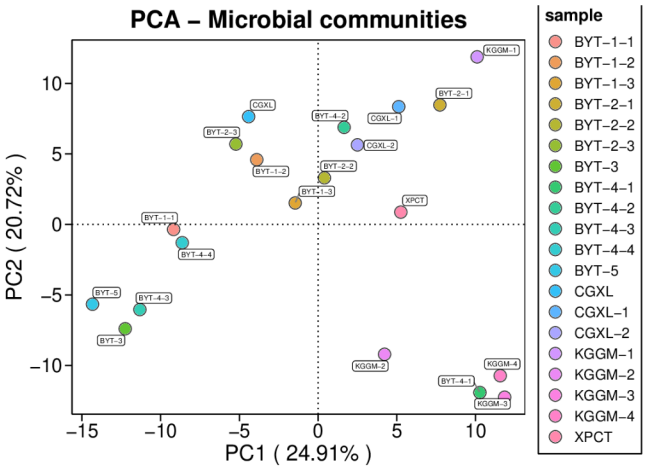

Figure 3. Principal component analysis of bacterial communities. samples clustered by building and substrate.

4. Discussion

Historic building surfaces share a common bacterial framework but exhibit local-specific variations. The four dominant phyla are widespread in heritage sites, shaped by harsh environmental filters of exposed architecture, yet core taxa do not eliminate building- and substrate-level differences. Ordination results indicate microbial communities vary with local conditions (ventilation, substrate, disturbances), so conservation planning requires fine-scale inspections rather than regional climate data alone, as microbial risk arises from interactions between regional exposure and local material properties. Wood-mineral substrate contrast strongly shapes communities: Wood favors biofilms while mineral substrates select for drought-adapted taxa, reflecting biodeterioration as a coupled process of material properties, microclimate, and microbial traits.

Repeated detection of Pseudomonas and Bacillus serves as warning markers for shifting microbial growth conditions (not direct damage indicators), particularly when combined with environmental and visual records. Heterotrophy dominates surface functions, and a tentative nitrification signal in Shanxi/Taiyuan samples requires further metagenomic or chemical verification. Study limitations include limited non-bacterial resolution, inferred functional data, uneven taxonomic comparison, and lack of continuous environmental and deterioration monitoring.

5. Conclusions

Across 72 samples from Foshan, Guangzhou, and Shanxi/Taiyuan, historic building surfaces showed both a shared bacterial core and strong local differentiation. Proteobacteria, Actinobacteriota, Firmicutes, and Bacteroidota dominated at phylum level, while Pseudomonas, Sphingomonas, Acinetobacter, and

Bacillus appeared repeatedly among the better-resolved taxa. PCA, Bray-Curtis PCoA, and PERMANOVA showed that building identity and substrate microenvironment structured the communities more clearly than broad regional grouping alone. Predicted functions were mainly chemoheterotrophic and aerobic chemoheterotrophic, with a modestly stronger nitrification signal in some northern samples. The conservation implication is direct: Microbial risk assessment should not stop at climate-zone classification, but should examine the particular buildings, materials, and microhabitats where microbial growth actually develops.

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