

Article

Metagenomics of the Surface of an Architectural Heritage Site: A Case Study of the Ji Family's Residence in the Southeast of Shanxi Province, China

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Abstract: Microbial corrosion poses a significant threat to architectural heritage worldwide. This study used metagenomics to investigate microbial diversity and taxonomic groups present in the door walls of the Ji family's residential houses, as well as their biological functions and chemical cycles. Taxonomic annotation revealed the predominant microbial taxa associated with wall corrosion, shedding light on their potential impact on structural integrity. Moreover, analyzing the metabolites and pathways present in these microbial communities allows for a thorough understanding of their functional capabilities. Our results revealed that areas with significant damage (dwelling bad door (DBD) and dwelling bad wall (DBW)) exhibited a higher microbial diversity compared to undamaged areas (dwelling good door (DGD) and dwelling good wall (DGW)), with variations in the occurrence of archaeal and bacterial species. The presence of bacteria was found to be connected with impaired function in DBW, whereas changes in the community patterns of *Sphingobium* and *Sphingomonas*, as well as a decrease in *Cercospora* proportion and an increase in *Fusarium* proportion, were correlated with damage in DBD. Both the Entner–Doudoroff (ED) route and sulfide oxidation processes were observed in both damaged locations (DBD and DBW). However, significant nitrogen-cycling mechanisms, including dissimilatory nitrate reduction to ammonium, were only found in DBW. Furthermore, DBD specifically detected the shift from methyl mercaptan (MMPA) to methyl mercaptan (MeSH). This research highlights the intricate interplay between microbial communities and the physical deterioration of residential structures, emphasizing the importance of understanding microbial ecology in mitigating such issues.

Keywords: built heritage; bio-weathering; microbiome function; nitrogen and sulfur cycling; metagenome



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1. Introduction

The Ji family's residential houses, located in Zhongzhuang Village, Gaoping City, southeastern Shanxi Province, China, are the research objects of this study [1]. The Ji family also refers to them as their "old houses". They were built in the 31st year of the Yuan Dynasty (1294). They are the earliest wooden residential buildings discovered in China so far, and they are still a unique example of residential buildings in this dynasty (A.D.1271–1368). The residence's architectural techniques and style, inherited from the Song and Jin Dynasties, are simple and a typical example of ancient rural architecture. On 20 November 1996, the announcement was made on the inclusion of the sixth group of national critical cultural relic protection units. As the environment changes, the microorganisms covering the surface of ancient architectural heritage also bring challenges to heritage protection. Despite the Ji family's residential houses being generally well preserved, their long history of over 700 years has also resulted in varying degrees of damage. Natural disasters like earthquakes have significantly tilted the four doorposts inward. Climate and environmental changes have also accelerated the natural weathering of residential buildings [2]. Furthermore, the burning of coal in Gaoping City and the emission of waste gas from chemical companies in Shanxi Province, a resource-rich province, have greatly affected the preservation and protection of architectural heritage [3]. The inevitable activities of humans in the thousand-year-old ancient villages and residential buildings will also intensify the challenge of safeguarding these architectural assets. The impact of microorganisms on architectural heritage is a crucial aspect to consider [4]. There is an intricate interaction between microbial communities and the physical deterioration of residential structures [5].

In 2007, the local government devised an overall maintenance plan for them. The thousand-year-old "Zhongzhuang Village", where the houses are located, has had residents for a long time [6]. To date, the protective measures that have been performed include structural reinforcements, treatments for weatherproofing, and normal maintenance procedures [7]. These steps have been implemented with the intention of preserving the physical integrity and historical significance of the houses that belong to the Ji family. Even though these attempts have been made, these solutions still face substantial problems when it comes to combating the persistent and pervasive impacts of microorganisms. Microorganisms are significantly eroding the Ji family's residential houses due to their long age and the multiple influences of nature and humans. The microbial community attaches to the walls of houses and the surfaces of wooden doors and windows, which not only affects the beauty of the architectural heritage but also causes erosion and damage [8]. The Ji family's residential houses must be protected immediately. Thus, we used metagenomics techniques to look at the different types of microbes and taxonomic groups in the homes of the Ji family. We also looked at the microbes' biological functions and chemical cycles and came up with ways to protect the homes based on the results of the experiments. This study has the potential to contribute new ideas and methods to the scientific protection of architectural heritage, particularly traditional residential architectural heritage. The current research clearly shows existing deficiencies in protecting traditional residential buildings, indicating a significant need for further research on the interaction between architectural heritage and microorganisms. This study hopes to gradually solve the problems of the physical deterioration of the Ji family's residential houses caused by microorganisms and provide a new research perspective for the protection and development of architectural heritage, further guiding the protection practices for architectural heritage in other regions. Simultaneously, we hope that the research ideas, methods, and viewpoints presented in this article will serve as valuable references for future related research.

2. Literature Review: Progress in Research on Microorganisms in Cultural Heritage

Since the beginning of human history, people have been constantly improving their living conditions and residential spaces through various technologies and means. Similarly, researchers are constantly inspecting and monitoring buildings, particularly those with historical value, to ensure their preservation or long-term use. In the past two decades, the methods for studying microorganisms in the built environment have become more diverse, and the research methods have become more scientific, such as those based on DNA sequencing. Kembel et al. believe that understanding the ecological and evolutionary processes of the diversity and composition of microbial communities living indoors is very important for determining the relationship between architectural design, biodiversity, and human health [9]. Scholars Verdier et al. outlined microbiological methods for evaluating microbial growth on different building materials and discussed different parameters for controlling it [10]. Adams and others explained the source and spatial distribution of microorganisms in buildings and believed that the composition and diversity of building microbial communities are the product of the interaction between buildings, residents, and air, with the layout of buildings directly or indirectly affecting the scale and direction of microorganisms [11]. Some scholars have also proposed linking design with the microbiology of the built environment, advancing knowledge of indoor microbiomes and transforming architectural practice to solve the current architectural design problems of sustainability and health [12].

The main research method of this study, metagenomics analysis, is often used in related research on marine life in many contexts [13–15], explorations in the development of human medicine [16,17], and the analysis of the role of microorganisms in various environments [18,19]. Of course, this technology is also gradually being used in the partial inspection and research of various cultural heritages, including architectural heritage. For example, by analyzing the metagenomics of microorganisms on the surface of smuggled marble statues, the storage environment and geographical transfer process in the past were inferred [20]; metagenomics analysis was used to analyze the stone building materials in cave temples to reveal the reasons for their biological degradation [21]; through the exploration of microbial biodegradation, possible methods and specific tools for diagnosis and treatment of the deterioration and degradation of artworks were sought [22]; and by analyzing the bacterial communities on the surfaces of a large number of cultural relics in museums, the differences in the bacterial distribution of cultural relics of different materials were obtained [23]. Despite the growing breadth of this technology's application, the threat of microbial biodegradation to global cultural heritage remains largely unexplored [24]. Using metagenomics analysis from an ecological point of view to look at and study microorganisms in cultural heritage will help in making correct and safe diagnoses for different types of historical buildings, ensuring their long-term protection.

3. Materials and Methods

3.1. Site Description and Sampling

3.1.1. Research Objects: The Ji Family's Residential Houses

Samples were collected from the surface of the dwelling belonging to the Ji family's residential houses, located in Zhongzhuang Village, Chenqu Town, 18 km northeast of Gaoping City, in the southern region of Shanxi Province, China (coordinates: 113°03'97" E, 35°85'05" N) (for specific location information, please refer to Appendices A and B). The residential structure was unearthed in the year 1986 as part of a comprehensive survey of cultural artifacts in Gaoping City. The inscription on the left door pier reads "In the 31st year of the Yuan Dynasty (A.D.1271–1368), the year of Jiawu, stonemason Feng of Tiandang

County built the house of the Ji family”. The exact date, more than 700 years ago, marks the construction of the residential building [1]. These words clearly record the construction time of the Ji family’s residential houses, the owner’s surname, and the engraver’s identity, which greatly enhances the historical value of this building. This dwelling represents one of the earliest examples of wooden residential buildings found in China and remains a unique representation of Yuan Dynasty (A.D.1271–1368) residential architecture [25].

3.1.2. Sampling

Samples were collected on 28 April 2023, using sterile cotton swabs to wipe the surfaces of both preserved and damaged areas of wooden door frames, lapis lazuli gate piers, and column bases (Figure 1). The four sampling points (A, B, C, D) are identified as the column base, door pier, door frame, and door window. In addition, A, B, C, and D also correspond to samples of four different situations as follows: A for dwelling good wall (DGW), B for dwelling bad wall (DBW), C for dwelling good door (DGD), and D for dwelling bad door (DBD). The DGW sample was collected from an exposed eave column made of sandstone with a square cross-section. The sampling area has a rough surface with a pronounced texture, showing signs of wear, scratches, and some areas that have faded to white. The DBW sample was taken from a square door pier made of limestone, which is relatively smooth overall, and features include carved characters and noticeable cracks [26]. A cat passage is left beside the door pier, and the sampling area shows some degree of wear. The DGD sample was collected from wooden window grilles, made of locally common pine. The sampling area is quite smooth with no clear signs of damage. The DBD sample was taken from a wooden door frame also made of pine. The sampling area has a rough and uneven surface, with visible cracks and round holes, as well as some areas where the wood bark has peeled off. All four sampling points are sheltered by eaves and are semi-exposed to sunlight and weather conditions. For each sampling point, three adjacent areas were wiped, and the samples were combined into a single sample for subsequent library preparation and sequencing analysis. These samples were subsequently placed in sterile self-sealing bags and stored in a dry refrigerator. They were then transported to Shanghai Biogenuintech Co. Ltd., where they were stored at -80°C until further processing and analysis.

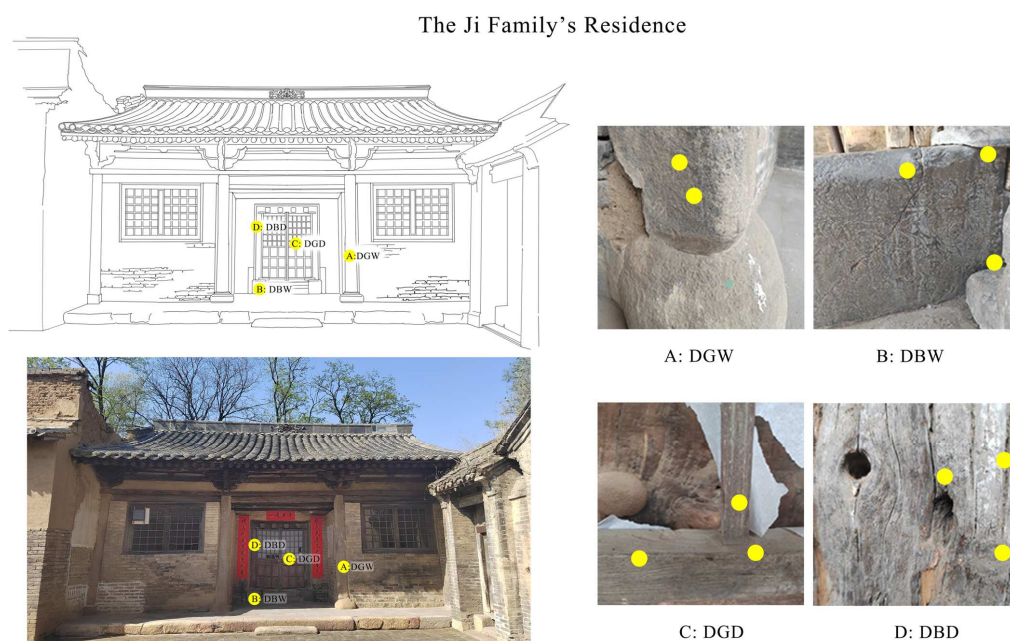


Figure 1. The Ji family residence’s facade displays the distribution of the sample collection. The four sampling points (A–D) are identified as the column base, door pier, door frame, and door window.

In addition, (A–D) also corresponds to samples of four different situations, namely dwelling bad door (DBD), dwelling good door (DGD), dwelling bad wall (DBW), and dwelling good wall (DGW).

3.2. DNA Extraction, Library Construction, and Next-Generation Sequencing

The extraction and purification of total genomic DNA from each sample were performed using the TIANamp Soil DNA Kit (TIANGEN BIOTECH, Beijing, China) in accordance with the instructions provided by the manufacturer. The concentration of the extracted DNA was assessed using the Qubit4 instrument manufactured by Thermo Fisher Scientific (Waltham, MA, USA). A small amount of extracted DNA from the sample, less than 1 ng (ranging from 0.1 to 2 ng/μL), was subjected to a transposase reaction mix for library construction. Briefly, the transposase reaction mix consisted of 5 μL of 2 × TD buffer, 2.5 μL of transposase obtained from BioGenuineTech in Shanghai, China, and 1.5 μL of nuclease-free water. The fragmentation process was conducted for a duration of 10 min at a temperature of 55 °C. The fragmentation mix was subjected to amplification for a total of 12–18 cycles using the Phusion enzyme, along with the MGI adaptor DNA library kit (manufactured by BioGenuineTech, located in Shanghai, China). The resulting amplified DNA samples were then sequenced using the DNBSEQ-T7 platform (developed by MGI) with a paired-end read length of 150 base pairs, resulting in a total sequencing capacity of 6 G.

3.3. Metagenome Assembly, Taxonomy, and Functional Annotation

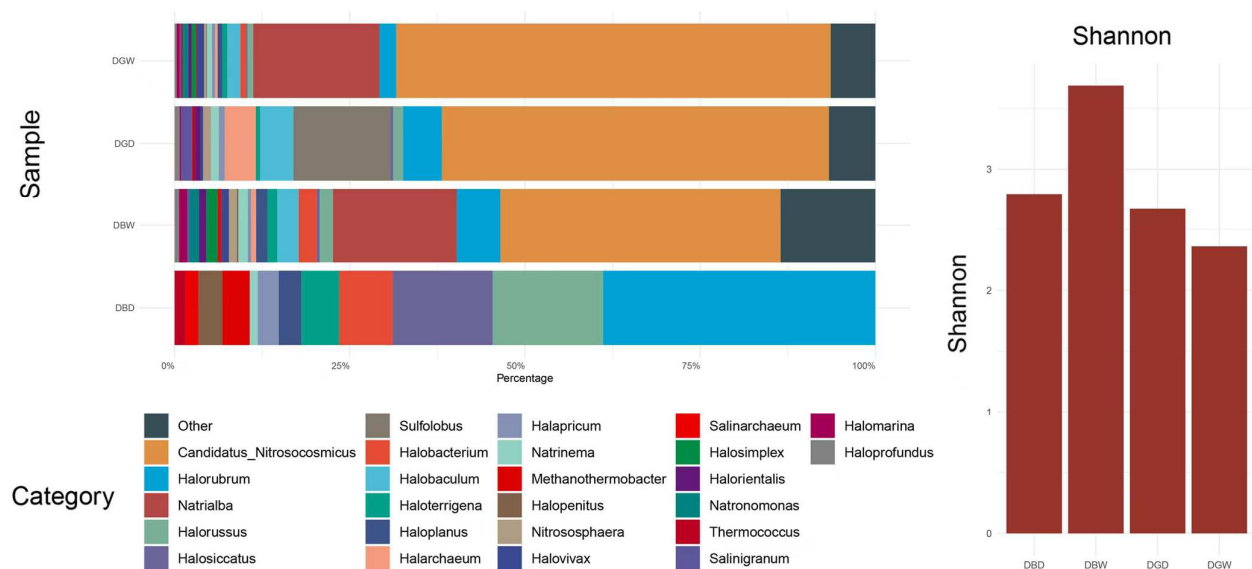
The MGI metagenomic reads underwent processing using fastp [27] with specific parameters (length required is 50, qualified quality phred is 15, unqualified percent limit is 40, n base limit is 10) in order to filter out low-quality reads and trim adapter sequences. Clean read assembly was performed using MEGAHIT (v1.2.9) as described for metagenome-assembled genomes [28], where the contigs with a length greater than 200 base pairs were employed for ultimate annotation and gene prediction. Then, clean read alignment was performed using the Burrows–Wheeler Alignment tool (BWA-mem2) [29]. Subsequently, a genome quality check was conducted and counts for unigenes were determined using checkm [30]. The taxonomic classification was obtained by Kraken2 [31], while the unigenes were subjected to blasting using diamond against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The functional annotation was further analyzed using EggNOG [32]. The main microbial communities were quantified by calculating the percentage of reads aligned to relevant species in the metagenome relative to the total number of reads. The Shannon index was utilized to assess the diversity of the microbial communities. Additionally, the Cluster of Orthologous Groups of proteins (COGs) database was employed to enrich the biological functions of the samples, allowing for the identification of key functional traits associated with the microbial populations. The utilization of DiTing was found to be more favorable in comparison to the analysis of biogeochemical cycles, as indicated in a recent study [33].

4. Results

4.1. Microbial Community Composition

To investigate the impact of the environment on the Ji family's residences, particularly near heavily damaged doors, we vigorously wiped the surfaces of both the good and decayed areas of the lapis lazuli gate piers and door frames with cotton swabs. As noted in previous studies, deadwood bacteria and fungi predominantly inhabit wooden structures while accompanying carbon and nitrogen cycles [34]. In contrast, stone surfaces are primarily associated with *Pseudonocardia*, *Sphingomonas*, and *Streptomyces*, along with a focus on nitrogen cycling [35]. The main microbial communities of archaea are illustrated

in Figure 2a. The prevalence of the archaea genus in DGW and DGD is mostly attributed to *Candidatus Nitrosocosmicus*, which accounts for almost 50%. The presence of *Candidatus Nitrosocosmicus* in DBW remains but with a notable decrease in occupancy. *Candidatus Nitrosocosmicus* has limited presence within the DBD dataset; however, there is a notable rise in the prevalence of *Halosiccatus* and *Halorussus*. This observation implies that the occurrence of breakage may be attributed, at least in part, to these aforementioned genera. The observed decline in *Candidatus Nitrosocosmicus* was concomitant with an elevation in both *Halosiccatus* and *Halorussus*. The distribution of bacterial communities of genera is displayed in Figure 2b. A disparity in bacterial communities between DGW and DBW can primarily be attributed to the heightened proportion of alternative genera. It is indicated to be plausible that bacterial contamination could be a contributing factor to the corrosion of DBW (Figure 1, B). A comparison of bacterial communities between DGD and DBD revealed a significant decrease in *Pseudomonas* proportion in DBD. Additionally, the proportion of *Serratia* was also reduced, while the proportion of *Sphingobium* and *Sphingomonas* exhibited a significant increase. These findings suggest that the altered community of *Sphingobium* and *Sphingomonas* may be the primary factor contributing to the breakage of DBD. An additional examination of the fungal communities of genera in various samples reveals that in comparison to bacteria and archaea, the presence of fungi exhibits a more pronounced diversity across different samples in DGW and DGD. Notably, there is an augmented proportion of *Ustilago* specifically in DBW. A comparative analysis of the fungal community in samples from individuals with DGD and DBD demonstrates a noteworthy reduction in *Cercospora* proportion and a notable elevation in the proportion of dominant microbial communities of *Fusarium*, which is associated with the observed damage in DBD. Additionally, a slight increase in the abundance of *Saccharomyces* and *Malassezia* is also evident.



(a)

Figure 2. Cont.

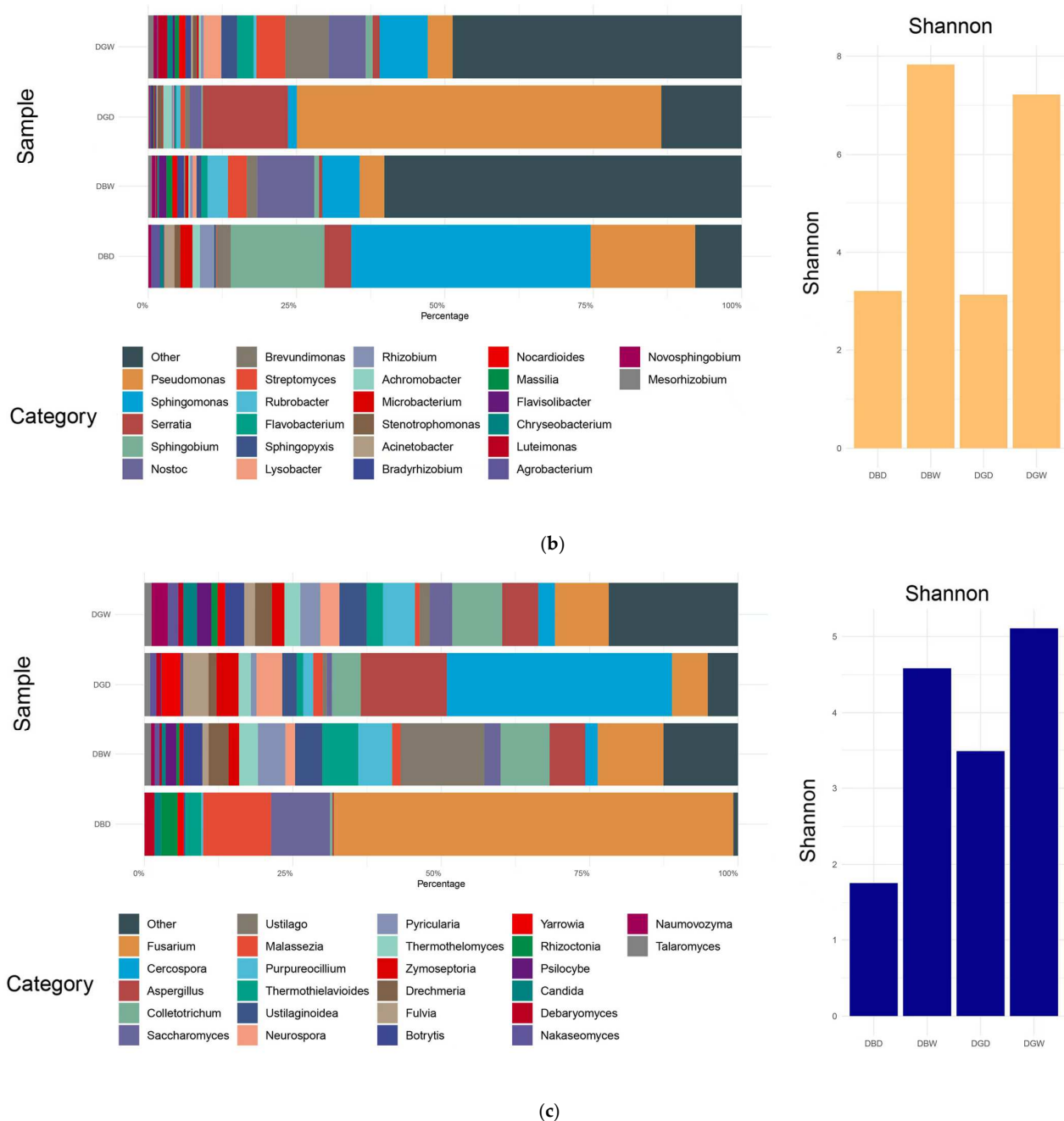


Figure 2. Genus-level identification of (a) archaeal, (b) bacterial, and (c) fungal communities and the alpha diversity from a dwelling of the Ji family's residence (ancient China).

4.2. Microbial Diversity

The taxonomic composition of bacteria, archaea, and fungi was obtained from metagenomic data and is categorized at the genus level. This information is visually shown in Figure 2. The Shannon Diversity Index was employed to assess the within-sample alpha diversity of each metagenome. This index provides insights into the community richness and evenness, hence highlighting variations observed across different sampling locations in this study. The Shannon diversity values were 3.22, 7.83, 3.14, and 7.22 for bacteria; 2.80, 3.68, 2.67, and 2.36 for archaea; and 1.76, 4.58, 3.48, and 5.11 for fungi from DBD, DBW, DGD, and DGW samples, respectively. As shown in the Shannon diversity values, overall, the biodiversity on the walls is higher than that of the wooden structures. In archaea, the diversity of damaged buildings is higher than that of intact buildings. Conversely, in fungi,

the diversity of damaged buildings is lower than that of intact buildings. No significant differences were found between good and bad buildings in bacteria.

4.3. Composition of Dwelling Microbial Communities in Phylum

The overall taxonomic composition of the dwelling microbial communities, determined from the metagenome data (see the Supplementary Materials for specific data), is summarized at the phylum level in Figure 3. The taxonomic compositions of microbial communities on stone surfaces are diverse and play a vital role in understanding their ecological activities and future biotechnological uses [36]. Other bacterial phyla detected in these communities included *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Chloroflexi*, and *Firmicutes* from all samples in this study. Ascomycota, the only major eukaryotic group in these stone metagenomes, accounted for 10 to 3.5% of the total microbiome, with the highest abundance in DBW and the lowest in DBD. The archaeal phyla *Euryarchaeota* and *Thaumarchaeota* were detected in the metagenome, but they contributed only approximately 1% or less. Comprehending the taxonomic makeup of these microbial communities is crucial for investigating their possible effects on stone deterioration, bioremediation, and other ecological processes [37,38].

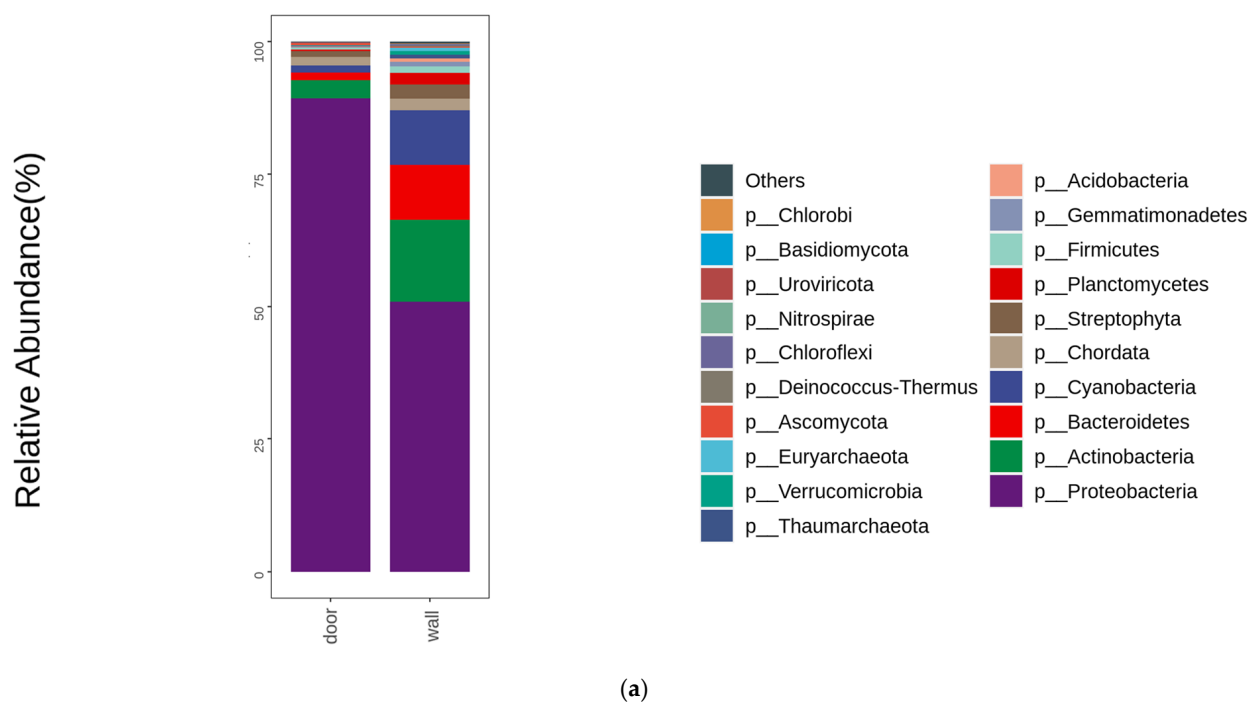


Figure 3. Cont.

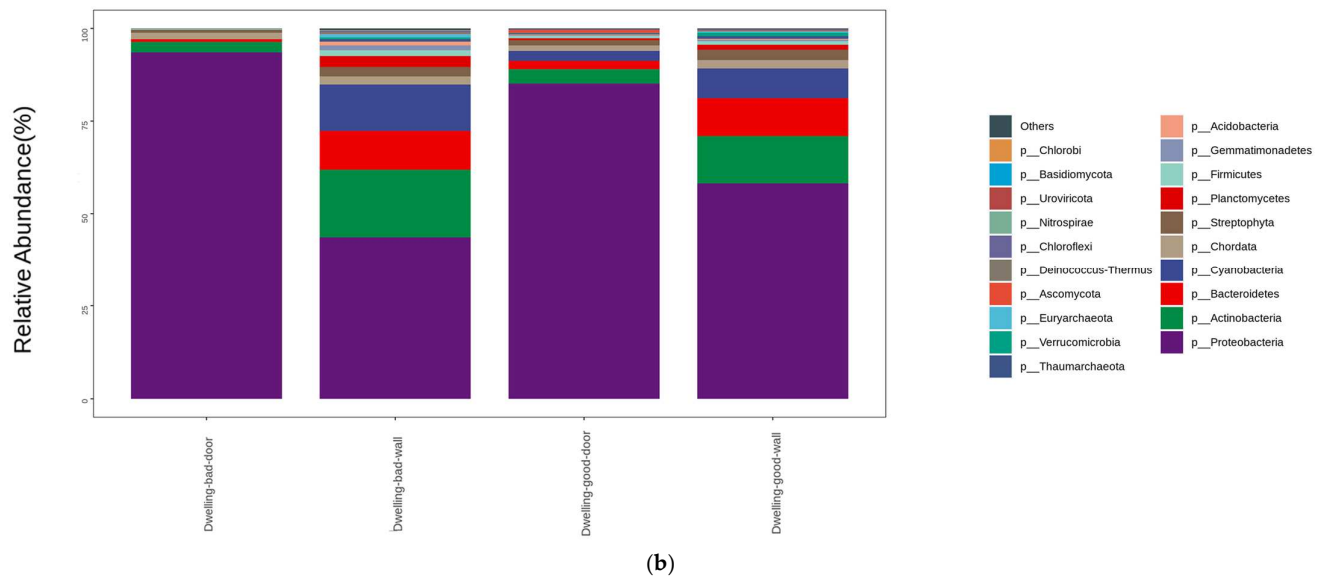


Figure 3. Metagenomic distribution from each sample. (a) Bar plot of phylum distribution between door and wall. (b) Bar plot of phylum distribution.

4.4. Microbial Functions Annotated by Cluster of Orthologous Groups of Proteins (COGs)

The enrichment analysis of the Cluster of Orthologous Groups of proteins (COGs) revealed the primary functions of microbial communities (Figure 4). Among these functions, metabolism constitutes the highest proportion, followed closely by cellular processes and signaling. Furthermore, it was observed that the metabolism associated with wooden structures has a higher functional representation compared to that associated with stone structures, with the difference being most pronounced in the DNA-binding domain (DBD). Additionally, the metabolism and cellular processes and signaling in damaged structures were found to be higher than those in well-preserved structures.

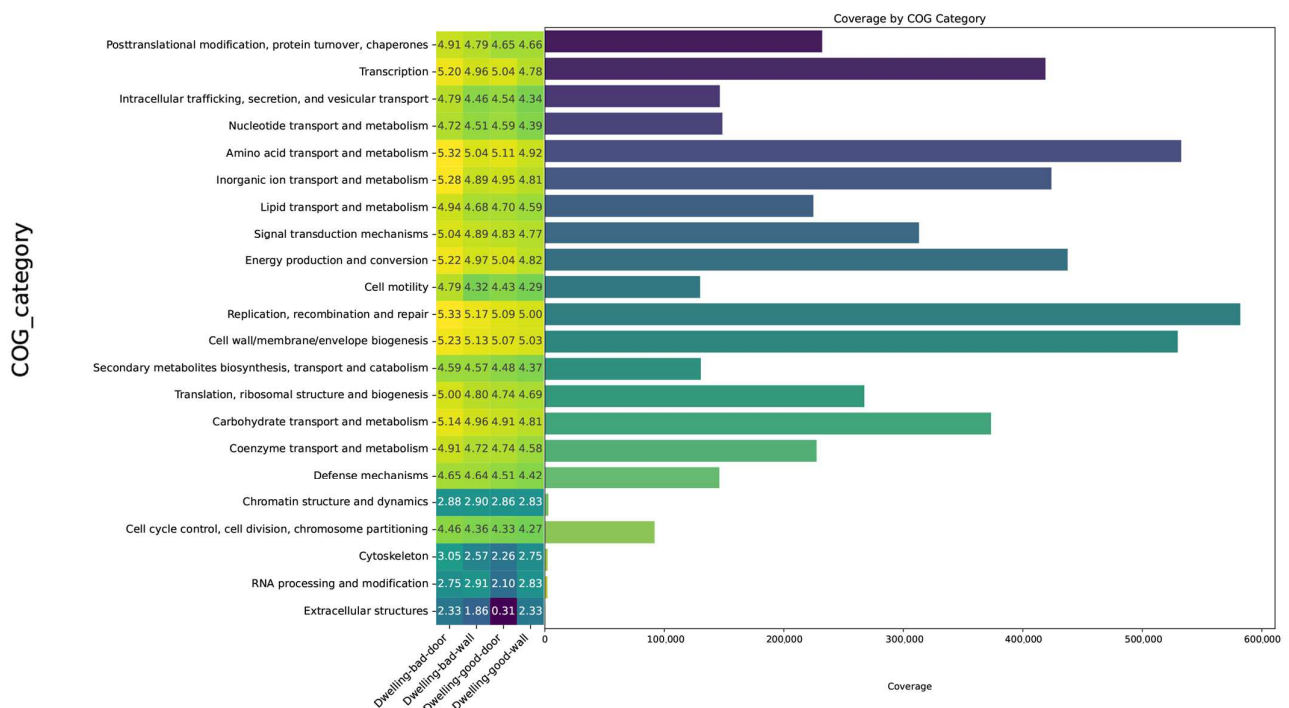


Figure 4. Enrichment of Cluster of Orthologous Groups of proteins (COGs) analysis in each sample, with heat map for log10 (coverage) from each sample and bar plot for total read coverage visualization.

4.5. Metabolic Mapping of Geomicrobiological Cycles

The metabolic mapping of geomicrobiological cycles can clarify that microbes play a crucial role in these cycles by mediating various biogeochemical reactions. It can help us identify the types of microorganisms present in a particular environment and understand how these microbes contribute to the cycling of elements in the ecosystem. With the development of techniques, we can analyze the genetic, transcriptomic, and proteomic profiles of microbial communities with various techniques, including metagenomics and metaproteomics [39]. This information is then used to map the metabolic pathways involved in geochemical cycles. Thus, the metabolic mapping of geomicrobiological cycles enhances our understanding of the intricate relationships between microorganisms and their environments, shedding light on the processes that drive geochemical cycles essential for the functioning of ecosystems. A summary sketch for the visualization of these pathways was generated by DiTing.

4.5.1. Carbon Cycling

The carbon cycle is widely acknowledged as a crucial element within biogeochemical processes, contributing to carbon fixation through processes such as photosynthesis with certain bacteria and archaea [40]. Photosynthetic autotrophic microorganisms transform inorganic carbon, typically carbon dioxide, into organic carbon compounds, while other microorganisms decompose complex organic matter into simpler forms, thereby releasing carbon dioxide back into the atmosphere. Here, a detailed KEGG analysis of the annotated metagenomic reads revealed that a substantial portion of the sequences aligned with prokaryotic CO₂ fixation pathways. Among these, genes linked to glycolysis and the tricarboxylic acid (TCA) cycle were particularly dominant (Figure 5). Additionally, less abundant pathways, including the Calvin–Benson–Bassham cycle, the 3-hydroxypropionate bi-cycle, and the reductive citric acid cycle, were also identified (Figure 5). Further analysis of the CO₂ fixation pathways highlighted a predominance of sequences associated with the conversion of pyruvic acid to lactic acid or acetic acid (Figure 5). Notably, the presence of the Entner–Doudoroff (ED) pathway was also detected (Figure 5), which contributes to the production of higher acid quantities, thereby accelerating the corrosion process on building surfaces.

By means of the production of acidic compounds, the study of the annotated metagenomic reads shows how microbial metabolic pathways speed up building damage. There were a lot of sequences found that were linked to the pyruvic-acid-to-lactic acid or acetic acid pathways, as well as the ED pathway. This shows that microbes are carrying out a lot of work to make organic acids. Because of these acids, like lactic and acetic acid, the pH level in the area around building materials decreases. When these acids come into contact with building materials like metals and concrete, they start chemical processes that break down the materials. This process not only weakens the concrete's structural matrix but also causes important minerals to leach out, which makes the concrete less stable. In another way, acids increase metal corrosion. Acidic conditions oxidize metals, causing rust in iron and steel and pitting in aluminum and copper. Corrosive processes weaken building structures, causing breakdowns and safety problems.

In addition, it was observed that the genes responsible for the transformation of carbon dioxide (CO₂) or acetic acid into methane (CH₄) and subsequently into formaldehyde, as well as the genes involved in the conversion of pyruvic acid to acetyl-CoA, were not detected in all of the metagenomes analyzed in this study.

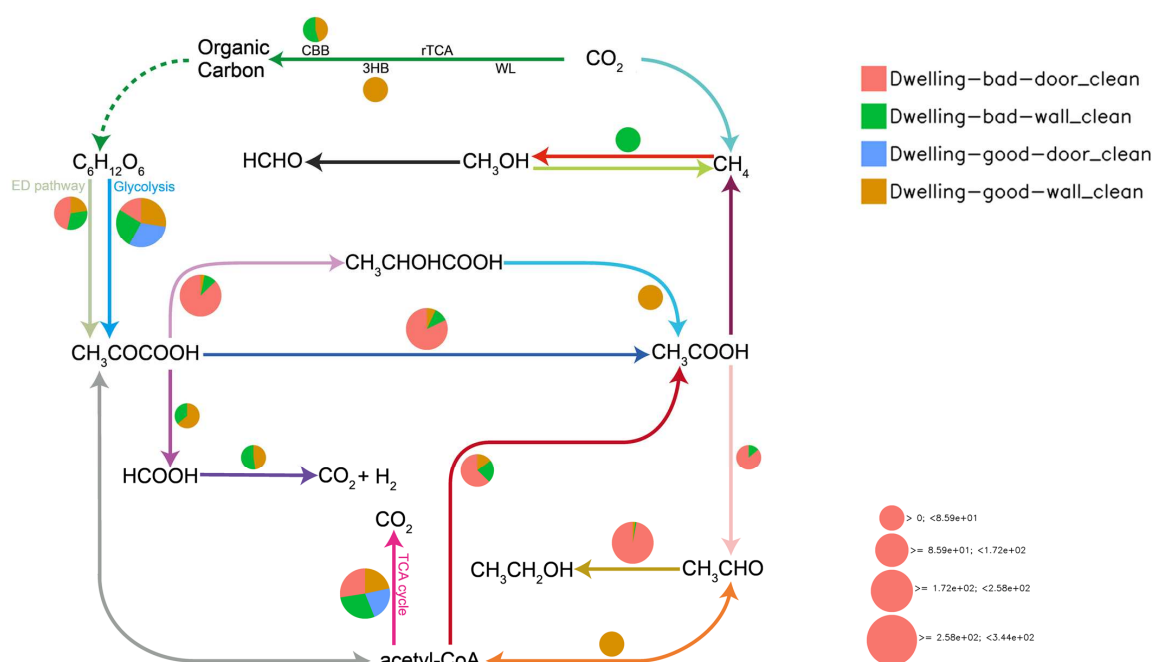


Figure 5. Carbon cycle diagram.

4.5.2. Nitrogen Cycling

Initially, it is important to note that ammonia-oxidizing/nitrifying bacteria, such as *Nitrosomanas* sp. and *Nitrobacter* sp., as well as ammonia-oxidizing archaea (AOA), engage in the process of oxidizing NH_3 [41]. This oxidation process serves to acquire energy or electrons, which are then utilized for the reduction of CO_2 into organic molecules that facilitate growth. The process of nitrification involves the conversion of NH_3 into nitrous and nitric acids by nitrifiers, which consist of bacteria and archaea. This conversion typically occurs through a two-step biochemical reaction, as illustrated in Figure 6. The damaged part of sandstone bas-reliefs in the samples exhibited a notable presence of nitrate, with a higher prevalence of ammonia-oxidizing archaea compared to bacteria on the sandstone surfaces, suggesting that archaea play a more significant role in the process of biodeterioration than bacteria (Figure 6).

The examination of the deteriorated sandstone bas-reliefs demonstrates a notable buildup of nitrate, suggesting ongoing nitrification. The greater abundance of ammonia-oxidizing archaea on the sandstone surfaces, as opposed to bacteria, indicates that archaea play a more significant role in the process of biodeterioration. This observation highlights the essential significance of AOA in the deterioration of sandstone [42,43].

The identification of genes involved in nitrogen metabolism can be inferred by the functional annotation of sequences obtained from the samples [44]. The metagenomic study reveals the metabolic process of ammonia oxidation, also known as nitrification. This process involves the entire conversion of ammonia to nitrate, as well as the subsequent transformation of nitrate into nitric oxide, dinitrogen oxide, and, ultimately, dinitrogen. The gene sequence discovered in the DBW sample exhibits a significant presence in many nitrogen-cycling processes, particularly demonstrating a notable abundance of dissimilatory nitrate reduction to ammonium [45]. The amount of nitric oxide, DBW, and DGW remained similar throughout the transformation processes of nitrate. The absence of DBD and DGD in the depicted figure suggests a potential correlation between nitrogen cycling and the specific sample locations and environmental conditions. Epilithic bacteria mostly obtain nitrogen via atmospheric pollution and animal waste, which contain ammonia and nitrogenous

oxides [46]. Nitrifying bacteria and archaea utilize these acids to generate nitrous and nitric acids, resulting in the subsequent erosion of samples with an acidic nature [47].

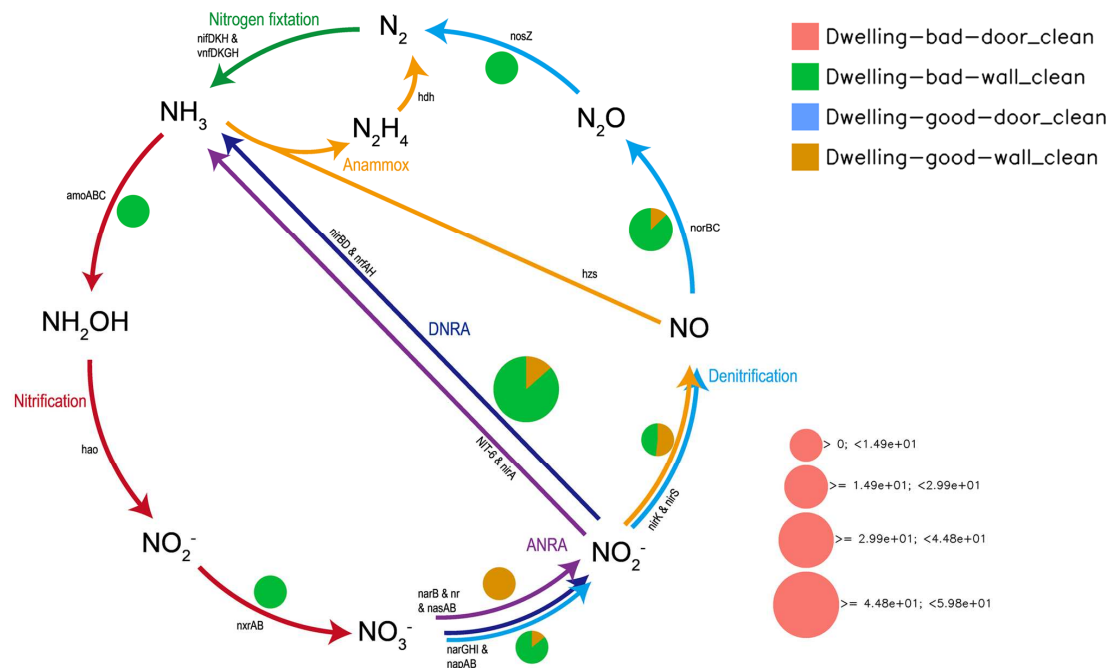


Figure 6. Nitrogen cycle diagram.

4.5.3. Sulfur Cycle

The sulfur cycle is intricately connected to microbial activity in the built environment. Sulfur-oxidizing and sulfur-reducing bacteria, which are diverse microorganisms, can be found in the built environment and have the ability to either oxidize or reduce sulfur. The former has the ability to convert sulfide into sulfuric acid, whereas the latter has the ability to convert sulfuric acid back into sulfide. These bacteria thrive and multiply in various ecological habitats and play a role in various phases of the sulfur cycle [48]. Additionally, they can absorb and accumulate sulfur compounds, which speeds up the corrosion of building surfaces. Microorganisms and building materials have multiple interactions. Microorganisms can adhere to building materials and alter their physicochemical qualities through their metabolites, thereby causing corrosion or damage. Additionally, the characteristics of construction materials might impact the development and biochemical processes of microorganisms, consequently altering the sulfur cycle and corrosion.

The presence of microorganisms can greatly accelerate the degradation of construction materials. Sulfur-oxidizing bacteria generate sulfuric acid as a byproduct of their metabolism, which can cause the corrosion of concrete and metal surfaces due to its acidic properties. This process is especially worrisome in sewer systems, water distribution networks, and buildings that are exposed to humid conditions. The formation of biofilms by these microorganisms intensifies this phenomenon by creating a sheltered environment for microbial communities, hence facilitating the buildup and concentration of corrosive substances. In addition, sulfur-reducing bacteria have the ability to convert sulfuric acid back into sulfide, thus contributing to the production of hydrogen sulfide gas. This gas exhibits both malodorous characteristics and potential hazards to human health and safety. Additionally, it has the ability to undergo chemical reactions with other compounds, resulting in the formation of corrosive substances such as iron sulfide. The interaction between these microbial processes and the materials they inhabit results in an intricate and frequently expedited breakdown route [49].

(1) Inorganic sulfur cycle

Geomicrobiological sulfur transformation processes are crucial in forming many terrestrial and marine ecosystems [50]. Sulfur, a necessary element, experiences diverse chemical and biological transformations assisted by microbial populations inhabiting specific environmental niches. Gaining knowledge of these processes is essential for understanding and reducing the deterioration of man-made structures, especially the corrosion of buildings.

Sulfides, particularly inorganic sulfur compounds, play a key role in the development of corrosion in buildings. The genomic analysis of samples revealed that areas exhibiting apparent damage (DBD and DBW) were mostly affected by the process of sulfide oxidation, where hydrogen sulfide is converted to sulfide, as well as reduction reactions involving sulfate being converted to hydrogen sulfide by assimilatory sulfate reduction (Figure 7). These reactions often occur in settings lacking oxygen, which are commonly found in groundwater and soil. The multi-step process entails utilizing organic or inorganic chemicals as electron donors to decrease microorganisms and convert sulfate ions into hydrogen sulfide. This mechanism is essential for the environmental sulfur cycle and has a vital function in both natural and constructed environments [51].

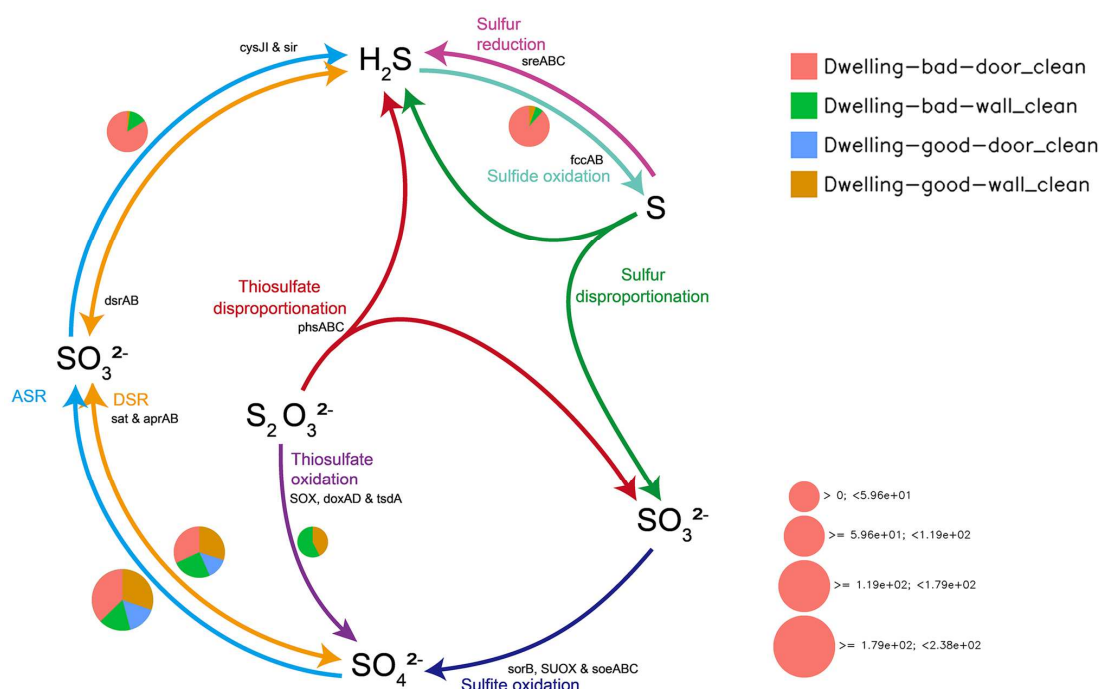


Figure 7. Inorganic sulfur elemental cycle diagram.

Specifically, doors found in residences have been linked to the process of thiosulfate oxidation, where $\text{S}_2\text{O}_3^{2-}$ is converted to SO_4^{2-} (Figure 7). Microbial communities have a crucial role in facilitating the process of thiosulfate oxidation, which is a significant alteration of sulfur [52]. Specific microbial communities facilitate the oxidation of thiosulfate to sulfate, hence playing a role in the total sulfur cycling in the environment. The existence of these sulfur conversion mechanisms signifies the active interplay between bacteria and sulfur compounds, which ultimately impacts the deterioration of structures.

(2) Organic sulfur cycle

Dimethyl sulfoniopropionate (DMSP) is primarily found in marine ecosystems and is a type of metabolite produced by marine microorganisms [53]. While DMSP itself is not typically directly engaged in the corrosion process of building materials, its oxidation product, dimethyl sulfide (DMS), may be associated with building corrosion, particularly when there is metal present in the building's structure. From the genome sequencing, we

can obtain the conclusion that the DBD showed a relationship with the transfer from MMPA to MeSH. From the results, we can conclude that DBD exhibits a significant association with the transfer from MMPA to methyl mercaptan (MeSH) (Figure 8). The changes in the makeup of the microbial community emphasize the necessity of carrying out targeted microbial management in order to combat the degradation of architectural heritage that is caused by organic sulfur [54]. Particularly vulnerable are the regions surrounding metal components, such as nails and fasteners on wooden doors and windows. These areas may be particularly sensitive to damage. Following the completion of the genome sequencing, we are able to draw the conclusion that DBD has a substantial correlation with the transfer of methyl mercaptan (MeSH) from methyl mercaptan (MMPA) (Figure 8). It is possible that the presence of metals in the building, such as nails or fittings, could make these microbiological activities easier to carry out, which would result in a more rapid deterioration of the structure. Dimethyl sulfide (DMS) undergoes oxidation in the environment, resulting in the production of sulfuric acid [55]. Sulfuric acid is a potent acid that has the potential to cause corrosion on metal surfaces. The DMSP cycle indirectly contributes to the process of building corrosion by means of its oxidation product DMS. Furthermore, the byproducts of DMSP degradation may also include sulfide, which is a prevalent contributor to the corrosion of buildings. In a moist environment, sulfide reacts with the metal surface, resulting in the formation of sulfide precipitation. By adding protective measures into current conservation techniques, it is feasible to reduce the influence of microbial populations on the architectural history of the Ji family's residential homes. The need for focused microbial control becomes apparent, and the utilization of metagenomic analysis is essential for the development and improvement of these techniques. This technique not only improves the protection of historical buildings but also offers vital knowledge for the preservation of other cultural heritage sites that encounter comparable difficulties.

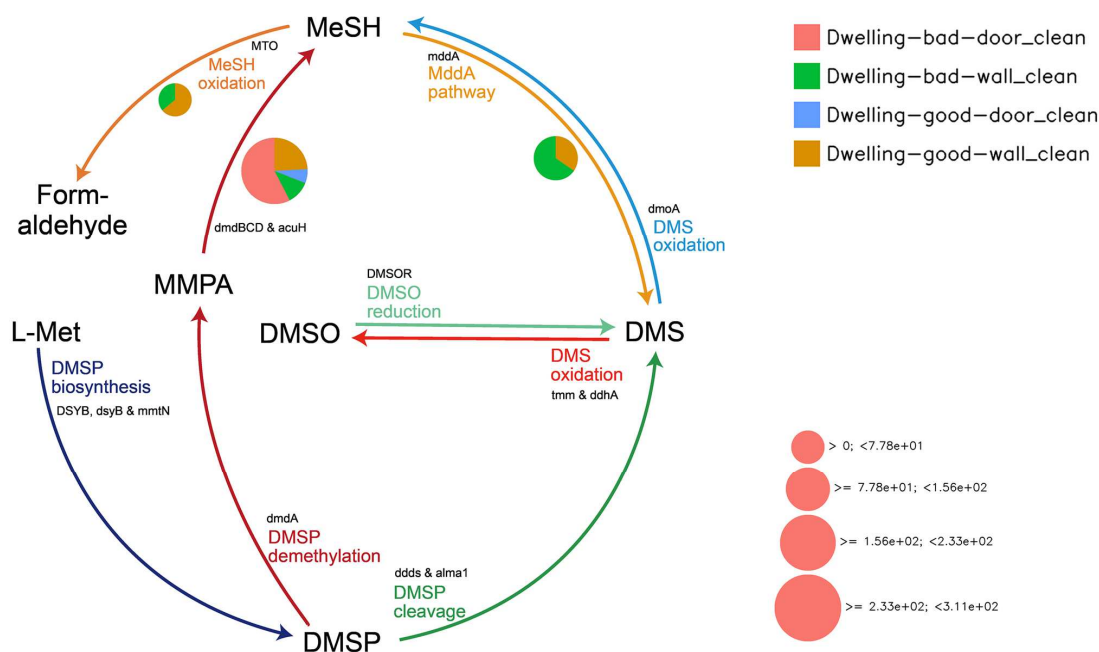


Figure 8. Organic sulfur elemental cycle diagram.

5. Discussion

Studying the microbial community composition and variety in relation to building corrosion yields useful information about the complex connections between microorganisms, geochemical cycles, and the decay of building materials [34,35,56]. This study utilized metagenomic sequencing to examine the taxonomic makeup of bacteria, archaea, and fungi

found in stone environments. As noted in previous studies, the composition of wooden doors in the Ji family's residences is primarily dominated by *Pinus densiflora*, while the stone columns are mainly associated with limestone and sandstone [1]. The examination of microbial community composition showed notable changes in the predominance of archaeal, bacterial, and fungal genera among different sampling locations. *Candidatus Nitrosocosmicus*, *Halosiccatus*, and *Halorussus* were discovered to be linked to the corrosion of building materials, indicating their probable involvement in the degradation process [57]. Furthermore, alterations in the manifestation of bacterial genera, including *Pseudomonas*, *Serratia*, *Sphingobium*, and *Sphingomonas*, were detected, suggesting their participation in the development of corrosion mechanisms [58]. In addition, the analysis of metabolic processes in geomicrobiological cycles has revealed the involvement of microorganisms in the cycling of carbon, nitrogen, and sulfur. These cycles play a crucial role in the corrosion of buildings [59]. The research on the carbon cycle showed that there are pathways for fixing CO₂ and certain metabolic pathways, such as glycolysis and the tricarboxylic acid (TCA) cycle, that could potentially contribute to the corrosion process [60].

The apparent damage detected in DBD and DBW samples had elevated levels of microbial diversity in comparison to undamaged areas, specifically in relation to archaeal and bacterial genera. The identification of bacteria such as *Sphingobium* and *Sphingomonas* in places that have been affected by damage suggests that they may play a significant role in breaking down organic carbon [61]. These bacteria had the capacity to break down intricate organic molecules, indicating that the breakdown of organic carbon might be a factor in the observed structural harm. The modified manifestation of these genera highlights the significance of monitoring and controlling microbial communities to alleviate carbon-related deterioration processes in architectural heritage.

In addition, the investigation of the nitrogen cycle emphasized the importance of ammonia oxidation and nitrification processes in the development of corrosion, with archaea potentially having a greater impact than bacteria [21,62]. This study found that damaged areas experience frequent nitrogen-cycling mechanisms, particularly a high number of dissimilatory nitrate reductions to ammonium in DBW. In a dry and high-temperature environment, nitric acid oxidation may produce ammonium, leading to the corrosion of stone columns. This process can result in the buildup of ammonium, which can potentially cause the removal of NO₃[−] and increase NH₄⁺ in building materials, causing the biodeterioration of sandstone [43]. The heightened nitrogen-cycling activity observed in the affected locations indicates that the microbial populations responsible for nitrogen transformations have a crucial impact on the degradation of the residential homes belonging to the Ji family. Implementing focused treatments to control these microbial processes could aid in maintaining the structural stability of the buildings.

Concerning the sulfur cycle, it was discovered that both inorganic and organic sulfur transformations are linked to the corrosion of buildings [63]. In the study of the acid-catalyzed gasification of pine wood, sulfuric and nitric acid can enhance the pyrolysis efficiency of pine wood, resulting in the formation of both lower and higher concentrations of formic acid, glycolaldehyde, and acetol [64], while in the process of sulfuric acid cave formation, H₂S-rich water interacts with oxygenated groundwater, which is replenished by meteoric infiltration or atmospheric oxygen, leading to its oxidation to sulfuric acid. This sulfuric acid then immediately reacts with the carbonate bedrock, forming gypsum (CaSO₄·2H₂O) and carbon dioxide (CO₂) [65]. Sulfide oxidation processes were detected in both DBD and DBW samples, indicating the active involvement of sulfur-oxidizing microorganisms in the damaged regions. The presence of these processes in evident damage highlights the potential for sulfuric acid production, which can further corrode building materials. Understanding the dynamics of sulfur-oxidizing microbial communities

is crucial for developing strategies to prevent sulfur-induced degradation [66]. Additionally, the transfer from MMPA to methyl mercaptan (MeSH) observed in DBD points to specific sulfur transformations that may exacerbate material decay. Inorganic sulfur compounds, specifically sulfides, have been identified as factors in corrosion processes, where the oxidation and reduction reactions of sulfides contribute to the deterioration of metal surfaces [67]. The organic sulfur cycle, specifically the DMSP cycle, indirectly impacts the corrosion of buildings by generating sulfuric acid and sulfide, both of which contribute to the corrosion of metal surfaces.

Furthermore, the examination of diversity using the Shannon Diversity Index revealed clear variations in microbial diversity patterns among samples collected from different locations [68]. Samples obtained from regions with evident damage (DBD and DBW) demonstrated greater levels of variety in comparison to undamaged regions (DGD and DGW), notably in relation to the presence of archaeal and bacterial species. These findings indicate a possible link between the variety of microorganisms present and the extent of damage to buildings caused by corrosion.

In summary, our study offers a thorough understanding of the intricate relationship between microbial communities, geochemical cycles, and the processes of building corrosion. Through comprehending these connections, we can formulate more efficient approaches for reducing building corrosion and safeguarding architectural history. Future research should prioritize the investigation of the precise processes through which microorganisms contribute to the development of corrosion and the exploration of innovative methods for controlling microbial activity in architectural conservation projects.

For further protection, it is recommended to implement coatings or biocides that specifically target bacteria that oxidize or reduce sulfur. These treatments can be customized to impede the proliferation of detrimental microorganisms while safeguarding the structural integrity of the building components. Controlling the environmental variables, such as humidity and temperature, can create an unfavorable environment for the growth of microorganisms. Enhancing the airflow in confined areas to minimize the buildup of moisture can effectively impede microbial growth. Sophisticated construction materials that exhibit enhanced resistance to corrosion caused by microorganisms can be employed. One possible approach is to integrate antimicrobial chemicals into construction materials or create new materials that are naturally resistant to microbial attack. Regular surveillance systems can be deployed to identify the initial indications of microbial colonization and corrosion. One such approach is to utilize sensors or conduct regular inspections to detect areas of concern prior to the occurrence of substantial harm or to investigate the utilization of advantageous microorganisms that can outperform or counteract detrimental microorganisms. This technique would entail the introduction or promotion of non-corrosive byproduct-free microbial populations to foster a harmonious microbial environment on building surfaces. Providing education to individuals responsible for the upkeep and preservation of historical structures regarding the impact of microorganisms on the process of corrosion and offering instruction on the most effective methods for inhibiting the growth of microorganisms can contribute to the durability of these constructions. By incorporating these tactics, we may establish a complete methodology to safeguard structures from biodeterioration and maintain their historical and cultural importance. This study underscores the significance of metagenomic analysis in informing and guiding effective conservation practices for historical buildings.

Biotechnology development provides a new research perspective for the protection and development of architectural heritage. This research can further guide the protection and practice of architectural heritage in other regions. Currently, there are only a few

interdisciplinary studies examining the interaction between architectural heritage and microorganisms, and these studies have numerous limitations.

This research still has a lot of room for improvement. The limitations primarily lie in two areas as follows: (1) Natural aspects: The interaction between microbial communities and the physical deterioration of architectural heritage is very complex. Microbial ecology has a certain role in alleviating such problems, but it may not achieve the expected results due to the influence of many natural geographical factors. (2) Human aspects: Traditional residential buildings represent a distinct category of architectural heritage, intimately intertwined with people's daily lives. It is impossible to achieve completely scientific and institutionalized protection. The pollution problems surrounding coal mines and chemical plants cannot be solved in a short time. Currently, especially when there is a lack of cross-disciplinary research, researchers cannot propose scientific response plans and strategies in a timely manner, and it is even more difficult to implement them.

Biotechnology development provides a novel approach to safeguarding and enhancing architectural heritage through study. This research can provide valuable guidance for the preservation and implementation of architectural history in different areas. Presently, there is a limited number of interdisciplinary studies that investigate the connection between architectural history and microorganisms, and these studies have multiple constraints. This research still has ample potential for enhancement. The relationship between microbiological populations and the physical deterioration of architectural heritage is a highly intricate process. The role of microbial ecology in mitigating these issues may be hindered by various natural geographical characteristics, perhaps limiting the attainment of anticipated outcomes. Another factor to consider is the humanistic nature of traditional residential buildings. These buildings hold a unique position in architectural heritage and are closely connected to the daily lives of people. Attaining comprehensive scientific and institutionalized protection is unattainable. The environmental issues associated with coal mining and chemical industries are not easily resolved within a short timeframe.

It is necessary to advocate for more collaboration among specialists in microbiology, materials science, conservation, and environmental science to formulate holistic approaches for safeguarding architectural heritage. Advanced analytical techniques and modern analytical methods, including metagenomics, proteomics, and metabolomics, can be used to obtain a more profound understanding of the microbial communities responsible for corrosion and their unique metabolic processes. Future research can make substantial progress in preserving the architectural legacy for future generations by overcoming these restrictions and exploring these potential approaches.

6. Conclusions

Our findings indicate that the areas with significant damage, DBD and DBW, exhibited a greater microbial diversity compared to undamaged areas, DGD and DGW, with notable variations in the presence of archaeal and bacterial genera. Specifically, the presence of certain bacteria was linked to functional impairment in DBW, while changes in community composition—such as shifts in *Sphingobium* and *Sphingomonas*, along with a decrease in *Cercospora* and an increase in *Fusarium*—were associated with damage in DBD. Both the ED pathway and sulfide oxidation processes were observed in the damaged areas, DBD and DBW. However, significant nitrogen-cycling mechanisms, including dissimilatory nitrate reduction to ammonium, were exclusively identified in DBW. Additionally, DBD showed a specific shift from MMPA to MeSH. This research underscores the complex interactions between microbial communities and the physical deterioration of residential structures, highlighting the critical role of microbial ecology in addressing such challenges.

Supplementary Materials: The source genome data related to this article have been submitted to the public database National Library of Medicine and can be accessed using the following link: <https://dataview.ncbi.nlm.nih.gov/object/PRJNA1170315?reviewer=936974ia6b8h9q8rkb8ii2ct18> (BioProject: PRJNA1170315) (accessed on 11 December 2024).

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Conflicts of Interest: Y.W. was employed by Shanghai Biogenuinotech Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. The Location Analysis of Ji Family's Residential Houses

The Ji family's residential houses face south and are built on a 42 cm high sandstone platform. The building is three bays wide and six rafters deep, with a gable roof. Sandstone forms the pillars, while four-layer brackets, without interlayer brackets, form the capitals. The main room in the center houses the door, but a corridor sets it back, aligning with the inner column. The secondary rooms, each featuring a large window, align with the eave column [26]. The building's roof is gently raised and folded, and the gable roof is not a five-ridge, six-beast roof but a ceramic ridge with flower patterns. The whole building gives people a feeling of vicissitude, stability, simplicity, and strictness. The Ji family's residential houses have a raised beam-type architectural structure. The structure of the two courtyards consists of four rafters and front-pressed nipples. The front and back eaves are of the three-column type. The sandstone used is all local, orange-yellow fine sandstone. It also features unique doors and windows [7]. The door is solid, with five cross-sections on the back and five iron door nails on the front, with six on each. Sandstone forms the ground, featuring a relief peony pattern on the front and a bluestone door pier on each side. An anvil directly sculpts the lower part of the door. Wood makes up the threshold, door cheeks, and door lintel. A frame adorns the outer side of the door cheeks and door lintel, uniting them in a "T" shape [26]. A 45-degree inclined lace decorates the right angle. The lace is based on a double-layer, superimposed, five-tooth arc-shaped petal wooden strip, and it is decorated with 45 openwork peony patterns on top. On the door lintel, there are four square door pins. The decoration of the window frame essentially mirrors

that of the door frame, with the exception of replacing the openwork with solid bamboo-shaped wooden strips, resulting in a slightly different decorative effect. The design divides the window core into two parts, left and right, and features a square window lattice for simplicity and practicality. The Ji family's residential houses, an important architectural legacy of the Yuan Dynasty in China, are clearly visible, and the daily monitoring and microbial analysis of the heritage are also very important. Ancient buildings in Shanxi, China, are primarily constructed of wood, brick, and other materials [69]. Wood contains components such as cellulose, hemicellulose, and lignin, which can provide nutrients such as carbon sources for microorganisms [70]. Some microorganisms, such as wood decay fungi, can decompose these components in wood, causing it to decay. Despite the relative stability of brick and stone materials, long-term weathering and rain erosion can cause tiny pores and cracks to appear on their surface. These pores and cracks can accumulate dust, moisture, and other impurities, creating an ideal environment for microorganisms to attach and grow. In addition, the structure of the Ji family's residential houses is complex, with many corners, gaps, and poorly ventilated areas [7]. For instance, the brackets, mortise, and tenon structures are susceptible to dust and moisture accumulation, making them difficult to clean, ventilate, and dry. Consequently, these areas may serve as a breeding ground for microorganisms [71,72]. Moreover, a blocked roof drainage system of the ancient building may lead to the accumulation of rainwater on the roof or under the eaves, thereby increasing local humidity and fostering microbial growth.

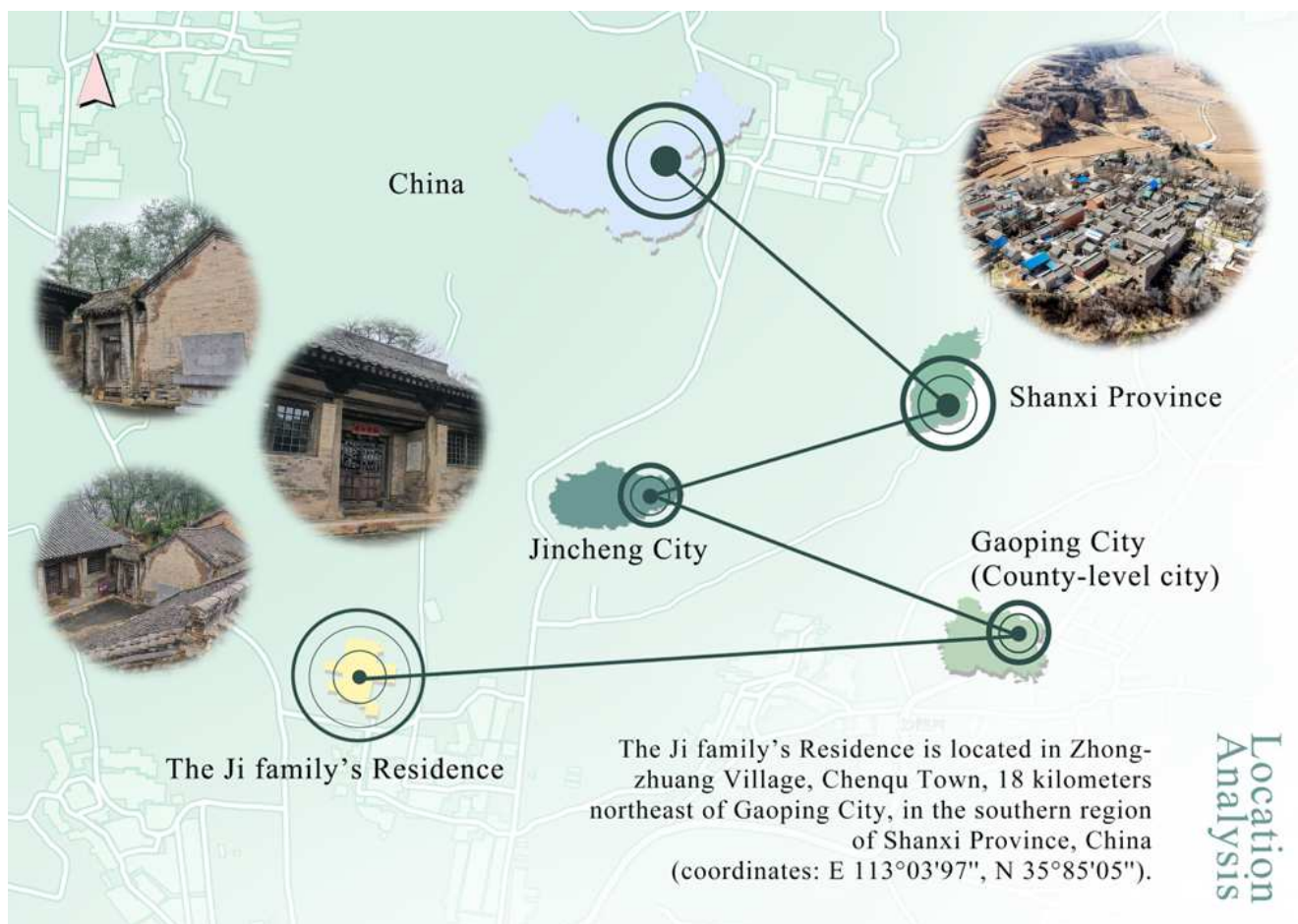


Figure A1. The location analysis of Ji family's residential houses.

Appendix B. Site Climate Analysis

The Ji family's residential houses are located in the hills, surrounded by mountains, overlapping mountains, ravines, and scattered villages, except for the southwest corner, which is an open area. From north to south, two seasonal rivers from the east and west flow through the whole area, merging into the Dongcang River and then into the Dan River. The climate in the surrounding region exhibits characteristics of a warm, temperate, humid continental monsoon climate, with an average annual temperature of 10 °C (Figure A2). The maximum temperature recorded in this area reaches 30 °C, while the minimum temperature can drop as low as −20 °C. The mean annual precipitation is from 500 to 550 mm, with the majority occurring throughout the months of July to September. Additionally, the duration of the frost-free period spans 185 days. Within the appropriate temperature range, the metabolic rate of microorganisms will increase accordingly for every 10 °C increase in temperature. For example, some bacteria grow and reproduce rapidly at temperatures between 20 and 37 °C. In the summer, the temperature in some parts of Shanxi is often within this range. If there are nutrients and moisture conditions suitable for bacterial growth on the surface of ancient buildings, bacteria may multiply in large numbers.

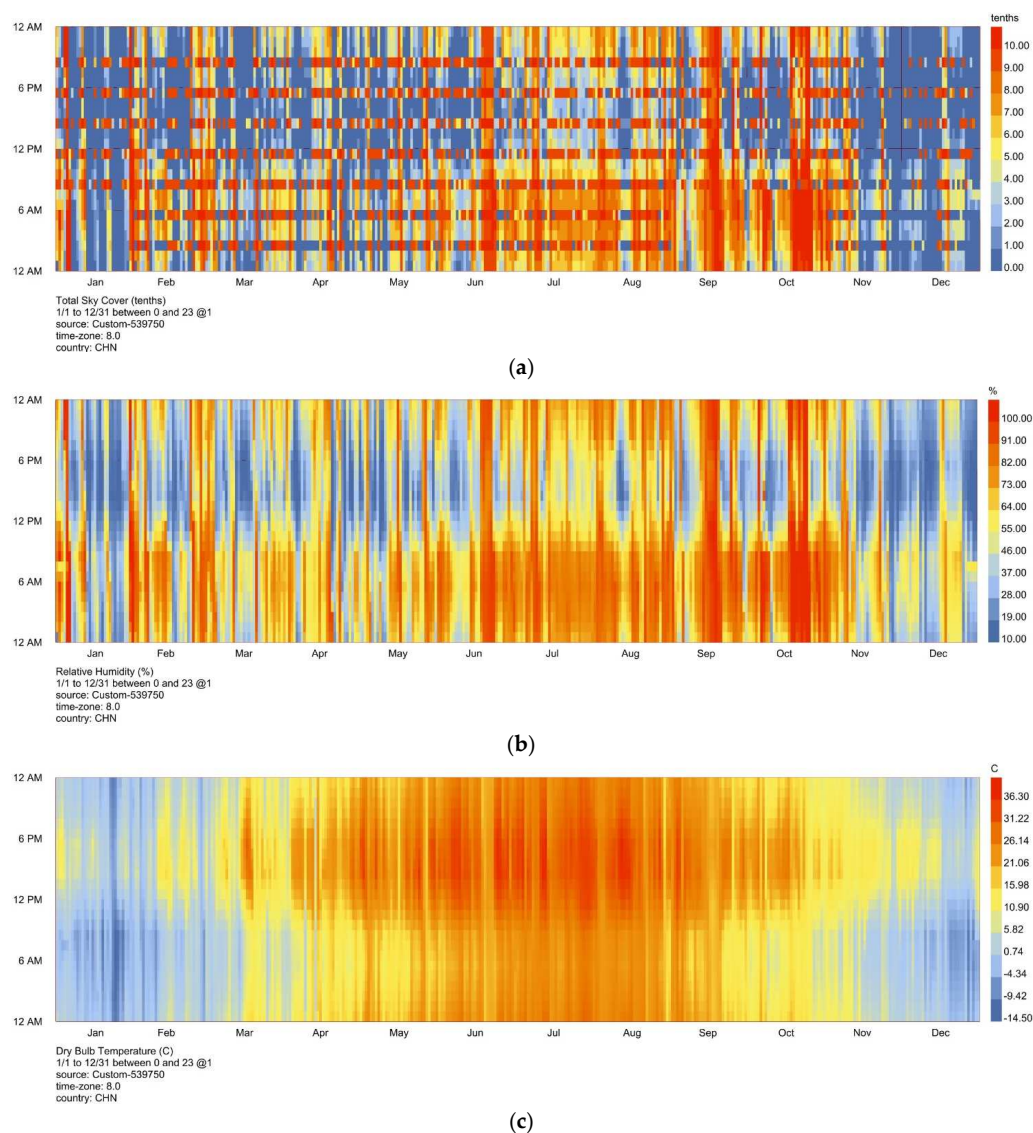


Figure A2. Cont.

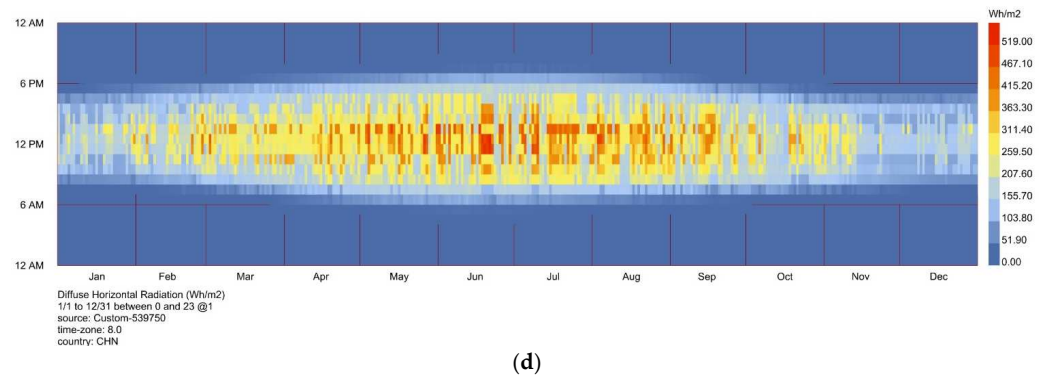


Figure A2. Site climate analysis in Ji family's residential houses. (a) Total sky cover; (b) relative humidity (%); (c) dry bulb temperature ($^{\circ}\text{C}$); and (d) diffuse horizontal radiation (Wh/m^2).

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