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Microbial Diversity Losses Constrain the Adaptive Capacity of cultural heritage to degradation --Manuscript Draft--

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Abstract:	<p>Since the industrial revolution, humans have emitted large amounts of polluting gases into the atmosphere, which has changed the Earth 's climate and pushed it toward an unprecedented state. In this context, it is very important to understand the weathering mechanism mediated by microbial geochemical cycle. However, previous studies have focused on the impact of microenvironment, but neglected the global pollution ecological perspective of heritage microorganisms. We analyzed the distribution of microbial diversity indicators and potential functions along the gradient of increased particle concentration, detecting over 6000 bacterial OTUs in 17 world cultural heritage sites. Remarkably, the results suggest that the effect of particulate matter pollution such as PM2.5 on the microbial bacterial community in the heritage site was not lower than that of temperature and precipitation. High particulate concentrations enhanced bacterial richness and introduced unique species. Among them, phototrophic bacteria (e.g., Cyanobacteria) and some heterotrophic bacteria (e.g., Actinobacteria and Proteobacteria) constitute the backbone of the microbial bacterial community in the heritage site. However, high particulate concentrations decrease the complexity and stability of microbial ecological networks, often favoring species that are tolerant to pollution. Additionally, high particulate concentrations can inhibit the expression of certain metabolic functions, particularly genes related to denitrification (such as nosZ and nirS). These findings infer that industrialization and urbanization have a profound impact on the global nitrogen cycle process and highlight the close link between biological deterioration and the nitrogen cycle.</p>

From: Xingyue Li
Chongqing University

Dear Editor(s):

The original manuscript "***Microbial Diversity Losses Constrain the Adaptive Capacity of cultural heritage to degradation***" is submitted to ***Science of The Total Environment***

A brief introduction: As industrialization and urbanization progress, understanding the interplay between air pollution, microbial community structures, and biological activity becomes essential for heritage preservation efforts. In this study, we analyzed the community composition, abundance, network patterns, and metabolic capabilities of microbial communities across different groups analysing data obtained using high-throughput sequencing (data from 17 global heritage sites). The results showed that the effect of particulate matter pollution such as PM 2.5 on the microbial bacterial community in the heritage site was not lower than that of temperature and precipitation. High particulate concentrations enhanced bacterial richness and introduced unique species. Among them, some phototrophic bacteria (e.g., Cyanobacteria) and some chemoorganotrophic bacteria (e.g., Actinobacteria and Proteobacteria) constitute the backbone of the microbial bacterial community in the heritage site. However, high particulate concentrations decrease the complexity and stability of microbial ecological networks, often favoring species that are tolerant to pollution. Additionally, high particulate concentrations can partially inhibit the expression of certain metabolic functions, particularly genes related to denitrification (such as *nosZ* and *nirS*).

A statement of the novelty, significance and relevance to journal: Most current studies typically investigate individual heritage sites, focusing on the microenvironments of these cultural heritage locations, lacking a global perspective on the microbial communities of cultural heritage. In particular, with the acceleration of industrialization and urbanization in recent decades, it is crucial to understand how particulate matter pollution affects the structure and metabolic capabilities of microbial communities at heritage sites. This study analyzes structure, function and ecological networks of bacterial communities using 16S rRNA literature high-throughput sequencing data from 66 samples across 17 global heritage sites. Viewing the microbial communities of cultural heritage from a global ecological perspective helps provide new insights for the sustainable protection of cultural heritage and the biological deterioration (or protection) of stone. Considering the degree of advance, broad implications and breadth and depth of this work, we believe it is suitable for '***Science of The Total Environment***'.

Our suggested reviewers are as follows:

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Moreover, we have not had any discussions with the editors of ***Science of The Total Environment*** regarding the work described in the manuscript, and note that there is no conflict of interest.

Most sincerely,

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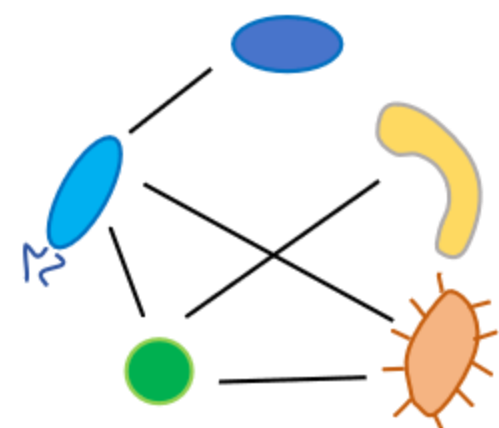
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Pollution environment



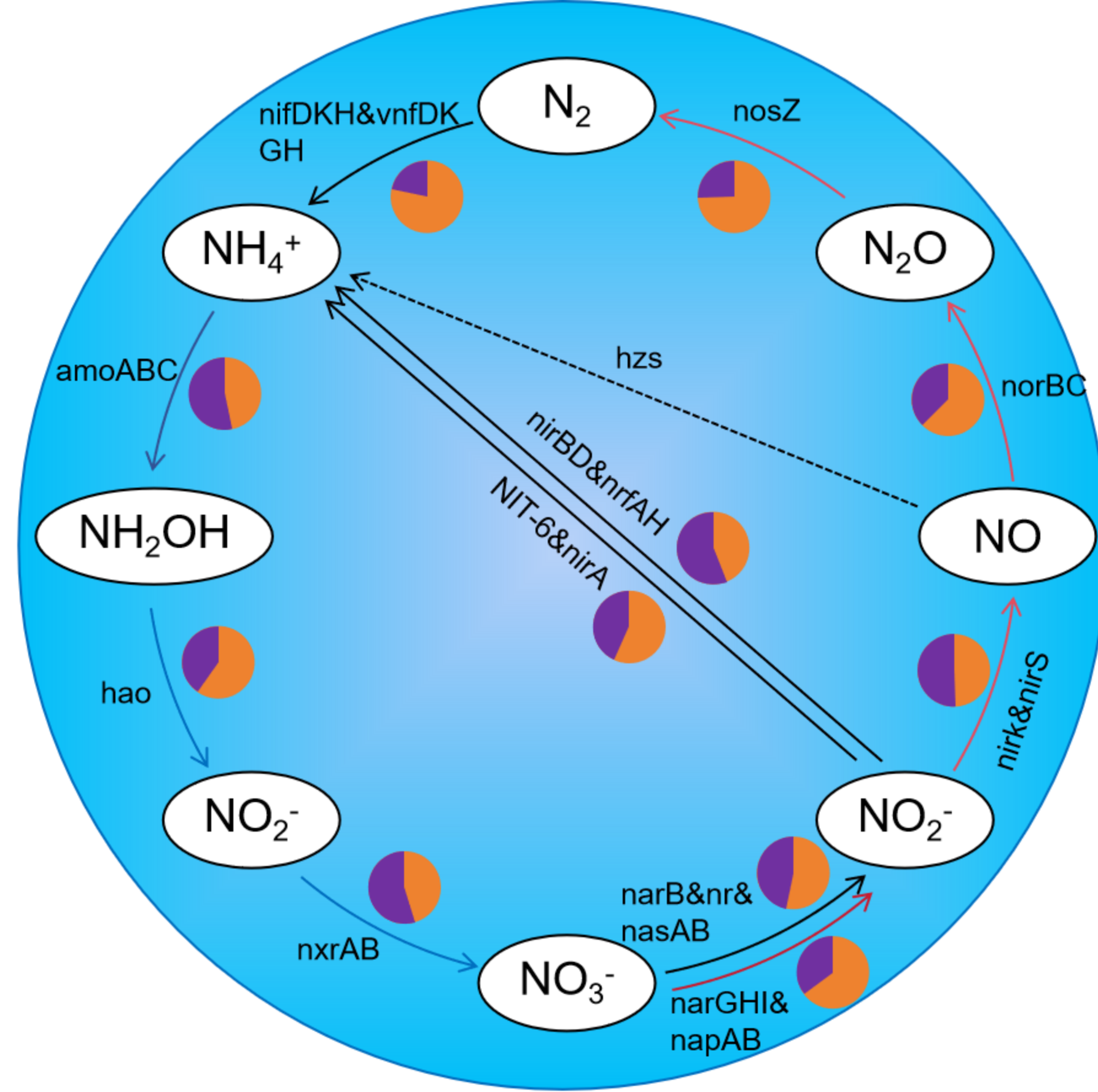
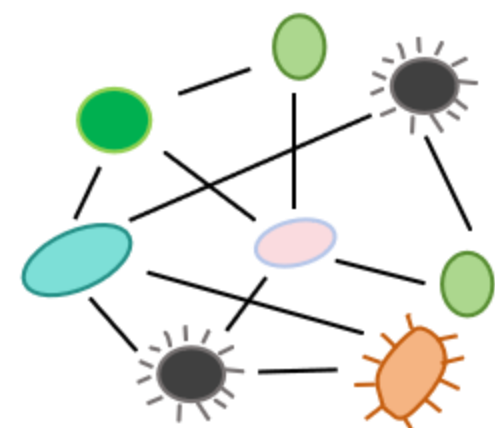
Simple network



Relatively low pollution environment



Complex network



Degradation adaptability decreased

Highlights

- The effect of particulate caused by industrialization on microbial communities cannot be ignored
- The high particle concentration makes many unique species appear on the surface of the heritage.
- High particle concentration reduced the complexity and stability of species network
- High particle concentration inhibits denitrification to reduce heritage tolerance.

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1 **Microbial Diversity Losses Constrain the Adaptive Capacity**
2 **of cultural heritage to degradation**

3

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13

1 **Abstract**

2 Since the industrial revolution, humans have emitted large amounts of polluting
3 gases into the atmosphere, which has changed the Earth 's climate and pushed it toward
4 an unprecedented state. In this context, it is very important to understand the weathering
5 mechanism mediated by microbial geochemical cycle. However, previous studies have
6 focused on the impact of microenvironment, but neglected the global pollution
7 ecological perspective of heritage microorganisms. We analyzed the distribution of
8 microbial diversity indicators and potential functions along the gradient of increased
9 particle concentration, detecting over 6000 bacterial OTUs in 17 world cultural heritage
10 sites. Remarkably, the results suggest that the effect of particulate matter pollution such
11 as PM_{2.5} on the microbial bacterial community in the heritage site was not lower than
12 that of temperature and precipitation. High particulate concentrations enhanced
13 bacterial richness and introduced unique species. Among them, phototrophic bacteria
14 (e.g., Cyanobacteria) and some heterotrophic bacteria (e.g., Actinobacteria and
15 Proteobacteria) constitute the backbone of the microbial bacterial community in the
16 heritage site. However, high particulate concentrations decrease the complexity and
17 stability of microbial ecological networks, often favoring species that are tolerant to
18 pollution. Additionally, high particulate concentrations can inhibit the expression of
19 certain metabolic functions, particularly genes related to denitrification (such as *nosZ*
20 and *nirS*). These findings infer that industrialization and urbanization have a profound
21 impact on the global nitrogen cycle process and highlight the close link between
22 biological deterioration and the nitrogen cycle.

1 **Keywords:**

2 Industrialization; Biodeterioration; Microbial ecology; Microbial nitrogen cycling;

3 Biogeography; Species co-occurrence

4

1 **1 Introduction**

2 Cultural heritage is not only a witness to history but also an important carrier of
3 civilization (Jroundi et al., 2017; Mosquera et al., 2023; Reimann et al., 2018). Their
4 unique artistic and historical value allows us to gain deeper insights into the
5 developmental trajectories of different societies and civilizations. The protection of
6 cultural heritage has garnered significant global attention. Especially with the
7 acceleration of industrialization, the impact of environmental pollution on heritage sites
8 has become a hot research topic (Gaylarde and Baptista-Neto, 2021). The consumption
9 of fossil fuels, traffic emissions, urbanization, and agricultural production significantly
10 increase the levels of particulate matter, aerosols, soot, and volatile organic compounds
11 in the air (Guo et al., 2024; Jandacka et al., 2017; Yu et al., 2024a). In this context,
12 understanding the impact of air pollution on the microbial communities of heritage sites
13 is crucial for revealing the mechanisms of microbiologically-mediated processes of
14 these sites.

15 Cultural heritage, particularly stone structures, monuments, and rock paintings that
16 are directly exposed to the natural environment, is often colonised by microorganisms
17 (Saiz-Jimenez et al., 2011; Yu et al., 2024b). Various microbial communities, including
18 bacteria (also of the domain Archaea), fungi, and algae form biofilms (Anthony et al.,
19 2020; Crispim and Gaylarde, 2005; Gorbushina, 2007; Sterflinger, 2010). Biofilm is a
20 kind of viscous film structure formed by microorganisms adhering to the solid surface
21 and secreting extracellular polymers substance (such as polysaccharides, proteins and
22 eDNA) (Berti et al., 2024). They can cause aesthetic alterations and deterioration of

1 stone heritage worldwide. Negative impacts of microorganisms, ranging from material
2 discoloration to structural damage, can result in serious losses to the historical and
3 aesthetic value of cultural heritage (Gadd, 2017; Liu et al., 2020). However, in the last
4 years, the many positive interactions of microorganisms with lithic materials
5 (bioprotection) has gained value and attention (Gulotta et al., 2018; Pinna, 2014;
6 Sanmartín et al., 2020; Villa et al., 2023). For example, (Villa et al., 2023) used a two-
7 species model system consisting of phototrophs and chemotactic organisms to simulate
8 the biofilm behavior on the stone heritage to determine its protective effect .

9 Microbial communities exhibit significant diversity across different heritage sites.
10 In addition to the stone type (Gambino et al., 2021), environmental factors are
11 considered the primary parameters influencing microbial community structure,
12 including temperature, humidity, light, salinity, and moisture (Mugnai et al., 2024; Nir
13 et al., 2019; Viles and Cutler, 2012; Zhang et al., 2021). Warm and humid climates
14 provide favorable conditions for the growth of most organisms (Zhu et al., 2023). It is
15 generally believed that warming can reshape microbial diversity and alter species
16 interactions, enhancing the complexity and stability of microbial networks (Li et al.,
17 2022; Yuan et al., 2021). Variations in precipitation impact the diversity and abundance
18 of microbial communities, reducing microbial and enzymatic activity (Xiao et al., 2018).
19 Due to the high levels of rainfall and humidity in Southeast Asia, a large number of
20 microorganisms often develop on the sandstone of Angkor Wat (Liu et al., 2018).
21 Additionally, salinity is a dominant force in the micro-geochemical processes within
22 soils and heritage rock bodies (Cheng et al., 2021; Li et al., 2024a; Mo et al., 2021).

1 (Wang et al., 2010) found a significant negative correlation between salinity and active
2 microbial communities and microbial diversity. An increase in salinity can lead to the
3 death of some microorganisms (Li et al., 2016). With the rapid advancement of
4 industrialization and urbanization, particulate matter pollution has become one of the
5 main sources of global air quality deterioration. These particulates can remain
6 suspended in the air for extended periods and spread to distant regions, affecting human
7 health and causing notable chemical corrosion effects on heritage materials (Gaylarde
8 et al., 2017; Nuhoglu et al., 2006). Regrettably, current research on how particulate
9 matter pollution affects the microbial structure of heritage sites is relatively lacking.

10 Microbial communities form biofilms on the surfaces of materials. The initially
11 colonizing microorganisms are often primarily phototrophic organisms, such as algae
12 and lichens. They can utilize CO₂ from the air as carbon source to produce
13 carbohydrates using sunlight (Liu et al., 2020; McNamara et al., 2006). These initial
14 biological colonizers facilitate the establishment of subsequent heterotrophic microbial
15 communities, including sulfate-reducing bacteria, denitrifying bacteria, halophiles, and
16 actinobacteria (Crispim and Gaylarde, 2005). As a result, the development of biofilms
17 often causes aesthetic alteration to materials due to microbial pigments. Because of
18 variations in the environmental conditions and state of conservation of the lithic
19 materials, heritage surfaces gradually select and recruit other microorganisms
20 (Gorbushina, 2007). Over time, biofilm can grow endolithically, either causing damage
21 to various heritage structures or protecting the heritage materials (Concha-Lozano et al.,
22 2012).

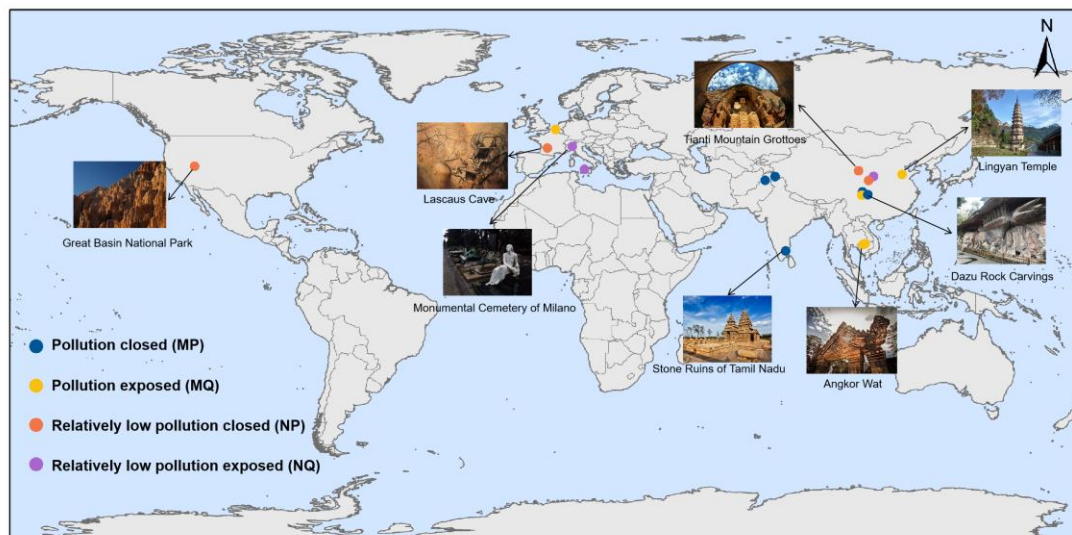
1 Existing research suggests many mechanisms of interaction between the lithic
2 materials and the biofilm community. For instance, different microorganisms can
3 extract suitable mineral ions from the rock matrix for growth, enriching the diversity of
4 microbial communities on the material's surface (Kusumi et al., 2013). These
5 microorganisms can contribute to biomineralization and lead to the microbial
6 degradation of heritage materials. In contrast, microbially induced carbonate
7 precipitation with external calcium added to the surface- has been used to consolidate
8 monuments (Benedetti et al., 2023). Notably, chemolithoautotrophs in the microbial
9 community, such as sulfur-oxidizing and nitrifying bacteria, can convert nitrogen and
10 sulfur compounds from the atmosphere into inorganic acids, such as nitric and sulfuric
11 acid. These acids are likely to cause erosion of stones and buildings if they reach the
12 heritage surface (Ding et al., 2020; Li et al., 2021; Meng et al., 2017).

13 Most current studies typically investigate individual heritage sites, focusing on the
14 microenvironments of these cultural heritage locations, lacking a global perspective on
15 the microbial communities of cultural heritage (Wu et al., 2023; Zhang et al., 2021). In
16 particular, with the acceleration of industrialization and urbanization in recent decades,
17 it is crucial to understand how particulate matter pollution affects the structure and
18 metabolic capabilities of microbial communities at heritage sites. What changes do
19 particulate matter pollution bring to the microbial structures and biologically-mediated
20 processes at these sites? This information provides insights into the biodiversity of
21 different cultural heritage locations.

22 To elucidate this topic, a global database containing from 17 heritage sites was

1 established (Table S1). Here, exposure conditions were considered and a classification
2 proposed. For example, stone grottoes, stone carvings, and monuments were considered
3 exposed environments, whereas caves were categorized as closed environments. The
4 level of environmental pollution was determined by PM_{2.5} concentrations. According
5 to the ambient air quality standards published by the Ministry of Ecology and
6 Environment of the People's Republic of China (GB 3095-2012), the study considered
7 0-35 µg/m³ as a relatively low polluted environment. A PM_{2.5} concentration higher
8 than 35 µg/m³ was classified as a polluted environment. Therefore, the samples were
9 categorized into four types based on their environmental conditions: pollution closed
10 (MP), pollution exposed (MQ), relatively low pollution closed (NP), and relatively low
11 pollution exposed (NQ). Although Ghent's PM_{2.5} is less than 35 µg/m³, it has been
12 clear in Laurenz's study (Schröer et al., 2020) that the sample is in a polluted
13 environment, so the samples BLS1-BLS4 belong to group MQ (Table S2).

14 In this study, we analyzed the community composition, abundance, network
15 patterns, and metabolic capabilities of microbial communities across different groups
16 analysing data obtained using high-throughput sequencing. The aim is to reveal the
17 impact of particulate matter pollution on the structure of microbial communities and
18 the biological degradation processes at heritage sites. Viewing the microbial
19 communities of cultural heritage from a global ecological perspective helps provide
20 new insights for the sustainable protection of cultural heritage.



1

2 **Fig. 1** Global distribution maps of the cultural heritage sampling sites considered in this
 3 study. The samples were divided into four groups according to the concentration of
 4 particulate matter in the city (PM_{2.5} concentrations) where the heritage site is located,
 5 namely, pollution closed (MP), pollution exposed (MQ), relatively low pollution closed
 6 (NP) and relatively low pollution exposed (NQ).

7

8 **2 Materials and Methods**

9 *2.1 Data collection*

10 Dazhu Rock Carvings, located in the Dazhu District of Chongqing, were listed as a
 11 UNESCO World Heritage Site in 1999 (Centre, 1999). Samples were collected from
 12 the outdoor statue of the Reclining Buddha at Baoding Mountain in Dazhu. Biofilm
 13 samples containing a small amount of weathered rock were carefully collected from the
 14 biofilm-sandstone interface using a sterile surgical knife. Each sample weighed about
 15 2g, stored in sterile centrifuge tubes. Additionally, this study analyzed high-throughput
 16 sequencing datasets from various historical monuments worldwide (Table S1). These

1 datasets are publicly available for download from NCBI. Data on the locations, heritage
2 types, materials, and preservation conditions for each study were also collected for
3 subsequent related analyses. The meteorological data used in the study were sourced
4 from the European Centre for Medium-Range Weather Forecasts
5 (<https://cds.climate.copernicus.eu/>), including PM2.5, PM10, CO, SO₂, NO₂, O₃, wind,
6 temperature, pressure, rainfall, humidity, Tmin (minimum temperature), Tmax
7 (maximum temperature), and solar radiation.

8 According to the detection scheme, DNA quantification was performed using a
9 Nanodrop. The quality of the extracted DNA was assessed through 1.2% agarose gel
10 electrophoresis. PCR amplification was completed using universal primers. The
11 primers 338F, 5'ACTCCTACGGGAGGCAG-3', and 806R, 5'-
12 GGACTACHVGGGTWTCTAAT-3', were used to amplify the v3-v4 region(Zeng et al.,
13 2011). The amplification products were verified using 2% agarose gel electrophoresis,
14 purified with a gel extraction kit (AXYGEN Co., China), and then further analyzed.

15 Since the data used in this study comes from 16S rRNA gene sequences of various
16 heritage sites worldwide, quality control of the downloaded sequence data is necessary.
17 In microbial diversity research, Operational Taxonomic Units (OTUs) are defined to
18 facilitate analysis by grouping sequences based on certain similarities. Using the Silva
19 database (Release 132) for alignment, sequences were classified into the same OTU
20 with a threshold of 97% similarity using USEARCH (version 7.1) (Cai et al., 2020).
21 Sequences that could not be mapped were labeled as 'unknown'. The matching full-
22 length sequences and their annotations were used as representative sequences for

1 further classification and analysis. MOTHUR (Schloss Patrick et al., 2009) was
2 employed to analyze similar sequences and group them into different OTUs. Excluding
3 unavailable samples, the remaining 66 bacterial samples were sourced from 17 heritage
4 sites globally.

5 *2.2 Statistical analysis*

6 In microbial diversity research, α diversity refers to the species diversity within a
7 single sample, commonly assessed using species richness (Chao 1, Ace) and diversity
8 indices (Shannon, Simpson). In this study, the 'vegan' package in R was used to
9 calculate the Chao 1, Shannon, Simpson, and Ace indices of bacterial community
10 diversity. One-way analysis of variance (ANOVA) was used to compare the significant
11 differences in microbial diversity indices between different groups. The analysis was
12 performed using SPSS software, with a significance level of $P < 0.05$. Non-metric
13 multidimensional scaling (NMDS) is an indirect gradient analysis method based on
14 dissimilarity or distance matrices, used for ordination analysis. In microbial community
15 studies, it can be employed to display community β diversity. The β diversity can be
16 seen with the Bray Curtis matrix. NMDS analysis was completed using Wekemo
17 Bioincloud (<https://www.bioincloud.tech>) (Gao et al., 2024). The principle of
18 PERMANOVA involves calculating the sum of squares of deviations between group
19 distances and the sum of squares of deviations within samples. The ratio of these values,
20 after dividing by their respective degrees of freedom, yields the F value (analogous to
21 the F value in traditional ANOVA). The pseudo-F values obtained through random
22 permutation are then compared to this F value; if the probability that the former is

1 greater than the latter is less than 5%, it indicates a significant difference between
2 groups ($P < 0.05$). This analysis was conducted using the *vegan* package. Additionally,
3 redundancy analysis (RDA) of microbial communities and environmental factors was
4 performed using Canoco 5.0. Spearman correlations between species and
5 environmental factors were calculated using the 'psych' package in R. LEfSe analysis
6 was completed using Wekemo Bioincloud (<https://www.bioincloud.tech>) (Gao et al.,
7 2024). The importance ranking of environmental factors was determined using the
8 random forest algorithm, with the analysis conducted in Python.

9 The correlation coefficient matrix between OTUs was calculated using the 'psych'
10 package in R, determining the Spearman correlation coefficients between OTUs.
11 Effective relationships were filtered by retaining those with correlation coefficients r
12 greater than $|0.9|$ and P values less than 0.01, based on the above parameters to generate
13 a co-occurrence network. The network was imported into Gephi (<https://gephi.org>) for
14 visualization using the Fruchterman-Reingold layout, and it was colored by module and
15 phylum. Topological features of the network were calculated at both the network level
16 (average node degree, clustering coefficient, average path length, modularity, density,
17 diameter, betweenness centrality, and degree centrality) and the node level (degree,
18 transitivity, betweenness centrality, and closeness centrality).

19 *2.3 Prediction of microbial community function*

20 Typically, 16S rRNA genes do not provide direct evidence of community function.
21 However, PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction
22 of Unobserved States) predicts 16S rRNA gene sequences across multiple functional

1 databases. This study utilized the annotation results from the most commonly used
2 KEGG database. The core of the KEGG database is the KEGG Pathway Database
3 (<http://www.genome.jp/kegg/pathway.html>), which classifies metabolic pathways into
4 six major categories: Metabolism, Genetic Information Processing, Environmental
5 Information Processing, Cellular Processes, Organismal Systems, and Human Diseases.
6 Each category of metabolic pathways is further divided into multiple levels.

7 The analysis of element cycles in the study was based on the DiTing tool (Cai et
8 al., 2020). This tool, which is built upon the KEGG database and a manually created
9 DMSP cycling gene database, has developed specific formulas for nearly 100
10 biogeochemical pathways related to C, N, P, and S cycles to calculate the relative
11 abundance of each pathway. Analyzing with DiTing provides rich information about
12 the biogeochemical cycles of microorganisms extracted from environmental omics data.
13 This analysis was completed using Wekemo Bioincloud (<https://www.bioincloud.tech>).

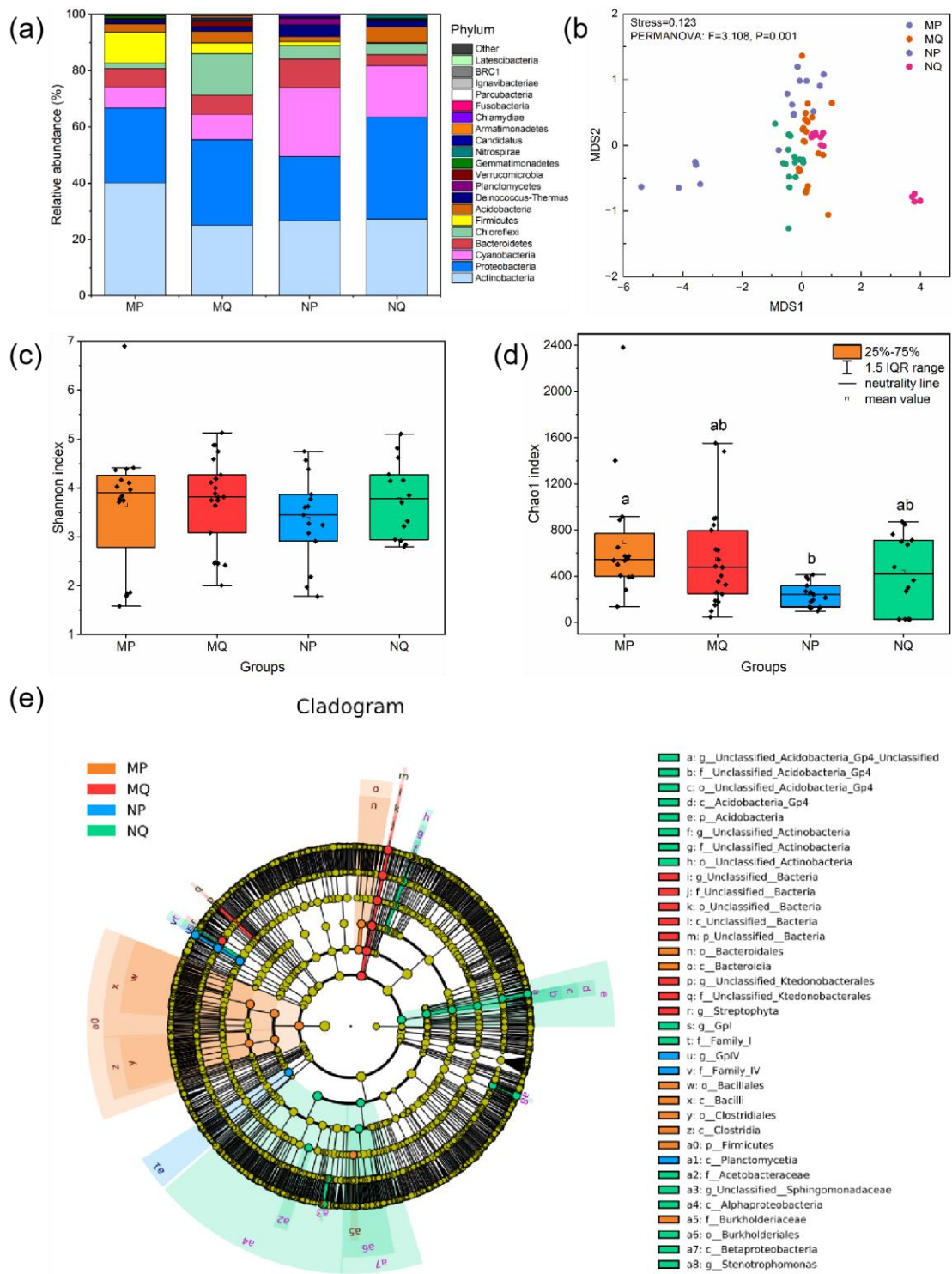
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15 **3 Results**

16 *3.1 Effect of particulate matter enrichment on microbial community structure in* 17 *heritage sites*

18 Since the industrial revolution, the gradual increase in the concentration of
19 particulate matter such as PM_{2.5} in the air has had a significant impact on the microbial
20 community structure of cultural heritage sites. These particles can accumulate on the
21 surface of heritage sites through deposition (Anaf et al., 2015), altering the habitat of
22 microorganisms and thus affecting their community structures. To predict the impact of

1 particulate matter on the microbial communities at heritage sites, high-throughput
2 sequencing of the 16S rRNA gene obtained from literature data was used to compare
3 the composition of bacterial communities at global scale. The results showed that the
4 microbial community structures of heritage sites vary across four habitats (Fig. 2). In
5 terms of community composition, the three phyla (Actinobacteria, Proteobacteria, and
6 Cyanobacteria) dominate the bacterial communities, with the total relative abundance
7 of ranging from 64.34% to 81.72% (Fig. 2a; Table S3). It is noteworthy that the relative
8 abundance of Cyanobacteria is significantly higher in relatively low-pollution areas
9 compared to polluted areas, while the Firmicutes shows the opposite pattern ($P < 0.05$).
10 Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance revealed
11 the diversity of microbial communities in different polluted environments (Stress=
12 0.123) (Fig. 2b). For bacteria in different heritage sites, the community structure in
13 different polluted environments was significantly different (PERMANOVA: $F = 3.108$,
14 $P < 0.001$) and there were a little overlap between the four microbial communities (Fig.
15 2b). Remarkably, the microbial communities of the two types of heritage (MP and MQ)
16 were more similar in a polluted environment, while the microbial communities in a
17 relatively low polluted environment (NP and NQ) were quite different.



1
2 **Fig. 2** Microbial community structure in heritage sites and indicator groups of microbial
3 community in different polluted environments. (a) the top 20 bacterial phyla. (b) Non-
4 metric multi-scale analysis of microbial communities based on Species. (c)-(d)
5 microbial community α diversity index (Shannon and Chao). (e) indicator groups in

1 different environments based on LEfSe (linear discriminant analysis effect size) with
2 the LDA score > 4.

3

4 In the diversity analysis of microbial groups (Figs. 2c and 2d), Shannon index and
5 Chao1 index were used to measure the alpha diversity of different groups. ANOVA
6 analysis of Shannon index showed that there was no significant difference in diversity
7 among these groups ($P > 0.05$), which means that they performed similarly in terms of
8 community diversity. The Chao1 index mainly measures species richness. Analysis of
9 variance showed that the species richness of NP group was significantly lower than that
10 of other groups ($P < 0.05$), especially with MP, MQ and NQ groups. These results
11 indicate that the microbial richness of the NP group is relatively low, while the other
12 groups are relatively rich in species richness. In addition, 32 indicator taxa identified
13 by LEfSe analysis belonged to different habitats (Fig. 2e). In polluted enclosed areas,
14 the indicator taxa were predominantly Firmicutes and Bacteroidia, while samples from
15 relatively low pollution areas included Acidobacteria, Actinobacteria,
16 Alphaproteobacteria, and Betaproteobacteria as indicator taxa. This suggests that the
17 particulate matter-enriched polluted environment can alter the structure of microbial
18 communities through selective pressure, resulting in specific indicators of pollution
19 levels among microbial taxa in different habitats.

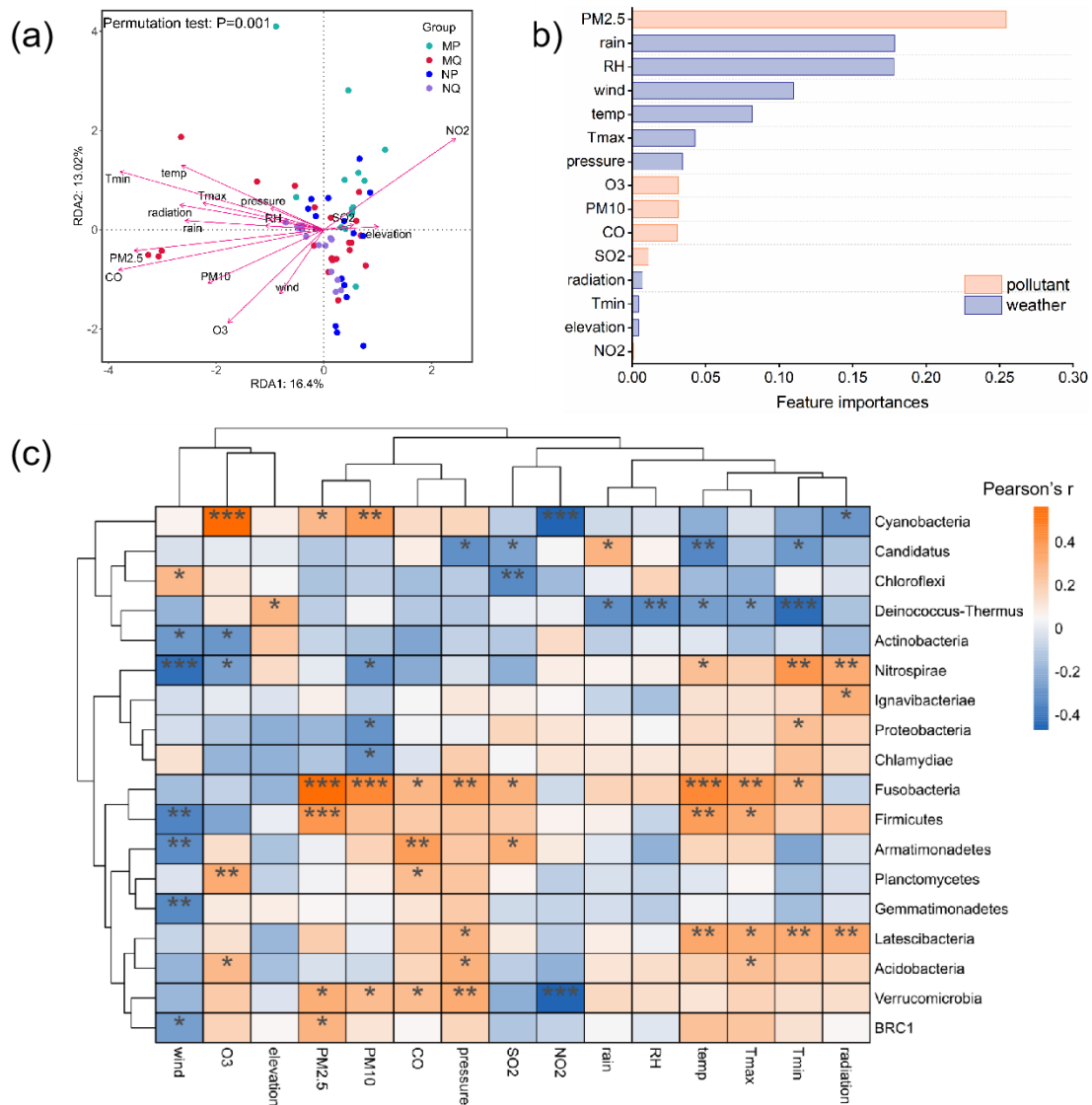
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21 *3.2 Environmental driving forces of cultural heritage microbiome*

22 Microbial communities in cultural heritage sites are driven by a combination of

1 environmental factors, including meteorological conditions and pollutant levels.
2 Redundancy analysis (RDA) results show that the first and second axes explain 16.40%
3 and 13.02% of the variance in microbial community changes, respectively (Fig. 3a;
4 Table S4). Among these, environmental factors such as PM2.5, CO, PM10, and
5 temperature play a significant role in shaping the microbial community structure
6 ($P < 0.001$). These factors explained 38.8 %, 41.7 %, 23.5 % and 19.8 % of the
7 community differences, respectively. Among them, PM2.5 had a significant effect on
8 Chloroflexi and Verrucomicrobia in the samples. Notably, compared to commonly
9 observed meteorological factors in other analyses, PM2.5 exhibited a stronger negative
10 impact. Its feature importance value was close to 0.3, significantly higher than other
11 variables (Fig. 3b). Specifically, Distance-decay relationships (DDR) analysis indicates
12 that bacterial communities in enclosed heritage sites typically exhibit less variation in
13 response to changes in PM2.5 concentrations compared to open heritage sites
14 (Supplementary Fig. 2). Particulate matter shows a significant positive correlation with
15 several microbial phyla, such as BRC1, Firmicutes, Fusobacteria, Verrucomicrobiota
16 and Cyanobacteria suggesting that high concentrations of PM2.5 may promote the
17 growth of these microorganisms (Fig. 3c). In contrast, some microorganisms, such as
18 Nitrospirae, Proteobacteria, and Chlamydiae, are negatively correlated with particulate
19 matter concentration, as high levels of particulates inhibit their abundance. The toxic
20 chemicals carried by particulates, such as heavy metals and organic pollutants
21 (Habebullah, 2016; Ji and Zhao, 2015; Wang et al., 2019), further exacerbate stress on
22 microbial communities, leading to functional degradation or a decrease in the number

1 of species.



2

3 **Fig. 3** Relationship between microbial community composition and environmental

4 factors. (a) redundancy analysis (RDA) between environmental variables and microbial

5 communities in heritage site samples. (b) the importance of ranking of environmental

6 factors based on a random forest algorithm. (c) Pearson correlation heatmap between

7 environmental factors and the top 20 microbial phyla. *: P < 0.05, **: P < 0.01, ***: P <

8 0.001.

9

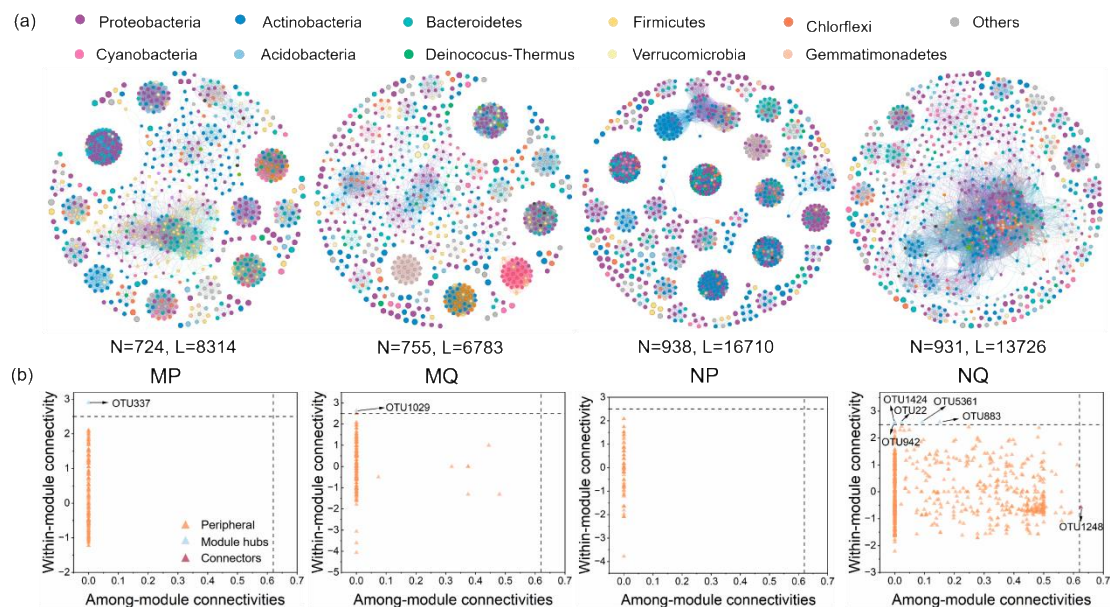
1 *3.3 Symbiotic network of microbial community in cultural heritage group*

2 To reveal the potential bacterial interactions in heritage sites under different
3 pollution levels, an OTU-based co-occurrence network was constructed for heritage
4 sites with varying pollution levels and their exposure conditions (Fig. 4). Microbial co-
5 occurrence analysis was used here as more and more studies have begun to explore the
6 co-occurrence relationships between microbial communities in different geographical
7 locations (Duan et al., 2022; Lin et al., 2017; Liu et al., 2024). The four network
8 diagrams illustrate the modular structure of microbial communities in different regions,
9 showing that pollution levels and openness significantly impact the modular
10 characteristics of these communities. The topological properties of the networks vary
11 significantly with particle changes; for example, the nodes (i.e., OTUs) and edges (i.e.,
12 correlations between OTUs), constituting all core networks, decrease as particulate
13 matter concentration increases (Table S5).

14 In the polluted MP and MQ areas, the modularity of microbial networks is higher,
15 displaying more complex connections, particularly with tighter within-module
16 connections in the MP area. The microbial networks in the MP and MQ areas with
17 higher particulate matter concentrations exhibit relatively lower modularity. Conversely,
18 the microbial networks in the relatively low pollution areas with lower particulate
19 concentrations show more complex connections, especially with tighter within-module
20 connections in the NQ area.

21 However, the Venn diagram indicates that environments with high particulate
22 matter concentrations have more unique OTUs (Supplementary Fig. 3). In polluted

1 areas, particulate matter provides unique survival conditions for specific microbial
 2 groups, allowing certain highly adaptive taxa to thrive in these environments, thereby
 3 forming distinct microbial communities. These species may be specific types that
 4 exhibit strong tolerance to particulate pollution. As a result, a relatively simple network
 5 structure emerges, leading to fewer nodes and edges. In areas with lower particulate
 6 concentrations, the environment is more diverse. This diversity may lead to more
 7 ecological interactions and complex species relationships, resulting in a higher number
 8 of nodes and edges. This suggests that interactions among species in relatively low
 9 pollution environments may be more intricate, resulting in richer ecological networks.
 10 Additionally, closed heritage sites have more unique OTUs compared to exposed
 11 heritage sites. This is because the environment in closed spaces is more stable,
 12 facilitating the adaptation and development of microbial groups.



13 **Fig. 4** Co-occurrence network analysis of microbial communities in heritage sites
 14 (based on Spearman's correlation between OTUs). (a) modular association network
 15 between microbial OTUs. The OTU used to construct the network exists in at least 60 %
 16

1 of the samples. All the connections have correlation coefficients $r > |0.9|$ and a $P < 0.01$.
2 Nodes were colored according to different phylum levels. N denotes the node and L
3 denotes the edge. (b) zipi key species search. OTU22 belongs to Solirubrobacterales of
4 Actinobacteria, and the remaining keys belong to Xanthomonadaceae of Proteobacteria.

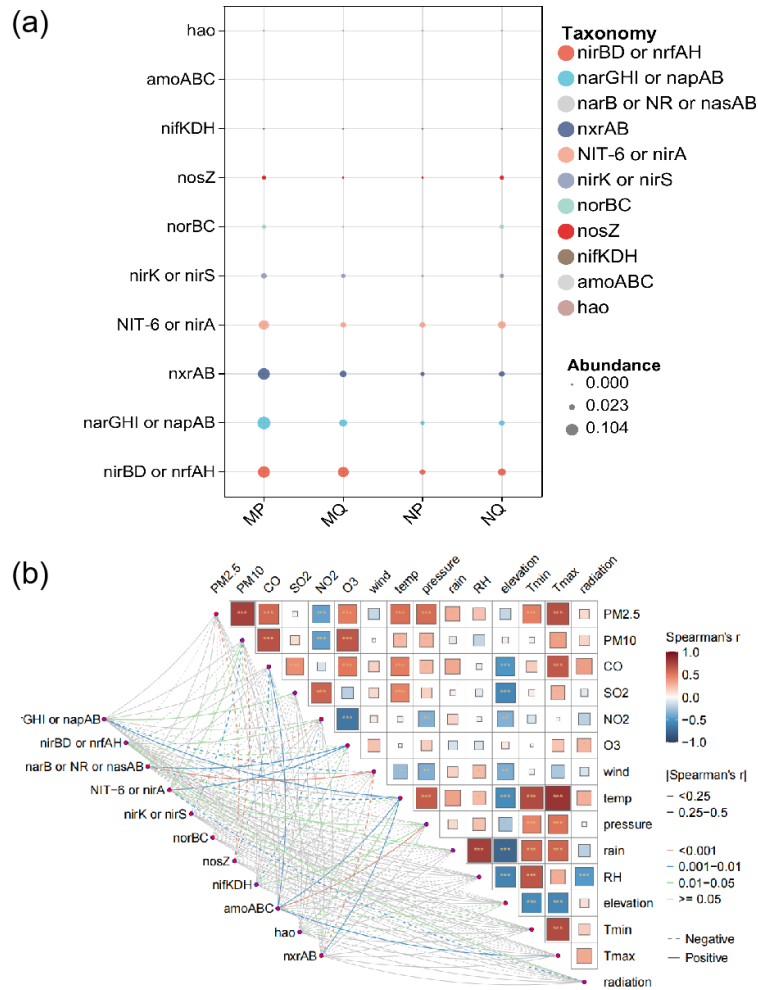
5
6 Fig. 4b shows the distribution of intra-module connectivity and inter-module
7 connectivity for each operational taxonomic unit (OTU). OTU22 belongs to
8 Solirubrobacterales of Actinobacteria, and the remaining keys belong to
9 Xanthomonadaceae of Proteobacteria. It can be observed that certain OTUs in the MP
10 and MQ regions (e.g., OTU337 and OTU1029) exhibit higher intra-module
11 connectivity, indicating that these OTUs play a key, central role within their respective
12 modules. In regions with relatively low pollution, particularly in the NQ region, the
13 modular structure of OTUs appears more dispersed, suggesting a lower degree of
14 modularity in microbial communities under relatively low pollution conditions.
15 Additionally, some OTUs (e.g., OTU1248 and OTU942) demonstrate higher inter-
16 module connectivity in the NQ region, implying that these microorganisms play
17 important roles in resource exchange or ecological functions across modules. Overall,
18 pollution levels and exposure conditions significantly affect the network structure and
19 modular characteristics of microbial communities.

20

21 *3.4 Effect of particulate matter concentration on microbial metabolic capacity*

22 On the surface of heritage sites, microorganisms almost always exist as a

1 community and work together to perform various tasks. Therefore, to understand the
2 impact of microbial communities on heritage sites, a functional analysis of the entire
3 population should be the starting point. PICRUSt2 provides predictive information on
4 the gene-level functional characteristics of microorganisms (Douglas et al., 2020). Fig.
5 5a shows the abundance distribution of specific functional genes in different
6 environments (MP, MQ, NP, NQ). As shown, the abundance of certain genes (e.g.,
7 *nirBD* or *nrfAH*) is higher in polluted environments (MP, MQ), indicating the
8 significant role of particulate matter pollution in shaping the nitrogen cycling function
9 of microbial communities. Fig. 5b shows the Pearson's correlation between
10 environmental factors and various microbial functional genes. The results indicate that
11 PM_{2.5} and PM₁₀ are negatively correlated with several nitrogen cycle-related
12 functional genes (such as *nosZ*, *norBC*, or *nirs*, *norBC*) ($r < -0.4$). Particulate matter
13 pollution significantly affects the expression of certain functional genes, suggesting that
14 increased particulate concentration reduces certain microbial metabolic functions
15 related to nitrogen cycling (Fig. 5b).

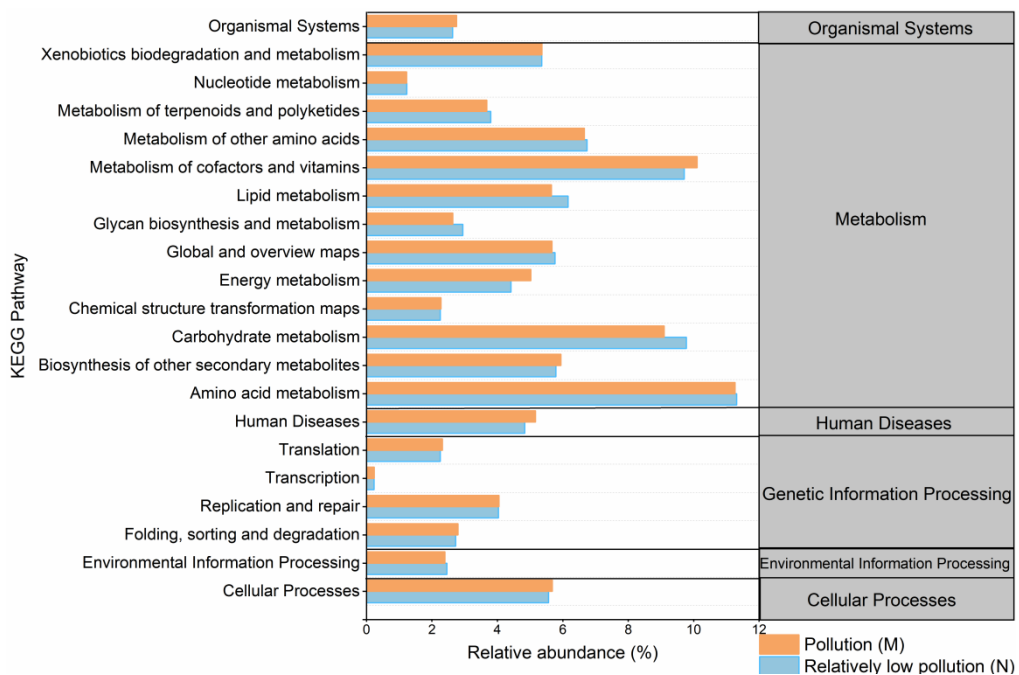


1

2 **Fig. 5** Analysis of microbial community metabolic capacity in heritage sites. (a) the
 3 abundance of nitrogen metabolism-related genes predicted by PICRUSt2. (b) the
 4 correlation between genes related to nitrogen metabolism in bacterial communities and
 5 environmental factors in heritage sites.

6 In the KEEG database, all OTU tables and species information are annotated and
 7 classified into the following functional groups (Fig. 6). The fundamental difference
 8 between the M group (pollution) and the N group (relatively low pollution) lies in the
 9 level of gene expression related to metabolism. Group M exhibits higher gene
 10 expression in "Metabolism of cofactors and vitamins," "Energy metabolism," and
 11 "Biosynthesis of other secondary metabolites," whereas group N shows higher gene

1 expression in "Metabolism of terpenoids and polyketides," "Lipid metabolism,"
 2 "Glycan biosynthesis and metabolism," and "Carbohydrate metabolism" (Fig. 6). The
 3 abundant genetic information expressed is related to environmental stress, while genes
 4 associated with metabolism are typically triggered by nutrient metabolism. This
 5 indicates that microorganisms in polluted environments may adapt to the stress of
 6 particulate matter by enhancing some metabolic activities, particularly in pathways
 7 related to pollutant degradation and energy conversion. In addition, particulate matter
 8 in polluted environments promotes the expression of certain microbial functional genes,
 9 especially those involved in nitrogen cycling and energy metabolism, while the
 10 ecological networks in mildly polluted environments exhibit more complex microbial
 11 interactions. High particulate matter concentration has profound effects on microbial
 12 function and diversity, potentially driving the formation of more adaptive microbial
 13 communities.



14

15 **Fig. 6** Comparison of gene function predicted by group M and group N at level 2.

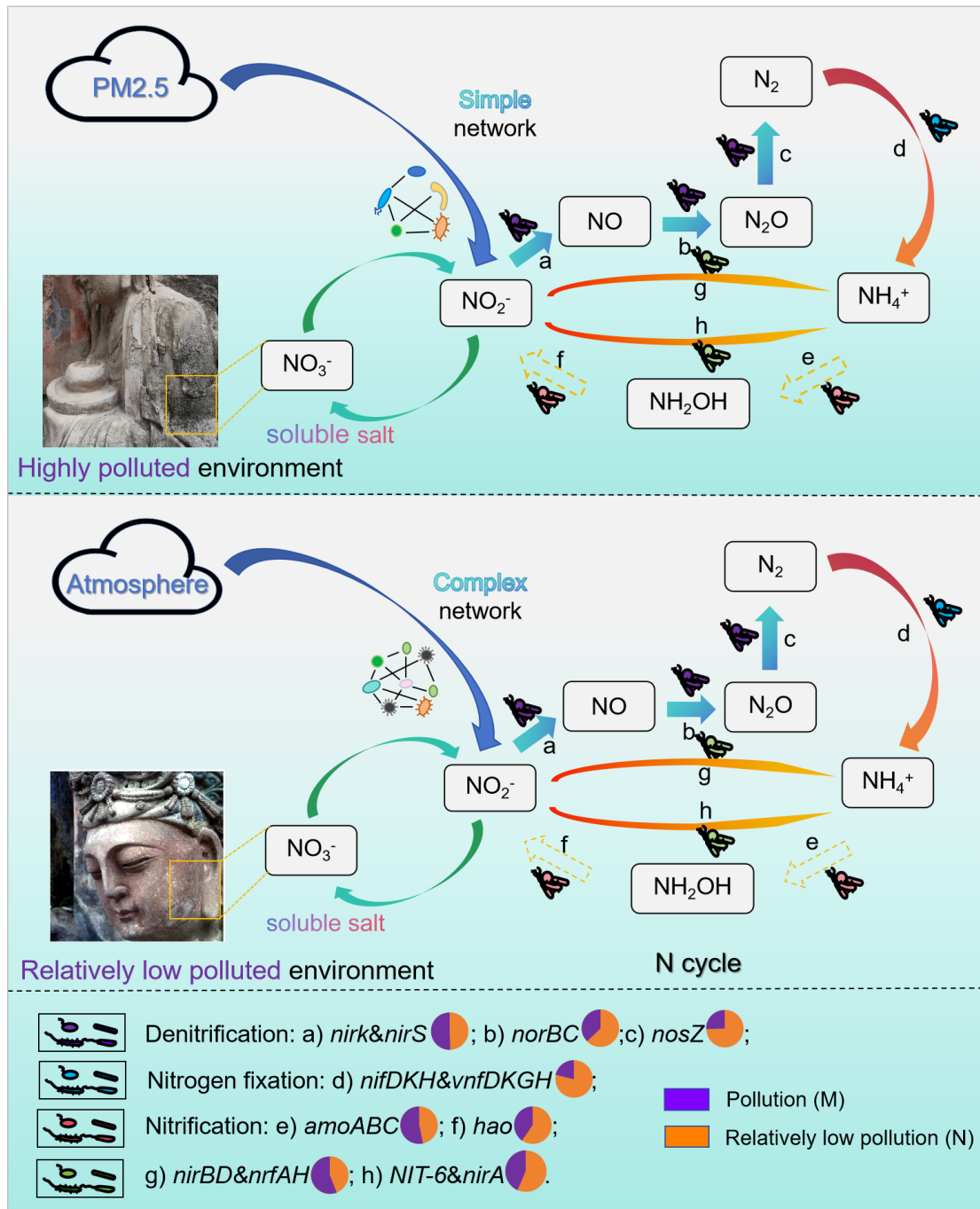
1 **4 Discussion**

2 *The effect of potential functional pathways of microorganisms on biological* 3 *deterioration*

4 Understanding the impact of particulate matter concentration on microbial
5 communities in heritage sites and the metabolic processes they govern is crucial for
6 explaining and addressing microbially induced biodeterioration or bioprotection. This
7 study helps to understand the relationship between microbial communities on stone and
8 pollution by analyzing the metabolic capacity of the communities in different polluted
9 environments.

10 Particulate matter pollution, particularly PM_{2.5} and PM₁₀, has a profound impact
11 on the physical and chemical structures of cultural heritage sites (Radulescu et al., 2021;
12 Vidovic et al., 2022). The sources of particulate matter are diverse, mainly including
13 industrial emissions, traffic pollution, and natural dust (Ogrizek et al., 2023). At cultural
14 heritage sites, especially in open heritage exposed to the external environment, these
15 particulates settle on the surfaces of artifacts through airflow and rainwater, affecting
16 their integrity and aesthetics (Bergin et al., 2015). This study found that pollution
17 environments with higher concentrations of particulate matter (MP, MQ) significantly
18 influenced the composition and functional metabolic pathways of surface microbial
19 communities at heritage sites, particularly showing stronger activity in gene expression
20 related to nitrogen cycling and energy metabolism (Fig. 7). High concentrations of
21 particulates can inhibit the growth of certain metabolic functions, particularly those
22 related to denitrifying microbial communities (Fig. 7). As the expression of

1 denitrification-related genes (such as *nosZ* and *nirS*) decreases, the accumulation of
2 nitrates increases. These nitrates may react with minerals on the heritage surface or with
3 moisture in the air to form soluble salts, such as nitrates. These soluble salts dissolve in
4 humid environments and crystallize under dry conditions. The repeated dissolution-
5 crystallization process generates salt crystals that exert physical stress on the heritage
6 structure (Li et al., 2024b; Sun et al., 2023; Yang et al., 2023), leading to surface erosion,
7 cracking, and even more severe weathering.



1

2 **Fig. 7** Nitrogen metabolism ability of microbial community in cultural heritage of
 3 different environments. The network structure of a polluted area (M) is simpler than
 4 that of a relatively low pollution area (N). The environment of high concentrations of
 5 particulate matter inhibited the expression of genes related to nitrogen metabolism,
 6 especially denitrification. This leads to the accumulation of soluble salts more easily,
 7 potentially further exacerbating the weathering of the heritage.

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The role of particulate matter in microbial communities at heritage sites can be explained from multiple perspectives. Firstly, particulate matter can carry a significant amount of organic matter, heavy metals, and other chemicals (Habeebullah, 2016; Ji and Zhao, 2015; Wang et al., 2019). These substances provide nutrients for microorganisms and may also act as selective pressures, altering the microbial community structure to favor specific microbial taxa that adapt to these pollutants. Studies have shown that in environments with higher concentrations of particulate matter, the expression levels of nitrogen cycling-related genes such as *nirBD* and *napAB* are elevated (Fig. 5a). This indicates that in these polluted heritage sites, microorganisms may adapt to the deposition of particulate matter and the ecological pressures it brings by enhancing nitrogen metabolic activity. On the other hand, the metabolic activities of microorganisms may also exert feedback effects on the chemical structures of cultural heritage sites (Zhang et al., 2021). Under high particulate matter concentrations, the metabolic function of bacteria tends to be enhanced, which may aggravate the weathering of heritage materials (Lazaridis et al., 2018). These phenomena suggest that particulate matter pollution is not merely an external physical deposition process. This study reveals the different impacts of particulate matter pollution on microbial communities by comparing the pollution levels in open and closed environments. The results indicate that in closed environments, areas with high particulate matter concentrations still exhibit more unique OTUs and higher metabolic efficiency. This may be attributed to the accumulation of pollutants and limited air

1 circulation in closed spaces, leading to the proliferation and adaptive evolution of
2 specific microbial populations. These communities often demonstrate strong tolerance,
3 allowing them to survive in high-pollution environments. Notably, genes related to
4 nitrogen cycling and pollutant degradation are more abundant in closed polluted
5 environments (MP), further indicating that these specific microbial communities may
6 mitigate the effects of particulate matter through their metabolic activities. The polluted
7 environment potentially promotes niche specialization and stability by selecting
8 specific microbial groups. On the other hand, Chao1 results showed that species
9 richness was lower in relatively low-polluted enclosed areas (NP). This may be because
10 the closed environment limits the flow of external substances and the interaction of the
11 environment, which in turn affects its diversity. However, the co-occurrence network
12 of relatively low-pollution closed environment is more complex than that of high-
13 pollution areas (Fig. 4). This may be because the competitive and cooperation
14 relationships of microbial communities relatively low-pollution closed environment is
15 more diversified, resulting in more complex network structure. In contrast, the pressure
16 is greater in highly polluted areas, so the limited species of microorganisms adapted to
17 pollution may lead to a relatively simple network.

18 Weathering is one of the primary causes of the degradation of cultural heritage
19 materials, and particulate matter deposition accelerates this process (Lin et al., 2017).
20 Common chemical pollutants in particulates, such as heavy metals and sulfur oxides,
21 can combine with moisture in the atmosphere to form acidic solutions that directly
22 affect cultural heritage surfaces, leading to accelerated weathering (Ding et al., 2020;

1 Liu et al., 2020; Meng et al., 2017). Meanwhile, these particulates can also engage in
2 complex chemical reactions with heritage materials, further exacerbating the
3 weathering and deterioration of heritage sites (Liu et al., 2020). Additionally, the results
4 of this study indicate that the relationship between particulate concentration and
5 microbial communities is one of the important factors in the weathering process.

6

7 **5 Conclusion**

8 This study analyzes the relationship between microbial communities and
9 particulate matter (PM_{2.5}) concentrations in World Cultural Heritage sites from a
10 global ecological perspective of heritage microbiology, revealing that particulate matter
11 pollution significantly impacts microbial structure, metabolic function, and ecological
12 network structure. Phototrophic bacteria (e.g., cyanobacteria) and certain heterotrophic
13 bacteria (e.g., Actinobacteria and Proteobacteria) form the backbone of bacterial
14 communities in the heritage site.

15 Among various parameters such as rainfall and relative humidity, PM_{2.5} emerges
16 as the most critical environmental factor. This indicates that the potential impact of PM
17 pollution on cultural heritage environments has been underestimated. High particulate
18 matter concentrations reduce the complexity and stability of microbial ecological
19 networks but introduce unique pollution-tolerant species. Additionally, the expression
20 of key functional genes related to nitrogen cycling and energy metabolism undergoes
21 significant changes. In polluted environments, particulate matter suppresses the
22 expression of genes associated with nitrogen cycling and metabolic pathways,

1 especially denitrification-related genes (e.g., *nosZ* and *nirS*), thereby affecting the
2 ecological functions of microbial communities. The weakening of denitrification may
3 lead to the accumulation of soluble salts, further exacerbating the weathering and
4 degradation of heritage sites. These findings underscore the profound impact of
5 pollution on heritage conservation and microbial ecosystems, highlighting the complex
6 interactions between microbial community functions and environmental pollution.
7 Viewing the microbial communities of cultural heritage from a global ecological
8 perspective helps provide new insights for the sustainable protection of cultural heritage
9 and the biological deterioration (or protection) of stone

10

11 **CRedit authorship contribution statement**

12 **Haiqing Yang** and **Francesca Cappitelli** conceived and directed the project; **Xingyue**
13 **Li** analyzed the experimental results and wrote the manuscript. **Xingyue Li** and
14 **Haiqing Yang** performed the visualisation. **Francesca Cappitelli** discussed the results
15 and commented on the manuscript.

16 **Data Availability Statement**

17 Data will be made available on request.

18 **Declaration of competing interest**

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

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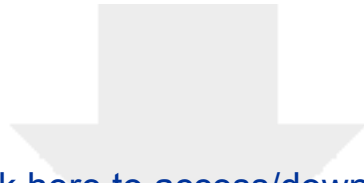
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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.