Red sandstone surface microbiota: functional convergence despite taxonomic divergence under dispersal limitation and hetero selection

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Abstract

Understanding the role of microbiota on stone surfaces is essential for developing effective grottoes conservation strategies. However, ecological feature of microbial communities on stone surfaces remains underexplored. In this study, we explored diversity, ecological feature, and functional profiles of microbial communities on the red sandstone surface of the Leshan Giant Buddha from microbial ecology perspective. The results show that Proteobacteria, Actinobacteria, and Cyanobacteria are the dominant prokaryotic phyla, and Ascomycota is the most dominant eukaryotic phylum. Interestingly, despite taxonomic divergence, the functional profiles of different types of the microbial communities remain convergent across all samples. As indicated by the ecological modelling, the interplay of dispersal limitation and hetero selection might have contributed to such species divergence, while selective pressure from the harsh environment on the stone surface promoted functional convergence. The assembly of visually distinct microbial communities is linked to a narrower ecological niche, higher proportion of habitat specialists, elevated complexity, and increased resilience of the prokaryotic network to disturbances. Microbial-mediated ammonium assimilation and nitrogen mineralization might be the two prominent processes that contribute to stone biodeterioration. This study deepens our understanding of microbial community assembly mechanism on stone cultural heritage surfaces and functional potentials, which provides microbial ecological insights for the conservation of these cultural treasures.

Keywords: Microbiota; Biodeterioration; Stone; Cultural heritage; Functional convergence; Community assembly; Microbial ecology

1. Introduction

Grottoes are a common form of stone cultural heritage. The stone surfaces of grottoes are integral components of ecosystems, providing critical habitats for microbial communities, serving as crucial habitats for microorganisms organized into diverse microbial communities[1-3]. These microbial communities not only diminish the aesthetic value of the artifacts by obscuring and deteriorating the carvings but may also, in some cases, compromise the structural integrity of the grottoes through severe erosion process[3-7]. Conversely, some studies have shown that under specific conditions, microbial communities on stone surfaces can contribute to the reinforcement of the stone through metabolic processes such as biomineralization[1,8-11]. Understanding the role of microorganisms on stone surfaces is essential for formulating better conservation strategies for grotto heritage. Current research in this field primarily focuses on the taxonomic diversity of microorganisms and their biogeochemical cycling and metabolic capabilities. These studies have revealed the key microbial groups involved in the biodeterioration of stone cultural heritage and their associated biogeochemical processes and metabolic responses[6,12-17]. However, ecological understanding of microbial communities on stone surfaces remains significantly limited.

The ecological mechanisms that govern microbial community assembly are one of the main scientific questions in ecological research[18-20]. It is generally believed that the assembly of local microbial communities is controlled by the combined effects of deterministic and stochastic processes[21-23]. Deterministic processes are used to describe the traditional niche-based theory, which hypothesize that species traits, interspecific interactions, and environmental factors govern community structure[24,25]. In contrast, stochastic processes are based on the neutral theory, which illustrate that community assembly is driven by stochastic events such as birth, death, colonization, extinction, and speciation[26,27]. A recent study on microbial communities on limestone surface revealed that prokaryotes and fungi in this environment are primarily dominated by the mutual effect of dispersal limitation (stochastic process) and homogeneous selection (deterministic process) [3]. However, it is currently unknown whether such community assembly mechanisms are specific to certain stone type, geographic location, or influenced by other factors.

The Leshan Giant Buddha, a world-renowned UNESCO World Heritage site, is a typical example of red sandstone grotto cultural heritage. The cleaning of stone surface is a crucial task for conservation of the grotto. However, despite regular cleaning of the stone surfaces by heritage conservators, microbial communities persistently reappear after each cleaning. To gain deeper insights into these continuously reappearing microbial communities on the stone surface of the Leshan Giant Buddha, several studies have explored their basic diversity and tested the abundance of certain nitrogen metabolic-related genes[28,29]. However, beyond this, our current knowledge regarding the microbial communities on the stone surface of the Leshan Giant Buddha, or even on other red sandstone surfaces, remains limited. The ecological mechanism of how microbes survive and form microbial communities within such ecosystem is still unknown. Therefore, this study uses the Leshan Giant Buddha, a UNESCO World Heritage site, as a case study to comprehensively evaluate the characteristics of microbial communities on the surface of this red sandstone cultural heritage from a microbial ecological perspective. Our goal is to gain ecological insights into the microbial communities on stone surfaces, providing a pioneering case study for understanding the

ecological roles of the stone surface microbiota. We aim to investigate how microorganisms on stone surfaces cope with frequent wet-dry cycles, nutrient limitations, and UV radiation stress, how they assemble microbial communities in such environments, and what functional potential and characteristics they exhibit. Our focus extends beyond the interactions within the microbiota to include the interactions among microorganisms, environmental factors, and the stone substrate.

2. Results

2.1. Taxonomic composition varies by the color of microbial communities

Samples of three different types of visually distinguishable microbial communities, along with blank samples, were collected from the stone surface of the Leshan Giant Buddha. In general, the total 27 samples were grouped and named according to their respective colors (Bla, black; Gre, green; Whi, white; and CK, blank control) (Fig. 1). We then performed amplicon sequencing of conserved phylogenetic marker genes (16S and ITS rRNA) to identify prokaryotes and eukaryotes within the microbial communities. After quality filtering and rarefaction, a total of 8928 and 2419 ASVs were obtained from the 16S and ITS sequences, respectively. Each group of data overlap with others, but they are not entirely identical or inclusive of each other (Fig. S1). In addition, metagenome sequencing was applied for each group and obtained 206, 491, 166 clean reads in total (Table S1).

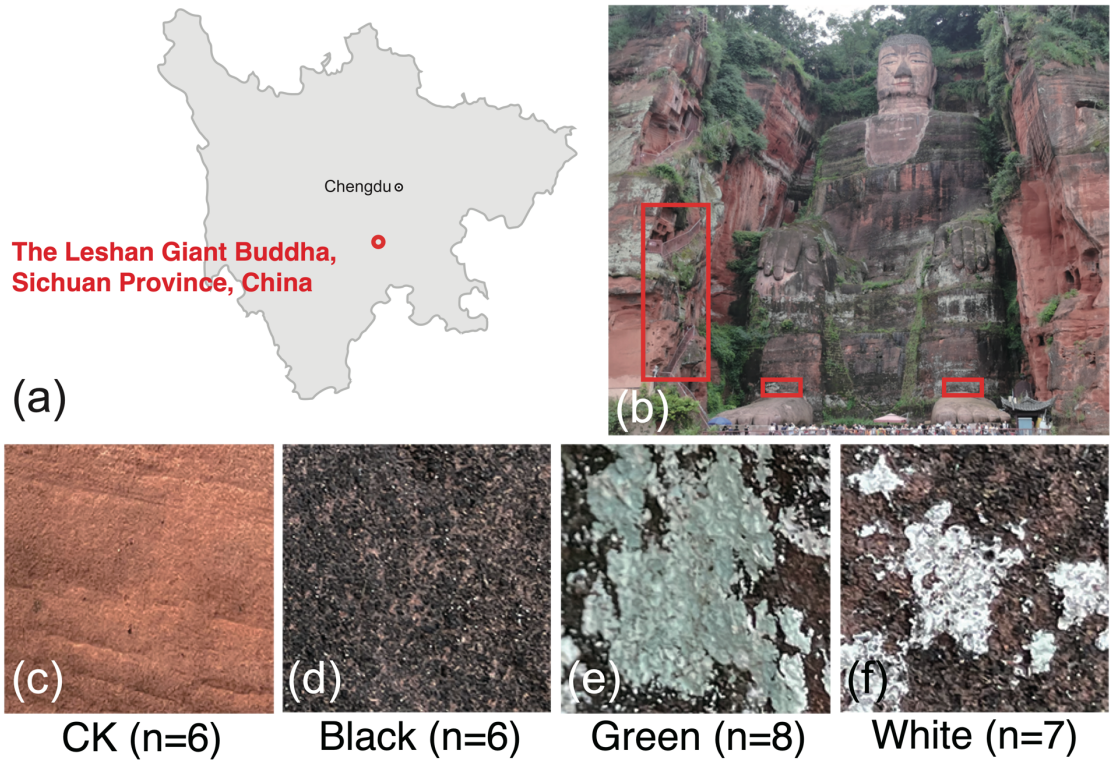

Figure 1. Representative examples and sampling of microbial communities on the red sandstone surface of the Leshan Giant Buddha. (a). Location of the Leshan Giant Buddha. (b). Sampling regions of the samples. Red boxes here indicate the specific sampling regions. (c). Representative examples of CK samples. (d). Representative examples of Black samples. (e). Representative examples of Green samples. (f). Representative examples of White samples. Notes: “n” in picture c-f represents the number of samples used in this study within each group.

To get taxonomic insights into the microbiota, we annotated the sequencing data, then visualized top 10 dominant phyla, top 20 dominant genus of prokaryotes (Fig. 2 a; Fig. S2 a; Table S2, S3) and eukaryotes (Fig. 2 b; Fig. S2 b; Table S4, S5) among all samples. We also constructed overall taxonomic community composition trees of top 5% dominant prokaryotes, and top 30% dominant eukaryotes (Fig. 2 c, d). The results show that among all samples, Proteobacteria, Actinobacteria, and Cyanobacteria are the dominant top three phyla in prokaryotes, comprising at least 70% of the total prokaryotic community in each sample. Ascomycota is the most dominant phylum in eukaryotes, comprising at least 50% of the total eukaryotic community in each sample.

To estimate the separation pattern between each group of microbial communities, non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity at the ASV level was performed and then conducted Anosim (Analysis of similarities) and PERMANOVA (i.e., adonis) tests (Fig. 2 e, f). The results show that both prokaryotes and eukaryotes among the four groups of samples reveal significant and major differences ($P < 0.001$). In summary, the structure of microbial communities varies according to the coloration of the samples, which indicates distinct microbial compositions among samples of different colors.

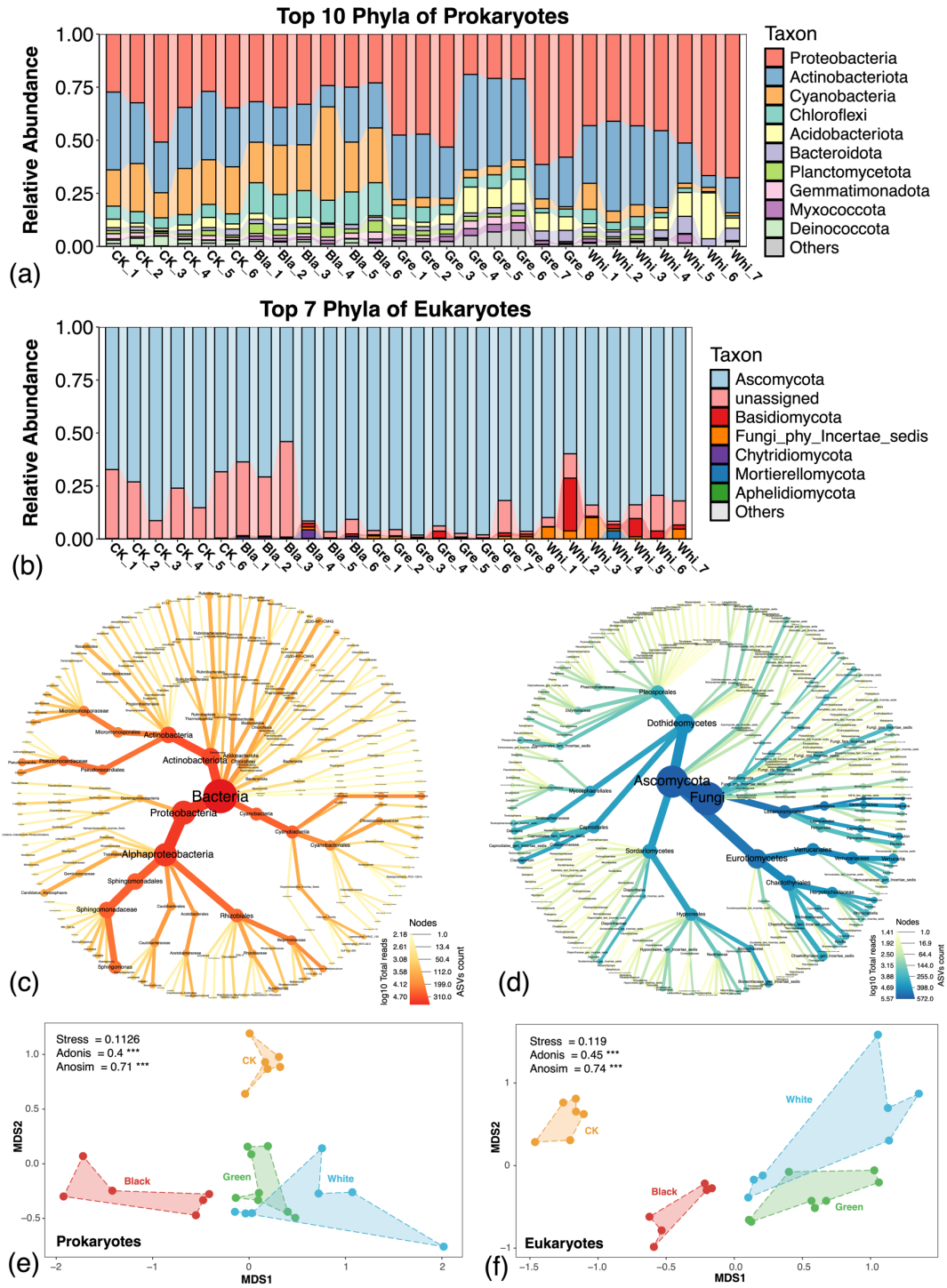

Figure 2. Taxonomic composition and distinct taxonomic structures of the microbial communities. (a). Relative abundances of dominant prokaryotes among all samples in phylum level. (b). Relative abundances of dominant prokaryotes among all samples in genus level. (c). The overall taxonomic community composition tree of top 5% dominant prokaryotes. (d). The overall taxonomic community composition tree of top 30% dominant eukaryotes. (e). Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities at the ASV level for prokaryotic communities. (f). Non-metric multidimensional scaling ordination based on Bray-Curtis dissimilarities at the ASV level for eukaryotic communities. Notes: The triple asterisks *** represents the $P < 0.001$.

138

139 **2.2. Dispersal limitation and hetero selection dominants the assembly of red sandstone surface**
140 **microbial communities**

141 To characterize the assembly mechanism of the red sandstone surface microbial communities,
142 we applied null model analysis[30,31] and dissimilarity-overlap curve (DOC) model analysis (Fig. 3)[32].

143 Null model analysis shows that both deterministic and stochastic processes controlled the
144 assembly of microbial communities, but the relative importance of the two processes in
145 community assembly vary among sample types (Fig. 3 a, b). In general, dispersal limitation
146 dominants the assembly of both prokaryotic communities (56.41%) and eukaryotic communities
147 (55.56%) on stone surface. Prokaryotes reveal overall elevated deterministic process than
148 eukaryotes. Hetero selection and dispersal limitation can explain about 91% of the assembly
149 mechanism of the prokaryotic communities. While hetero selection and dispersal limitation are
150 also main process for the assembly process of eukaryotic communities; drift plays a more pivotal
151 role in the assembly process of eukaryotic communities (20.23%) than in the prokaryotic ones
152 (5.41%). Prokaryotic samples reveal significantly higher value of betaNTI than eukaryotic ones (Fig.
153 3 c). No significant change of betaNTI is detected among any groups of prokaryotic samples
154 comparing with CK (Fig. 3 d). On the contrary, significant elevation of betaNTI is detected among
155 Black as well as Green groups of eukaryotic samples comparing with CK (Fig. 3 e).

156 In addition, the DOC model analysis demonstrates a significantly negative correlation
157 between overlap and dissimilarity in both prokaryotic and eukaryotic communities ($P < 0.001$),
158 which indicates a general dynamic and interspecific interaction within these microbial
159 communities (Fig. 3 f, g). These complex dynamic interactions among microorganisms in the
160 microbiota might lead to competition within the shared niche for survival.

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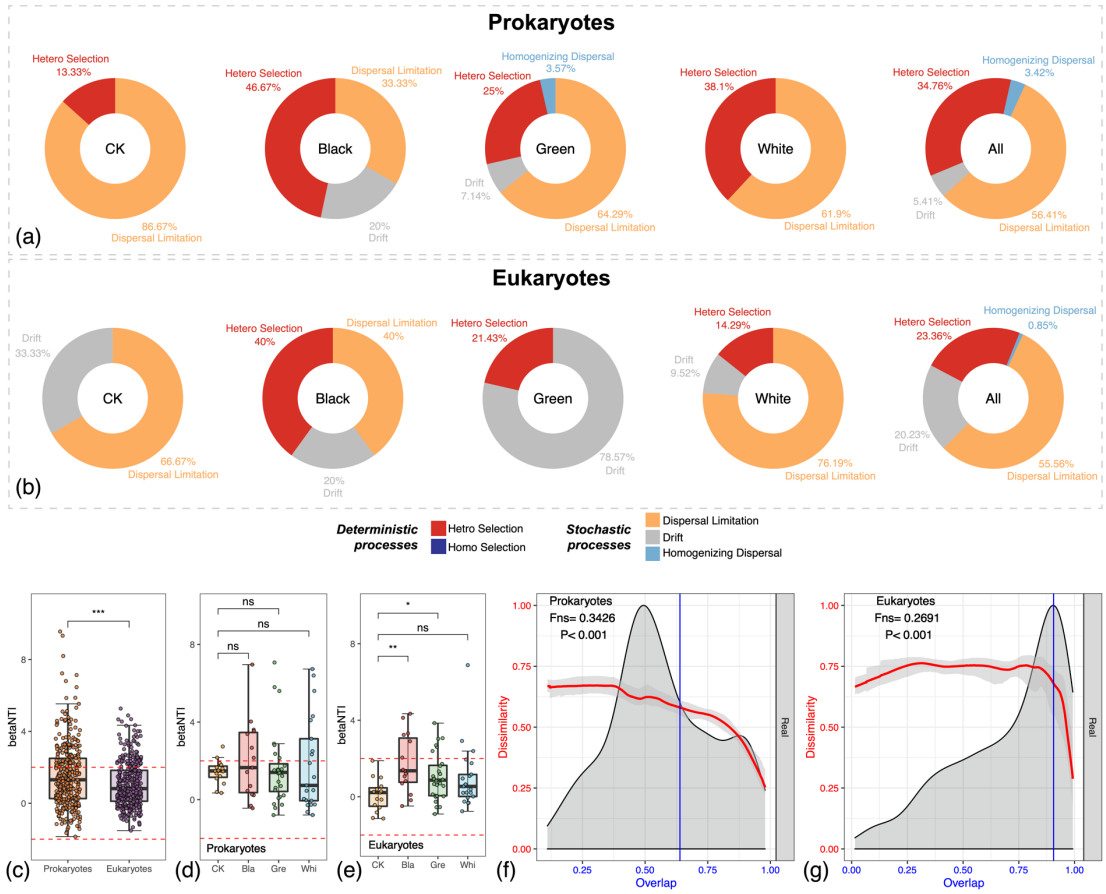

Figure 3. Assembly dynamics of the red sandstone surface microbial communities. (a). The relative importance of assembly processes in prokaryotic communities. (b). The relative importance of assembly processes in eukaryotic communities. (c). The differences of betaNTI between prokaryotic and eukaryotic communities. (d). Changes of betaNTI among three groups of prokaryotic samples comparing with CK. (e). Changes of betaNTI among three groups of eukaryotic samples comparing with CK. (f). Dissimilarity-overlap curve (DOC) analysis of the universality of prokaryotic dynamics among samples. (g). DOC analysis of the universality of eukaryotic dynamics among samples.

2.3. Specialization prevails over flexibility in microbial community formation on red sandstone surfaces

To delve deeper into the dynamic interactions among microorganisms within the microbiota, we further investigated the niche characterization of microbial communities on red sandstone surfaces. According to Levins' niche breadth theory, the distribution of microbiota composition can be used as an indicator of the environmental conditions[33]. Here, we calculated niche breadth (*Bcom*) and niche overlap (*Ocom*) as indicators to describe the habitat niche characterization of both prokaryotes and eukaryotes making up the stone surface microbial communities. First, the *Bcom* was calculated with Levins' index (Fig. 4 a-c). The results show that the visually distinguishable microbial communities reveal decreased overall *Bcom* comparing with CK group. Such results were also further validated with Shannon index (Fig. S3 a-c). Then, the *Ocom* was calculated with Schoener index. The *Ocom* for both prokaryotes and eukaryotes displayed a similar

trend that CK samples had the highest *Ocom*, followed by Black, Green, and White, in descending order. All pairwise comparisons were statistically significant, indicating distinct niche overlaps among the different samples. When comparing *Ocom* across all samples, prokaryotes had a significantly higher *Ocom* than eukaryotes (Fig. 4 d-f). Such results were also further validated with Pianka index (Fig. S3 d-f). In summary, both prokaryotic and eukaryotic communities exhibit significant variations in *Bcom* and *Ocom* across different sample types (CK, Black, Green, White). In general, CK samples displayed widest niche breadth and highest overlap level compared with the rest groups of samples. These results suggest that the formation of visually distinguishable microbial communities on rock surfaces requires species to be more specialized, rather than relying on diverse and flexible species with metabolic flexibility and redundancy.

To further explore the inference, we also calculated the proportion of generalists and specialists among the microbial communities (Fig. 4 g-k). Generalists are species that are better suited to survive in diverse and challenging environments and often play pivotal roles within their communities. Specialists, on the other hand, have a more limited range of habitats and are specifically adapted to environmental conditions[34,35]. The results show that at the ASVs level, the proportion of specialists in each type of sample is higher than that of generalists in both prokaryotes and eukaryotes (Fig. 4 g). To determine whether there are differences between all prokaryotes and eukaryotes, we also compared the proportion of specialists and generalists within these groups. The results show that in prokaryotes, the proportion of specialists is lower than that of generalists. Conversely, in eukaryotes, the proportion of specialists is higher than that of generalists (Fig. 4 g). At the phylum level, up to 70.22 % of the prokaryotes are classified as the middle; the proportion of specialists (29.26 %) is higher than that of generalists (0.52 %) (Fig. 4 i, Table S6). Myxococcota have the largest fraction of specialists in prokaryotes (50%) among the top 10 dominant phyla. (Fig. 4 h, Table S6). For the eukaryotes, 59.5% are classified as middle, 40.5% as specialists, and none of the ASV is classified as generalists. (Fig. 4 k, Table S7). This suggests that specialists prevail over generalists in the dominant phyla within the assembly of microbial communities. Notably, the non-dominant phyla (others) of both prokaryotes and eukaryotes exhibit the highest proportion of specialists (Fig. 4 h, j; Table S6, S7). This suggests that the non-dominant phyla within the community might have even lower metabolic flexibility compared to the dominant phyla.

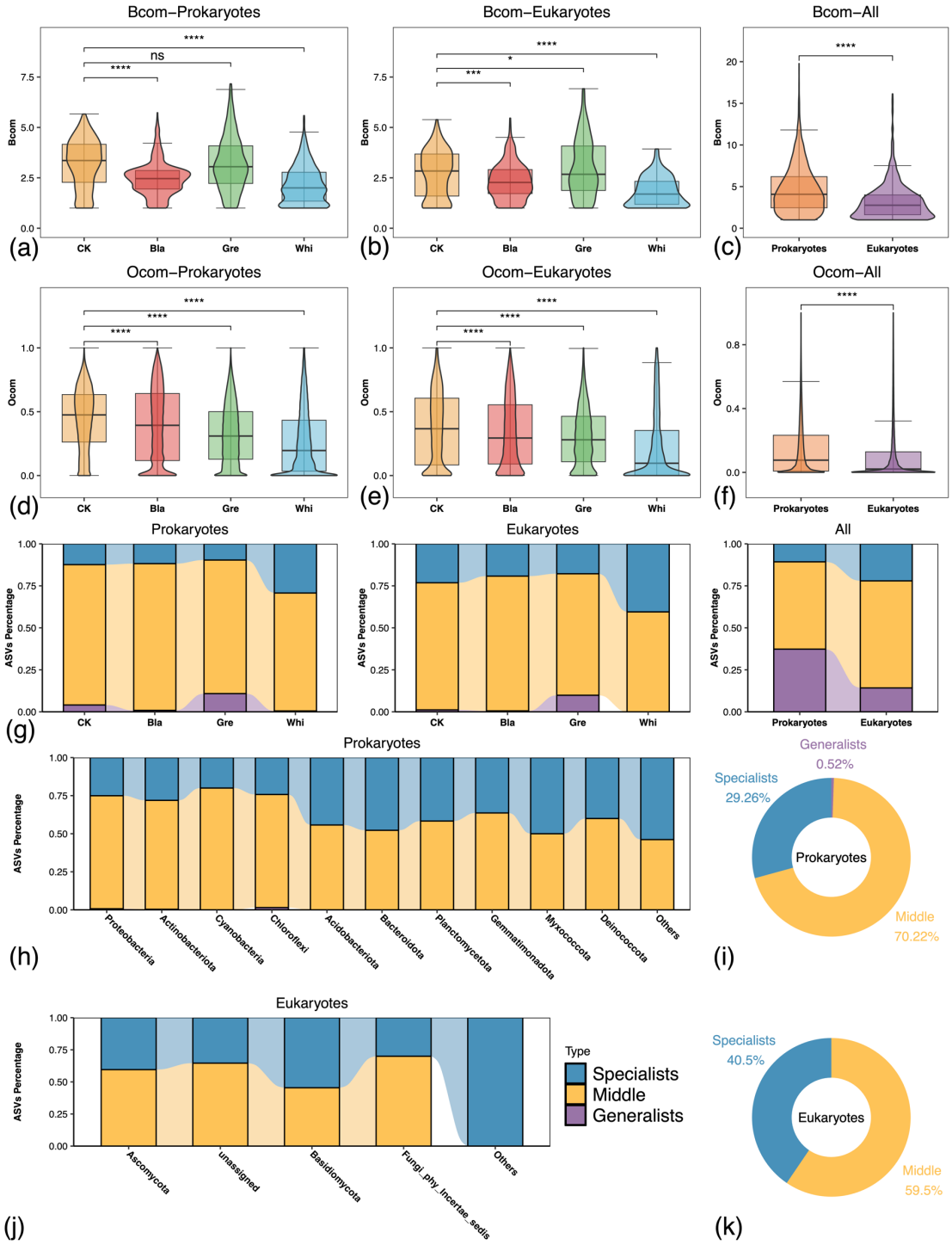

Figure 4. Niche characterization of the red sandstone surface microbial communities. (a). Average habitat niche breadth (*Bcom*) differences among three groups of prokaryotic samples comparing with CK in prokaryotes. (b). *Bcom* differences among three groups of prokaryotic samples comparing with CK in eukaryotes. (c). *Bcom* differences between prokaryotes and eukaryotes. (d). Average habitat niche breadth (*Ocom*) differences among three groups of prokaryotic samples comparing with CK in prokaryotes. (e). Average *Ocom* differences among three groups of prokaryotic samples comparing with CK in eukaryotes. (f). Average *Ocom* differences between prokaryotes and eukaryotes. (g). Proportional distribution of specialists, middle, and generalists in each sample group, and between prokaryotes and eukaryotes. (h). Proportional distribution of

specialists, middle, and generalists of prokaryotes at phylum level based on Levins' niche breadth index. (i). Overall proportion of the habitat niche breadth of prokaryotes at phylum level. (j). Proportional distribution of specialists, middle, and generalists of eukaryotes at phylum level. (k). Overall proportion of the habitat niche breadth of eukaryotes at phylum level. Notes: The *Bocm* was calculated with Levins' index. The *Ocom* was calculated with Schoener index. All ASVs were defined as specialists, middle or generalists according to Levins' niche breadth index. The asterisks in the boxplots stand for the significance of the Wilcoxon signed-rank test. The “ns” stands for no significance, single asterisk stands for $P < 0.05$, double asterisks stand for $P < 0.01$, Triple asterisks stand for $P < 0.001$, Four asterisks stand for $P < 0.0001$.

2.4. Elevated network complexity and resilience to disturbances during the formation of visually distinguishable microbial communities on red sandstone surface

Given the non-random assembly patterns observed in red sandstone surface microbial communities, we proceeded to construct microbial co-occurrence networks of both prokaryotes and eukaryotes in different types of microbial communities to further explore the interaction patterns within these communities (Fig. 5).

Topological features analysis of the networks reveals that prokaryotic network showed higher total nodes and links (Fig. 5 a). This indicates that the elevation of the complexity of the prokaryotic network is necessary for the formation of visually distinguishable microbial communities. To evaluate the resilience of the prokaryotic network to disturbances, we also analyzed the robustness of the network with different approaches including targeted and randomly removal of key species, removal of nodes, as well as the calculation of negative correlation ratio. The results show that the prokaryotic network robustness decreases as both targeted and randomly removal of the key species, with Black, Green, and White groups showing greater vulnerability compared to CK (Fig. 5 b, c). The prokaryotic networks of the three visually distinguishable microbial communities also have greater vulnerability to the removal of nodes than CK (Fig. 5 d). In addition, the CK network also revealed higher negative correlation ratio than the other three networks of visually distinguishable microbial communities, especially higher than both the Black and the White samples (Fig. 5 e). This implies the formation of visually distinguishable microbial communities could mitigate the mutual exclusion of microbial populations in resource competition.

In contrast, the topological features of eukaryotic networks differ from those of prokaryotic networks. The number of links in the Green group decreased to half compared to the CK group, while the number of links in the White group increased by approximately half (Fig. 5 f). The network robustness analysis results indicate that only the White group demonstrated higher stability across all test categories (Fig. 5 g-j). This suggests that the role of prokaryotes in community formation might be more complex and variable depending on the morphology of communities.

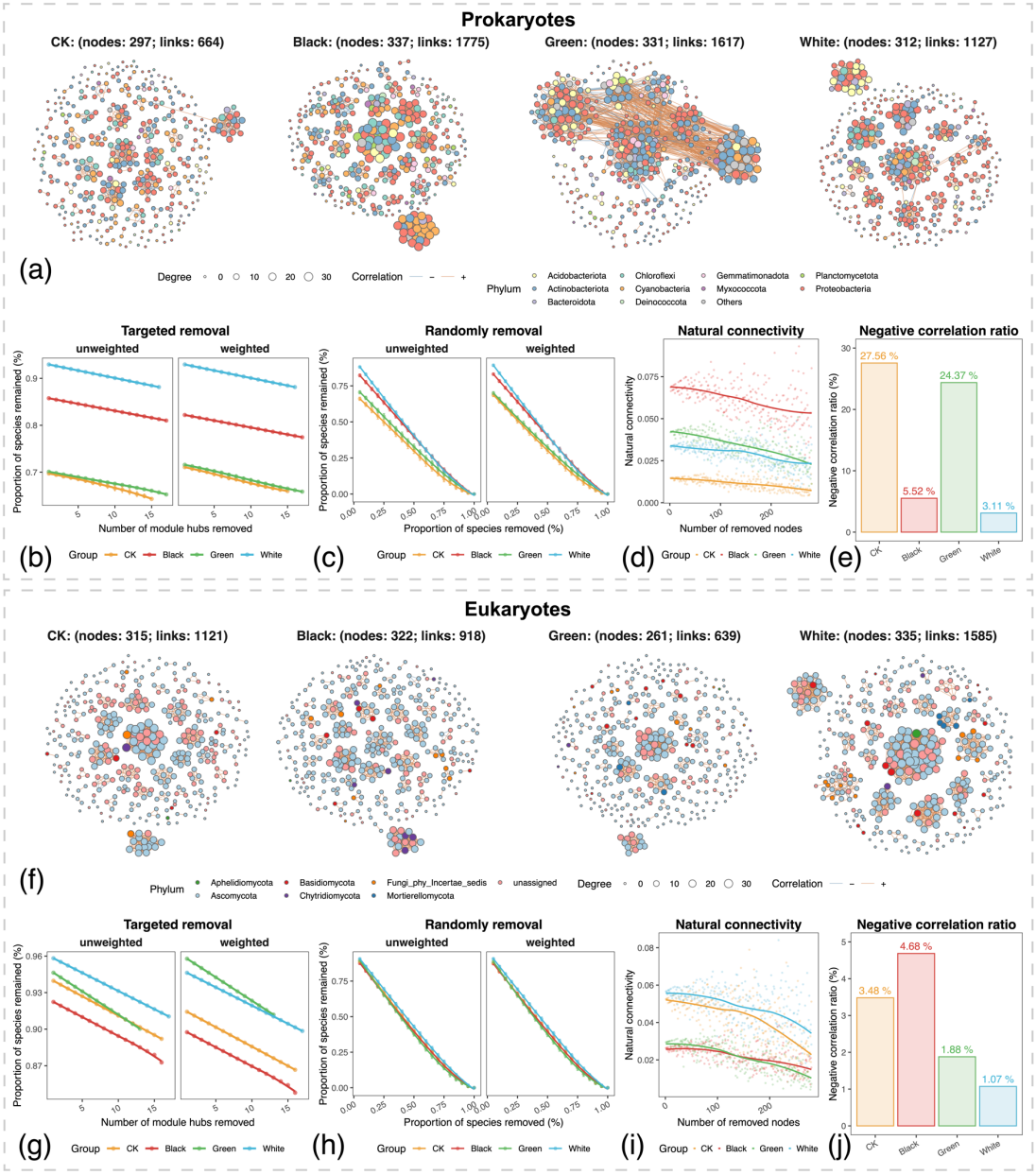

Figure 5. Topological features of the co-occurrence network within microbial communities on the stone surface. (a). Co-occurrence network of prokaryotic communities. (b). Robustness of prokaryotic networks under targeted removal of key species. (c). Robustness of prokaryotic networks under random removal of key species. (d). Vulnerability of prokaryotic networks under removal of key node. (e). Negative correlation ratio of the prokaryotic co-occurrence networks within each group. (f). Co-occurrence network of eukaryotic communities. (g). Robustness of eukaryotic networks under targeted removal of key species. (h). Robustness of eukaryotic networks under random removal of key species. (i). Vulnerability of eukaryotic networks under removal of key node. (j). Negative correlation ratio of the eukaryotic co-occurrence networks within each group.

279 **2.5. Functions of the microbial communities are convergent despite taxonomic variations**

280 To elucidate the metabolic processes that enable the survival of microbial communities on
281 the stone surface, we performed metagenomic sequencing to each type of microbial communities.
282 Ten Gene Ontology (GO) terms that could potentially contribute to the assembly of microbial
283 communities on red sandstone surfaces were identified in all four types of microbial communities[3].
284 These terms are associated with functions related to organism interactions, microbial survival, and
285 trophic processes. (Fig. 6 a). We then performed more detailed insight into nitrogen cycle
286 processes because they have been broadly reported as closely related with the deterioration of
287 stone[3,5,36-38]. The results showed that despite the variation in taxonomic structure, relative gene
288 abundance within each nitrogen cycle pathway remained consistent across all four types of
289 microbial communities (Fig. 6 b). Notably, the specific high-abundance genes regulating the four
290 major nitrogen cycling processes (ammonium assimilation, nitrogen mineralization, ammonia
291 assimilatory nitrate reduction, and dissimilatory nitrate reduction) might vary among different
292 groups. However, each group consistently possesses high-abundance genes capable of regulating
293 these respective nitrogen cycling processes. Afterwards, we analyzed the overall abundance
294 distribution of the genes involving in the nitrogen cycle process (Fig. 6 c). The results showed that
295 ammonium assimilation and nitrogen mineralization were the two dominant processes among the
296 nitrogen cycle. The two processes possessed accounted for up to 38.52% and 31.39% of the overall
297 abundance of reads within the nitrogen cycle pathways, respectively, which imply the intensive
298 nitrogen-exchanging interactions between microbial communities and stone surface substrate. In
299 addition, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment
300 annotation of all the genes to gain further insights (Fig. S4 a). The result showed that stone surface
301 microbial communities revealed activating metabolic processes in environmental information
302 processing, cellular processes, and basic metabolism, *etc*. Notably, about 2.4 % of unigenes were
303 annotated as human diseases-related functions, which suggests that the potential human health
304 risks posed by these microorganisms on stone surfaces might worth a thorough evaluation.
305

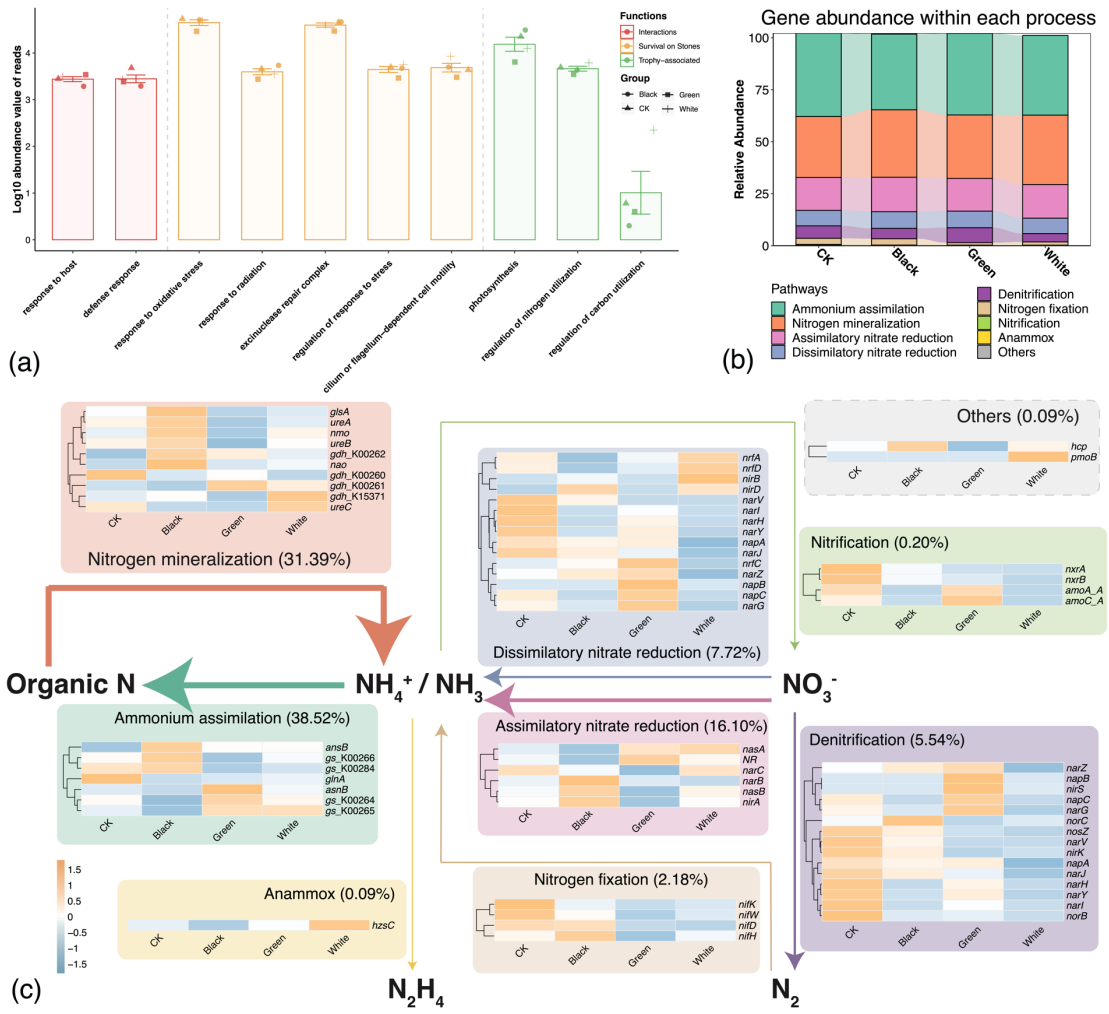

Figure 6. Functional profiles of the stone surface microbiota. (a). GO functional annotation of the stone surface microbiota. (b). Gene abundance within each nitrogen cycle pathway of the stone surface microbiota. (c). Nitrogen cycling potential of the stone surface microbiota. Arrow thickness indicates the proportion of functional genes for corresponding metabolic processes.

To get further insights into the potential human health risks from the stone surface microbiota, we further analyzed the human diseases-related unigenes (Fig. S4 b, c; Table S8). The results showed that the 7763 unigenes belongs to 166 types of antimicrobial genes and bacterial infectious disease-related genes by the KEGG annotation (Fig S4 b). The most abundant genes include the *mrcA*, encoding penicillin-binding protein; *lepA*, encoding GTP-binding protein; and *namH*, encoding UDP-MurNAc hydroxylase, *etc*. In addition, we annotated the virulence factors within metagenome sequencing data using the Virulence Factor Database (VFDB). The results showed that there are in total 1,324,923 unigenes can be annotated as the virulence factors. These unigenes can be classified into 14 different categories including biofilm formation, motility, immune modulation, and notably, antimicrobial activities (Fig. S4 c). Similar to the genes in the nitrogen cycle pathway, each category of virulence factors is evenly distributed across the four different types of stone surface microbial communities.

Environmental antimicrobial resistance (AMR) genes poses a rising global threat, stemming from human activities and the widespread use of antimicrobials, leading to contamination and

326 proliferation of resistant bacteria and genes in nature[39]. On this basis, we then further annotated
327 the AMR genes within the stone surface microbiota using the Comprehensive Antibiotic Resistance
328 Database (CARD). The results showed that in our sequencing data, 26,862 unigenes were
329 annotated as 166 AMR genes, classified into 9 different resistance mechanisms (Fig. S5, Table S9).
330 These genes, especially the dominant genes with antibiotic efflux, antibiotic target alteration, and
331 antibiotic target protection mechanism are evenly distributed across the four different types of
332 red sandstone surface microbial communities. The presence of mobile genetic elements (MGEs) is
333 closely linked to the horizontal gene transfer of AMR genes among microbial communities[40-42].
334 Hence, we also performed a metagenome-wide search against the MGEs within the stone surface
335 microbiota using the Mobile Genetic Element Database. In total, 483 unigenes were annotated as
336 the MGEs. These unigenes can be further annotated as 57 genes, classified into 14 different types
337 such as transposase, integrase, insertion sequences and plasmids, *etc*. (Fig. S6, Table S10). The
338 most abundant gene, *tnpA*, encodes the only known transposase that facilitates both transposition
339 and target immunity[43,44]. This gene, similar to all the above-mentioned genes within the stone
340 surface microbiota, are also evenly distributed across all the four different types of microbial
341 communities.
342

343 ## 3. Discussion

344 Uncovering the diversity, assembly mechanisms, and functional traits of microbial
345 communities is a central focus in microbial ecology[18,45,46]. Recently, the role of microbial
346 communities on stone surfaces has garnered increasing attention, particularly in the field of stone
347 cultural heritage conservation[47]. In this study, we present a pioneering case study on the ecological
348 roles of microbiota on red sandstone surfaces, using the Leshan Giant Buddha as an example. The
349 results indicate that despite the taxonomic composition of microbial communities varies according
350 to their coloration, their functions are convergent, particularly in terms of nitrogen cycle-related
351 biogeochemical processes. Dispersal limitation and heterogeneous selection dominate the
352 assembly of stone surface microbial communities. Habitat specialists play a more significant role
353 than generalists during the formation of microbial communities on rock surface. As visible
354 microbial communities form on stone surfaces, network complexity increases, and resistance to
355 disturbances is enhanced. By comparing our findings with previous studies on microbial
356 communities on stone surfaces, we inferred both commonalities and unique characteristics in the
357 ecological features of stone surface microbial communities.
358

359 **3.1. Harsh habitant on stone surface might have facilitated the functional convergence of**
360 **microbial communities**

361 In this study, the microbial diversity analysis shows that Proteobacteria, Actinobacteria,
362 Cyanobacteria, Chloroflexi, and Acidobacteriota dominate the top phyla among the prokaryotes in
363 all samples, while Ascomycota stands as the most dominant phylum in eukaryotes, despite the
364 distinct microbial community morphology across different sample groups. Such phylum-level
365 diversity patterns are largely consistent with previous reports on taxonomic diversity of microbial
366 communities on the surface of other stone types, but the diversity differ significantly at the genus
367 level[2,3,8,29,48-50]. Interestingly, in this study, despite the distinct taxonomic structures observed
368 among different sample types based on NMDS analysis, there is a high degree of functional

consistency across all sample groups. By comparing the results of studies on microbial communities on the surfaces of several other types of stone, such characteristic of functional convergence despite taxonomic divergence is widely observed across these surfaces. These microbial communities all possess the capability to withstand pressures such as fluctuations between wet and dry conditions, endure UV stress on stone surfaces, and facilitate ion exchange with the stone substrate[3,5].

Previous study in limestone surface microbial communities reveals that nitrifying bacteria genera *Nitrospira* and *Nitrosomonas* were significantly enriched in such habitant[3]. In another case study involving regular sandstone, *Nitrosopumilus* and *Nitrospira* were also identified from deteriorated sediments[5]. However, in our study, several dominant genera with ability to participate in nitrogen cycling were also identified from the red sandstone microbial communities, yet their taxonomy differs from that of the microorganisms in previously studies in other types of stones. For example, in this study, a dominant prokaryotic genus, *Sphingomonas*, possesses the ability to remove ammonia nitrogen and nitrite[51]. Additionally, species from the genus *Sphingomonas* are involved in nitrification processes under low dissolved oxygen conditions[52]. Furthermore, another dominant genus in our study, *Methylobacterium*, includes species known as plant growth-promoting rhizobacteria, whose genomes contain enzymes involved in nitrogen metabolism and ammonia assimilation[53]. Notably, the two dominated types of nitrogen cycling genes in limestone surface microbial communities are nitrogen assimilation and mineralization, which is coherent with what we found in red sandstone surface microbial communities in this study[3]. In summary, this characteristic - functional convergence despite taxonomic divergence - might be widely applicable among microbial communities inhabiting various types of stone surfaces.

Functional convergence despite taxonomic divergence is not unique to microbial communities on stone surfaces. Several gut microbiota studies have reported functional, but not compositional, convergence of gut microbiomes[54-57]. Some soil microbiota studies also reported the stable functional structure despite high taxonomic variability[58-60]. In addition, microbial functional genes of ocean microbiota that execute certain functions, such as B_{12} biosynthesis, are widely distributed, while the microbial taxa that carry these genes vary significantly[61]. These results corroborate the ecological hypothesis that environment truly selects for function, rather than taxonomy[62]. Different taxonomic groups could reveal convergent functions as a result of functional redundancy among microbial systems[63]. On stone surface, such functional convergence phenomenon might be attributed to the selective pressure imposed by the harsh living conditions on the stone surface, favoring the survival of microbial communities with specific functions[58,64-66]. This study suggests that the interplay of deterministic and stochastic processes might have led to significant variations in the taxonomic structure of the stone surface microbiota, which is consistent with previous cases regarding crude oil microbial community assembly[66].

3.2. Assembly of stone surface microbial communities requires a general pattern of dynamic interactions

In this study, we witnessed the extensive microbe-microbe and microbe-environment interactions within the microbial communities on the stone surface. The mutual effect of hetero selection and dispersal limitation controls the assembly process of both prokaryotic and eukaryotic communities on red sandstone surfaces. Such results differ from previous studies on the assembly process of microbial communities on limestone surfaces, where the assembly process is driven by

413 hetero selection and dispersal limitation[3]. This suggests that the assembly mechanisms of
414 microbial communities on stone surfaces might vary based on stone types, geographical locations,
415 climatic characteristics, or other environmental factors. In addition, the results of the DOC model
416 analysis suggest a general pattern of dynamic interspecific interactions within the communities on
417 the red sandstone surface and high selective pressure on the red sandstone surface[32,67-69]. Such
418 complexity was further testified in this study by the topological feature analysis of the microbial
419 networks. The active biogeochemical metabolism such as ammonium assimilation and nitrogen
420 mineralization processes by the microbial communities also suggests their active interactions with
421 the red sandstone substrate[70].
422 Here, we propose a hypothesis that a possible model capable of precisely interpreting the
423 assembly processes of stone surface microbial communities is the classic MacArthur consumer-
424 resource model, where species-species interactions are mediated through resource dynamics[71-73].
425 Because the nutrient-poor nature of stone surfaces, the colonization by pioneer species is
426 primarily driven by deterministic processes linked to nutrient availability, which aligns with
427 commonly observed among natural communities from various locations[74]. Some pioneer species
428 utilize inorganic energy from the stone to establish themselves. These species then leak metabolic
429 by-products into the environment, increasing the diversity of resources and enhancing the
430 biocompatibility of the stone surface. Other species can subsequently utilize these leaked
431 metabolites, in turn releasing new by-products into the environment, further enhancing the
432 biocompatibility of the stone surface, and facilitating the formation of microbial communities. The
433 observed decrease in ecological niche breadth among the visually distinguishable microbial
434 communities in this study also suggests that the utilization of specific resources is more efficient
435 within these communities. We also observed that specialization prevails over flexibility during the
436 formation of microbial communities on red sandstone surfaces. This is consistent with a previous
437 study on the assembly of microbial communities in a wide range of well-defined resource
438 environments, where habitat specialists also drive the increase of diversity in such microbial
439 communities[19].
440
441 **3.3. Potential resources and bio-risks in stone surface microbial communities**
442 The elimination of deterioration-causing microorganisms is one of the fundamental goals in
443 the conservation of stone cultural heritage. This study demonstrates that the microbial community
444 assembly on the surface of red sandstone is driven by dispersal limitation and hetero selection
445 processes. The harsh conditions on the stone surface create a challenging environment, exerting
446 strong selective pressure. Additionally, ecological modelling from this study reveals widely
447 negative correlation between species, all of which suggest that intense competition is occurring in
448 these microbial communities. One possible source of the negative correlation between species in
449 microbial communities is that certain species can gain a population advantage in competitions
450 during community formation by secreting antagonistic substances. Recently, there have been
451 numerous reports of metagenomic studies that explore new antagonistic substances as
452 antimicrobial resources from various habitats, including novel antibiotics, and antimicrobial
453 peptides, among others[75-77]. If machine learning and other methods can be used for in-depth
454 mining of metagenomic sequencing data from microbiota on red sandstone, it is possible to
455 develop novel antimicrobial agents for the removal of stone deterioration-causing microorganisms
456 in the future.

The "One Health" concept emphasizes the interconnectedness between human health and environmental health[78]. Previous study provided a description of AMR genes in the microbial communities on the surface of two stone cultural heritage sites[12,79], which indicates that stone surfaces might be an overlooked ecosystem capable of harboring AMR genes as well as other potentially risky factors for human health. In this study, an in-depth analysis of the potential human health risks associated with microbial communities on stone surfaces was conducted. As expected, virulence factors, AMR genes, and MGEs were observed from red sandstone surface. We suggest that AMR genes could aid microorganisms in gaining an advantage in intense competition within the microbial communities, both within and between species competition, thereby promoting the enrichment of AMR genes within the stone surface microbial communities. Human activities and the extensive use of antimicrobials might have facilitate this process, notably through mechanisms like the water cycle[79-82]. Furthermore, the presence of MGEs on stone surfaces indicates the potential for horizontal gene transfer of AMR genes among microbial communities, potentially facilitating the dissemination and persistence of resistant microorganisms on stone surface as in previous cases across various environments[83-85]. This underscores the potential for stone surface microbial communities to be reservoirs for antibiotic-resistant bacteria. Currently, natural environmental health assessments predominantly focus on habitats including soil, water, and air, *etc*[86-88]. Although stone surfaces are also vital components of ecosystems, research on whether these stone surface microbes pose health risks remains limited. Our study suggests that more detailed assessments of the environmental health of stone surfaces are necessary in future research.

3.4. Summary

In summary, this study focuses on microbial communities on the surface of red sandstones of the Leshan Giant Buddha, which reveals their ecological and functional characteristics. Under the interplay of dispersal limitation and hetero selection, the microbiota on the red sandstone were assembles into distinct types of visually distinguishable microbial communities., significantly differentiate the taxonomic composition of these communities. However, under the harsh habitant on red sandstone surface, such as UV radiation, fluctuations between dry and wet conditions, and limited nutrient availability, the functions of the microbe among different types of microbial communities are convergent. These functions are particularly enriched in biogeochemical processes related to nitrogen cycling and responses to environmental stress. The formation of visually distinguishable microbial communities is associated with a narrower niche width, elevated complexity, and increased resilience of the prokaryotic network to disturbances. Furthermore, a higher proportion of habitat specialists are found in microbes forming these visually distinguishable microbial communities. Given the nutrient limitations of the red sandstone surface, the classic MacArthur consumer-resource model might provide a potential framework to further explain the assembly mechanisms of these microbial communities. A significant accumulation of AMRs and MGEs within these communities might help enhances their competitive advantage during community formation, enabling them to resist antagonistic substances secreted by competing organisms. The sources of these AMRs and MGEs might include water cycles and human activities (Fig. 7). Future research into the ecological traits and functional potential of these microbial communities will provide critical insights for managing microbial communities on red sandstone surfaces. Moreover, microbial deterioration affects not only stone cultural heritage but

also a wide range of different cultural heritage materials[89-92]. The microbial ecology-based approach used in this study will help advance research on microbial deterioration mechanisms in other cultural heritage materials, providing a model for future work.

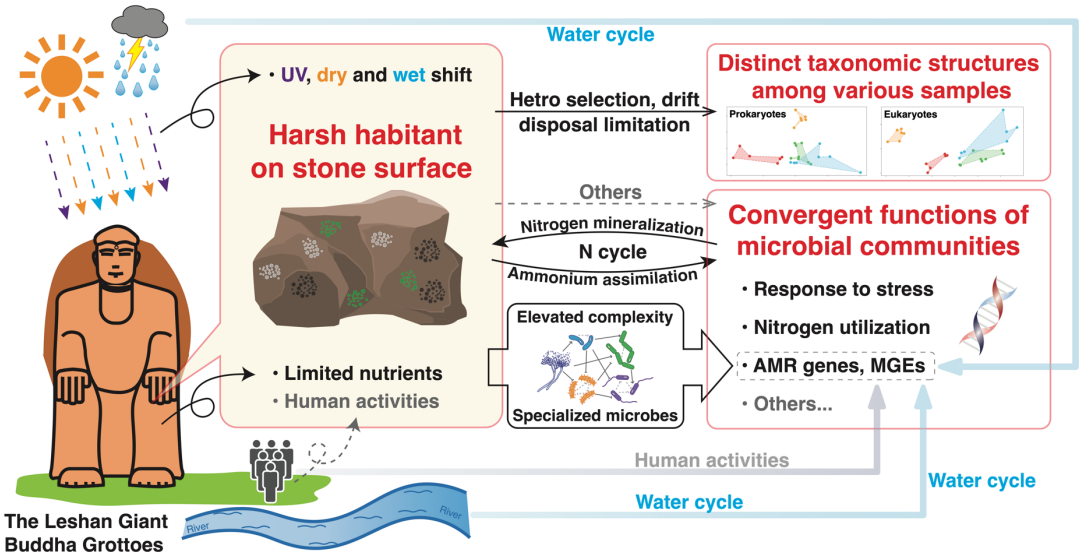

Figure 7. Schematic diagram illustrates the characteristics of microbial communities on the red sandstone surface of the Leshan Giant Buddha

4. Materials and Methods

See detailed materials and methods in **Supplementary information 1**. The authors used ChatGPT to check the English grammar during the writing of this manuscript. Authors are fully responsible for the content of this manuscript, even those parts produced by ChatGPT, and are thus liable for any breach of publication ethics.

Supplementary Files

Supplementary information 1. Supplementary Materials and Methods

Figure S1. The overall taxonomic community composition among all the samples.

Figure S2. Taxonomic community composition of the microbial communities in genus level.

Figure S3. Niche characterization of the stone surface microbial communities based on Shannon niche breadth index and Pianka niche overlap index.

Figure S4. Human health risk potentials within the stone surface microbiota.

Figure S5. Antimicrobial resistance (AMR) genes within the stone surface microbiota.

Figure S6. The mobile genetic elements (MGEs) within the stone surface microbiota.

Table S1. Summaries for the details from the metagenome sequencing

Table S2. Relative abundances of dominant prokaryotes among 27 samples at the phylum level.

Table S3. Relative abundances of dominant prokaryotes among 27 samples at the genus level.

Table S4. Relative abundances of dominant eukaryotes among 27 samples at the phylum level.

Table S5. Relative abundances of dominant eukaryotes among 27 samples at the genus level.

Table S6. Habitat niche breadth of prokaryotes at the phylum level.

Table S7. Habitat niche breadth of eukaryotes at the phylum level.

Table S8. Human disease-related genes within the stone surface microbiota based on KEGG annotation.

Table S9. AMR genes within the stone surface microbiota.

Table S10. The mobile genetic elements within the stone surface microbiota.

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