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Metagenome Across a Geochemical Gradient of Indian Stone Ruins Found at Historic Sites in Tamil Nadu, India

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Abstract

Although stone surfaces seem unlikely to be habitable, they support microbial life. Life on these surfaces are subjected to many varying harsh conditions and require the inhabitants to exhibit resistance to environmental factors including UV irradiation, toxic metal exposure, and fluctuating temperatures and humidity. Here we report the effect of hosting stone geochemistry on the microbiome of stone ruins found in Tamil Nadu, India. The microbial communities found on the two lithologies, granite and granodiorite, hosted distinct populations of bacteria. Geochemical composition analysis of sampled stones revealed quartz mineral content as a major driver of microbial community structure, particularly promoting community richness and proportions of Cyanobacteria and Deinococcus-Thermus. Other geochemical parameters including ilmenite, albite, anorthite, and orthoclase components or elemental concentrations (Ti, Fe, Mn, Na, and K) also influenced community structure to a lesser degree than quartz. Core members of the stone microbiome community found on both lithologies were also identified and included Cyanobacteria (Chroococcidiopsaceae and *Dapisostemonum* CCIBt 3536), *Rubrobacter*, and *Deinococcus*. A cluster of taxa including *Sphingomonas*, *Geodermatophilus*, and *Truepera* were mostly found in the granodiorite samples. Community diversity correlated with quartz mineral content in these samples may indicate that the microbial communities that attach to quartz surfaces may be transient and regularly changing. This work has expanded our understanding of built-stone microbial community structure based on lithology and geochemistry.

Keywords Microbiome · Metagenome · Stones · Ruins · Climate · Geochemistry · Geodermatophilaceae · Actinobacteria · Stone-dwelling bacteria

Introduction

Once thought uninhabitable, stone surfaces have been found to support microbial life. Life on these surfaces are subjected to numerous and varying harsh conditions including limited availability of nutrients and water, extended periods of desiccation, lethal UV irradiation, exposure to toxic metals and metalloids, and fluctuations in temperature [1–6]. Because these severe environmental conditions constantly and significantly vary over time, stone-dwelling microbial communities must adapt to extreme changes [7]. In spite of these conditions, it has been shown that microbial communities are found worldwide on stone surfaces [8–13]. These communities form biofilms, or assemblages of microbes that stick to each other and to a surface that promotes community survival by retaining water and nutrients [7, 8, 13–15]. Like any ecological community, the members of these biofilms fulfill specific roles that help promote the survival of the entire community. Actinobacteria, fungi, and other filamentous microbes extend the growth of their filaments beyond the surface deeper into the stone, not only to promote biofilm adherence to the stone but also to gain access to minerals within the stone [13, 16–18]. In contrast, nonfilamentous microbes, such as various Proteobacteria and Firmicutes, form aggregates that aid

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biofilm development and community cohesion [7, 19]. Autotrophs, like Cyanobacteria, Chloroflexi, and Algae, are primary producers of the community providing a carbon source through photosynthesis [20–22]. Although these biofilms or assemblages contain a wide range of microbes, members of Actinobacteria and Cyanobacteria are considered to be primary microbial colonizers of stone surfaces due to hyphal attachment to stones and providing a carbon source through photosynthesis, respectively, and by promoting the growth of successive microbial colonizers [13, 17, 23–26]. Members of the Actinobacteria family Geodermatophilaceae [27], which have been frequently isolated from both stone interiors and surfaces, have been described as multi-resistant microbes and are particularly radioresistant [4, 28–33].

Stone-colonizing microbes degrade the materials that they inhabit [7, 34] and inflict physical and chemical damage upon the stones [35]. Both hyphal growth and biofilm development can cause physical disruptions and cracks in stone materials [1, 16–18]. Organic acids produced by microbial respiration can cause indirect mineral leaching [36, 37]. Stone deterioration via microorganisms may also be a natural part of geochemical cycling, requiring a deeper understanding of the rock microbiome.

Recently, we investigated the effects of environmental conditions on the microbial community structure of the stone microbiomes found in North Africa, all with similar lithology [8]. In this study, we investigated the structure of stone-dwelling microbial communities in relation to the geochemical composition of stones from ancient ruins of Tamil Nadu, India. We used high-throughput amplicon-based (targeting the 16S rRNA gene) sequencing on stone ruins from temples, rock forts, and caves in Tamil Nadu, India, to achieve our objectives. These stone samples were collected from two different lithologies and represent the first investigation of the microbiome of granite and granodiorite stone walls and their geochemical relationship of any historic sites in Tamil Nadu, India. Our primary focus was the prokaryotic community structure of the stones from different lithologies.

Methods

Sampling and DNA Extractions

In the month of October 2016, stone samples were collected from seven different sites in Tamil Nadu, India, consisting of historic Hindu temples and forts (Fig. 1 and Fig. S1 Online Resource 1). The stone samples from Fort Dindigul (DF), Valikandapuram Sivan Temple (PS), Fort Ranjankudi (PR and PRA), Thanjavur Big Temple (TB), and Fort Thirumayam (TM and TMA) were composed of granite (monzo-granite), while stone samples from Tiruchirappalli Rockfort (TF and TFA) and Sittanavasal Cave Temple (PV and PVA) were composed of granodiorite. The Koppen-Geiger climate classification system [38] was used to define the climate for each sampling location. All of the stone samples were defined as "built" stone condition (Table 1). The "built" stone condition describes stone samples collected from man-made stone structures, while "natural" describes stone samples collected from stones of environmental origin. For each location, about 10 g of stone was collected aseptically from the stone ruins using a sterile rock hammer or chisel and then stored in sterile collection bags. All stone samples were prepared for DNA extractions by aseptically crushing the stone with a sterile rock hammer and further reducing to a powder by grinding with a sterile mortar and pestle. DNA was extracted from 0.5 g of pulverized stone using a DNeasyPowerSoil Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). Extracted DNA was treated with 0.5 μL of RNase A (10 ng/mL) for 30 min at 37 °C to remove RNA from the sample (Qiagen, Valencia, CA). For each sample, extractions were performed in triplicate.

Climate and Geochemical Data

Quantitative climate variables, obtained from the World Weather Online database [39], were used to interpret variations observed in the microbial stone community structure. Monthly averages for the temperature, precipitation, humidity, UV index, wind speed, elevation, snowfall, and air pressure for the distinct timeframe of sampling at each location are summarized in supplementary Table S1 (Online Resource 2).

Geochemical characterizations of the specimens were also assessed to investigate if elemental concentrations and mineral content influence the diversity and structure of the stone microbial communities. For each specimen, 1–5 g aliquots of sample were powdered and whole-rock geochemical composition was determined via X-ray fluorescence following procedures outlined previously [40] at the University of Massachusetts, Amherst. The percentages of the following oxides within the sampled stones were determined: SiO_2, TiO_2, Al_2O_3, Fe_2O_3, MnO, MgO, CaO, Na_2O, K_2O, and P_2O_5. Raw values (in %) of the measured oxides are shown in Table S2 (Online Resource 3) and were converted into ppm and mmol/kg of stone material during subsequent ecological analyses. Percentages of oxides were converted into percentages of minerals, as described by Kelsey [41], and were also incorporated into ecological analyses.

Amplicon (16 S rRNA) Sequencing of Stone Microbial Communities

To investigate the prokaryotic community profiles of the extracted stone, the V4 hypervariable region of the 16S subunit rRNA gene was amplified and sequenced following the Earth Microbiome Project 515F/806R protocol [42]. For each

Table 1 Summary of stone samples

Sample	Specific site of collection	Location	Coordinates (DMS)	Climate type	Stone type	Stone condition	Approximate stone age (years)
DF	Temple wall outside	Fort Dindigul, Dindigul, Tamil Nadu, India	10° 21′ 39″ N, 77° 57′ 42″ E	Tropical wet and dry	Granite	Built	250–500
PS	Inside temple wall damage area	Valikandapuram Sivan Temple, Perambalur, Tamil Nadu, India	11° 18′ 55″ N, 78° 54′ 55″ E	Tropical wet and dry	Granodiorite	Built	250–500
PR	Fort upper wall damage area	Fort Ranjankudi, Perambalur, Tamil Nadu, India	11° 20′ 45″ N, 78° 56′ 20″ E	Tropical wet and dry	Granodiorite	Built	250–500
PRA	Fort upper temple damage area	Fort Ranjankudi, Perambalur, Tamil Nadu, India	11° 20′ 45″ N, 78° 56′ 20″ E	Tropical wet and dry	Granodiorite	Built	250–500
TB	Temple wall outside damage area	Thanjavur Big Temple, Thanjavur, Tamil Nadu, India	10° 46′ 58″ N, 79° 7′ 54″ E	Tropical wet and dry	Granite	Built	1000–1500
TM	Fort upper damage area	Fort Thirumayam, Thirumayam, Tamil Nadu, India	10° 14′ 49″ N, 78° 45′ 2″ E	Tropical wet and dry	Granite	Built	250–500
TMA	Temple inside damage area	Fort Thirumayam, Thirumayam, Tamil Nadu, India	10° 14′ 49″ N, 78° 45′ 2″ E	Tropical wet and dry	Granite	Built	250–500
TF	Outside rock damage area	Fort Tiruchirappalli Rock, Tiruchirappalli, Tamil Nadu, India	10° 49′ 40″ N, 78° 41′ 49″ E	Tropical wet and dry	Granite	Built	1000–1500
TFA	Temple wall outside	Fort Tiruchirappalli Rock, Tiruchirappalli, Tamil Nadu, India	10° 49′ 40″ N, 78° 41′ 49″ E	Tropical wet and dry	Granite	Built	1000–1500
PV	Cave inside	Sittanavasal Cave Temple, Pudukkottai, Tamil Nadu, India	10° 27′ 15″ N, 78° 43′ 28″ E	Tropical wet and dry	Granite	Built	1000–1500
PVA	Outside of the cave	Sittanavasal Cave Temple, Pudukkottai, Tamil Nadu, India	10° 27′ 15″ N, 78° 43′ 28″ E	Tropical wet and dry	Granite	Built	1000–1500

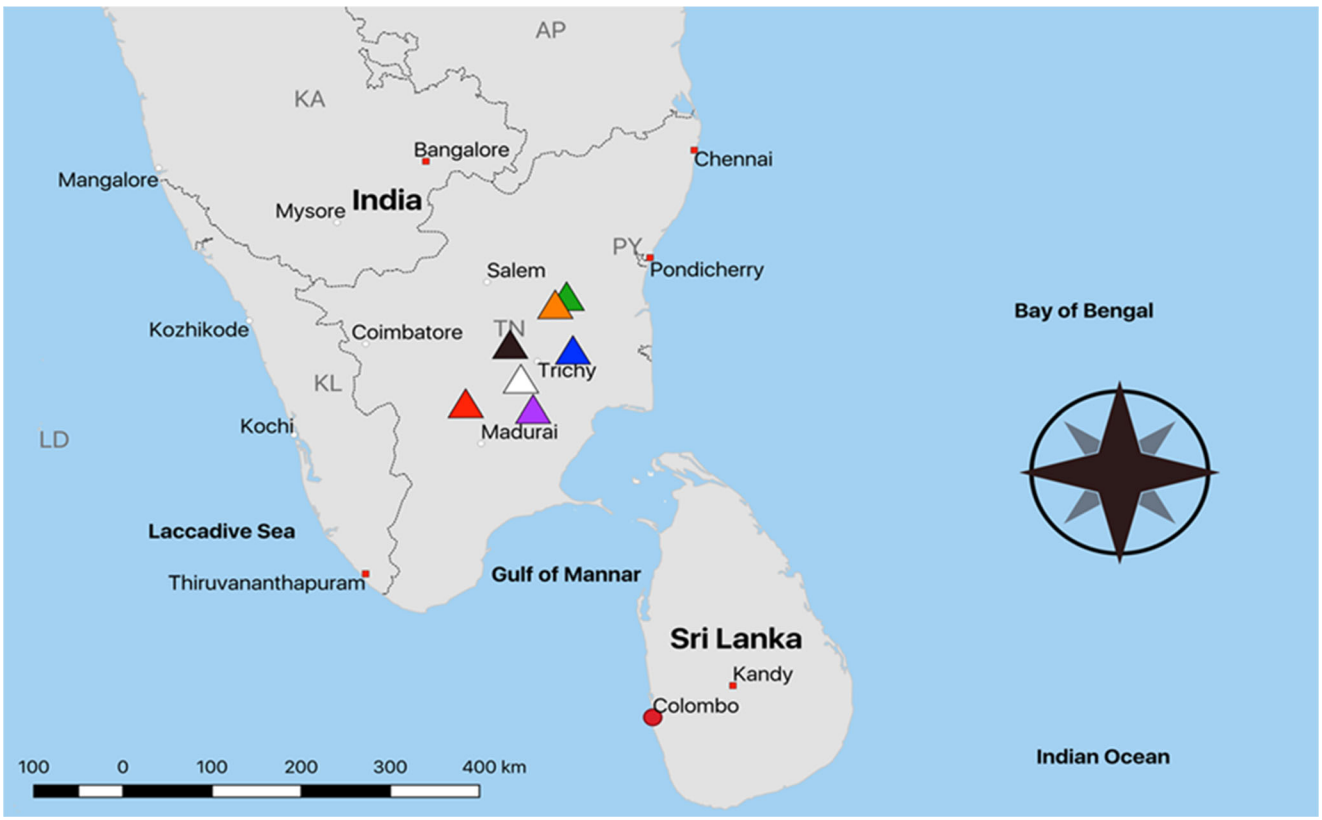

Fig. 1 Map of Indian sampling sites. Triangles denote the approximate locations of Fort Dindigul (red), Valikandapuram Sivan Temple (orange), Fort Ranjankudi (green), Thanjavur Big Temple (blue), Fort Thirumayam (purple), Fort Tiruchirappalli (black), and Sittanavasal Cave Temple (white)

sample replicate, triplicate amplifications were performed. The triplicates were pooled, and all samples were normalized to equal DNA concentrations. Paired-end sequencing of the amplification products (250 base-pair read length) was performed using the Illumina HiSeq2500 platform (Illumina Inc., San Diego, CA).

Amplicon Sequence Data Processing and Analysis

The 16S rRNA amplicon sequences of each sample were imported into the Quantitative Insights into Microbial Ecology (QIIME) 2 environment (version qiime2-2017.12) for processing and downstream analysis [43]. Utilizing DADA2 [44], unique sequence variants and per sample counts of each sequence variant were identified from the raw paired-end reads at truncation lengths of 249 and 238 base pairs for forward and reverse reads, respectively. For sake of continuity with previous amplicon-based microbial community studies, we will refer to sequence variants as operational taxonomic units, or OTUs [45]. Taxonomy was assigned to each OTU using a Naïve-Bayes classifier [46] trained with the SILVA_132_QIIME_release 16S rRNA database [47] at a 97% identity threshold. The resulting feature table was further filtered to exclude all singleton OTUs and those that were assigned to mitochondrial or chloroplast taxonomy.

Feature tables were normalized by rarefying to the smallest number of reads in a single replicate. Although rarefying limits the amount of data used for downstream analyses, other more recently developed normalization methods have been shown to introduce both type I and type II errors in datasets with small sample sizes and variable sample library sizes [48, 49]. For these reasons, rarefying samples to the lowest library size was deemed the most reliable normalization method.

Alpha diversity for each sample was calculated based on the Shannon Diversity Index [50], and significant differences in diversity based on climate type were determined using ANOVA. Community beta diversity between climate types was measured by the UniFrac distance metric [51] and was ordinated through principle coordinates analysis (PCoA) using EMPeror [52]. Correlations between the PCoA ordination axes and measured environmental variables were determined through Pearson's correlation and Kendall's rank correlation tests [53, 54] using PC-ORD Version 6.22 [55]. Multi-response permutation procedures (MRPP) were used to determine if there are significant differences in stone microbial community structure based on climate type [56, 57] through PC-ORD. Significance was calculated by permutation of the Bray-Curtis distance 4999 times to determine if the groups had within-group distances that were different to what would be expected by chance. To determine differences in stone microbial community structure in relation to climate, a two-way cluster analysis was performed through QIIME 2 using the Bray-Curtis distance [58]. Samples were grouped by hierarchical clustering using the average linkage method and only OTUs that comprised greater than 0.5% of the total community were displayed.

Results

Indian Stone Microbial Community Analysis

The microbial community profiles for stone ruin samples (Table 1) collected from seven locations in India (Fig. 1) of two different stone lithologies were determined to test the hypothesis that stone lithology influences microbial community structure. Initially at the site, these stone samples were identified as three different lithologies (granite, quartz, and quartzite). However, we subsequentially classified these samples based on geochemical analyses and reconstructed normative mineralogic data (Table S2; Online Resource 3) into two lithotypes: granite and granodiorite. Lithologies were defined based on modeled mineral contents interpreted via the IUGS Classification [59]. The 16S rRNA amplicon sequences from the seven sample sites were processed and filtered through the QIIME 2 pipeline and summarized in Table S3 (Online Resource 4). Based on a total of 717,090 paired-end reads, a total of 4947 OTUs were identified. For each sample including replicates, the library size ranged from 1158 to 149,852 reads per sample and averaged 22,409 reads per sample. Rarefaction plots of the samples showed that the curves approached a plateau at a sequencing depth of about 1100 reads (Fig. 2). These results indicate that the sequencing provides a complete community structure for the two lithologies.

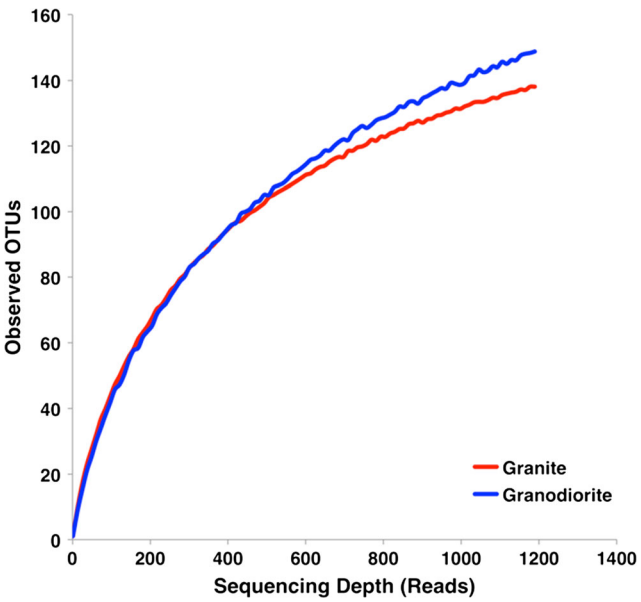

Fig. 2 Rarefaction plots of Indian samples grouped by stone type. Samples were rarefied to a sequencing depth of 1158 reads but appeared to reach diversity saturation at about 1100 reads

Additionally, the plateau of each curve represents the average richness level for each sample, or the amount of unique OTUs counted in each sample. These rarefaction curves showed that microbial communities of granodiorite stones were slightly more diverse than those of granite stones.

Alpha Diversity Analyses

The Shannon Diversity Index was used to measure the within-sample (alpha) diversity of samples. Lithologies had the following Shannon Diversity values: granite: 4.53 ± 0.55 and granodiorite: 4.12 ± 1.52 (Fig. S2; Online Resource 5). The alpha diversity for each stone grouping was not significantly different from each other (Student's T test, $p > 0.05$). Although these Shannon Diversity values approximately reflect the diversity patterns shown in the rarefaction plots (Fig. 2), there were more notable differences in alpha diversity seen in the rarefaction plots. This contrast in diversity may be explained by difference in the diversity metrics. The rarefaction plots only measure OTU richness, while the Shannon Diversity Index measures both richness and evenness [57]. Therefore, while the microbial communities across stone types may vary in OTU richness, the OTUs present may be evenly distributed approximately to the same level across the two lithologies.

The overall taxonomic composition of these stone microbial communities at the phylum level is summarized in Fig. 3 and indicates that Proteobacteria, Cyanobacteria, and Actinobacteria on average collectively accounted for more than 60% of the communities for both lithologies. The relative abundances of the following major stone-dwelling Actinobacteria families were determined: Geodermatophilaceae, Rubrobacteriaceae, Solirubrobacteraceae, and Micrococcaceae (Fig. S3A; Online Resource 6). Geodermatophilaceae was present at relatively constant levels in both lithologies, representing 0.8%, and 1.3% of the microbial communities of granite and granodiorite stones, respectively. Rubrobacteriaceae was most abundant within the granodiorite microbial communities and composed 6.6% of the community but was reduced to 1.5% in granite communities. Both Solirubrobacteraceae and Micrococcaceae had very low relative abundances in both lithologies. Both of these families were most abundant at 0.4% within the granodiorite microbial communities.

The relative abundances of the following major stone-dwelling Cyanobacteria families were also determined: Chroococcidiopsaceae, Nostocaceae, and Xenococcaceae (Fig. S3B; Online Resource 6). Chroococcidiopsaceae was most abundant in granodiorite and represented 10.7% of the microbial communities, while abundance was reduced to 8.8% in granite. Nostocaceae comprised a very small portion of the microbial communities of both lithologies, representing a maximum of 0.4% of the communities in granite stones. Xenococcaceae was completely absent from granite but comprised 4.1% of the granodiorite microbial communities.

Beta Diversity Analyses

To determine how samples differed by lithology, the beta diversity of the Indian stone microbial communities was analyzed. Both the weighted and unweighted UniFrac distance metrics of the rarefied feature table were calculated [51] and ordinated using PCoA. Figure 4 shows the results of this analysis. In both ordinations, the samples formed distinct groupings based on lithology. Axis 1 accounted for the largest

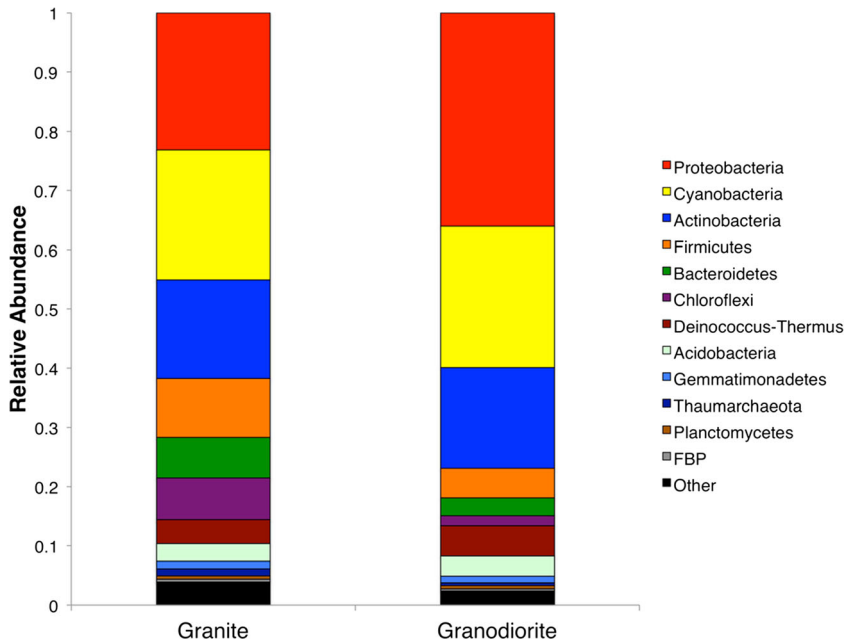

Fig. 3 Phylum level taxonomy of the stone microbial communities grouped by stone type. While the 12 phyla listed in the legend compose over 60% of the stone microbial communities of each stone type, the samples were clearly dominated by Proteobacteria, Cyanobacteria, and Actinobacteria for all stone types

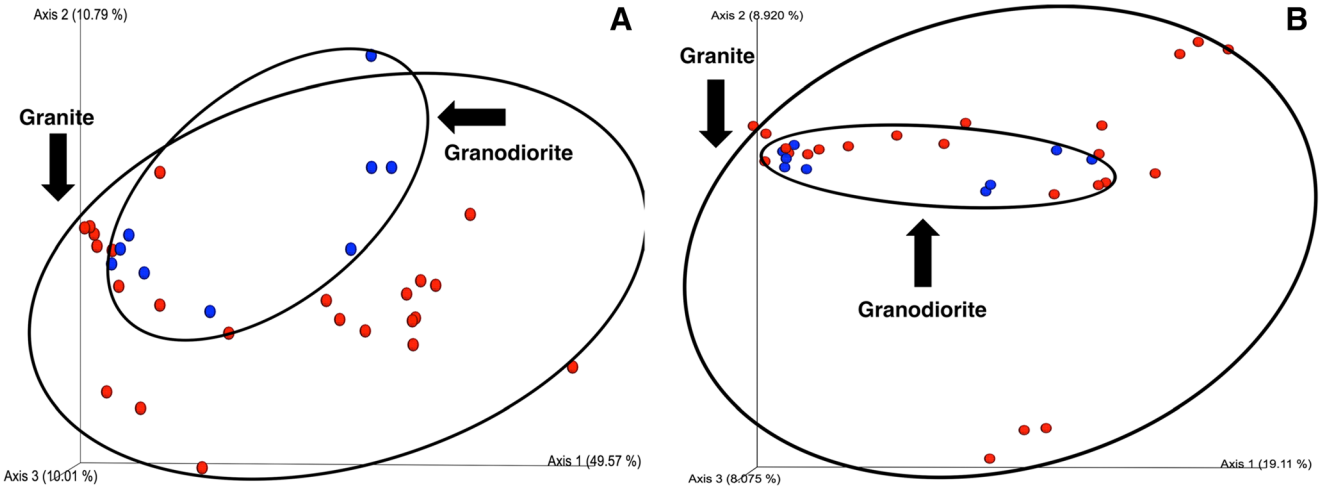

Fig. 4 PCoA ordinations of Indian stone microbial communities. Ordinations of Indian stone microbial communities were produced based on the weighted UniFrac (**a**) and unweighted UniFrac (**b**) distance metrics. Samples approximately separate into distinct groupings based on stone type through both distance metrics, which is supported by the MRPP results. For both ordinations, all three axes account for a total of 70.37% and 36.11% of the observed variation among samples in the weighted and unweighted UniFrac ordinations, respectively

amount of community variation in both ordinations (49.57% and 19.11% variation in weighted and unweighted UniFrac ordinations, respectively). To determine their correlation with variations of microbial diversity, monthly averages for temperature, precipitation, humidity, UV index, wind speed, elevation, snowfall, and air pressure were recorded for each sampling site during the exact month of sampling (Table S1; Online Resource 2). Furthermore, the data were assessed for correlations between the community structure and geochemistry of the stone (Table S2; Online Resource 3). Supporting information Table S4 (Online Resource 7) shows correlation values for Pearson's correlation tests for all tested variables. Quartz mineral content was the environmental variable that most strongly negatively correlated with Axis 1, suggesting that the quartz may be a significant driver in community variation among stone microbial communities. Titanium, iron, and manganese concentrations were also negatively correlated with Axis 1. On average, quartz mineral content promoted higher abundances of Acidobacteria, Armatimonadetes, Cyanobacteria, and Deinococcus-Thermus, and was associated with lower levels of Actinobacteria, Firmicutes, and Proteobacteria. Interestingly, community diversity (measured by observed OTUs) was also negatively correlated with Axis 1, indicating that stones with higher quartz content hosted richer microbial communities. Microbial communities of granite stones showed a more variable grouping than those found in granodiorite. Axis 2 represented 10.79% and 8.92% of the variation observed in the weighted and unweighted UniFrac ordinations, respectively. Ilmenite mineral content was most strongly correlated with Axis 2, but anorthite mineral content and titanium, iron, and manganese concentrations were also correlated. The concentrations of these minerals and elements were associated with lower abundances of Bacteroidetes and Chloroflexi within the stone microbial communities. Axis 3 also accounted for levels of variation comparable to Axis 2 (10.01% and 8.08% in weighted and unweighted UniFrac ordinations, respectively) and was most strongly correlated with sodium concentrations and albite and anorthite mineral content. Potassium concentrations and orthoclase mineral content were also strongly negatively correlated with Axis 3. The abundances of Proteobacteria and Verrucomicrobia were influenced by the differences in concentrations of these minerals and elements and were associated with higher levels of sodium, albite, and anorthite. Interestingly, silica concentrations were negatively correlated with both Axes 2 and 3. These ordinations show that quartz mineral content was the strongest driver of microbial community diversity and structure on Indian stones, but additional community variation was also influenced by finer differences in stone mineral content (ilmenite, albite, anorthite, and orthoclase) and elemental concentrations (Ti, Fe, Mn, Na, and K).

Multi-response permutation procedure (MRPP) analysis was used to determine the significance of the PCoA ordination groupings and showed that the microbial communities were distinct based on lithology, with a $p < 0.05$. The within-group agreement (A) for granite versus granodiorite was 0.03199449 with a p value of 0.00876782.

To further test sample groupings, a UPGMA consensus tree, based on the unweighted UniFrac distance, was generated (Fig. S4; Online Resource 8). Samples did not cluster very coherently, similar to the overlapping groupings of the PCoA ordinations (Fig. 4). This is likely due to the similar geochemical composition of these two lithologies, as both granite and granodiorite are considered to be granitic stones. In addition, the heterogeneous nature of these stones, especially granite, may be similar and result in comparable microbial community

structures among distinct lithologies. Despite the less clear groupings observed in the PCoA ordinations and UPGMA consensus tree, MRPP supported the idea that the Indian stone samples contained distinct microbial communities based on lithology.

Analysis of Significant Taxa Associated with Stone Type Differences

A two-way cluster analysis was performed to determine which taxa were most responsible for the observed grouping patterns (Fig. 5). Clustering was performed by calculating the Bray-Curtis distance of the rarefied feature table summarized to the genus level with taxonomy annotations. While clustering was performed on all genera, only taxa that represented more than 0.5% of the total community are displayed in Fig. 5. The heat map of this analysis showed that the samples did not form coherent clusters grouped by lithology based on their taxonomic composition, similar to the PCoA ordinations (Fig. 4) and UPGMA consensus tree (Fig. S4; Online Resource 8). From this cluster analysis, four different clusters were formed. Cluster 1 contained a small number of low abundance taxa and included Chloroflexi, Proteobacteria, and Cyanobacteria such as *Xenococcus*. These taxa were only found in very few samples and therefore were not considered to be part of the core microbial community. Cluster 2 contained a large number of taxa that were present in both lithologies, indicating that this cluster is most representative of the core stone microbial community of all four clusters. Many of the taxa in this cluster belonged to Cyanobacteria, particularly an unknown Chroococcidiopsaceae and *Dapisostemonum* CCIBt 3536. Other notable taxa within this cluster include *Rubrobacter*, *Deinococcus*, an unknown Sphingomonadaceae, and an unknown Enterobacteriaceae. A diverse range of bacteria was found in cluster 3, including *Sphingomonas*, *Geodermatophilus*, and *Truepera*. The diversity of this cluster reflects the alpha diversity measured previously (Fig. 2 and Fig. S3; Online Resource 6), as the taxa of this cluster were mostly found in the generally more diverse granodiorite samples. Cluster 4 contained the most taxa of any cluster, but they were mostly Proteobacteria, Actinobacteria, and Firmicutes. Specific notable taxa of this cluster include *Pseudomonas*, *Moraxella*, *Staphylococcus*, *Streptococcus*, *Lactococcus*, and *Frankia*. Similar results on the overall microbial community profiles are shown above (Fig. 3), where most of the taxa belonged to the phyla Actinobacteria, Cyanobacteria, and Proteobacteria. The presence of *Geodermatophilus* primarily within the granodiorite samples of cluster 3 indicates that the specific geochemical composition of the stones may limit or promote the growth of members of Geodermatophilaceae, which may in turn influence the microbial colonization and subsequent deterioration of the stones they inhabit. The four clusters that formed from this two-way cluster analysis support the microbial community profile and diversity patterns described earlier and provide insight into the specific taxa that shape the community variation observed among lithologies.

Discussion

Prominent Microbial Groups of Stone-Dwelling Microbial Communities

The microbial communities associated with Indian stone ruin surfaces were dominated by Actinobacteria, Cyanobacteria, and Proteobacteria. These three phyla fulfill the major ecological roles needed for community survival on stones that have been described previously: Actinobacteria grow their filaments deeper into stones to promote community attachment and to access a wider range of minerals within the stone, Cyanobacteria provide a carbon source for the community through photosynthesis, and Proteobacteria aid in biofilm development for community cohesion and water retention [15, 17–19, 21, 22]. Although these communities contain additional members, the significant presence of these three phyla within the stone microbiome is consistent with previous research and the ecological expectations needed to survive on stone surfaces [7, 17].

Lithology and Geochemistry Influence Stone Microbial Communities

Lithology and geochemical composition influenced the structure of the Indian stone microbial communities. Although the microbial communities were distinct by lithology, these comparisons are inherently categorical and are difficult to infer quantitative, measurable drivers of microbial diversity. In addition, the geochemical heterogeneity of some stones, particularly granite, resulted in variable microbial community profiles within the same lithology (Fig. 4). For these reasons, it is best for future studies of stone microbial communities to characterize sampled stones by geochemical composition (i.e., trace elements, mineral content) rather than by approximate lithology or geologic classification. The percentage of quartz, which is well known for its hard crystal structure [60, 61], was the most significant geochemical driver of variation in the Indian stone microbiome. It is possible that the non-porous crystal structure typical of quartz minerals may limit microbes to only attach to its surface instead of growing into the interior of the stone, which may explain why filamentous Actinobacteria were negatively associated with quartz mineral content [17]. In contrast, microbes that are autotrophic and desiccation or UV tolerant would likely survive best on stones that would have limited access to its interior, which may explain why bacteria such as Cyanobacteria and Deinococcus-

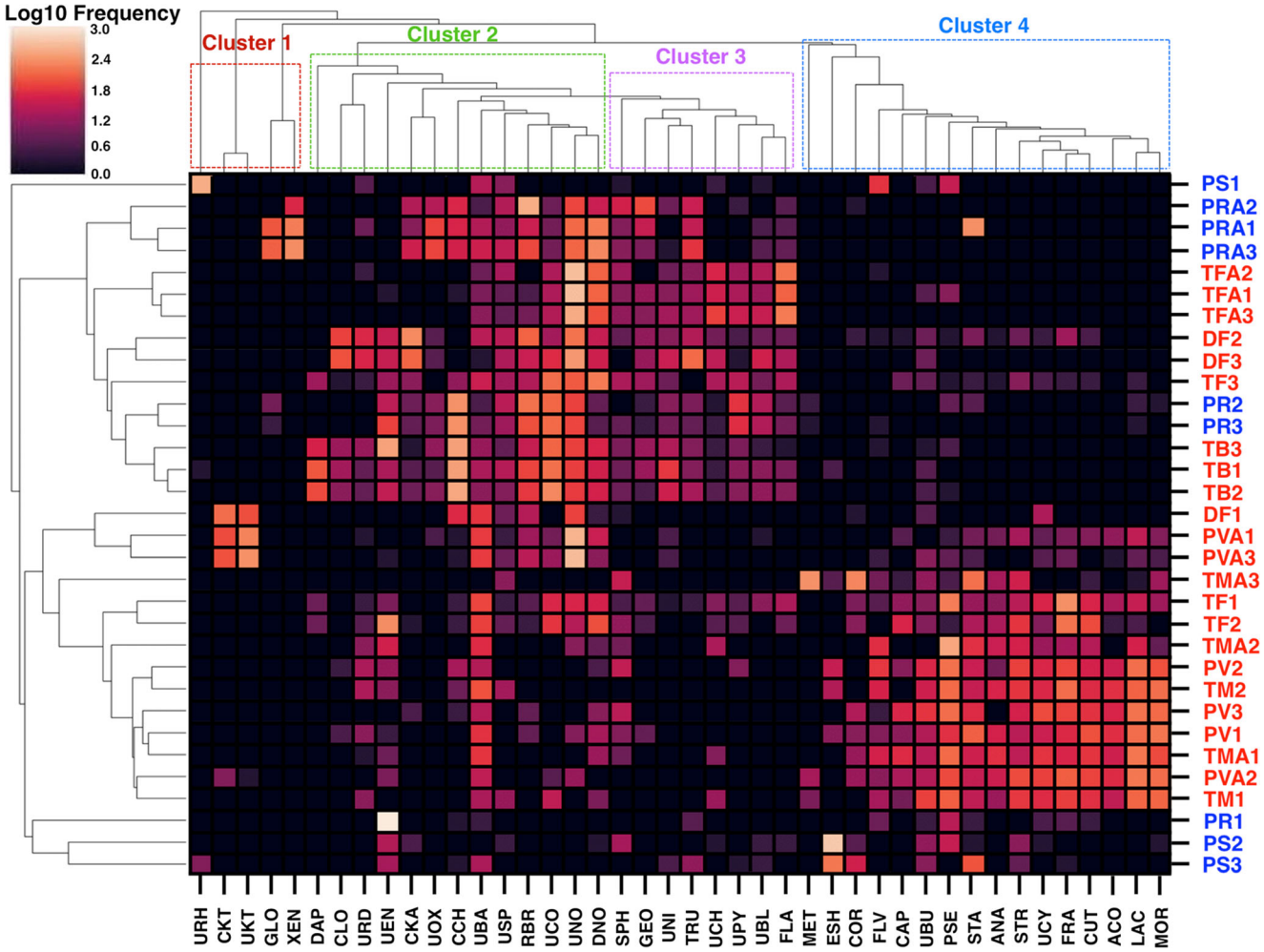

Fig. 5 Two-way cluster analysis of Indian stone microbial communities. Hierarchical clustering using the average linkage method was performed on the rarefied feature table using the Bray-Curtis distance metric. Rows represent how samples relate by community composition, as arranged by the vertical dendrogram. Columns represent OTUs present within the stone-dwelling community, as arranged by the horizontal dendrogram. The color of each box represents the abundance (normalized by taking the log10 value of feature table relative abundances) of each OTU present within each sample—dark colors indicate lower relative abundance, and lighter colors indicate higher relative abundance of each OTU within each sample. Clustering was performed on the rarefied feature table summarized to the genus level with taxonomy annotation, but only OTUs that represented more than 0.5% of the total community are displayed here ($N = 42$). Sample labels are colored by stone type—granite (red), quartz (orange), and quartzite (blue). The four taxa clusters of the horizontal dendrogram are outlined and color labeled. All OTU names are represented by a three-letter code, described by the following OTU Key: URH, unknown Rhodocyclaceae (Proteobacteria); CKT, uncultured Ktedonobacteraceae (Chloroflexi); UKT, unknown Ktedonobacteraceae (Chloroflexi); GLO, Gloeocapsa PCC-7428 (Cyanobacteria); XEN, Xenococcus CRM (Cyanobacteria); DAP, Dapisostemonum CCIBt 3536 (Cyanobacteria); CLO, uncultured Longimicrobiaceae (Gemmatimonadetes); URD, unknown Rhodobacteraceae (Proteobacteria); UEN, unknown Enterobacteriaceae (Proteobacteria); CKA, uncultured Kallotenuales (Chloroflexi); UOX, unknown Oxyphotobacteria (Cyanobacteria); CCH, uncultured Chroococcidiopsaceae (Cyanobacteria); UBA, unknown bacteria; USP, unknown Sphingomonadaceae (Proteobacteria); RBR, Rubrobacter (Actinobacteria); UCO, unknown Chroococcidiopsaceae (Cyanobacteria); UNO, unknown Nostocales (Cyanobacteria); DNO, Deinococcus (Deinococcus-Thermus); SPH, Sphingomonas (Proteobacteria); GEO, Geodermatophilus (Actinobacteria); UNI, unknown Nitrosphaeraceae (Archaea); TRU, Truepera (Deinococcus-Thermus); UCH, unknown Chitinophagaceae (Bacteroidetes); UPY, unknown Pyrinomonadaceae (Acidobacteria); UBL, unknown Blastocatellaceae (Acidobacteria); FLA, Flavisolibacter (Bacteroidetes); MET, Methylobacterium (Proteobacteria); ESH, Escherichia-Shigella (Proteobacteria); COR, Corynebacterium (Actinobacteria); FLV, Flavobacterium (Bacteroidetes); CAP, Capnocytophaga (Bacteroidetes); UBU, unknown Burkholderiaceae (Proteobacteria); PSE, Pseudomonas (Proteobacteria); STA, Staphylococcus (Firmicutes); ANA, Anaerococcus (Firmicutes); STR, Streptococcus (Firmicutes); UCY, unknown Corynebacteriaceae (Actinobacteria); FRA, Frankia (Actinobacteria); CUT, Cutibacterium (Actinobacteria); ACO, Ambiguous Corynebacteriaceae (Actinobacteria); LAC, Lactococcus (Firmicutes); MOR, Moraxella (Proteobacteria)

Thermus were correlated with quartz mineral content. Community diversity was also correlated with quartz mineral content in these samples, which may indicate that the microbial communities that attach to quartz surfaces may be transient and regularly changing. Further sampling over time is required to determine if this is a temporal response.

Core Members of the Stone Microbiome

These Indian stone ruins represent built stone samples, which are similar to those included in our previous study of the stone microbiome sampled from North African ruins [8]. The core microbial members of North African and Indian stone communities were similar and included Actinobacteria, Cyanobacteria, Proteobacteria, and Deinococcus-Thermus. However, the proportion of Actinobacteria in the stone microbiome was reduced under the hot humid conditions of southern India compared with hot arid North Africa, while the proportion of Proteobacteria increased. Although lithology and geochemistry of these stone sampling sites differed, the humid climate of India similarly showed to have an impact, when compared with the North Africa samples, on the structure of stone microbial communities.

Conclusions

This study provides insight on the ecology of built-stone microbiomes through amplicon-based metagenomic analysis and geochemical assessment. Geochemistry influenced microbial community structure, likely by impacting rates of microbial colonization and limiting access to minerals and nutrients required for growth. Core members of the stone microbiome consisted of diverse but specific members of Actinobacteria, Cyanobacteria, Proteobacter, and Dienococcus-Thermus families.

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Authors' Contributions NJE, DD, and LST conceived the original idea for the study; NJE, DD, and LST jointly designed the approach and experimental plan; NJE and DD performed the sampling and DNA isolation; NJE prepared the sequenced libraries and analyzed the sequence data; NJE and JB designed the geochemical studies, prepared the samples for analyses, and interpreted and modeled the resulting data; and LST, NJE, JB, and DD wrote the manuscript. All authors read and approved the final manuscript.

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Data availability All sequence data (16S rRNA gene datasets) presented in this article is available in the repository of NCBI under Bioproject number PRJNA545121.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for Publication Not applicable

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