Supplementary Information for
Multi-kingdom interactions govern the microbiome in subterranean
cultural heritage sites

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Figure S1. Sampling (Samples T1-T12) and air collection points (A-D) of the tomb (A) as well as surrounding soils (Samples T13-T24) (B). In total, 10 tomb (Samples T1 and T2 are undetected due to the low DNA concentration) and 12 surrounding soils samples are used in this study.
Figure S2. Amplicon reads indicate that phylum Actinobacteria and Arthropoda respectively dominate bacterial and eukaryotic communities (A and B). At higher resolution, family Pseudonocardiaceae and order Collembola exhibit the highest percentages of phylum Actinobacteria and all eukaryotic taxa (in term of OTU) in the tomb, ranging from 73.26% to 95.01% and 41.24% to 98.62% respectively (C and D).
Figure S3. Constituents of phylum Actinobacteria, family Pseudonocardiaceae-dominated bacterial (A and B) and Arthropoda-dominated eukaryotic (C) communities of tomb samples revealed by shotgun metagenomic sequencing.
Figure S4. Copy numbers of 16S rRNA, ITS rRNA and 18S rRNA gene fragments of tomb samples are quantified by real-time quantitative polymerase chain reaction (qPCR).
Figure S5. The phylogenetic identification of eighteen screened Actinobacteria strains, using bacterial 16S rRNA gene fragments (~1300 bp in length). There are eight Pseudonocardiaceae strains. The insets are the colony morphologies of the 5-day-old culture of screened Actinobacteria strains on Yeast Malt Extract medium.
Figure S6. Redundancy analysis (RDA) reveals the environmental factors shifting bacterial and eukaryotic communities as well as dominant species between the tomb and surrounding soils (A and B). Black and blue lines indicate environmental factors and dominant species respectively. Note: humidity and temperature stability use the constancy index of humidity and air temperature respectively (Fig. S7); the presence and absence of illumination use “1” and “0” respectively; geosmin uses measured concentrations (Table S2) within the tomb, while “0” (i.e., not detected) outside the tomb. The bacterial and eukaryotic community compositions between the tomb and surrounding soils are significantly different (P<0.01) (confirmed by Permutational multivariate analysis of variance (PERMANOVA) tests), as visualized by the Bray-Curtis distance-based heatmap (C and D).
Figure S7. Monitoring points of air temperature and humidity within and outside the Dahuting Han Dynasty Tomb (A). Herein, No. 1 represents the condition of surrounding environments, while No. 2, 3, 4 and 5 represent tomb conditions. The constancy index of air temperature and humidity for both surrounding environments and the tomb are based on the six-month data (2020/11/25 to 2021/4/25) (B).
Figure S8. Co-occurrence networks of significantly positively interacting Pseudonocardiaceae OTU1 and Collembola (A) as well as significant negative interactions with other bacteria (B). Positively and negatively interacting taxa are connected by green and red lines, respectively. The stacked bar diagrams present the distributions of Pseudonocardiaceae and Collembola taxa among 22 samples. Herein, Pseudonocardiaceae OTU1 with the highest percentages in family Pseudonocardiaceae and 7 highest-abundance Collembola taxa are used for the construction of multi-kingdom co-occurrence network (Fig. 3A).
Figure S9. Fourier Transform Infrared (FTIR) Spectrometer result indicates that the main chemical components of the surface of walls are large molecular-weight cellulose-like substances.
For all OTU-based analyses, the original OTU table was rarified to the depths of 29,066 bacterial and 1,108 eukaryotic reads per sample to measure community compositions and beta diversities. Amplicon sequences indicate that the phyla Actinobacteria and Arthropoda taxa (in terms of OTUs) dominate bacterial and eukaryotic communities in the tomb, which are up to 67.20% and 90.67% respectively. At higher resolution, the family Pseudonocardia and order Collembola exhibit the highest percentages of phylum Actinobacteria and all Arthropoda taxa, accounting for 91.79% and 74.90% respectively. Venn analyses indicate the shared and specific bacterial and eukaryotic taxa within and between the Dahuting Han Dynasty Tomb and surrounding soils. Herein, 98.99% of bacterial and 70.12% of eukaryotic taxa in the tomb are detected in surrounding soils, indicating that the species in the tomb are just subsets of those in surrounding environments.
Figure S11. Amplicon sequences indicate that phylum Actinobacteria and Arthropoda respectively dominate bacterial and eukaryotic communities (A and B). Herein, Actinobacteria taxa (in terms of OTUs) are the dominant bacterial group in the Dahuting Han Dynasty Tomb, accounting for 57.82% to 74.20% (67.20% in average). At higher resolution, family Pseudonocardiacae exhibit the highest percentages of phylum Actinobacteria (in term of OTU), ranging from 82.10% to 95.66% (C). In addition, most eukaryotic sequences belong to phylum Arthropods (90.67%), order Collembola (71.59%) in tomb sample. Order Collembola exhibit the highest percentages of all eukaryotic taxa, ranging from 39.50% to 98.89% (except for T4) (D).
Figure S12. Redundancy analysis (RDA) reveals the environmental factors shifting bacterial and eukaryotic communities as well as dominant species between the tomb and surrounding soils (A and B). Black and blue lines indicate environmental factors and dominant species respectively. Note: humidity and temperature stability use the constancy index of humidity and air temperature respectively (Fig. S7); the presence and absence of illumination use “1” and “0” respectively; geosmin uses measured concentrations (Table S2) within the tomb, while “0” (i.e. not detected) outside the tomb. The bacterial and eukaryotic community compositions between the tomb and surrounding soils are significantly different (P<0.01) (confirmed by Permutational multivariate analysis of variance (PERMANOVA) tests), as visualized by the Bray-Curtis distance-based heatmap (C and D).
Table S1. Concentrations of geosmin and 2-methylisoborneol in the air of tombs

| Concentration | Geosmin (ng/m³) | 2-methylisoborneol (ng/m³) |
|---------------|-----------------|---------------------------|
| A (chamber)   | 16.1            | 5.9                       |
| B (chamber)   | 5.9             | 5.9                       |
| C (atrium)    | 2.9             | 4.4                       |
| D (corridor)  | 8.8             | 22.0                      |
Table S2. Concentrations of geosmin and 2-methylisoborneol in the air of the headspace of screened Actinobacteria strain cultures with Yeast Malt Extract medium for 5 days

| Family      | Genera     | Strain | Geosmin (ng/ml) | 2-methylisoborneol (ng/ml) |
|-------------|------------|--------|-----------------|-----------------------------|
| Pseudonocardiaeae | Amycolatopsis |        |                 |                             |
|              | 10         |        | 1.40            | 8.60                        |
|              | 11         |        | 3.13            | 32.80                       |
|              | 29         |        | 4.00            | 429.67                      |
|              | 30         |        | 0.80            | 94.60                       |
|              | 35         |        | 3.47            | 36.73                       |
|              | 36         |        | 6.00            | 75.80                       |
|              | 37         |        | 3.73            | 40.00                       |
|              | 38         |        | 3.93            | 43.73                       |
| Nocardiaceae | Rhodococcus | 27     | 0.20            | 0.33                        |
|              | Nocardia    | 39     | 25.93           | 0.53                        |
|              | 40         |        | 27.87           | 0.13                        |
| Microbacteriaceae | Microbacterium | 17     | 0.20            | 0.13                        |
| Streptomycetaceae | Streptomyces | 24     | 0.40            | 76.53                       |
| Nocardiodaceae | Nocardioides | 42     | 0.40            | 0.13                        |
|              | 45         |        | 0.27            | 0.20                        |
| Kribbellaceae | Kribbella   | 25     | 0.47            | 0.53                        |
|              | 28         |        | 0.13            | 0.27                        |
|              | 32         |        | 1.27            | 5.33                        |
Table S3. Cellulases activities of 18 Actinobacteria strains after growing on CMC-Na medium for 3 days

| Family            | Genera      | Strain | CMC (unit/ml) |
|-------------------|-------------|--------|--------------|
| Pseudonocardiaeae | Amycolatopsis | 10    | 0.53±0.08    |
|                   |             | 11    | 0.49±0.10    |
|                   |             | 29    | 0.37±0.05    |
|                   |             | 30    | 0.45±0.07    |
|                   |             | 35    | 0.58±0.04    |
|                   |             | 36    | 0.43±0.01    |
|                   |             | 37    | 0.50±0.06    |
|                   |             | 38    | 0.46±0.05    |
|                   | Rhodococcus | 27    | 0.40±0.05    |
| Nocardiae         | Nocardia    | 39    | 0.45±0.06    |
|                   |             | 40    | N.D.         |
| Microbacteriaceae | Microbacterium | 17   | 0.56±0.10    |
| Streptomycetaceae | Streptomyces | 24    | 0.62±0.07    |
| Nocardioidaceae   | Nocardioides | 42    | 0.31±0.04    |
|                   |             | 45    | 1.57±0.30    |
| Kribbellaceae     | Kribbella   | 28    | 0.30±0.06    |
|                   |             | 32    | 0.36±0.08    |

Note: One enzymatic unit of activity was defined as the amount of enzyme that released 1 μmol of reducing sugar equivalents per minute. N.D. indicates not detected.
Movie S1 (separate file). The video of springtails, *Folsomia candida* actively feasting upon Pseudonocardiaceae. The white is the colony of screened Pseudonocardiaceae strains. The typical post of springtails feasting upon prey.

Movie S2 (separate file). The video of springtails, *Folsomia candida* actively bringing Pseudonocardiaceae around. It is obvious that the Pseudonocardiaceae adhere to the skin of springtails. And springtails bring them around.

Dataset S1 (separate file). The detailed information on geosmin metabolic pathway in the pivotal Actinobacteria bin from metagenomics of tomb samples.

Dataset S2 (separate file). The detailed information on sequences encoding cellulases in the pivotal Actinobacteria bin from metagenomics of tomb samples.

Dataset S3 (separate file). The detailed information on gene clusters for the biosynthesis of antimicrobial substances in the pivotal Actinobacteria bin from metagenomics of tomb samples.