

Changes in soil microbial communities at Jinsha earthen site are associated with earthen site deterioration

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Abstract

Background: Earthen sites are immobile cultural relics and an important part of cultural heritage with historical, artistic and scientific values. The deterioration of features in earthen sites result in permanent loss of cultural information, causing immeasurable damage to the study of history and culture. Most research on the deterioration of earthen sites has concentrated on the physicochemical factors, and information on microbial communities in earthen sites and their relationship with the earthen site deterioration is scarce. We used high-throughput sequencing to analyze bacterial and fungal communities in soils from earthen walls with different degree of deterioration at Jinsha earthen site to characterize the communities and their correlation with environmental factors, and compare community structures and the relative abundances of individual taxa associated with different degree of deterioration for identifying possible marker taxa.

Results: The relative abundances of Proteobacteria and Firmicutes were higher and that of Actinobacteria lower with higher degree of deterioration. At the genus level, the relative abundances of *Rubrobacter* were highest in all sample groups except in the most deteriorated samples where that of *Bacteroides* was highest. The relative abundance of the yeast genus *Candida* was highest in the severely deteriorated sample group. The bacterial phylum Bacteroidetes and genus *Bacteroides*, and fungal class Saccharomycetes that includes *Candida* sp. were specific for the most deteriorated samples. For both bacteria and fungi, the differences in community composition were associated with differences in EC, moisture, pH, and the concentrations of NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} and SO_4^{2-} .

Conclusion: The microbial communities in soil with different degree of deterioration were distinctly different, and deterioration was accompanied with bigger changes in the bacterial than in the fungal community. In addition, the deteriorated soil contained higher concentrations of soluble salts. Potentially, the accumulation of *Bacteroides* and *Candida* plays an important role in the deterioration of earthen features. Further work is needed to conclude whether controlling the growth of the bacteria and fungi with high relative abundances in the deteriorated samples can be applied to alleviate deterioration.

Background

Earthen sites are mainly soil formations produced by ancient activities. These immobile cultural relics are one part of cultural heritage with historical, artistic and scientific values [1]. In China, the earthen sites with significant archaeological value include for example heritage sites along the Silk Road, the Great Wall remains and Beacon Tower in Gansu province [2, 3]. Preservation of these sites is essential for studying history and culture. However, for thousands of years, earthen sites have been subject to environmental impacts such as erosion due to severe winds and heavy rainfall, earthquakes, and fluctuation in temperature and humidity[3]. The deterioration of features in earthen sites include loose efflorescence, weathering, cracks, and collapses [4-6]. The deterioration, mediated by physical, chemical and biological processes [4-7] results in permanent loss of cultural information, causing immeasurable damage to the study of history and culture.

Jinsha earthen site was discovered in Chengdu, Sichuan, in the southwest of China in February 2001. Jinsha was the capital of ancient Sun Kingdom that dates back to 12th to 7th century BCE (approximately 2900-3200 years ago), considered as an ancient civilization center along Yangtze River. So far, archaeologists have

unearthed important features of large-scale palace foundation, sacrificial area, residential area, and a burial site. On the site, numerous artifacts have been unearthed, including more than 5,000 articles of gold, bronze, jade and stone, as well as millions of potsherds, tons of ivory and thousands of boar tusks and deer horns (Figure S1a). The Jinsha Site Museum has built the Relics Hall in the excavation site for the protection, study and exhibition of Jinsha culture and ancient Shu civilization (Figure S1b). Even though the Relics Hall has alleviated damage by external factors, such as wind, sun and rain, the earthen features in the in semi-open Relics Hall have suffered different degrees of deterioration, including salinization, efflorescence, and cracking.

Most research on the deterioration of earthen sites has concentrated on the physicochemical factors [1, 2, 8-10]. Microorganisms play an important role in weathering of stone monuments [11], and probably cause serious damage to earthen sites as well. However, information on microbial communities in earthen sites and their relationship with the earthen site deterioration is scarce. We used high-throughput sequencing to analyze bacterial and fungal communities in soils from earthen walls with different degree of deterioration at Jinsha earthen site. The objectives were to (1) characterize the communities and their correlation with environmental factors, and (2) compare community structures and the relative abundances of individual taxa associated with different degree of deterioration for identifying possible marker taxa. We hypothesized that community composition would vary depending on deterioration degree. The results were expected to pave way for means to alleviate the deterioration of earthen features.

Results

The physicochemical properties

The physicochemical properties of the soil samples varied with the deterioration degree of the earthen wall (Table 1). Moisture was higher in the moderately and severely deteriorated samples S3 and S4 than in the not or slightly deteriorated samples S1 and S2 ($p > 0.05$), and pH ranged from 7.32 in S4 to 7.48 in S1 ($p > 0.05$). EC value and the concentrations of Mg^{2+} , Ca^{2+} and SO_4^{2-} were higher in the more deteriorated samples ($P < 0.05$), whereas the concentrations of NH_4^+ and K^+ were lower ($p > 0.05$). In the SEM-EDS spot analysis, the relative proportions of C, S, O and Mg elements were higher in samples S3 and S4 than in samples S1 and S2, and those of Al, Si and K were lower ($p > 0.05$) (Additional File 1: Table S1).

The diversity of microbial community

The bacterial and fungal communities in the soil samples were analyzed using amplicon sequencing targeting the 16S rRNA gene and ITS, respectively. The 816336 16S rRNA gene amplicons were divided into 2555 bacterial operational taxonomic units (OTUs) at $\geq 97\%$ similarity. The goods coverage was above 99.7% and 99.9% for 16S rRNA gene and ITS amplicons, respectively, and all the rarefaction curves reached an asymptote, showing that the amplicons represented well the sampled populations (Figure S2). The average number of bacterial OTUs per sample group ranged from 553 to 718 (Table 2). The 957322 ITS amplicons were divided into 899 fungal OTUs. The average number of fungal OTUs per sample group ranged from 249 to 355 (Table 2). For bacteria, richness was higher in S4 than in the other sample groups, and diversity was higher in S3 and S4 than in S1 and S2 ($p < 0.05$) (Table 2). For fungi, richness was higher in S1 than in S4 ($p < 0.05$) (Table 2), and diversities were on the same level in all the sample groups.

Altogether 330 bacterial and 200 fungal OTUs were detected in all the four sample groups (Figure 1). The highest number of unique bacterial OTUs was detected in S4 and the lowest in S2. The highest number of unique fungal OTUs was detected in S4 (Figure 1). Both the bacterial and fungal communities in the not deteriorated and severely deteriorated sample groups S1 and S4, respectively, were clearly separated in the principal component analysis (PCA) (Figure 2).

Distribution of microbial community in sample groups

The bacterial OTUs were assigned into 36 phyla and 617 genera. Actinobacteria, Bacteroidetes, Proteobacteria and Firmicutes were the most abundant phyla (Figure 3a). The relative abundances of Actinobacteria were highest in the sample groups S1 and S2, and those of Proteobacteria and Firmicutes in S3 and S4 ($p < 0.05$) (Additional File 1: Table S2).

At the genus level, the relative abundances of *Rubrobacter* were highest in all sample groups except S4 where that of *Bacteroides* was highest (Figure 3b). Compared with the S1 and S2, the relative abundances of *Shewanella* were higher in S3 and S4 ($p > 0.05$). The results showed that the bacterial community compositions in sample groups with different degree of deterioration were significantly different (Additional File 1: Table S3).

The fungal communities were assigned into 5 phyla and 205 genera. In all sample groups, Ascomycota was the most abundant phylum and Basidiomycota the second most abundant with relative abundances ranging from 95.9% to 98.8% and 1.1% to 2.7%, respectively (Figure 4a, Additional File 1: Table S4). At the genus level, *Toxicocladosporium* and *Alternaria* were more abundant in sample groups S1 and S3 than in S2 and S4 ($p < 0.05$), *Fusarium* was the most abundant in S2 ($p < 0.05$) and *Candida* in S4 (Figure 4b, Additional File 1: Table S5).

Liner discriminant analysis (LDA) coupled with effect size (LEfSE) was used to identify differentially abundant taxa. A total of 46 bacterial taxa were differentially abundant among the four sample groups. Three taxa were significantly more abundant in S1 than in the other three sample groups, six taxa in S2, sixteen taxa in S3, and 21 taxa in S4 (Additional File 1: Figure S3a). A total of 30 fungal taxa were differentially abundant. Fifteen taxa were significantly more abundant in S1 than in the other three sample groups, ten taxa in S3, and five taxa in S4 (Additional File 1: Figure S3b). The LDA scores of bacterial phylum Bacteroidetes and genus *Bacteroides*, and that of fungal class Saccharomycetes that includes *Candida* sp. were approximately 5 in the S4 samples, suggesting that these organisms were specific for the most deteriorated samples.

The correlation between the microbial community and environmental factors

The relationship between community compositions and environmental factors was analyzed using redundancy analysis (RDA). For bacteria, the RDA axes 1 and 2 accounted for 24.81% and 14.77%, respectively, of the total variation (Figure 5a); for fungi, 30.01% and 14.96%, respectively (Figure 5b). For both bacteria and fungi, the differences in community composition were associated with differences in EC, moisture, pH, and the concentrations of NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} and SO_4^{2-} .

Discussion

In this study, we analyzed bacterial and fungal community composition in soils from an earthen wall with different degree of deterioration in Jinsha earthen site, Chengdu, China, using high-throughput sequencing approach. For bacteria, the relative abundance of Actinobacteria was four times lower in the moderately and severely deteriorated sample groups S3 and S4 than in the not or mildly deteriorated sample groups S1 and S2; those of Proteobacteria and Firmicutes were highest in S3 and S4. However, it should be noted that changes in absolute abundances cannot be concluded from the relative abundance data [12]. Actinobacteria have been frequently found as a dominant group in subterranean micro-niches, including cultural relics in caves and wall paintings in catacombs [13-17]. Previous work demonstrated that some Actinobacteria are potentially harmful to the preservation of cultural relics [18-20]. Proteobacteria are commonly the most abundant bacteria in soil [21, 22], and Firmicutes that are tolerant to extreme temperatures and low humidity are often found in extreme environments [17]. These bacteria may play an important role in the microecological balance of earthen sites.

Rubrobacter was the most abundant genus in all but the most deteriorated sample group. *Rubrobacter* was considered connected with the biodeterioration of cultural relics and the rosy discolouration of masonry and lime wall paintings in historical buildings in Austria and Germany [23]. Resistance to desiccation might be a selective advantage for *Rubrobacter* growth and efflorescence on walls might be due to *Rubrobacter* strains [24]. Therefore, *Rubrobacter* may play a crucial role at the early stage of deterioration of earthen sites. However, in the moderately and severely deteriorated sample groups S3 and S4, the relative abundances of *Rubrobacter* were remarkably lower than in S1 and S2. *Bacteroides* were specific for the most deteriorated samples. *Bacteroides* have the capability to produce acid [25], and the acid produced may dissolve minerals and further damage earthen features.

The distribution of the fungal genera in the four sample groups seemed more random than that of bacteria. The communities in S1 and S3 were similar, indicating that the fungal composition varied only little with the deterioration degree. The filamentous fungi *Cladosporium*, *Fusarium* and *Toxicocladosporium* were the most abundant genera in sample groups S1, S2 and S3, respectively. These genera are widely distributed in wall paintings in caves, catacombs and churches and have been isolated from severely decayed areas of stone artwork [15, 26, 27]. *Cladosporium* and *Fusarium* have been reported to produce extracellular enzymes and abundant mycelia that contribute to the mineral dissolution and mechanical destruction of soil structure [15, 26, 27]. The yeast *Candida* was most abundant in the severely deteriorated sample group S4, and Saccharomycetes that includes *Candida* was specific for the most deteriorated samples. *Candida* species have the ability to secrete extracellular metabolites and to acidify soil [28]. Potentially, the accumulation of *Candida* plays an important role in the deterioration of earthen features. Further work is needed to conclude whether controlling the growth of the bacteria and fungi with high relative abundances in the deteriorated samples can be applied to alleviate deterioration, especially since information on *Candida* species in cultural relics is scarce.

The diversity and distribution of microorganisms in soil are affected by environmental factors. The microbial community structure can rapidly change in response to altered environmental conditions [29, 30]. In our study, the differences in microbial communities were associated with differences in moisture, pH, EC and concentrations of soluble salt ions. Moisture has been found a major factor in affecting microbial communities and their activities [31, 32]. The higher moisture in the moderately and severely deteriorated sample groups S3 and S4 was associated with higher microbial diversity. Soil pH is another major factor connected with soil microbial community structure. In our study, the pH was higher in the moderately and severely deteriorated

sample groups S3 and S4 than in the not or mildly deteriorated sample groups S1 and S2. Bacterial communities have been found more sensitive to changes in pH than fungal communities [33]. This could partly explain the more pronounced difference in bacterial communities than in fungal communities along the difference in deterioration degree.

EC is apparently associated with soil salinity [34]. As in an earlier study [35], the relative abundance of Bacteroidetes was found to correlate positively with EC. Soluble salts are considered to cause damage on earthen sites [5, 8]. We found that the differences in microbial communities were associated with differences in soluble salt concentrations. The bacterial and fungal communities in samples with no obvious deterioration correlated positively with K^+ concentration, and those in severely deteriorated samples with Mg^{2+} , Ca^{2+} and SO_4^{2-} concentrations. Since the deterioration of earthen features releases nutrients [36], the nutrients released may have influenced the microbial diversity. Minerals are the main component of soil, the primary constituent of earthen features, accounting for more than 90% of the total solid phase of soil [37]. The dissolution of minerals could cause deterioration of earthen features by destroying soil structure and reducing stability. Plausibly, the dissolution of the Jinsha site mineral components lead to higher concentrations of Mg^{2+} and Ca^{2+} in the moderately and severely deteriorated sample groups S3 and S4. The nutrients released by soil mineral dissolution could support the growth and metabolism of microorganisms. As the growth and metabolism of microorganisms results in damage for cultural heritage relics [38], the preservation of earthen features may potentially benefit from preventing increases in diversity and possibly more active metabolism.

Conclusions

In this study, high throughput sequencing was applied to explore microbial community structures in earthen wall with different degree of deterioration from Jinsha earthen site. The bacterial communities varied more than the fungal communities along the differences in deterioration degree. The differences in community composition were associated with differences in soil physicochemical properties. Microbial diversity, soil moisture and the concentrations of Mg^{2+} , Ca^{2+} and SO_4^{2-} were higher and soil pH was lower with the increased degree of earthen wall deterioration. The results may potentially benefit the preservation of earthen sites.

Methods

Sampling

Jinsha Site Museum is in No.2 Jinsha Site Road (30.68091 N, 104.01362 E), Chengdu, Sichuan, China (Figure 6a). The earthen wall of the Ivory pit, formed during excavating in a sacrificial area with a lot of buried ivory, had undergone different degree of deterioration. Triplicate soil samples were randomly taken from the same cultural deposit layer (15C) in the Ivory pit wall with no obvious, mild, moderate and severe deterioration, and referred to as S1, S2, S3 and S4 sample groups, respectively (Figure 6b, Figure 6c). Samples were taken using minimally invasive sampling techniques and aseptic procedures, and transported on ice to the laboratory.

Physicochemical properties analyses

Due to the minimal intervention principle in sampling in an archaeological site, the sample quantities were too low to meet the requirements of routine soil property analyses. We analyzed pH, moisture, electrical conductivity (EC) and soluble salt contents that are considered to play significant roles in the deterioration of earthen sites [1, 3, 8, 39]. The samples were air-dried, crushed and sieved through a ϕ 1 mm sieve. The determination of pH and EC were made on 1:1 slurry of air-dried soil and water [40]. Moisture was determined by oven drying method [41]. Soluble salt contents were measured using ion chromatography (IC): 2 g sample was suspended into 20 mL deionized water, the suspension was shaken for 30 minutes at 150 rpm, filtered first through medium pore sized filter paper and finally through 0.2 μ m pore size syringe membrane filter [42]. Ions in the extracts were determined using a Dionex ICS-3000 ion chromatography system with an anion suppressor, a cation suppressor and a conductometric detector (Dionex Corporation, Sunnyvale, USA). Anions and cations in 25 μ L of extract were analyzed using 4 mm \times 50 mm guard columns AG11-11C and CG12A, respectively, 4 mm \times 250 mm analytical columns IonPac AS11-HC and IonPac CS12A, respectively, and 20 mmol L⁻¹ sodium hydroxide solution and methanesulfonic acid solution, respectively, at 1 mL min⁻¹. The main elements in the samples were analyzed using scanning electron microscope EVO18 and energy dispersive spectrometer X-Max^N (SEM-EDS) (Carl Zeiss, Jena, Germany). The main minerals in Jinsha earthen site are quartz, feldspar, illite, montmorillonite and chlorite, with SiO₂, Al₂O₃, Fe₂O₃, K₂O, MgO, and CaO as the main chemical components (Additional File 1: Table S6) [43].

DNA extraction

DNA was extracted from 0.5 g fresh sample using Fast DNA[®] SPIN for Soil Kit (MP BIO Laboratories, California, USA) according to the manufacturer's instructions. The concentration and purity of DNA were checked by electrophoresis in 1.0% agarose gel and NanoDrop spectrophotometer (Thermo Scientific Inc., USA). DNA samples were stored at -20 °C.

16S rRNA and ITS amplicon sequencing

The DNA samples were sequenced at Novogene Bioinformatics Technology, Co., Ltd. (Beijing, China). The V3-V4 regions of 16S rRNA genes were amplified using the primers 341F (5'-CCT AYG GGR BGC ASC AG-3') and 806R (5'-GG ACT ACN NGG GTA TCT AAT-3') [44]. The ITS2 region of ITS was amplified using the primers ITS3-2024F (5'-GCA TCG ATG AAG AAC GCA GC-3') and ITS4-2409R (5'-TCC TCC GCT TAT TGA TAT GC-3') [45]. The primers included sequencing specific adaptor sequences. Amplification was done in 30 μ L reactions with 15 μ L of Phusion[®]High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μ M of forward and reverse primers, and approximately 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, and final elongation at 72 °C for 5 min and cooling at 4 °C.

Amplification was checked by mixing equal volume of 1X loading buffer with SYBR green and PCR product, and subjecting the mixture to electrophoresis in 2% agarose gel. A bright band at 400-500 bp indicated successful amplification. PCR products were purified with GeneJET Gel Extraction Kit (Thermo Scientific). Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) was used to generate sequencing libraries following manufacturer's recommendations. The quality of the libraries was assessed on the Qubit 2.0 Fluorometer

(Thermo Scientific). Finally, the libraries were sequenced on a Thermo Fisher Scientific Ion S5 XL platform and 600 bp single-end reads were generated.

Bioinformatic and statistical analyses

Single-end reads were assigned to samples according to their unique barcodes and primers and barcodes were cut off. Low-quality sequences and reads with ambiguous nucleotides were removed using Cutadapt V1.9.1 [46]. Chimeric reads (sequences with ambiguous base and average base quality score <30) were filtered out using UCHIME v. 4.2.4.0 [47]. Sequences were assigned to operational taxonomic units (OTUs) at $\geq 97\%$ similarity using UPARSE v7.0.1001 [48]. The representative sequences of the 16S rRNA OTUs were assigned to taxa using Silva 132 database and Mothur v1.36.1 [49, 50]. The representative sequences of the ITS OTUs were assigned to taxa using Unite database v7.2 (<https://unite.ut.ee/>) and QIIME v1.9.1 [51]. Chao1 and Shannon indices were calculated using the phyloseq package [52]. Venn diagrams were done at VennDiagramWeb [53]. Principal component analysis (PCA) was done using CANOCO 5 [54]. Differential taxa at phylum to species levels were identified using linear discriminant analysis coupled with effect size (LEfSe) [55]. To analyze the bacterial and fungal community distribution and their correlation with environmental factors, redundancy analysis (RDA) was carried out using CANOCO 5 [54]. Statistical differences among groups were analyzed by One-Way ANOVA with repeated measures followed by a post hoc least significance difference test (SPSS 17.0) [56]. Differences were taken statistically significant at $p < 0.05$.

The amplicon sequencing data were deposited into the NCBI Sequence Read Archive (SRA) under the accession numbers SRR9678166-SRR9678177 and SRR9678184-SRR9678195.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The raw sequence data on 16S rRNA gene and ITS amplicons have been submitted to the NCBI Sequence Read Archive (SRA) database with accession numbers SRR9678166-SRR9678177 and SRR9678184-SRR9678195.

Competing interests

The authors declare that they have no competing interests.

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in the study design, sample collection, data collection and analysis, and manuscript preparation.

Authors' contribution

KZ, LX, TY, QC and XPZ conceived and designed this experiment. JL, XYZ, YL, ZWZ collected samples and performed the study. JL, XYZ, KZ, PP, KL and XLA analyzed the data. JL, ZK, DCL, YFG, MGM, XMY, QJX and JC discussed the results. JL wrote the manuscript. ZK and PP revised the manuscript finally. All authors read and approved the final manuscript.

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Abbreviations

16S rRNA: 16S ribosomal RNA; ITS: Internal transcribed space; OTU: Operational Taxonomic Units; PCA: Principal component analysis; RDA: redundancy analysis; One-Way ANOVA: One-Way analysis of variance; LEfSe: Linear discriminant analysis coupled with effect size; LDA: Linear discriminant analysis; SEM-EDS: Scanning electron microscope - energy dispersive spectrometer; EC: Electrical conductivity; IC: Ion chromatography.

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Tables

Table 1

Sample	Moisture (%)	pH	EC ($\mu\text{s cm}^{-1}$)	Contents of soluble salts (mmol kg^{-1})							
				Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	SO_4^{2-}
S1	4.56 \pm 0.11 ^b	7.48 \pm 0.04 ^a	6.74 \pm 0.05 ^d	12.53 \pm 3.66 ^b	3.80 \pm 0.02 ^a	4.94 \pm 0.04 ^a	193.933 \pm 0.99 ^d	4.45 \pm 0.08 ^d	21.12 \pm 0.19 ^c	10.44 \pm 0.46 ^b	111.39 \pm 0.91 ^d
S2	4.69 \pm 0.04 ^b	7.41 \pm 0.03 ^{ab}	10.45 \pm 0.40 ^c	18.59 \pm 0.02 ^a	3.62 \pm 0.02 ^b	4.70 \pm 0.02 ^b	668.29 \pm 1.44 ^c	11.25 \pm 0.02 ^c	51.43 \pm 0.56 ^a	33.46 \pm 0.42 ^a	205.04 \pm 0.92 ^c
S3	4.84 \pm 0.10 ^a	7.35 \pm 0.09 ^{ab}	11.44 \pm 0.67 ^b	10.41 \pm 0.05 ^c	2.45 \pm 0.04 ^c	4.56 \pm 0.02 ^c	682.74 \pm 2.19 ^b	43.15 \pm 0.33 ^b	22.80 \pm 0.79 ^b	7.25 \pm 0.16 ^c	326.42 \pm 2.24 ^b
S4	4.98 \pm 0.04 ^a	7.32 \pm 0.08 ^b	13.92 \pm 0.63 ^a	10.40 \pm 0.16 ^c	0.0014 \pm 0.0003 ^d	4.55 \pm 0.04 ^c	1519.70 \pm 1.56 ^a	143.07 \pm 0.94 ^a	13.67 \pm 0.30 ^d	9.87 \pm 0.08 ^b	946.48 \pm 2.12 ^a

The results are average \pm standard deviation (n=3). Different superscript letters in a column indicate statistical significant difference ($p < 0.05$) in the least significant difference test. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Table 2

Sample	Bacteria			Fungi		
	OTUs	Chao1	Shannon	OTUs	Chao1	Shannon
S1	570±76 ^b	641±70 ^b	3.456±0.25 ^b	355±26 ^a	392±15 ^a	5.86±0.05 ^a
S2	553±163 ^b	598±158 ^b	3.76±0.974 ^b	288±29 ^{ab}	344±61 ^{ab}	4.603±0.49 ^a
S3	656±128 ^b	672±128 ^b	6.771±0.046 ^a	325±18 ^{ab}	361±11 ^{ab}	5.67±0.088 ^a
S4	718±375 ^a	741±362 ^a	6.649±1.142 ^a	249±80 ^b	264±83 ^b	4.255±1.863 ^a

The results are average ± standard deviation (n=3). Different superscript letters in a column indicate statistical significant difference ($p < 0.05$) in the least significant difference test. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Figures

Figure 1

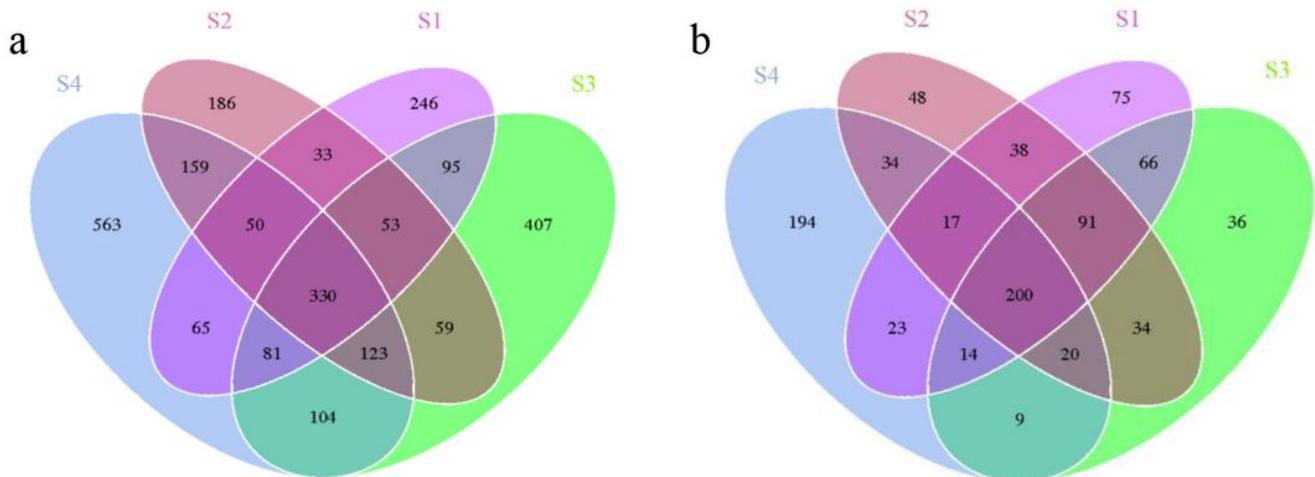


Figure 1

The unique and shared bacterial (a) and fungal (b) OTUs in soil. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Figure 2

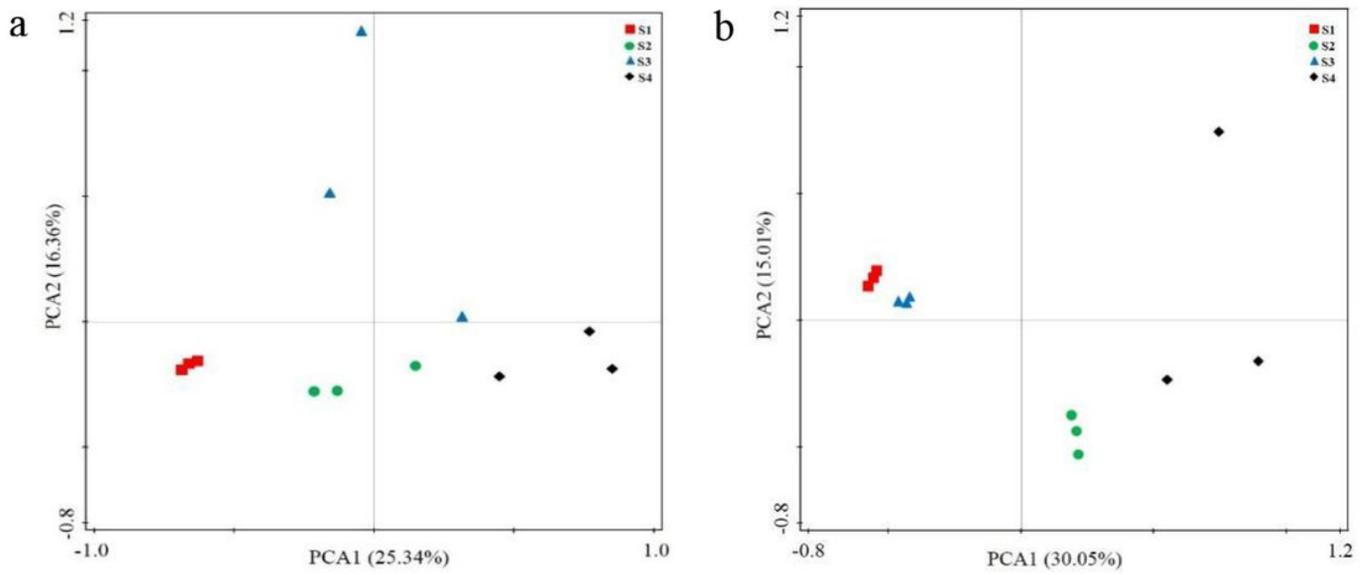


Figure 2

Principal component analysis (PCA) of bacterial (a) and fungal (b) communities in soil. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Figure 3

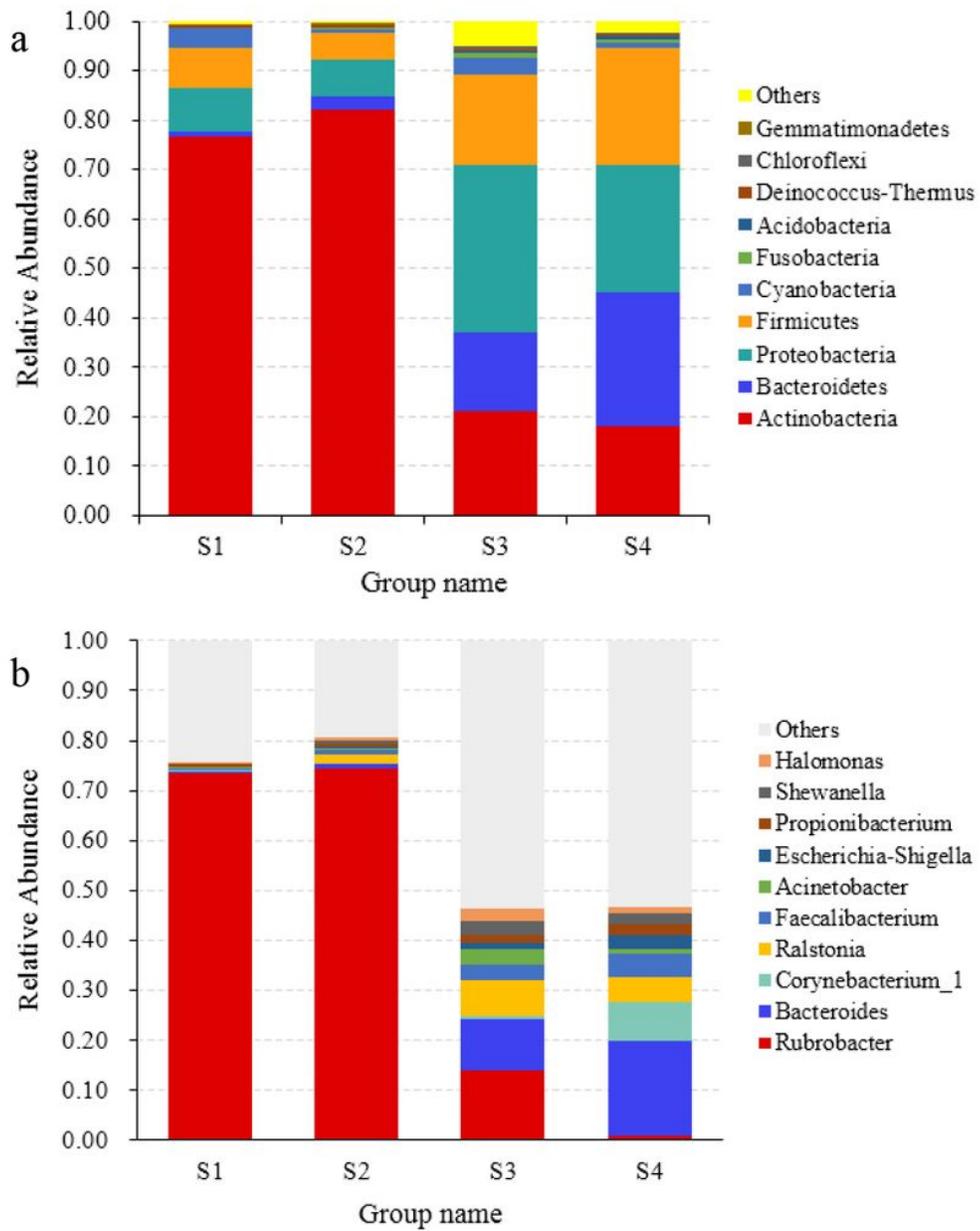


Figure 3

Relative abundances of bacterial phyla (a) and genera (b) I in soil. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Figure 4

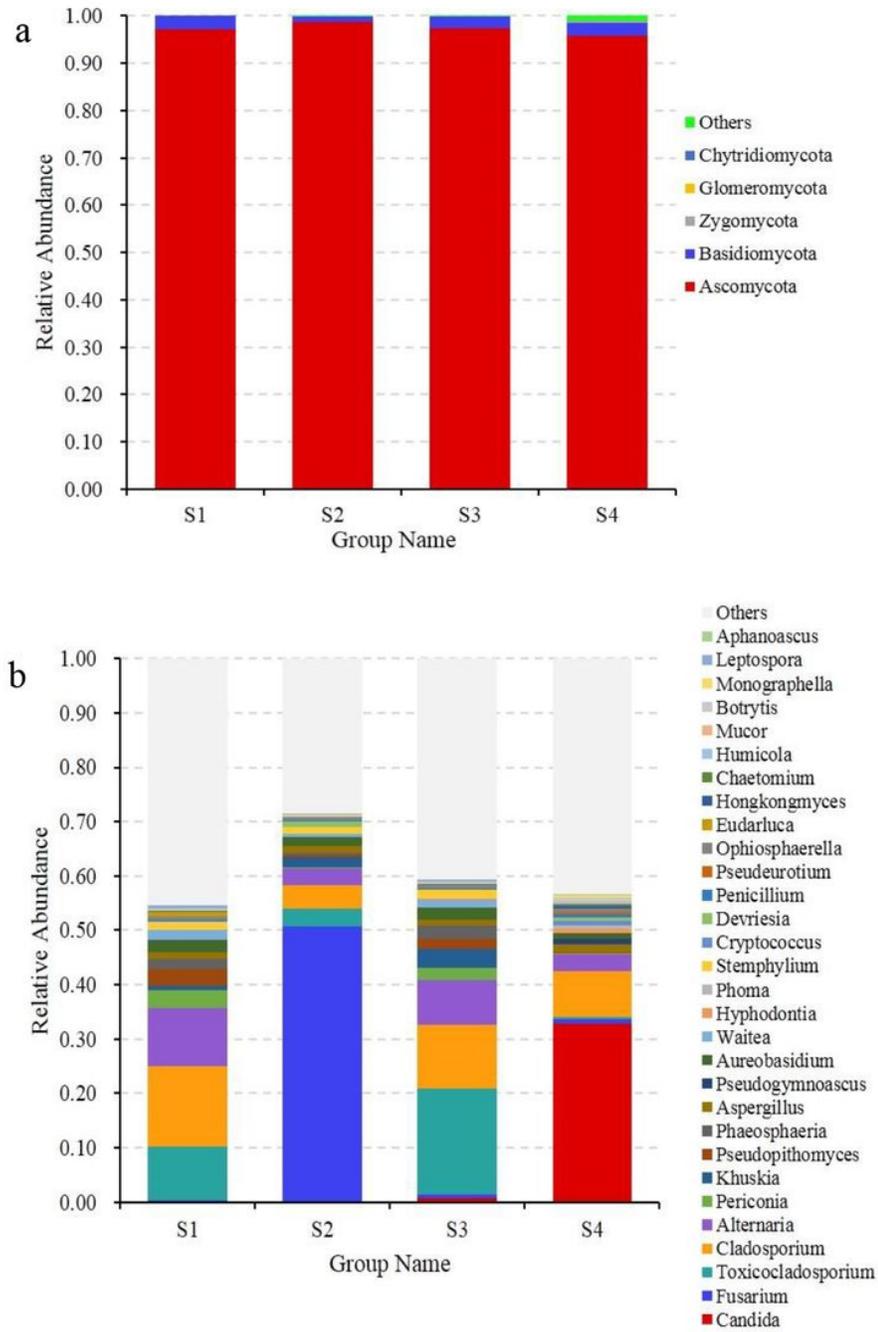


Figure 4

Relative abundances of fungal phyla (a) and genera (b) in soil. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Figure 5

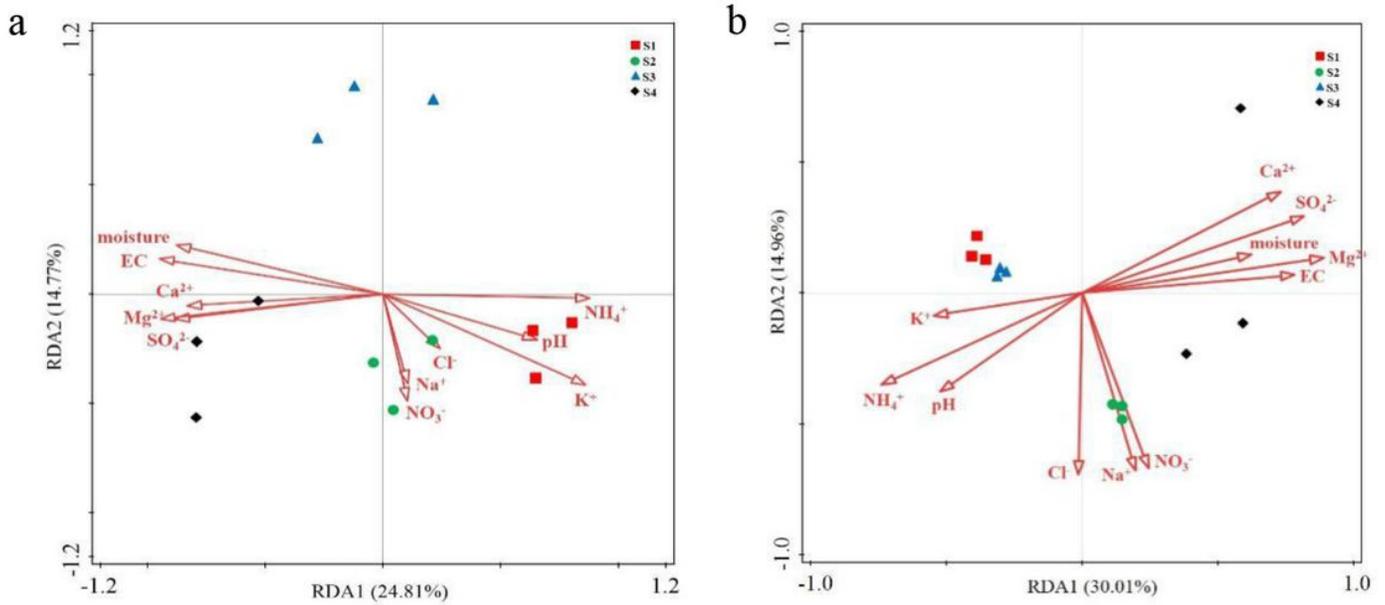


Figure 5

The relationships of environmental factors with bacterial (a) and fungal (b) communities in soil. S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

Figure 6

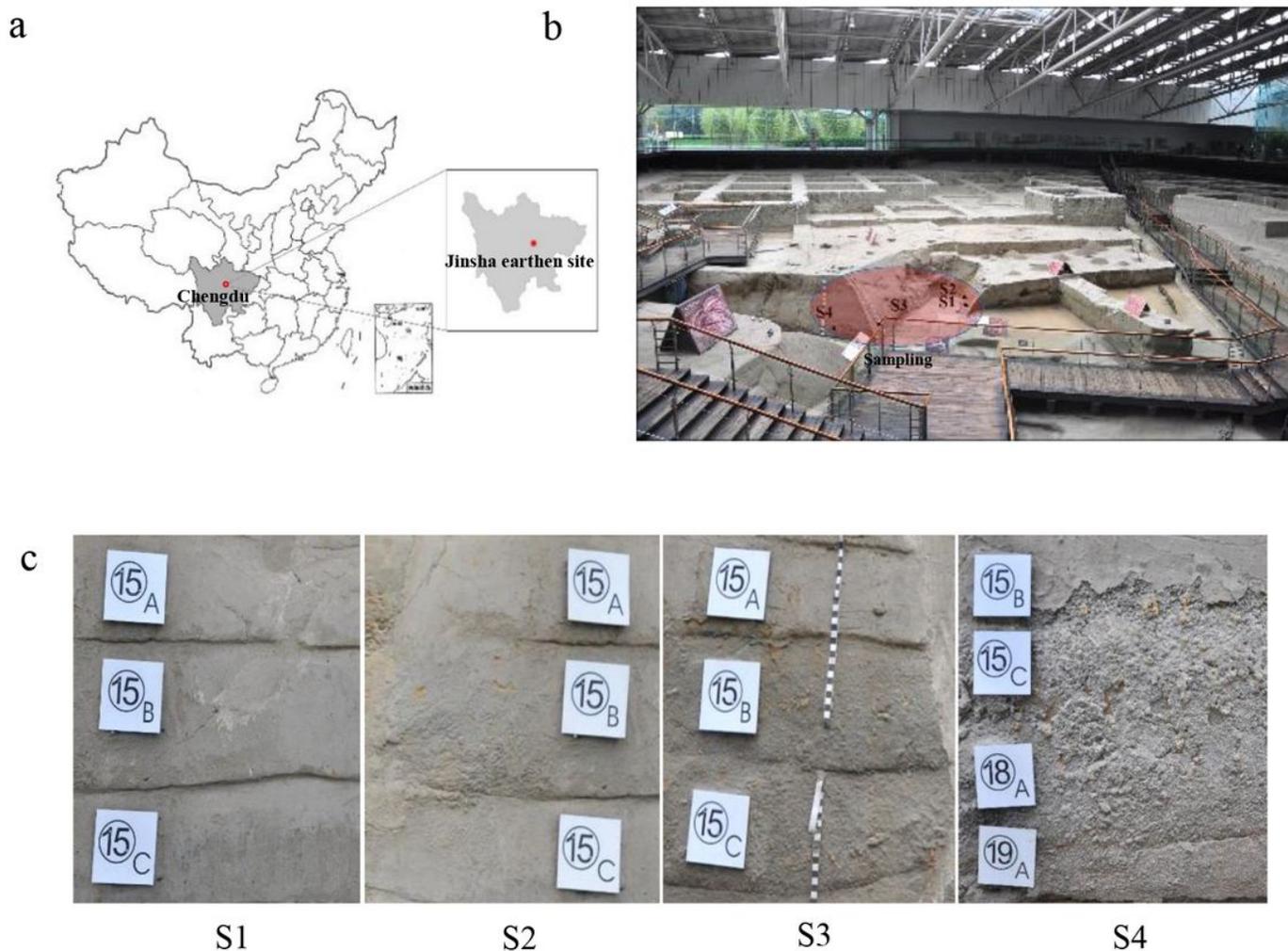


Figure 6

The locations of Jinsha site museum in Chengdu, Sichuan, China (a), sampling sites in the Relics Hall at Jinsha earthen site (b), and the individual samples (c). S1, no obvious deterioration; S2, mild deterioration; S3, moderate deterioration; S4, severe deterioration.

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