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

Jing Li
Sichuan Agricultural University
Xiaoyue Zhang
Sichuan Agricultural University
Lin Xiao
Chengdu Istitute of Cultural Relics and Archaeology
Ke Liu
Jinsha site Museum
Yue Li
Sichuan Agricultural University
Ziwei Zhang
Sichuan Agricultural University
Qiang Chen
Sichuan Agricultural University
Xiaolin Ao
Sichuan Agricultural University
Decong Liao
Sichuan Agricultural University
Menggen Ma
Sichuan Agricultural University
Yunfu Gu
Sichuan Agricultural University
Xiumei Yu
Sichuan Agricultural University
Quanju Xiang
Sichuan Agricultural University
Ji Chen
Sichuan Agricultural University
Xiaoping Zhang
Sichuan Agricultural University
Tao Yang
Chengdu Institute of Cultural Relics and Archaeology
Petri Penttinen
Sichuan Agricultural University
Ke Zhao (zhaoke82@126.com)
Sichuan Agricultural University

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Abstract

Background: Jinsha earthen site in Chengdu, China, plays an important role in understanding the ancient culture and history of Shu civilization. The site is undergoing soil degradation due to physical, chemical and biological factors, while very little is known about the influence of biological factors on earthen sites. To investigate the biological factor, we analyzed microbial communities and physicochemical properties from samples with no obvious, mild, moderate and severe degradation, referred to as S1, S2, S3 and S4 sample groups, respectively.

Results: Amplicon sequencing targeting the 16S rRNA gene and ITS for bacteria and fungi, respectively, revealed high bacterial and relatively low fungal diversity; the bacterial OTUs were assigned into 36 phyla and 617 genera and the fungal OTUs into 5 phyla and 205 genera. The relative abundances of Bacteroidetes, Proteobacteria and Firmicutes were higher and that of Actinobacteria lower with higher degree of degradation. In the genus level, the relative abundances of Bacteroides and Ralstonia were higher and that of Rubrobacter lower with higher degree of degradation. The distribution of the fungal genera in the four sample groups seemed more random than that of bacteria; however, the relative abundance of the yeast genus Candida was highest in the severely degraded sample group. 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: Taken together, the microbial communities in soil with different degree of degradation were distinctly different at Jinsha earthen site, and degradation was accompanied with bigger changes in the bacterial than in the fungal community.

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, fluctuation in temperature and humidity, and the growth and metabolism of microbes [3, 4]. The problems in earthen sites include weathering, cracks, alkali-bleaching, collapses and shedding [4–6].

The soil of earthen sites is different from farmland or forest soil and characterized by low mechanical properties and overturning resistance [7]. Over time, the cohesive forces between soil particles have weakened or disappeared, and the distance between particles has increased, which has changed the surface of earthen sites. Erosion in earthen sites is mediated by physical, chemical and biological processes that cause degradation and result in the loss of cultural information [8–10]. The loss will be permanent, 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 burial site. On the site, various and numerous artifacts have been unearthed, including more than 5,000 articles of gold, bronze, jade and stone, as well as millions of pottery potsherds, tons of ivory and thousands of boar tusks and deer horns (Figure S1B). For the protection, study and exhibition of Jinsha culture and ancient Shu civilization, the Jinsha Site Museum has built the Relics Hall in the excavation site (Figure S1A). Even though the Relics Hall alleviates damage by external factors, the earthen sites in the Relics Hall have undergone different degrees of degradation.

Most research has concentrated on degradation of earthen sites due to the physicochemical environmental factors [1, 2, 11–13]. Soil microorganisms play an important role in weathering, soil formation and development, and probably cause serious damage to earthen sites [14]. However, information on microbial community structures in earthen sites is scarce. In particular, very little is known about the relationship between these communities and the degradation of earthen sites.

In this study, we used high-throughput sequencing to analyze bacterial and fungal communities in soil with different degree of degradation at Jinsha earthen site. The objective was to (1) characterize the communities, (2) compare overall community

structure and the relative abundances of individual taxa in soil with different degree of degradation, and (3) determine the correlation of microbial communities with environmental factors. We hypothesized that community composition would vary depending on degradation degree.

Results

The soil physicochemical properties

Soil characteristics varied with the degradation of earthen site (Table 1). Soil moisture was higher in moderately and severely degraded S3 and S4 samples than in not or slightly degraded S1 and S2 samples ($p > 0.05$), and pH ranged from 7.32 in S4 to 7.48 in S1 to S4 ($p > 0.05$). EC value and the concentrations of Mg^{2+}, Ca^{2+} and SO_4^{2-} were higher in the more degraded 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 S3 and S4 samples than in S1 and S2 samples, and those of Al, Si and K were lower ($p > 0.05$) (Additional File 1: Table S1).

Table 1

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

The diversity of microbial community

The bacterial and fungal communities in soil from Jinsha earthen site 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 ≥ 97% similarity. 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). 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 α diversity was estimated using Chao1 and Shannon indices, in order to determine the richness and diversity of the microbial community in soil with different degree of degradation from Jinsha earthen site. Chao1 index reflect the microbial community richness and Shannon index was used to characterize the microbial diversity. 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 not degraded and severely degraded sample groups 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).

On 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 *Bacteroides* and *Corynebacterium* were higher in S3 and S4 ($p>0.05$). The results showed that the bacterial community compositions in sample groups with different degree of degradation 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). Glomeromycota and Chytridiomycota were detected only in sample S4. At the genus level, *Toxicocladosporium*, *Cladosporium* and *Alternaria* were the most abundant genera in sample groups S1 and S3, *Fusarium* in S2 and *Candida* in S4 ($p < 0.05$) (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 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-}.

Table 2

	Bacteria			Fungi		
Sample	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 degradation; S2, mild degradation; S3, moderate degradation; S4, severe degradation.

Discussion

In this study, we analyzed bacterial and fungal community composition in soil with different degree of degradation 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 degraded sample groups S3 and S4 than in the not or mildly degraded 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 [15]. Actinobacteria have been frequently found as a dominant group in subterranean micro-niches, including cultural relics in caves and wall paintings in catacombs [16–20]. Previous work demonstrated that some Actinobacteria are potentially harmful to the preservation of cultural relics [21–23]. Proteobacteria are commonly the most abundant bacteria in soil [24, 25], and Firmicutes that are tolerant to extreme temperatures and low humidity are often found in extreme environments [20]. These bacteria may play an important role in the microecological balance of earthen sites.

Rubrobacter was the most abundant genus in S1, S2 and S3. Rubrobacter was considered connected with the biodegradation of cultural relics and the rosy discolouration of masonry and lime wall paintings in historical buildings in Austria and Germany [26]. Resistance to desiccation might be a selective advantage for Rubrobacter growth and efflorescence on walls might be due to Rubrobacter strains [27]. Therefore, Rubrobacter may play a crucial role at the early stage of degradation of earthen sites. However, in the moderately and severely degraded sample groups S3 and S4, the relative abundances of Rubrobacter were remarkably lower than in S1 and S2, and those of Bacteroides, Ralstonia, Bacillus and Acinetobacter were higher. Bacteroides have the capability to produce acid [28], and the acid can dissolute minerals and further damage the structure of soil. Moreover, Bacillus and Acinetobacter in other cultural heritage sites have been found to possess efficient degradation ability [20, 29]. Therefore, the higher relative abundances of Bacillus and Acinetobacter may potentially accelerate the rate of degradation at Jinsha earthen site.

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 degradation degree of earthen site. 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 [18, 30, 31]. Cladosporium, Fusarium and Toxicocladosporium have been reported to produce extracellular enzymes and abundant mycelia that contribute to the mineral dissolution and mechanical destruction of soil structure [18, 30, 31]. The yeast Candida was most abundant in the severely degraded sample group S4. Candida species have the ability to secrete extracellular metabolites and to acidify soil [32]. Potentially, the accumulation of Candida plays an important role in the degradation of Jinsha site. Since information on Candida species in cultural relics is scarce, further work is needed to understand the role of this fungal genus.

The diversity and distribution of microorganisms in soil are affected by environmental variables. The microbial community structure can rapidly change in response to altered environmental conditions [33, 34]. 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 [35, 36]. The higher moisture in the moderately and severely degraded 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 degraded sample groups S3 and S4 than in the not or mildly degraded sample groups S1 and S2. Bacterial communities have been found more sensitive to changes in pH than fungal communities [37]. This could partly explain the more pronounced difference in bacterial communities than in fungal communities along the difference in degradation degree.

EC is apparently associated with soil salinity [38]. As in an earlier study [39], the relative abundance of Bacteroidetes was found to correlate positively with EC. Soluble salts are considered to cause damage on earthen sites [5, 11]. 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 degradation correlated positively with K^+ concentration, and those in severely degraded samples with Mg^{2+}, Ca^{2+} and SO_4^{2-} concentrations. Since the weathering of earthen relics soil releases nutrients into the soil [40],

the nutrients released by weathering may have influenced the microbial diversity in Jinsha earthen site. The main minerals in Jinsha earthen site are quartz, feldspar, illite, montmorillonite and chlorite, with SiO_2, Al_2O_3, Fe_2O_3, K_2O, MgO, and CaO as the main chemical components [8]. Plausibly, the weathering of these components lead to higher concentrations of Mg^{2+} and Ca^{2+} in the moderately and severely degraded sample groups S3 and S4. The nutrients released by weathering could support the growth and metabolism of microorganisms. As the microbially caused damage for cultural heritage relics is the result of microbial growth and the corresponding metabolism [41], the higher microbial diversity and possible more active metabolism may have potential harm to the preservation of Jinsha earthen site.

Conclusions

In this study, high throughput sequencing was applied to explore microbial community structures in soil with different degree of degradation from Jinsha earthen site. The dominant bacterial phyla and genera showed more variability than fungal phyla and genera. Furthermore, differences in bacteria and fungus 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 site soil degradation.

Material And Methods

Sampling

Jinsha Site Museum is in No.2 Jinsha Site Road (30.68091 N, 104.01362 E), Chengdu, Sichuan, China (Figure 6a). Soil in the sacrificial area with a lot of buried ivory, called as the Ivory pit, had undergone different degree of degradation. Triplicate samples were randomly taken from the same cultural deposit layer (15C) in the Ivory pit with no obvious, mild, moderate and severe degradation, 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 not enough 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 degradation of earthen sites [1, 3, 11, 42]. The samples were air-dried, crushed and sieved through a ø 1 mm sieve. The determination of pH and EC were made on 1:1 slurry of air-dried soil and water [43]. Moisture was determined by oven drying method [44]. 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 μm pore size syringe membrane filter[45]. 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 μL of extract were analyzed using 4 mm×50 mm guard columns AG11-11C and CG12A, respectively, 4 mm×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).

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'). 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'). The primers included sequencing specific adaptor sequences. Amplification was done in 30 μL reactions with 15 μL of Phusion®High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM 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 were filtered out using UCHIME v. 4.2.4.0 [47]. Sequences were assigned to operational taxonomic units (OTUs) at ≥ 97% similarity using UPARSE v7.0.1001 [48]. The representative sequences of the OTUs were assigned to taxa using Silva Database based on Mothur algorithm [49]. Chao1 and Shannon indices were calculated using the phyloseq package [50]. Venn diagrams were done at VennDiagramWeb [51]. Principal Component Analysis (PCA) was done using CANOCO 5 [52]. Differential taxa at phylum to species levels were identified using linear discriminant analysis coupled with effect size (LEfSe) [53]. To analyze the bacterial and fungal community distribution and their correlation with environmental factors, redundancy analysis (RDA) was carried out using CANOCO 5 [52]. 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) [54]. 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

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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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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.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Department of Microbiology, College of Resource, Sichuan Agricultural University, Yaan 625000, P. R. China. [2] Chengdu Institute of Cultural Relics and Archaeology, Chengdu Sichuan 610072, China. [3.]Jinsha site museum, Chengdu Sichuan 610072, China.

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