Seasonal Variation of Airborne Fungi of the Tiantishan Grottoes and Western Xia Museum, Wuwei, China

Yulong Duan
Northwest Institute of Eco-Environment and Resources
Fasi Wu
Dunhuang Research Academy
Dongpeng He
Dunhuang Research Academy
Ruihong Xu
Dunhuang Research Academy
Huyuan Feng
Lanzhou University School Of Life Sciences
Tuo Chen
Northwest Institute of Eco-Environment and Resources
Guangxiu Liu
LANZHOU
Wanfu Wang (wwanfu@hotmail.com)
Northwest Institute of Eco-Environment and Resources

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Abstract

Background: The deposition of the airborne fungi onto cultural heritage is associated closely with the subsequently biodeterioration taking place. In this study, a systematic survey of the culturable airborne fungi was carried out in the occurrence environments of the wall paintings that preserved in the Tiantishan Grottoes and the Western Xia Museum, China. Bio-aerosol sampler was used for sampling in four seasons of 2016. Culture-dependent and -independent methods was taken to acquire the airborne fungal concentration and purified strains; by the extraction of genomic DNA, amplification of fungal ITS rRNA gene region, sequencing, and phylogenetic analysis, thereafter the fungal community composition and distribution characteristics of different study sites were clarified. Finally, we disclosure the main environmental factors which may responsible for the dynamic changes of the airborne fungi at the sampling sites.

Results: The concentration of culturable airborne fungi was in a range from 13 to 1576 CFU/m^3, no significant difference between the two sites at the Tiantishan Grottoes, with the obvious characteristics of the seasonal variation, in winter and spring were higher than in summer and autumn. Meanwhile, a significance difference in fungal concentration between inside and outside of the Western Xia Museum, the outside of the museum was far more than the inside of the museum in the four seasons, particularly in the winter. Eight fungal genera were detected, including *Cladosporium*, *Penicillium*, *Alternaria*, and *Filobasidium* as the dominant groups. The airborne fungal community structures of the Tiantishan Grottoes showed a distinct characteristic of seasonal variation and spatial distribution. Relative humidity, temperature and seasonal rainfall all have influences on the airborne fungal distribution. Some of the isolated strains have potential to cause biodeterioration of the ancient wall paintings.

Conclusions: The prominent seasonal variation of airborne fungal concentrations, community structures and distribution patterns were found in this study, that have close relationship with local climate conditions of the Tiantishan Grottoes. Therefore, it is necessary to carry out the long period monitoring of airborne fungal flora in the future, and integrated it into a pre-warning monitoring and conservation system.

Background

The microorganisms, including bacteria and fungi, are pervasively found in almost all types of habitats in cultural heritage sites on earth, such as rock caves, cave temples, archaeological sites and museums, the growth of microorganisms may contribute to the severe biodeterioration of fragile artworks [1–5]. Some of the previous studies had shown us microbial outbreaks causing aesthetic and mechanical damage of mural paintings like pigments discoloration, cracking and disintegration of painted layers caused by contamination and biofilm formation on the painted surface, and flaking of the painted layer from the underlying support layer because of binders' degradation [6–11]. Actually, atmosphere is an important microbe's reservoir with ca. 1,200 species of bacteria and actinomycetes and 40,000 species of fungi [12], what's more, air-exchange is the main reason for the transportation and dispersion of microorganisms. In

many cases, they can be attached to the surface of cultural relics such as murals and can bring risk to the biodeterioration of the substratum materials. Particularly under environmental conditions of rich nutrient supply, favorable temperature, relative humidity, pH or sunlight for the growth and proliferation of microbes [13]. Therefore, studies that have been carried out on the airborne microflora in various historical sites may help us understand the process of biological degradation of artworks and provide data as supporting for preventive heritage conservation and tourism management [14].

Ancient wall paintings are kind of valuable cultural heritage and can be divided into *in-situ* conservation and *ex-situ* conservation according to their storage venue, the former refers to the primary environment of mural paintings such as caves, temples and catacombs. If the original environment of these paintings was drastically altered and even threatened their conservation, they were often removed and transported to museum for *ex-situ* conservation [15]. Airborne microorganisms usually exist as microbial aerosols which attached to floating dust and other soil particles, most of them have natural and anthropogenic sources, such as plants, soils, vegetables, animal feeding, agricultural activities, and transportation industry [16]. Colony forming units (CFU) is commonly used to calculate the numbers of culturable airborne microorganism in per cubic meter of air (CFU/m^3), it's an important ecological indicator for early warning and environmental control of microbial outbreaks which refers to biodeterioration of cultural relics at the various heritage sites [17–21].

Recently, both culture-based methods and DNA-based molecular approaches such as clone library method based on 16S ribosomal DNA/RNA or ITS (Internal transcribed spacer) regions, PCR-DGGE, NGS (Next-Generation Sequencing) have been widely employed on biological identification of the airborne microbes in various historical sites [22–24]. Previous studies revealed that airborne microbial concentration and community composition varied greatly among heritage sites, particularly for indoor and outdoor of the museums [23, 25–27]. Meanwhile, the microbial concentration and community composition in the atmosphere are influenced by various factors, mainly natural environment variation (e.g. temperature, relative humidity, sunlight, wind and rainfall) and artificial disturbance (e.g. tourists' activities) [28, 29]. For example, when tourists get into the cave, catacomb and museum, that may break primitive conservation environment of the cultural relics and cause issues such as increasing of CO_2 concentration, temperature and relative humidity [30, 31]. Furthermore, tourists may transferring particulate materials and carried exogenous microbes into cave ecosystem, lead to an increase of the particle materials concentration and change of airborne microbial communities [32, 33]. When it concerned to the public health, many of the airborne microbes act as opportunistic pathogens lead to human diseases [29, 34].

Up to now, there is few studies on the discrepancy of airborne microbial community characteristics which caused by the change of cultural heritage preservation environment. In this study, we aimed to compare the concentration and community structure difference of airborne fungi at two sites where mural paintings preserved, the Tiantishan Grottoes and the Western Xia Museum. Combined with environmental monitoring data, to disclosure the main factors which responsible for the dynamic changes of the airborne fungi at two sites.

Results

Fungal concentrations

Considering all four sampling sites, the concentration range of culturable fungi was from 13 to 1576 CFU/m^3, and the mean and median values were 603 ± 57 CFU/m^3 and 525 CFU/m^3, respectively (Table 1). The mean concentration value was 559 ± 81 CFU/m^3 in TT18, 504 ± 43 CFU/m^3 in TT13, 1057 ± 117 CFU/m^3 in TMO, 291 ± 74 CFU/m^3 in TMI. The highest fungal concentration appeared in TMO, and significantly higher than those in TT18, TT13, and TMI (P<0.05).

Table 1 Total airborne fungi (CFU/m^3) enumerated on PDA agar plates from the four sites

Sampling sites	Mean	Median	Minimum	Maximum
TT18	559 ± 81b	561	226	887
TT13	504 ± 43bc	475	374	689
TMO	1057 ± 117a	900	852	1576
TMI	291 ± 74c	238	13	674
Sum	**603 ± 57**	**525**	**13**	**1576**

Note: a, b and c mean significant difference.

The culturable airborne fungal concentrations of the four sites show obvious seasonal variation characteristics (Fig. 1). The highest concentration was observed in winter at TT18 with 887 CFU/m^3 and TT13 with 689 CFU/m^3, and in autumn at TMO with 1576 CFU/m^3 and TMI with 674 CFU/m^3. The lowest concentration was observed in autumn at TT18 with 226 CFU/m^3 and TT13 with 374 CFU/m^3, spring at TMO with 852 CFU/m^3, and in winter at TMI with 13 CFU/m^3.

In the same season, there was no significant difference in airborne fungal concentration between the TT18 and TT13 in the two sampling sites of the Tiantishan Grottoes (P>0.05). Meanwhile, the airborne fungal concentration in the square outside of the Western Xia Museum (TMO) was significantly higher than in the warehouse inside of the museum (TMI), especially in winter (P<0.05).

To evaluate the relationship between environmental parameters (T, RH and rainfall) and fungi concentrations, Pearson correlation analysis was performed (Table 2). It is observed that T, RH and rainfall had negative effects on the fungal concentration at the two sampling sites of cave 18 (TT18) and cave 13 (TT13), while these environmental parameters had positive effects on the fungal concentration at the outside of the Western Xia Museum (TMO). Meanwhile, we also observed that T and RH had positive effects on the fungal concentration at the inside of the museum (TMI).

Table 2 Pearson correlation analysis for fungal concentrations (CFU/m^3) and environmental parameters

Factors	T/℃	RH/%	Rainfall/mm
TT18	- 0.787	- 0.778	- 0.843
TT13	- 0.246	- 0.332	- 0.181
TMO	0.124	0.837	0.531
TMI	0.361	0.536	NA

T for temperature (℃), RH for relative humidity (%); NA for data no available.

Phylogenetic analysis of the airborne fungal communities

A total of 34 sequences were classified into 8 different fungal genera corresponding to the GenBank database, and among them, seven genera were affiliated to Ascomycota and the rest one was Basidiomycota. The phylogenetic tree is shown in Fig. 2. *Cladosporium* (45.38%) was most predominant fungal genus, followed by *Penicillium* (38.62%), *Alternaria* (8.09%) and *Filobasidium* (6.25%), these four fungal genera accounted for 98.20% of the entire community (Fig. 3). The rest of the members including the genera *Epicoccum*, *Aspergillus*, *Didymella*, and *Microdochium* that accounted for 1.80% of the entire community.

The Shannon-Weiner index represents diversity of fungal communities. The changes in the Shannon-Weiner index of fungal communities at the four sampling sites are shown in Fig. 4. The mean value of the Shannon-Weiner index differed among the four sampling sites (1.12 in TT18, 0.93 in TT13, 0.71 in TMO, 0.74 in TMI) and fluctuated markedly among different seasons. The highest diversity index values were as follows: spring in TT18 (1.73), autumn in TT13 (1.43), summer in TMO (1.07), and winter in TMI (0.96). The lowest diversity index was Spring in TMO (0.33).

The structure of culturable fungal communities varied greatly at different sampling sites in four seasons, the most prevalent fungal genera are presented in Fig. 5. *Cladosporium* and *Penicillium* were the most frequently discovered airborne fungi at four sampling sites. In spring, *Penicillium* (35.90%) was the major genus in TT18, followed by *Cladosporium* (29.06%), *Epicoccum* (11.97%), *Filobasidium* (11.97%) and *Alternaria* (9.40%). *Penicillium* (56.41%) and *Cladosporium* (43.59%) were the main genus in TMI. Meanwhile, *Cladosporium* accounting for 83.33% of the whole community in TT13, and the rest of the members includes *Didymella*, *Epicoccum*, and *Filobasidium* collectively accounted for only 16.67%. *Cladosporium* was the largest genus accounting for 95.38% in TMO. In summer, the proportion of *Penicillium* and *Filobasidium* was significantly increased to 55.25% and 37.74% in TT18, respectively. Meanwhile, in TT13, the proportion of *Cladosporium* was sharply decreased to 47.20%, and the genera of *Alternaria* and *Penicillium* was accounting for 19.88% and 29.19%, respectively. In addition, *Cladosporium* was still the largest genus both in the TMO and TMI, and its proportion was very close to each other with 76.99% and 80.00%, respectively. In autumn, *Alternaria* and *Cladosporium* dominated in TT18 and TT13, both of that accounted for 72.00%, 24.00% and 60.98%, 21.95%, respectively. The proportion of *Cladosporium* was sharply decreased to 21.55% and 13.41% in TMO and TMI, respectively. Meanwhile,

the proportion of *Penicillium* was increased to 78.45% and 81.01% in TMO and TMI as the dominant genera, respectively. In winter, the proportion of *Alternaria* was sharply decreased to 0.00% and 19.23% in TT18 and TT13, but the proportion of *Cladosporium* was sharply increased to 64.58% and 80.77% in TT18 and TT13, respectively. *Cladosporium* and *Penicillium* were making up to 56.46% and 43.54% in TMO, respectively. In addition, *Alternaria*, *Cladosporium*, and *Didymella* were reaching to 57.14%, 28.57%, and 14.29% in TMI, respectively.

The contributions of environmental parameters to community structure of airborne fungi

The relationships between fungal community structure and environmental parameters were determined by canonical correlation analysis (CCA). The results showed that the community structure of culturable fungi varied greatly among the different sampling sites in different seasons (Fig. 6). The contribution rate of the environmental parameters to the fungal community distribution were rainfall (0.097), T (0.049), and RH (0.042) by descending order. Among seasons, the fungal community structure was relatively similar at the four sites between summer and autumn, as well as in spring and winter, respectively. Moreover, the community structure of the four seasons was more similar in TMI.

Discussion

In this study, we explored the temporal-spatial dynamics of airborne fungal community in two different occurrence conditions for wall paintings of the Tiantishan Grottoes, China. The results shown that the culturable fungal concentration in the atmosphere was very close between TT13 (504 CFU/m^3) and TT18 (559 CFU/m^3) due to their close proximity position, within noticeable seasonal fluctuation, and higher fungal concentrations were recorded in winter and spring than those in summer and autumn. Meanwhile, the airborne fungal concentration at TMO was far higher than that TMI through all four seasons ($P < 0.05$), autumn in particular, the fungal concentration at the two sites were all reached the highest level with 1576 CFU/m^3 in TMO and 674 CFU/m^3 in TMI, that's may be attributed to the optimal nature conditions (high temperature and relative humidity, etc.) for fungal spores' germination in autumn [35, 36].

In general, local climatic conditions, including but not limited to temperature and relative humidity, may be provide an optimal environment conditions for fungal growth. For temperature, some previous studies revealed that airborne fungal concentrations at outdoor sites increased with the surrounding temperature rises [35–38]. For air humidity or rainfall, some studies believed that the fungal spore concentrations, including ascospores and basidiospores increased with humidity or rainfall [36, 39]. However, some argued that higher humidity may also means a rainfall condition, which could remove ambient fungal spores, especially for dry-air spore [40, 41]. Thus, the effects of relative humidity on airborne fungi were inconsistent in different studies, particularly considering more climatic factors, e.g. temperature and rainfall. Interestingly, pearson correlation analysis revealed that there was a positive correlation between airborne fungal concentration and three domestic climatic parameters, including temperature, relative humidity and rainfall both in the TMO and TMI, but on the other way a negative correlation on that at

TT18 and TT13. In Wuwei city, low temperature and low rainfall due to the high-cold and semi-arid climatic conditions might be the primary reason for the positive correlation on total fungal level with temperature, relative humidity and rainfall at the outside and inside of the museum, respectively. The co-efficient of the temperature rising and seasonal rainfall could result in the increasing of the relative humidity, which was beneficial to the germination and proliferation of the airborne fungal spores. Meanwhile, low temperature and high relative humidity due to the special microhabitat of the Tiantishan Grottoes maybe responsible for negative correlation on fungal concentrations with three climatic parameters at the TT18 and TT13, respectively. These two sites are located on the upstream of an artificial lake, named Huangyang River Reservoir, core microbial groups in the atmosphere may be insensitive to high relative humidity and that more adapted to lower temperatures. Of course, the reasons for this phenomenon still need further study in the future. In addition, human activities also have pronounced effect on airborne fungal concentration, especially between the outside and inside of the Western Xia Museum, which was located on the urban areas of Wuwei city with multitudinous people, vehicles, floating dust and microbial concentration in the atmosphere [31, 32]. On the contrary, inside of the museum with low airborne fungal concentration depends on its own barrier effect on external air pollutants and strict environmental control by staffs, especially during the winter, the restoration of the damaged murals was suspended and the internal environment without human intervention [42], the fungal concentration was falling to 13 CFU/m^3.

The airborne fungal communities of all sites that investigated in this study showed differences in their structures, but without any detected fungal genus that is unique and associated especially to one site. Eight fungal genus were picked from the PDA plates with the aid of ITS-based biological identification method, *Cladosporium* was the most frequently found fungi, followed by *Penicillium*, and *Alternaria*. They were the most frequently isolated genera in the air of the Tiantishan Grottoes and the Western Xia Museum, and these results were consistent with previous studies which carried on urban and caves [43–46]. The abundance of airborne fungi in the Tiantishan Grottoes and Western Xia Museum were influenced by both research sites and sampling periods. Among the four sites, TT18 showed the highest Shannon index value and TMO showed the lowest. Meanwhile, not only the mean value of the Shannon-Weiner index at TT18 was closed to TT13, but also the TMO and TMI (Fig. 5). In addition, the highest diversity index values were observed in four seasons, namely spring (TT18), autumn (TT13), summer (TMO), and winter (TMI). This suggests that airborne fungal diversity at our study site was depended on both the local environment and sampling seasons.

Fungi play a considerable role for the deterioration of cultural heritage [47], the research in Lascaux Cave declared that metabolic activity of fungi is a larger order of magnitude than bacteria; therefore, they have considerably higher biodeteriorative potential than bacteria [9]; Their ability to form hyphae, fungi may colonise and damage precious historical objects such as books, mural paintings and architectural surfaces [3, 48–50]. It is demonstrated *In vitro* biodegradation potential test of airborne *Aspergilli* and *Penicillium* that they have an excellent capacity for carbonate dissolution, acid excretion, alkaline metabolites, cellulolytic activity, and extracellular pigment production [51]. Meanwhile, many members of

the airborne fungi, such as *Cladosporium*, *Penicillium*, *Alternaria*, and *Aspergillus* were considered as ubiquitous opportunistic pathogens to humans and may induce respiratory diseases as rhinitis, sinusitis, asthma and alveolitis, meanwhile, may pose potential threats on visitors [34, 43]. Therefore, when they appeared in some relatively enclosed space like caves and museums, a periodic ventilate measures should be taken in these places to alleviate the potential dangers for cultural relics and staffs.

The airborne fungal dynamics of the Tiantishan Grottoes and Western Xia Museum show a notable seasonal periodicity and fluctuations, which mainly caused by the local environmental factors includes temperature, relative humidity, solar radiation, wind speed and rainfall, as well as human disturbance and adaptability of airborne microorganisms to local environments [22, 28, 52]. Canonical correlation analysis results show that the factors of the rainfall, temperature, and relative humidity all have some effects on the airborne fungal communities among sampling sites, briefly speaking, they have a positive effect on three sampling sites of TT18, TT13, and TMO in summer and autumn, which mainly attributed to favorable external conditions for microorganisms' grown and proliferation due to the warm and wet weather conditions during the periods. However, environmental parameters including wind speed, wind direction, solar radiation, and human disturbances, were not taken into consideration in whole analysis due to the data absence of these factors, which may also have vital impact on the composition and distribution of airborne fungi, their roles still need further study.

Conclusions

The prominent seasonal variation of airborne fungal concentrations, community structures and distribution patterns were found in this study, that have close relationship with local climate conditions of the Tiantishan Grottoes. It is necessary to carry out the long period monitoring of airborne fungal flora in the future, and integrated it into a pre-warning monitoring and conservation system. This study could provide supporting information for the scientific protection of cultural relics that preserved at the local sites and the museums.

Methods

Sites description

Tiantishan Grottoes, known famously as "Liangzhou Grottoes" in the history, are located in the north of the Tiantishan, approximately 60 km south of the Wuwei City, which is a typical northwest city located in the eastern Hexi Corridor in Gansu Province, China (Fig. 7 A). The caves of the Tiantishan Grottoes were built in the Northern Liang (401-439AD) of sixteen kingdoms period about 1,600 years ago. It not only played an important role in spreading Buddhism and propagating Buddhist art in the Hexi Corridor region on the Silk Road, but also had profound influence on art characteristics of the grottoes in later generations, including the World Heritage Sites Yungang Grottoes and Longmen Grottoes, both of them are representative grottoes in China. Therefore, Tiantishan Grottoes was called the "Origin of Grottoes" by academic field.

Tiantishan Grottoes are inland and situated in the basin valley between the Qilian Mountains and the south mountains of Hexi corridor. It has a cold climate of semi-arid and high land with an average annual temperature of 4.9 ℃, annual rainfall of 159 mm. Because of the historical factors [53], most priceless historical objects including wall paintings and painted sculptures of the Tiantishan Grottoes were relocated to the Gansu Provincial Museum for the *ex-situ* protection in 1959, only a few mutilated remains of the painted sculptures or wall paintings were left in the original Tiantishan Grottoes. Began from 2006, these historical objects were returned gradually. However, due to seismic damage, festinate relocation, immature exfoliating technology, poorly conservation environment in the history, these priceless cultural objects suffered from various diseases, including paint losses, detachment, flaking, and microbial deterioration. In this case, the immediate and salvageable restoration is under way. Therefore, these objects which transferred to the Western Xia Museum of Wuwei city from Gansu Museum would then return to the Tiantishan Grottoes after completion of restoration.

Sampling process

Four sites were selected for the sampling of aerosol in four seasons (represented by April, June, October, and December) in 2016, including Cave 13 and Cave 18 of the Tiantishan Grottoes, warehouse inside and square outside of the Western Xia Museum, sample sites are namely TT13, TT18, TMI, and TMO, respectively (Fig. 7 B-D). In Cave 13, there was a giant sitting Shakyamuni Statue of 23.8 m which protected from the water by a semi-enveloping cofferdam constructed by reinforced concrete. Cave 18, only a central tower with a Buddha Statue remained its position is higher than Cave 13 with an open and flat landform. Cave 13 and cave 18 were two sites of the Tiantishan Grottoes which is still with surviving wall paintings. Western Xia Museum is located in the southeast of the Confucius' temple in Wuwei City, the square outside of it and the surrounding areas are always with activities of people and vehicles, however, the warehouse inside of the museum has been employed to store the uncovered wall paintings of the Tiantishan Grottoes, in where these artworks have been gradually restored in recent years. It should be noted that the restoring work of the cultural relics was only carried out during the period from April to November in a year and suspended at rest for the months without human disturbance due to the local bitter-cold weather.

Buck Bio-Culture™ Model B30120 sampler (A.P. Buck Inc, USA) was used to sampling from the atmosphere in Maijishan Grottoes. At each site, the sampler was installed 1.5 m above ground level with a supporting platform. Samples were carried out for 2 min, with three parallel repetitions. For each sampling, the bio-aerosol sampler loaded with 90 mm Petri dishes containing Potato Dextrose Agar (PDA) medium. Exposed culture dishes were incubated for 7-15 days at room temperature with 25 ℃.

Fungal identification

Colony forming units (CFU) on the plates were enumerated, and fungal concentrations were expressed as per cubic meter of air (CFU/m^3). CFU/m^3 was calculated as below:

$$C\ (\mathrm{CFU/m^3}) = \frac{T \times 1000}{t\ (min) \times F\ (L/min)}$$

In the equations, ***C*** is the airborne fungi concentration; ***T*** is the total colonies on the PDA plates; ***t*** is the sampling time; ***F*** is the airflow rate.

After incubating, microbial colonies were counted and fungi were preliminary identified according to morphological characteristics, including shape, size, color, and refractivity, etc. The isolates were identified by using a molecular method as described below. Each isolate was homogenized in liquid nitrogen and then genomic DNA was extracted by using a commercially available extraction Kit (Tiangen Co., Beijing, China) according to the manufacturer's protocols. The internal transcribed spacer (ITS) region of fungal rRNA genes was amplified with the following universal primer set: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [54]. The reaction mixture (25 μL) consisted of 2.5 μL 10 × PCR buffer, 1-unit *Taq* polymerase (Tiangen Co., Beijing, China), 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.2 μM each primer, and 2.5 μL (ca. 10 ng DNA) template. The PCR program included an initial denaturation at 94 ℃ for 5 min, 30 cycles at 94 ℃ for 40 s, annealing at 55 ℃ for 40 s, an extension at 72 ℃ for 40 s, and a final extension step at 72 ℃ for 10 min. The PCR products were detected by electrophoresis on 1.0% agarose gel.

The similarities among PCR products were determined by restriction fragment length polymorphisms (RFLP) analysis. The PCR products were digested with the double restriction endonucleases *BsuR* I and *Hinf* I (MBI, Fermentas). And then, the digested fragments were differentiated into several clusters according to their spectral patterns on 2.5% agarose gel.

Cloning of PCR product of distinctive strain was performed with the pGEM-T Vector System (Tiangen Co., Beijing, China) following their manufacturer's instructions, and the ligation product was subsequently transformed in *Escherichia coli* DH5α competent cells (Tiangen Co., Beijing, China), which allows Blue-White selection. The transformants were plated on LB medium with ampicillin (100 mg/ml), X-Gal (20 mg/ml) and IPTG (200 mg/ml). Positive clones were identified by PCR amplification with the pGEM-T vector primer pairs (T7/SP6) by using the same program as ITS amplification.

Fungal suspensions of expected clones were sequenced by the Shanghai Majorbio Bio-technology Co., Ltd. (Shanghai, China). A total of 34 fungi representative sequences were obtained (ca. 600 bp) and then analyzed via National Center for Biotechnology Information (NCBI) Blast program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The most similar sequences were extracted from the GenBank database. A phylogenetic neighbor-joining tree was constructed via the MEGA software 7.0. The sequences retrieved during this study could be accessed under the data range of MH042805-MH042838.

Data of environmental parameters

The meteorological data were collected in all four sampling sites for one year (from April 16, 2016 to April 16, 2017). Temperature (T, °C) and relative humidity (RH, %) were collected by HOBO® U23-001 Temp/RH

data logger (Onset Computer Corporation, USA). Data of rainfall was provided by local weather bureaus. For all environmental parameters, the values used in data analyses were 10-day averages from before and after the sampling days.

Statistical analysis

All the experiments were analyzed by one-way analysis of variance (ANOVA) via SPSS version 16.0. The relationships between airborne fungi concentration and environmental parameters were tested via Pearson correlation analysis. Shannon-Weiner diversity index of fungal communities were generated using the Vegan packages in R (4.0.0). Canonical correlation analysis (CCA) for airborne fungal communities and environmental parameters were then analyzed using CANOCO version 4.5.

Abbreviations

16S rRNA
16S ribosomal RNA; ITS:Internal transcribed spacer; CFU:Colony forming units; NGS:Next-Generation Sequencing; PDA:Potato Dextrose Agar; CCA:Canonical correlation analysis; ANOVA:one-way analysis of variance.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The sequences retrieved during this study could be accessed under the NCBI data range of MH042805-MH042838.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Data curation: YLD, FSW, and DPH. Funding acquisition: WFW and FSW. Methodology: YLD, FSW, RHX, WFW, TC, and GXL. Project administration: YLD and FSW. Writing-original draft: YLD, FSW and WFW. Writing-review & editing: TC and GXL. All authors have read and approved the manuscript.

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Figures

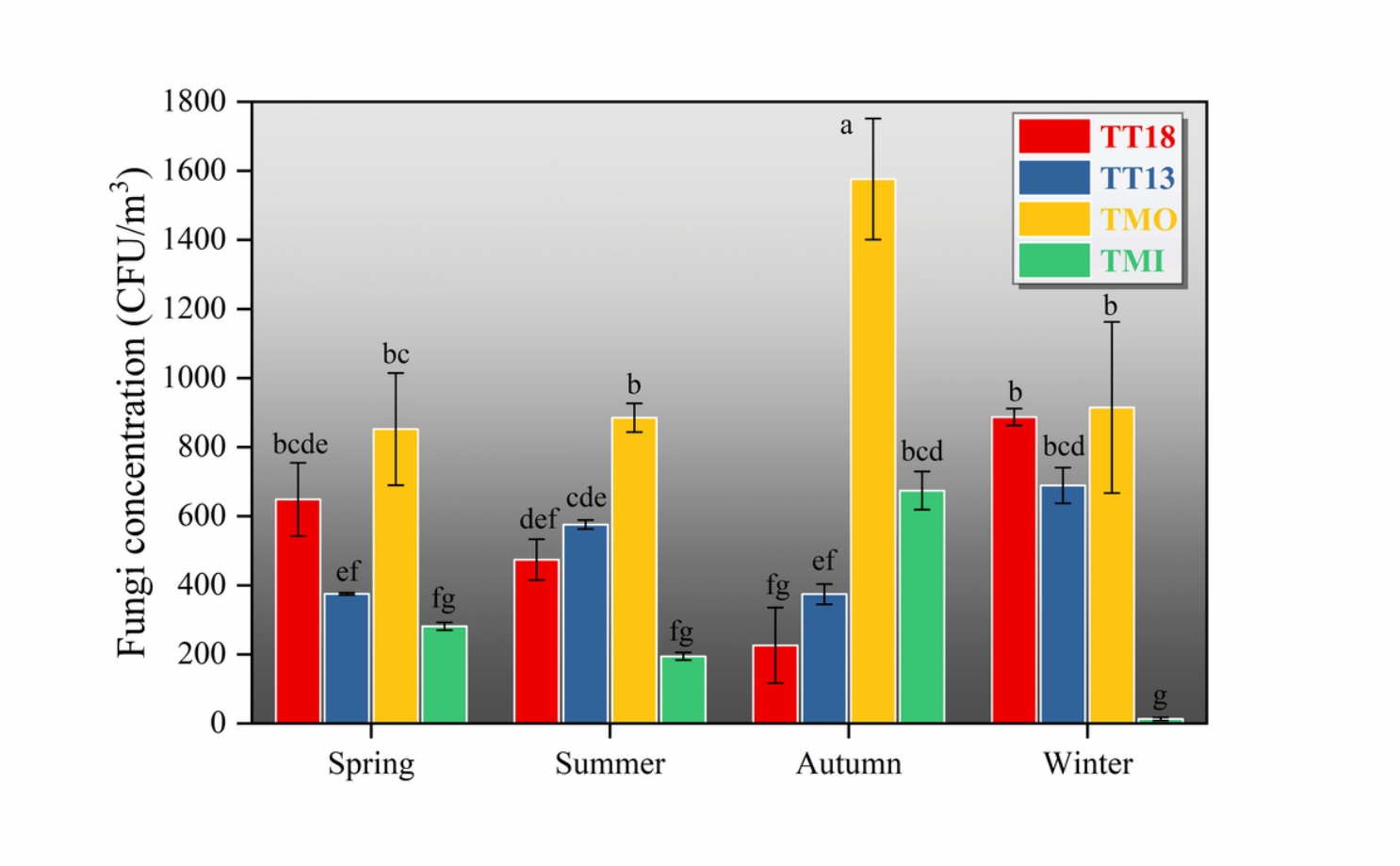

Figure 1

The fungi concentrations (CFU/m3) at the four sites in different seasons (April, June, October, December).

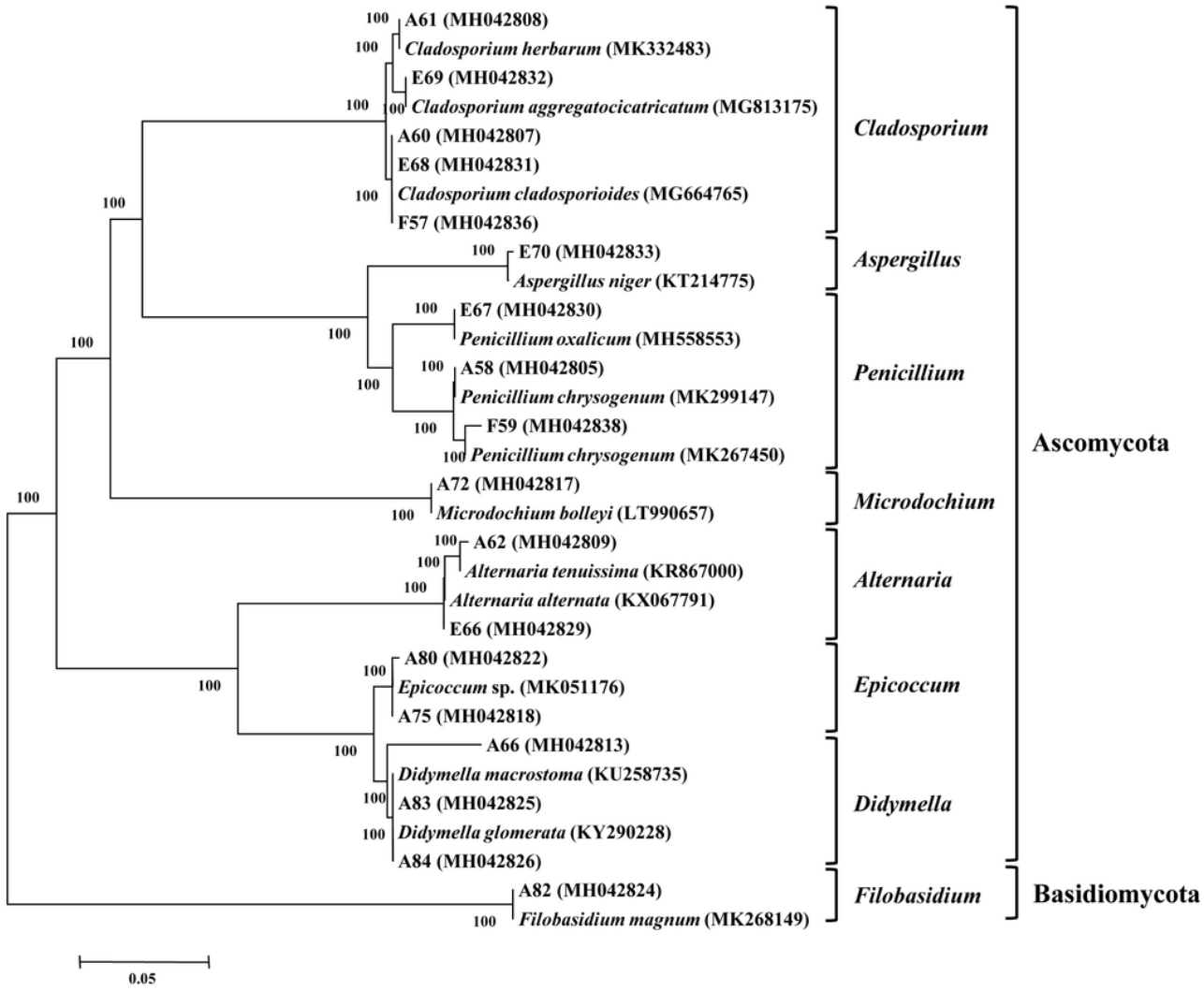

Figure 2

Phylogenetic tree of airborne fungi based on ITS sequences.

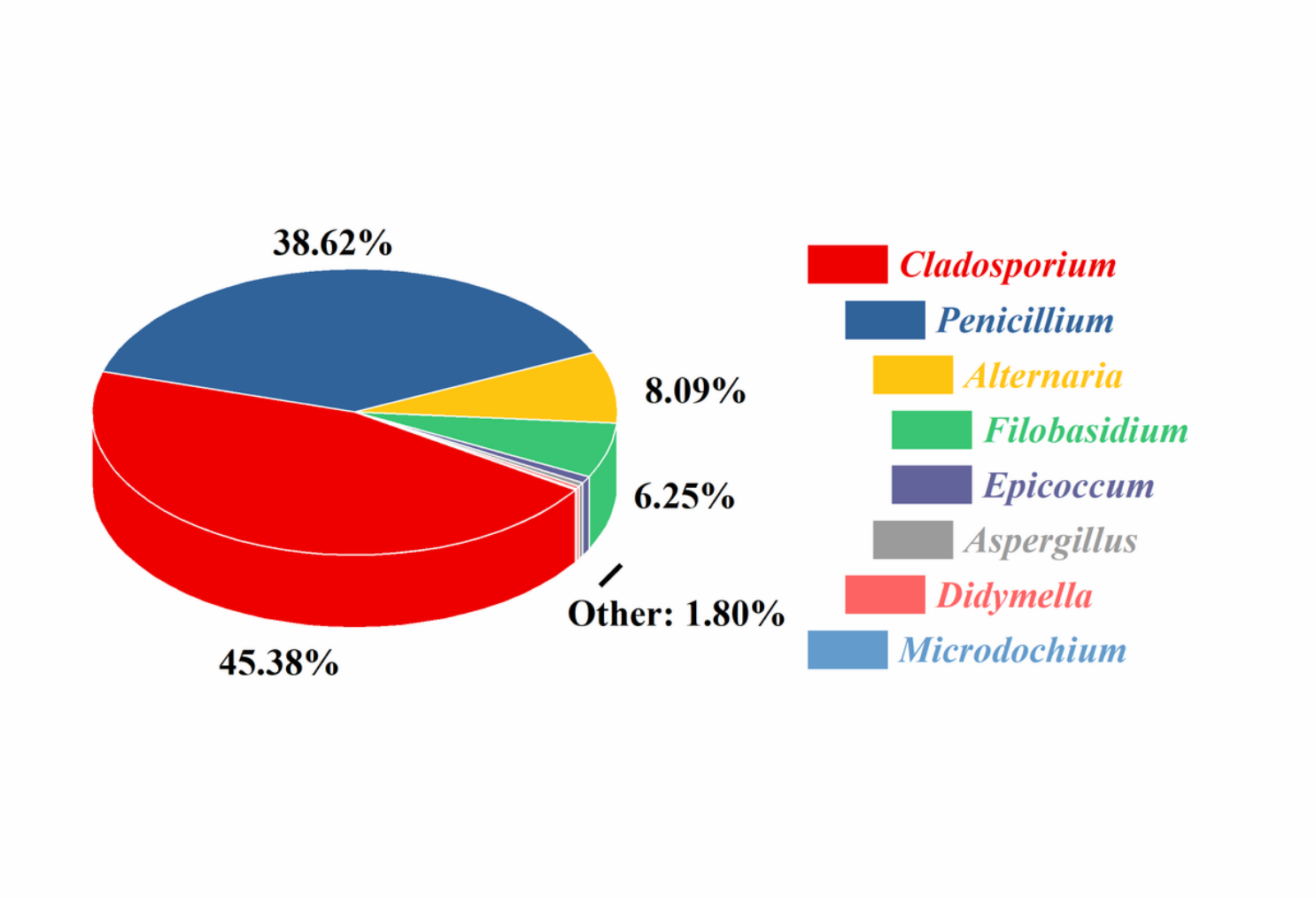

Figure 3

Proportions of fungal genera detected in atmosphere environment of the Tiantishan Grottoes and the Western Xia Museum.

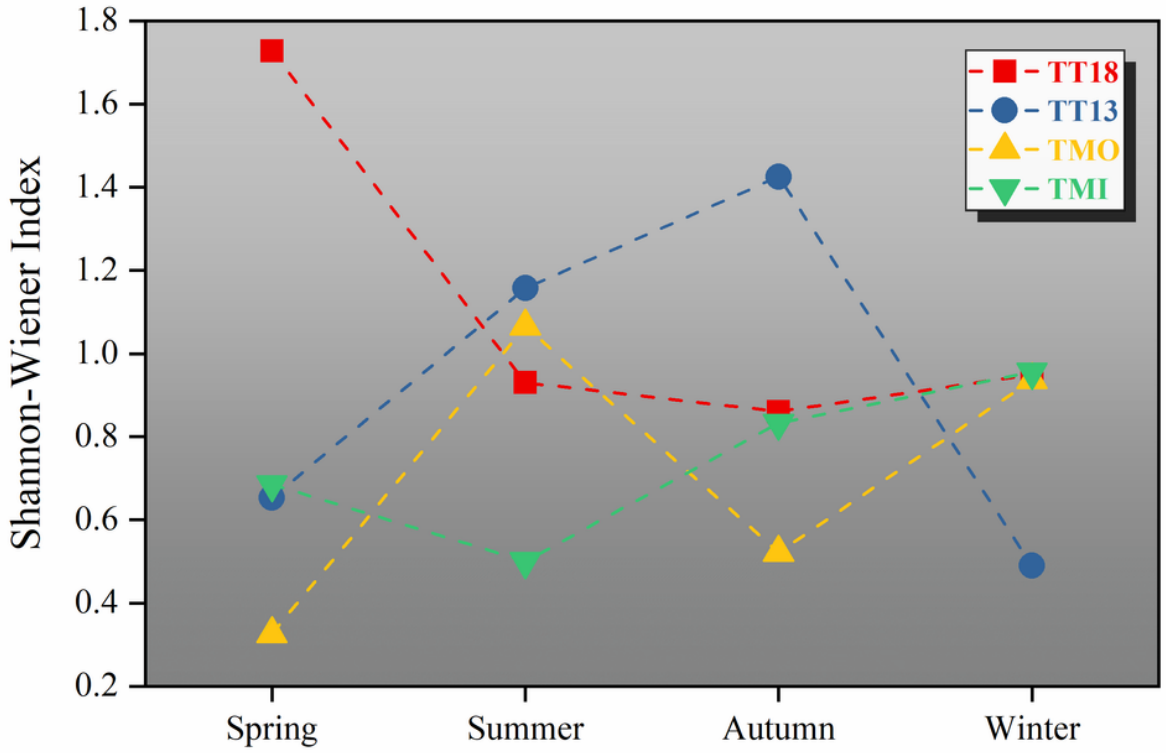

Figure 4

Seasonal changes in Shannon-Weiner index of airborne fungal communities at the four sampling sites.

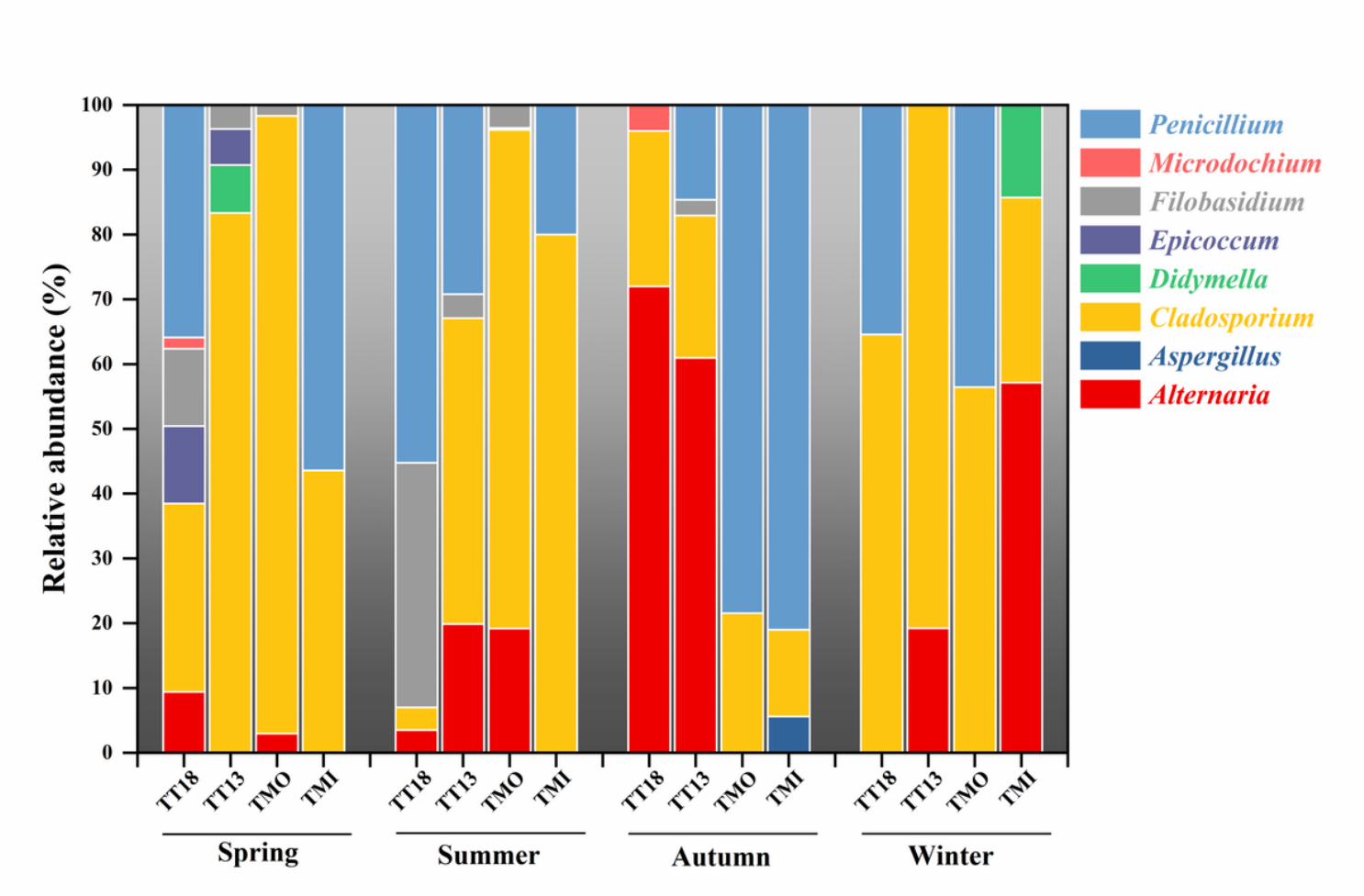

Figure 5

Relative abundance of the dominant fungal genera at the four sites.

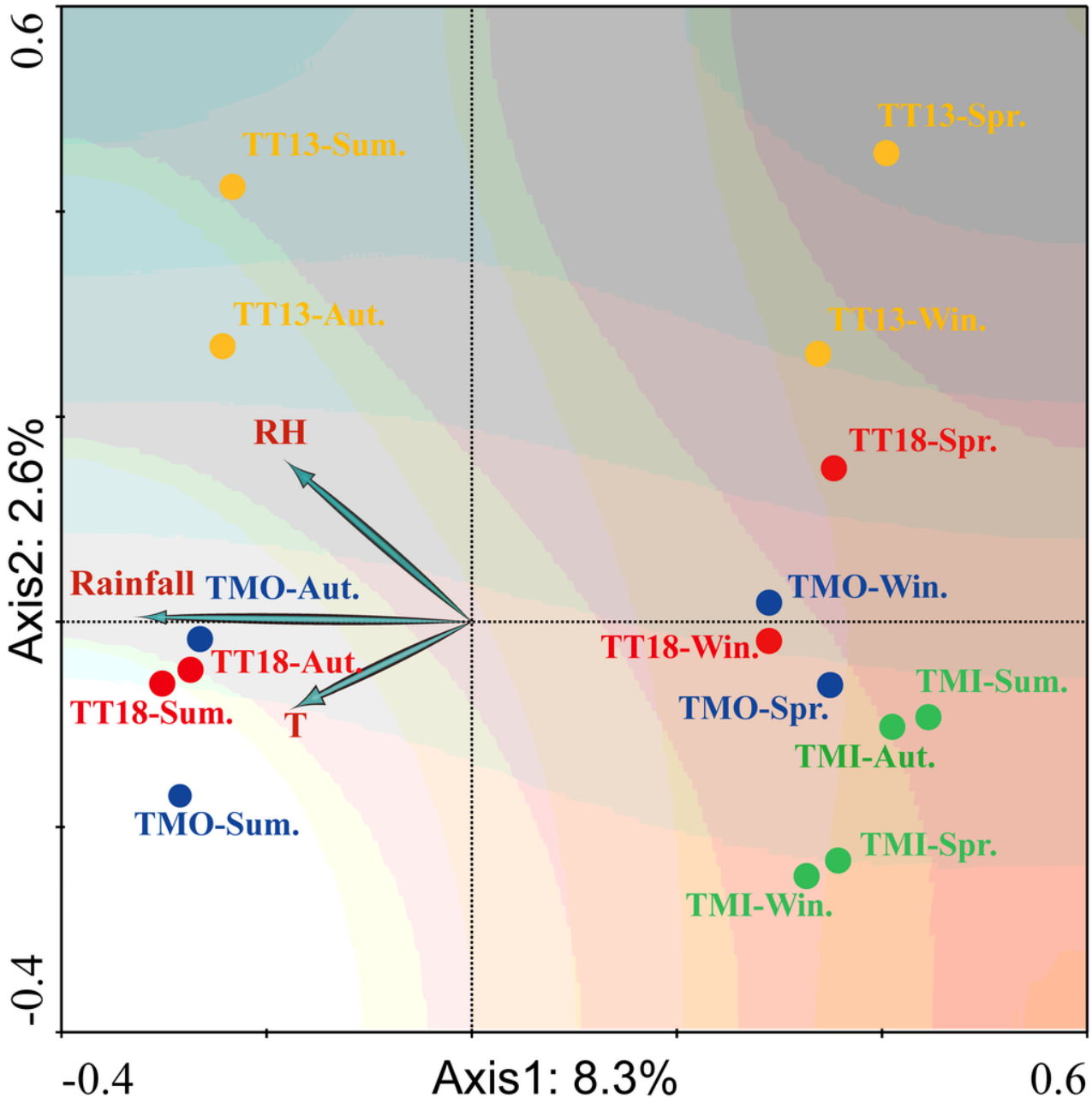

Figure 6

Canonical correlation analysis for fungal communities and environmental parameters from the four sites in different seasons.

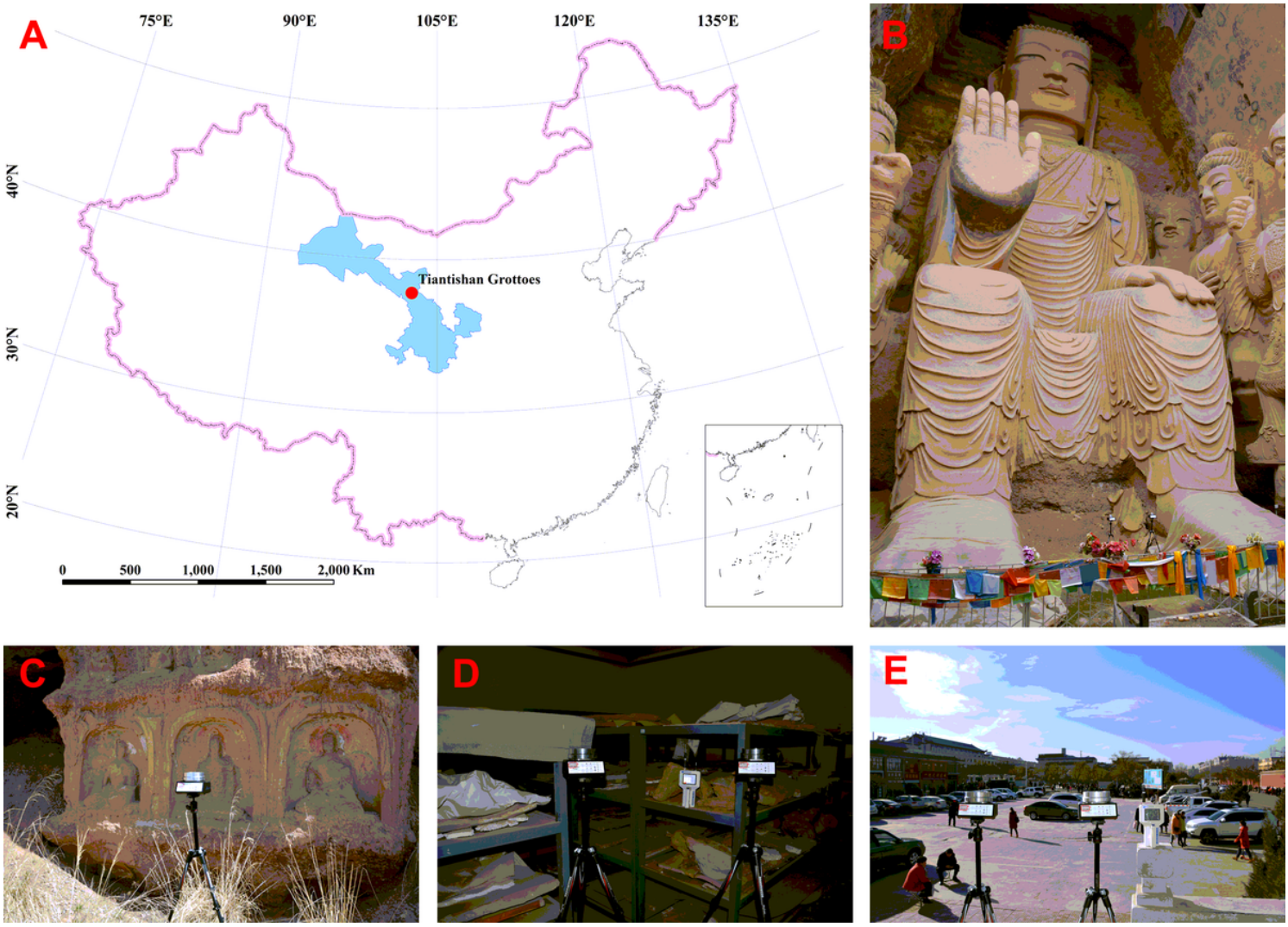

Figure 7

The four sampling sites of the airborne fungi at the Tiantishan Grottoes and the Western Xia Museum. A: location of the Tiantishan Grottoes in China; B, C: Cave 13 and cave 18 of the Tiantishan Grottoes, respectively; D, E: The inside and outside of the Western Xia Museum, respectively. Note: the map of Tiantishan Grottoes in China (A) was exported by the software ArcGIS Pro 2.6 Stabilization (https://developers.arcgis.com/). The images of the sampling sites were all provided and authorized by the Administrative office of the Tiantishan Grottoes for this article.