

读书报告

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通过对真菌ITS区域测序确定我国中部和尚洞洞穴真菌群落的多样性

IF=4.019

Introduction

- Mycobiomes at global-scale indicated a regional endemism patterns (Meiser et al., 2014) and strongly influenced by bio-geographical factors such as climates (Tedersoo et al., 2014) and geographical isolation (Talbot et al., 2014). Few NGS studies focused on systematic investigations of the diversity of mycobiomes in pristine karst caves (Vaughan et al., 2015)
- Fungi have long been recorded in caves 225 years ago (Dobat, 1967), however, they have received far less attention and limited our deeper understanding for the diversity and potential roles of fungi in subterranean ecosystems.
- Sequencing data of mycobiomes quickly increased during the past decade and the internal transcribed spacer (ITS) region of nuclear DNA was usually used as the phylogenetic marker for fungi (Schoch et al., 2012).

Introduction

Two goals:

In-depth explore the hidden mycobiomes of the air, weathered rocks, bat guanos, sediments, and drip waters ;

Reveal the diversity of mycobiomes and community differences in five habitats of Heshang Cave and address a new insight into fungal communities under nutrient-limited solution cave ecosystems.

Study Sites and Sample Collection

Material & Methods

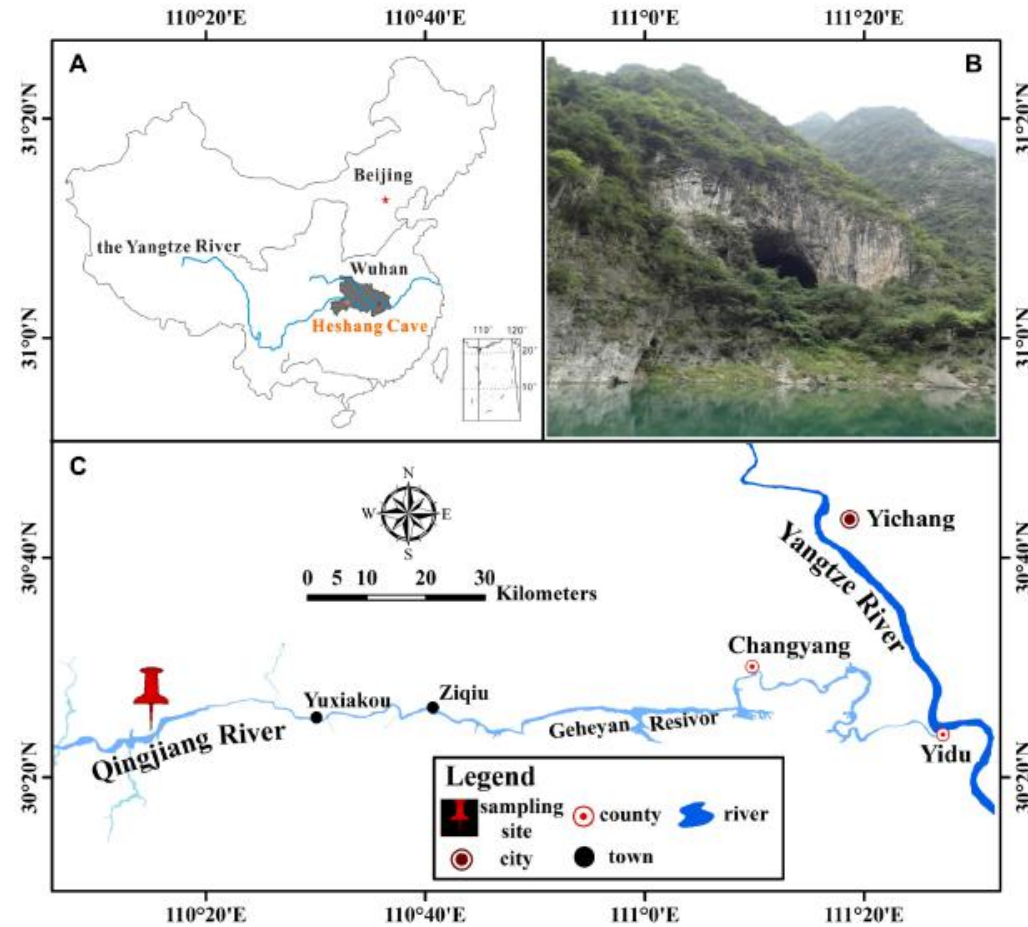
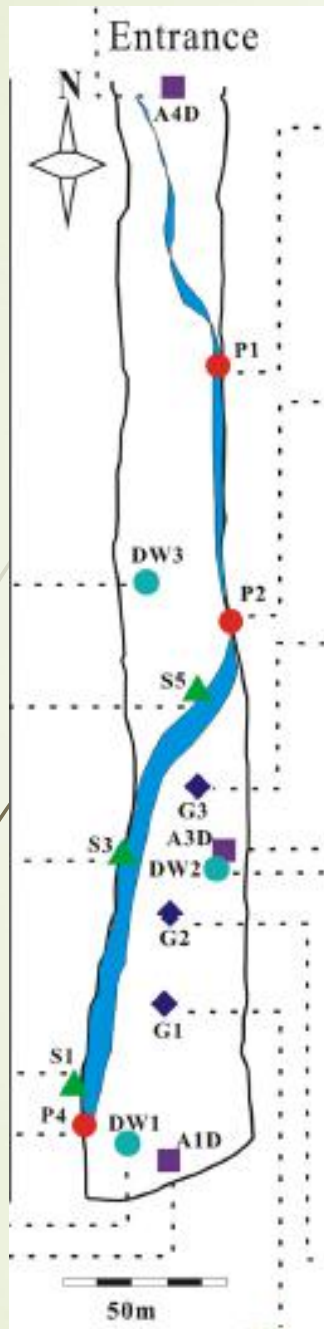


FIGURE 1 | Location of Heshang Cave, central China. **(A)** The dark gray area shows the location of Hubei province. **(B)** Outside view of Heshang Cave. **(C)** Location of Heshang Cave.

Heshang Cave, a pristine carbonate cave, locates in the south bank of Qingjiang Valley in the middle reaches of the Yangtze River, south-western karst region, China. 250m

Material & Methods



15 sampling sites, five habitats, including sediments, weathered rocks, bat guanos, drip waters, and air in Heshang Cave. Triplicate samples were collected for solid samples, only three samples of drip waters were collected due to lack the active dripping sites.

50 ml sterile plastic centrifuge tubes: **sediments** (S1/S3/S5)

bat guanos (G1/G2/G3) **weathered rocks** (twilight: P1; aphotic zone: P2/P4)

aerosol samples (A1D/A3D: aphotic zone; A4D: photic zone)

10-L sterile plastic bottles: **drip waters** (aphotic zone:DW1/DW2/; twilight zone: DW3)



Physicochemical Parameters

Material & Methods

pH

drip waters: in situ, multiparameter water quality detector (HACH, Loveland, CO, United States).

solid samples: UB-7 pH meter (Denver Instrument) in the laboratory

Temperature, humidity, the concentration of carbon dioxide

anions and cations: ICS-900 ion chromatograph (Thermo Fisher, USA)

weathered rocks and sediments: X-ray diffraction (XRD, Bruker AXS D8-Focus, Germany)

Genomic DNA Extraction

genomic DNA extraction: sediments, weathered rocks, bat guanos: the FastDNA SPIN Kit for Soil (MP Bio, Santa Ana, CA, United States)

air and drip waters: Power Water DNA Isolation Kit (Mobio Laboratory, Carlsbad, CA, United States).

Amplicon Preparation and Illumina HiSeq Sequencing

Illumina

Bioinformatics Analysis

Results Physicochemical Parameters in Heshang Cave

mean temperature : 18°C,

relative humidity and concentrations of CO₂ : increasing from entrance (96%,418 ppm) to the innermost part (100%, 494 ppm).

pH: 7.74 - 8.15, except the sample of bat guanos(G).

NO₃⁻: the highest is Bat guano, 11.90 - 38.22mM

Supplementary Table 2 Physicochemical parameters of samples in Heshang Cave.

Sample	pH	Ca ²⁺ (mM)	Mg ²⁺ (mM)	Cl ⁻ (mM)	NO ₃ ⁻ (mM)	SO ₄ ²⁻ (mM)
S1	8.08	1.28	2.52	1.05	4.81	0.18
S3	8.15	3.53	7.95	2.81	4.04	2.41
S5	8.05	1.17	0.73	0.33	2.01	0.37
P1	7.74	5.60	24.25	1.65	6.38	19.77
P2	7.84	2.34	2.65	1.79	4.60	2.04
P4	7.80	4.97	2.65	0.81	8.33	9.59
G1	6.60	10.65	12.37	1.81	11.90	3.02
G2	6.39	8.35	8.46	2.09	13.45	2.41
G3	6.63	11.90	13.08	4.11	38.22	4.18
DW1	7.86	0.89	1.50	0.08	0.14	0.33
DW2	7.96	0.87	1.52	0.06	0.22	0.28
DW3	7.76	0.68	1.85	0.05	0.20	0.33

Results

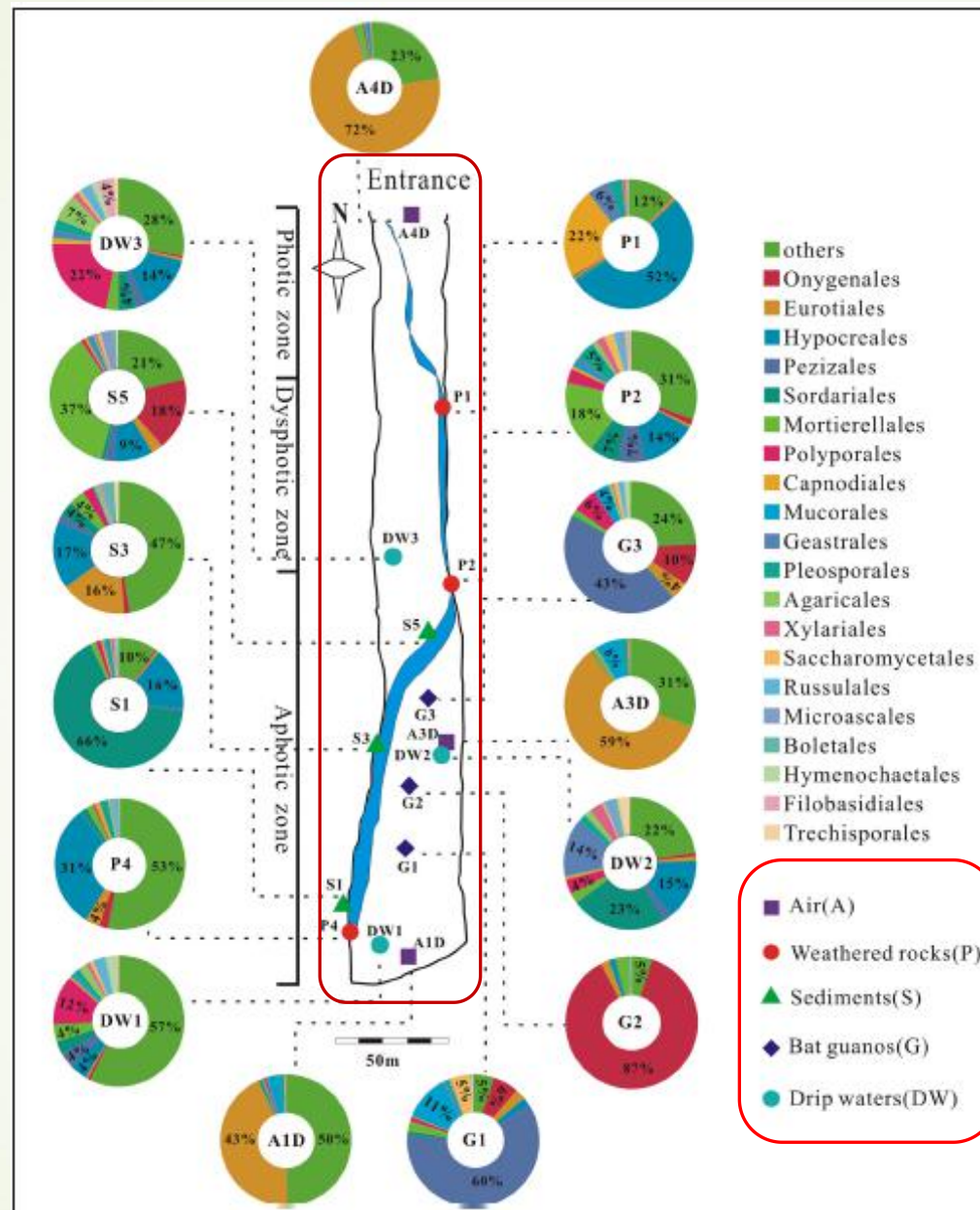


FIGURE 2 | Schematic map of sampling sites and the relative abundance ($\geq 4\%$) pie chart of the top 20 known fungal orders detected in 15 samples in Heshang Cave. Others in each pie chart indicate the sum of the relative abundance of the rest orders.

Results Taxonomy and Community Composition

In total: 2,179 OTUs,
mean OTU numbers (in 5 Sample sites): 249 to 1067 with 5% cut off.

Fungi

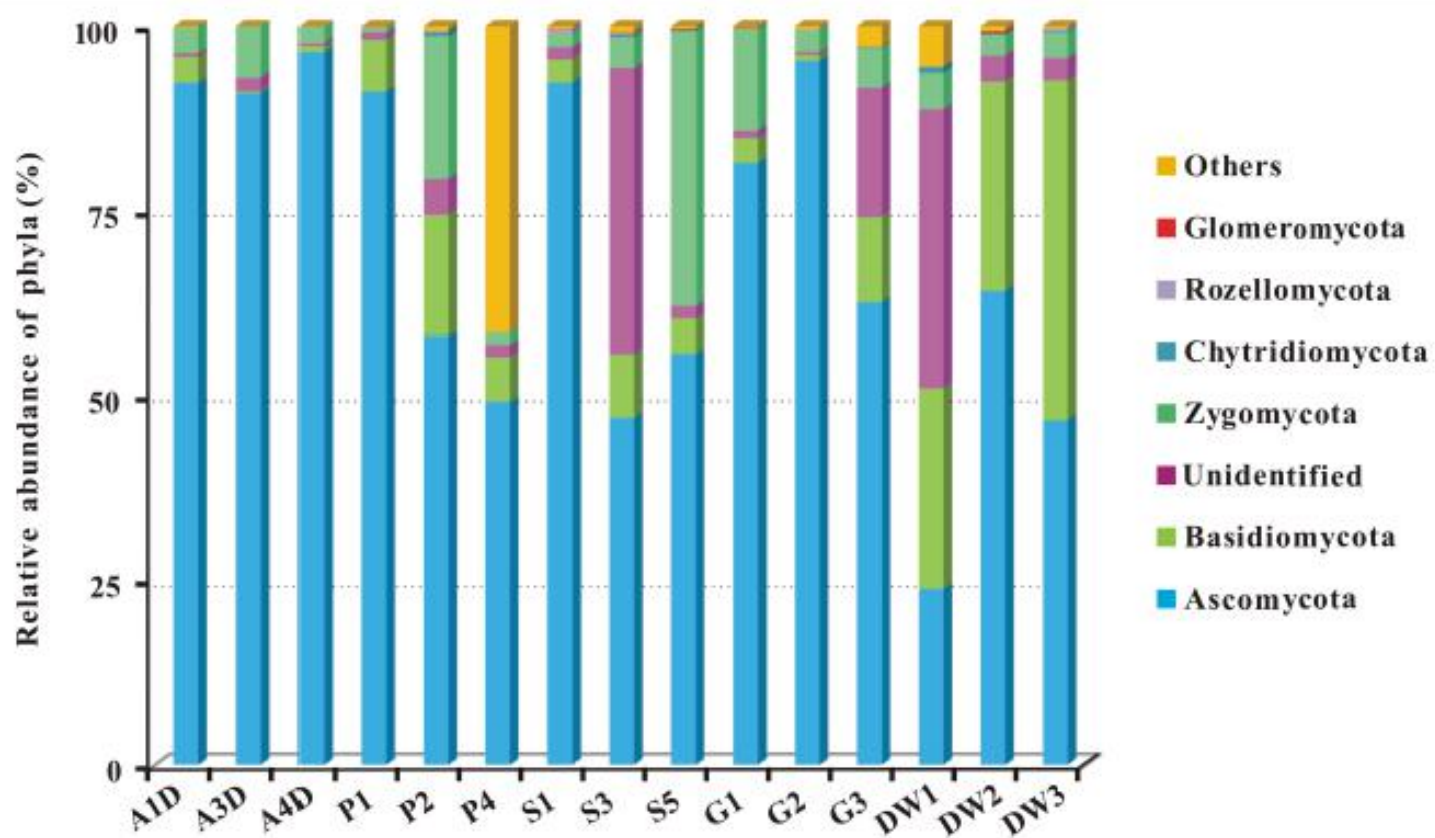


FIGURE 3 The relative abundance of mycobiomes at the phylum level in Heshang Cave.

0.06% (498 reads, 11 OTUs)
0.09% (706 reads, 15 OTUs)
0.14% (1,081 reads, 32 OTUs)
8.02% (63,647 reads, 54 OTUs)
10.66% (84,614 reads, 653 OTUs)
72.25% (573,274 reads, 916 OTUs)

Core Genera of Mycobiomes

Results

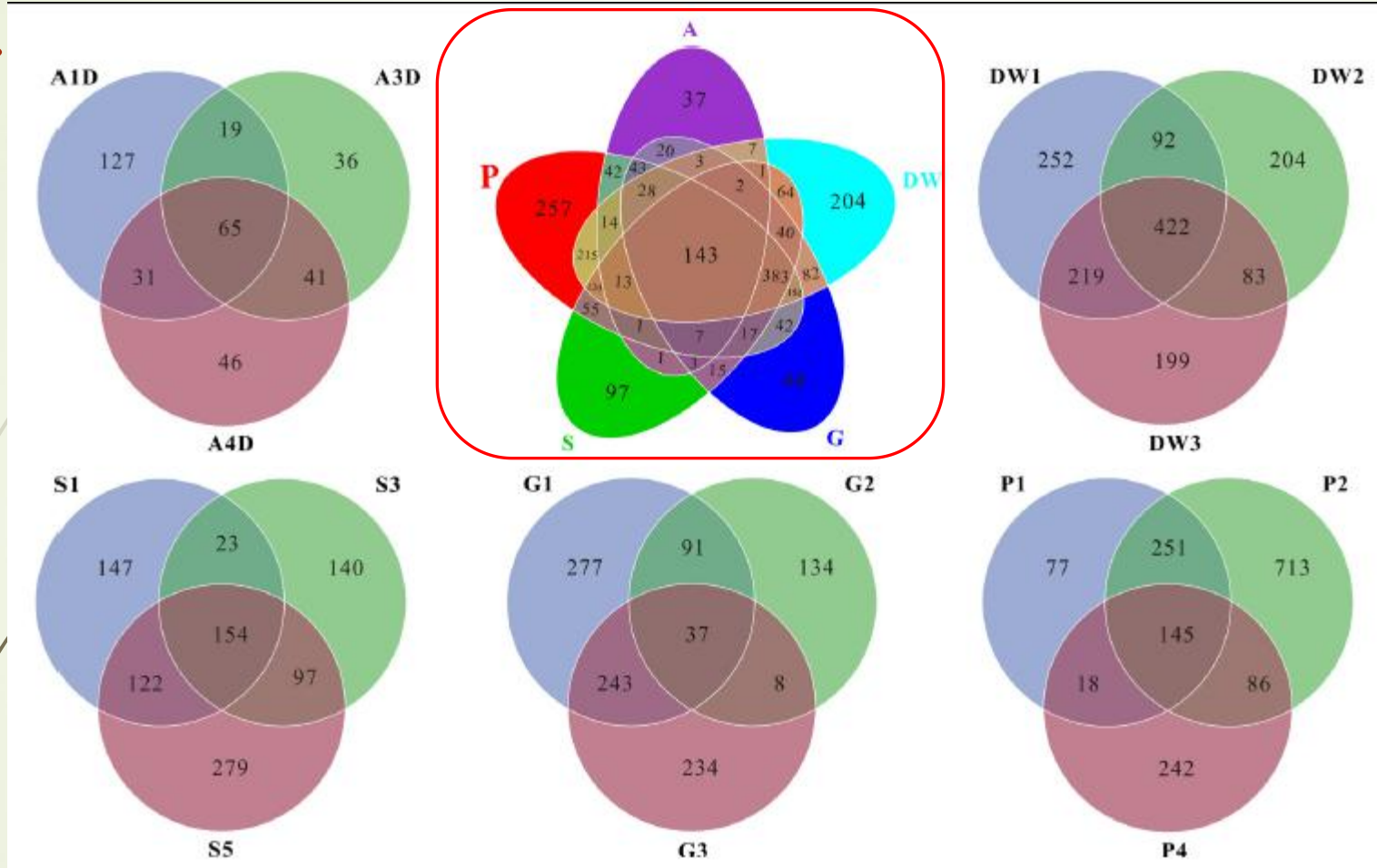


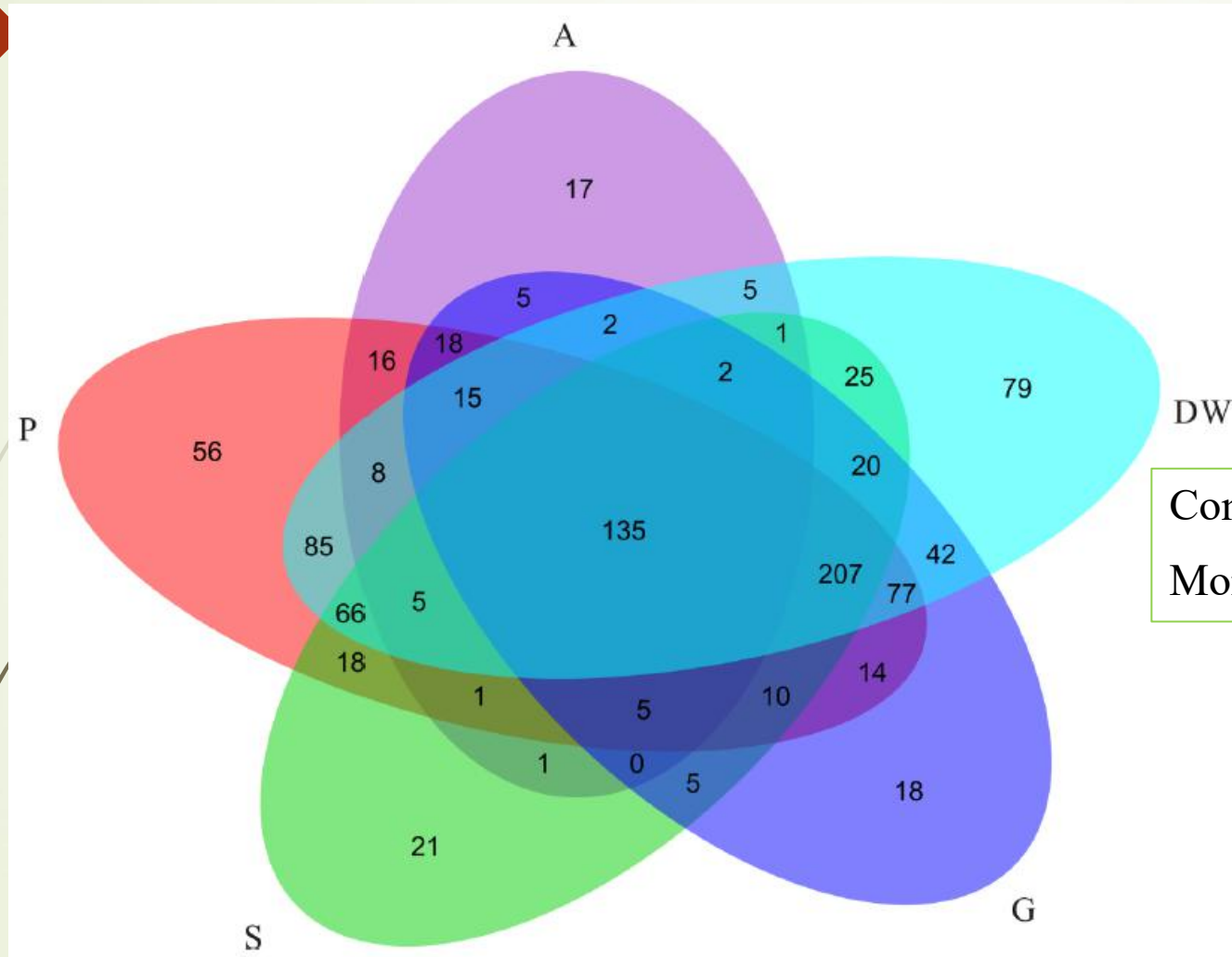
FIGURE 4 | Venn diagram displayed the number of common and distinct OTUs for mycobiomes of five habitats in Heshang Cave.

Common: 6.60% core OTUs
weathered rocks (P) : 11.90%
drip waters (DW) : 9.36%

Top 10 genera

Ascomyceta: *Penicillium*,
Chaetomium, *Neogymnomycetes*
Aspergillus, *Mallochia*
Gymnoascus
Zygomycota: *Mortierella*,
Mucor, *Circinella*
Basidiomycota.

Results



Common(genus level):135 OTUs, 6.2%
More unique : P & DW samples,56 & 79 OTUs

Supplementary Figure 1. Venn diagram displayed the number of OTUs at the genus level for mycobiomes of five habitats in Heshang Cave.

Biodiversity Assessment of Mycobiomes

Results

Sample	Shannon		Simpson		Chao1		ACE		Good's coverage (%)	
	3%	5%	3%	5%	3%	5%	3%	5%	3%	5%
A1D	3.06	2.94	0.75	0.74	337.62	329.57	352.91	341.10	99.70	99.70
A3D	3.11	2.76	0.81	0.75	235.56	207.58	237.42	232.51	99.80	99.80
A4D	3.32	3.11	0.84	0.81	307.53	257.29	310.24	261.24	99.80	99.70
Mean A	3.16	2.94	0.80	0.88	293.57	264.81	300.19	278.28	99.77	99.73
P1	4.65	4.56	0.90	0.90	718.54	648.12	704.41	666.89	99.50	99.40
P2	7.78	7.62	0.99	0.99	1464.27	1299.84	1535.05	1349.12	99.20	99.10
P4	4.97	4.81	0.88	0.86	620.16	540.76	626.54	521.29	99.80	99.70
Mean P	5.80	5.66	0.92	0.92	934.32	829.57	955.33	845.77	99.50	99.40
S1	2.81	2.78	0.56	0.56	556.29	557.69	576.88	566.67	99.60	99.60
S3	5.24	5.22	0.94	0.94	488.92	464.28	492.41	479.23	99.70	99.70
S5	5.42	5.33	0.94	0.94	924.03	911.92	937.48	879.13	99.30	99.20
Mean S	4.49	4.44	0.81	0.81	656.41	644.63	668.92	641.68	99.53	99.50
G1	3.47	3.47	0.67	0.68	1006.99	877.36	1033.58	924.95	99.20	99.10
G2	1.33	1.25	0.25	0.24	388.33	355.21	389.95	368.15	99.70	99.70
G3	4.17	4.11	0.81	0.81	716.11	687.07	722.44	696.38	99.40	99.40
Mean G	2.99	2.94	0.58	0.58	703.81	639.88	715.32	663.16	99.43	99.40
DW1	6.37	6.20	0.93	0.93	1358.93	1146.15	1354.10	1189.98	99.20	99.00
DW2	6.41	6.32	0.95	0.95	1049.60	998.06	1066.23	981.10	99.40	99.30
DW3	7.38	7.27	0.98	0.98	1265.34	1075.88	1250.65	1080.88	99.40	99.20
Mean D	6.72	6.60	0.95	0.95	1224.62	1073.36	1223.66	1083.99	99.33	99.17
Total Mean	4.63	4.52	0.81	0.83	762.55	690.45	772.69	702.58	99.51	99.44

Community diversity was significantly different between drip waters and bat guanos (Shannon and Simpson indices, Wilcoxon's test, $p < 0.01$), while difference between weathered rocks and bat guanos was also observed (Shannon and Simpson indices, Wilcoxon's test, $p < 0.05$).

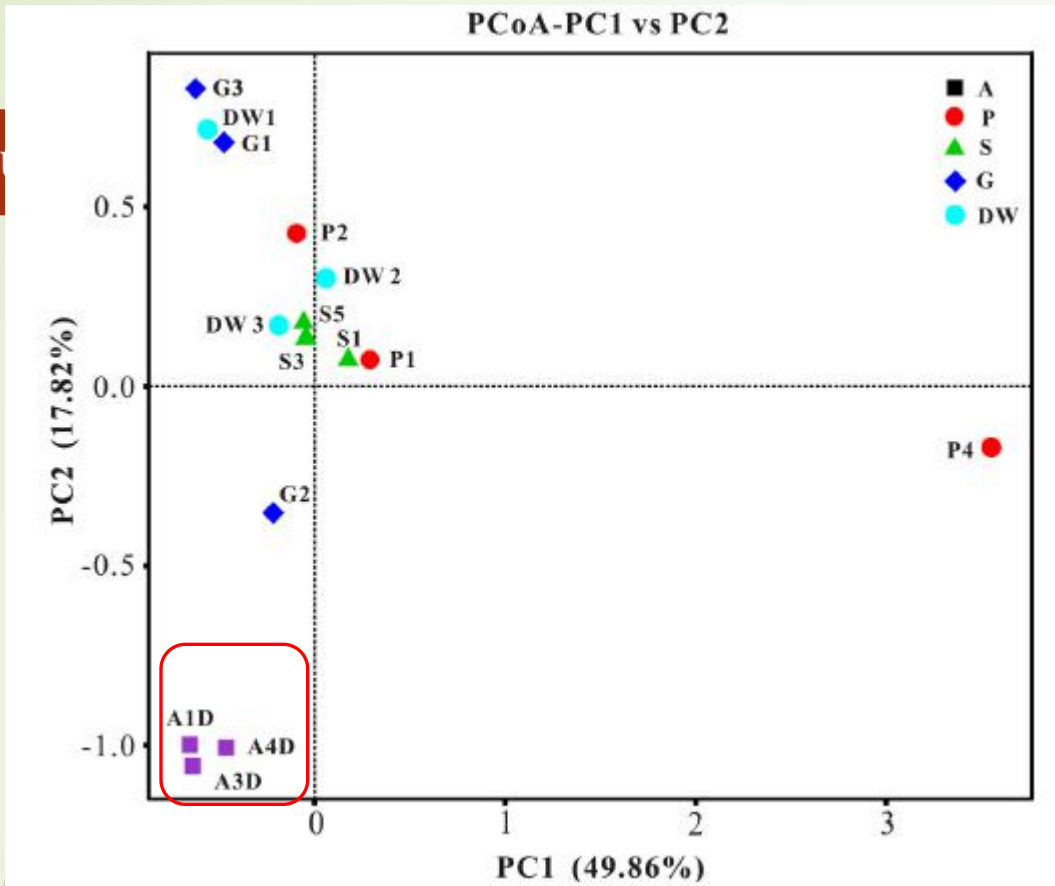


FIGURE 5 | Results of principal coordinate analysis (PCoA) based on weighted-UniFrac distance matrix of mycobiomes in Heshang Cave.

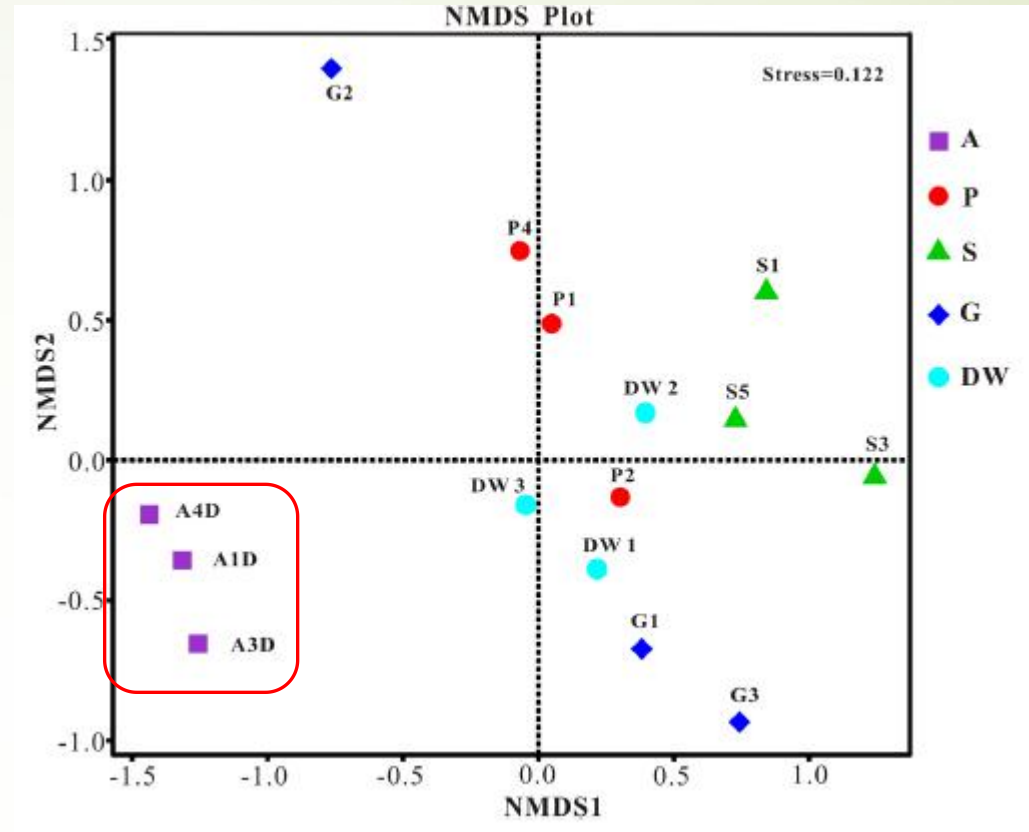


FIGURE 6 | Nonmetric Multidimensional Scaling (NMDS) analysis based on the OTU level of mycobiomes in Heshang Cave.

Mycobiomes in air samples were distinctive from those of other samples. Overlaps of mycobiomes were observed between the other four habitats, but mycobiomes can be better separated by multivariate community analysis with NMDS. However, the observation of a few exceptional samples via PCoA (G2 and P4) and NMDS (G2 and P2) analysis suggested the heterogeneity of mycobiomes in a specific habitat.

Result

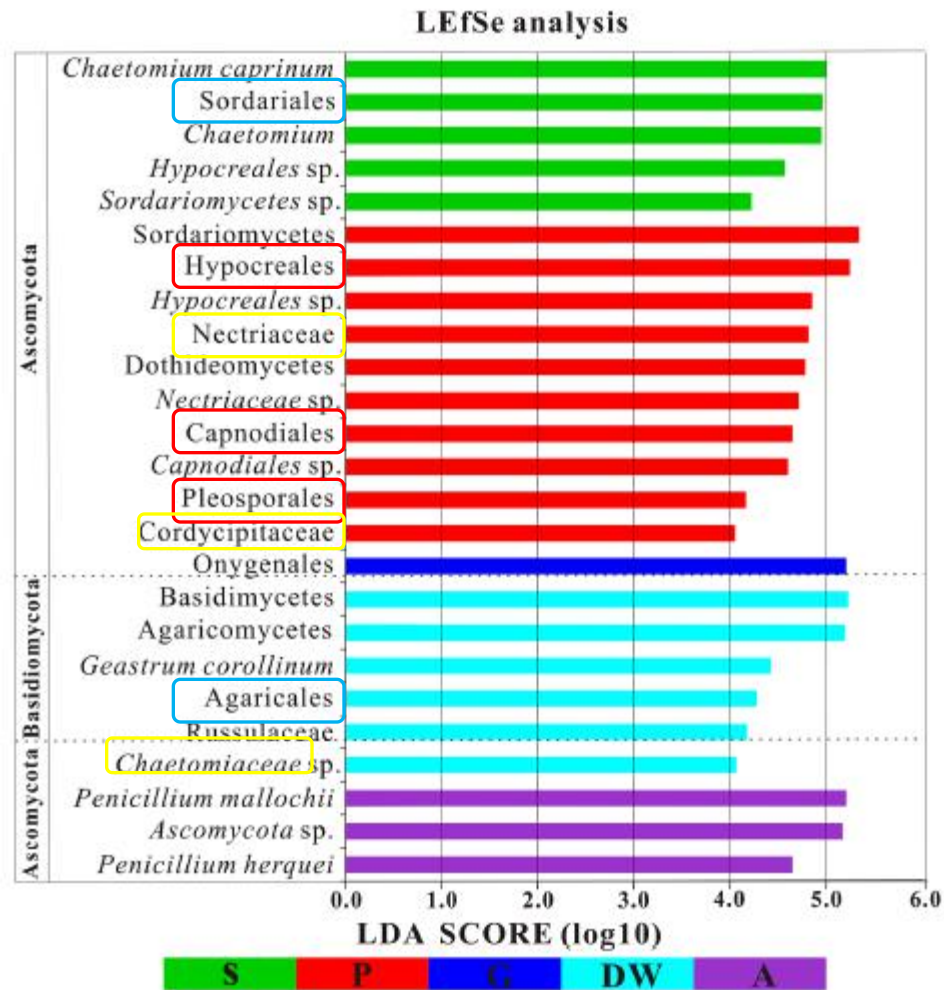


FIGURE 7 | Results of Linear discriminant analysis Effect Size (LEfSe) analysis of mycobiomes in Heshang Cave (LDA Score > 4). Abbreviations are the same as those in **Figure 2**.

In total, 25 indicator groups were identified from the five habitats, 7 indicator groups have high LDA scores ($LDA > 5$, $p < 0.05$) illustrating their specificity and high abundance in a given habitat.

Order level:

P: Capnodiales, Pleosporales, Hypocreales

DW & S: Sordariales, Agaricales

Family level:

DW & P: Nectriaceae, Cordycipitaceae

Chaetomiaceae

Discussion

- ◆ Ascomycota (72.25% reads, 42% OTUs) was the most abundant phylum across the five habitats. This result is consistent with previous reports (Vanderwolf et al., 2013), which showed a predominance of Ascomycota (69%) in caves and mines worldwide.
- ◆ Despite the prevailing harsh and hostile environments, rock surfaces still serve as reservoirs for specialized fungi. The class Sordariomycetes (relative abundance of 43%) and the order Hypocreales (relative abundance 34.62%) dominated in weathered rocks. 35% of the 20 most common families reported from cave environments (Vanderwolf et al., 2013) were members of Sordariomycetes.
- ◆ In this study, the beta diversity based on weighted Unifrac analysis showed that air mycobiomes significantly differed from those of four other habitats and clustered as a single group as indicated by PCoA (Figure 5) and NMDS (Figure 6) analysis.

Conclusion

- ◆ High fungal diversity in Heshang Cave, 453 genera, 72 orders and 19 classes in 6 phyla.
- ◆ Community richness and diversity were highest in drip waters and lowest in air samples.
- ◆ The authors first identified Sordariomycetes (43%) in weathered rocks to be dominant for rock in-habiting fungi.
- ◆ The results will facilitate a better understanding of the highly diverse mycobiomes in Heshang Cave, enabling better



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