Supplementary Materials: Variation in the microbiome, trichothecenes, and aflatoxins in stored wheat grains in Wuhan, China

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Figure S1. PCR amplicons of ITS2 and V3V4 in wheat samples during storage. I, II, and III represent three repeats (silos) of the experiments, 0, 3, 6, 9, and 12 represent the storage times in months in silos.



Figure S2. Distribution of bacteria at the phylum level in wheat stored for 0–12 months at different silos positions. Top, middle, and bottom indicate silo positions. 0, 3, 6, 9, and 12 represent the storage times in months in silos. The top panel represents the variation of the number of classified phyla, the values represent the means of three replicates with the standard deviation (SD); the bottom panel represents the distribution of bacteria at the phylum level.



Figure S3. Distribution of bacteria at the genus level in wheat stored for 0–12 months at different silos positions. Top, middle, and bottom indicate silo positions. 0, 3, 6, 9, and 12 represent the storage times in months in silos. The top panel represents the variation of the number of classified genera, the values represent the means of three replicates with the standard deviation (SD); the bottom panel represents the distribution of bacteria at the genus level.



Figure S4. Distribution of bacteria at the species level in wheat stored for 0–12 months at different silo positions. Top, middle, and bottom indicate silo positions. 0, 3, 6, 9, and 12 represent the storage times in months in silos. The top panel represents the variation of the number of classified species, the values represent the means of three replicates with the standard deviation (SD); the bottom panel represents the distribution of bacteria at the species level.

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Figure S5. Correlation of *Fusarium* sp. abundance with other species. Static data of relative abundance of each fungus species at the top, middle, and bottom silo positions, and at different storage time points, and analysis of their relationships. The circular diagrams and flat circles represent lower and higher correlations, respectively; the sample spot distribution represents the significance.

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Figure S6. Correlations of *Aspergillus* sp. abundance with other species. Static data of relative abundance of each fungus species at the top, middle, and bottom silo positions, and at different storage time points, and analysis of their relationships. The circular diagrams and flat circles represent lower and higher correlations, respectively; the sample spot distribution represents the significance.



Figure S7. Temperature and relative humidity in Wuhan Storeage areas.

Sample	Reads Length	Clean Boads	Read Utilization	Tags Without	Tag Utilization	Average Length	OTU Number				
IName	(bp)	Reaus	Ratio (%)	Primer	Ratio (%)	And SD	Number				
OI	293:294	24243*2	95.01	21863	90.18	366/24	200				
OII	299:294	25397*2	90.77	22709	89.42	365/21	202				
OIII	298:294	30735*2	87.7	28470	92.63	308/21	186				
3BI	297:294	30091*2	95.31	25573	85.02	372/21	221				
3BII	296:294	30252*2	96.38	27330	90.31	366/12	151				
3BIII	294:294	29859*2	91.4	26982	90.3	368/26	186				
3MI	297:294	30091*2	95.31	25583	85.02	371/21	241				
3MII	296:294	30252*2	96.38	27320	90.31	367/12	197				
3MIII	294:294	29859*2	91.4	26962	90.3	368/26	143				
3TI	297:294	30091*2	95.31	25583	85.02	371/21	152				
3TII	296:294	30252*2	96.38	27220	90.31	367/12	121				
3 TIII	294:294	29859*2	91.4	26162	90.3	368/26	183				
6BI	300:294	30784*2	81.13	28199	91.6	336/33	266				
6BII	298:294	30960*2	84.4	27930	90.21	328/31	289				
6BIII	300:299	23851*2	96.72	21087	88.41	370/15	196				
6MI	300:294	30114*2	95.84	26903	89.34	367/13	283				
6MII	299:294	30565*2	88.75	27469	89.87	330/34	279				
6MIII	297:294	31142*2	84.96	27660	88.82	323/31	370				
6TI	293:294	29816*2	97.29	26599	89.21	367/12	104				
6TII	296:299	31842*2	77.95	28864	90.65	347/28	129				
6TIII	297:293	30297*2	94.75	27166	89.67	366/12	71				
9BI	294:294	31271*2	78.48	28252	90.35	327/29	334				
9BII	299:300	29359*2	92.2	26744	91.09	369/23	195				
9BIII	296:300	29580*2	95.8	26099	88.23	364/20	404				
9MI	296:294	30588*2	81.69	27410	89.61	335/32	437				
9MII	295:298	32024*2	60.79	29151	91.03	333/24	98				
9MIII	293:294	31424*2	71.4	28064	89.31	333/31	466				
9TI	294:293	23641*2	91.25	21382	90.44	359/28	69				
9TII	293:300	29975*2	97.94	27295	91.06	370/8	20				
9TIII	298:300	29380*2	95.2	27062	92.11	365/14	48				
12BI	300:294	30244*2	44.32	27138	89.73	340/31	235				
12BII	298:299	25496*2	96.39	22604	88.66	367/15	184				
12BIII	294:299	29935*2	96.81	26925	89.94	365/17	349				
12MI	293:300	27571*2	95.1	24367	88.38	367/20	392				
12MII	299:299	29435*2	96.93	26695	90.69	369/13	196				
12MIII	296:299	26063*2	95.89	20882	80.12	367/18	249				
12TI	294:300	30779*2	91.34	28080	91.23	330/15	55				
12TII	293:299	30119*2	98.15	27360	90.84	368/8	31				
12TIII	297:299	29697*2	83.88	26604	89.58	338/22	57				
Average	293:300	29409*2	89.44	26324	89.47	355/21	205				

Table S1. Data Statistics of ITS sequence

T, M, and B represent the top, middle, and bottom silo positions, respectively; I, II, and III represent the repeat silos of the experiments. Read lengths are the range in each sample, the number of pairs of clean reads following removal of low quality reads. Tags are assembled from clean read pairs, and the tags were clustered to Operational Taxonomic Unit (OTU) by scripts in the program USEARCH (v7.0.1090) at a 97% threshold value.

Sample Name	Reads Length (bp)	Clean Reads	Read Utilization Ratio (%)	Tags Without Primer	Tag Utilization Ratio (%)	Average Length And SD	OTU Number				
01	297:300	30205*2	92.57	29874	98.9	428/3	89				
OII	296:300	30601*2	92.17	30271	98.92	428/2	85				
OIII	295:300	30629*2	92.29	30303	98.94	427/5	94				
3BI	294:300	30634*2	92.89	30477	98.83	427/3	107				
3BII	293:300	31044*2	91.27	30641	98.7	428/3	57				
3BIII	300:300	30165*2	91.59	29654	98.97	426/2	66				
3MI	294:300	30634*2	92.89	30277	98.83	428/3	104				
3MII	293:300	31044*2	91.27	30641	98.7	428/3	122				
3MIII	300:300	30165*2	91.59	29854	98.97	428/2	76				
3TI	294:300	30634*2	92.89	30077	98.83	428/3	77				
3TII	293:300	31044*2	91.27	30841	98.7	428/3	142				
3TIII	300:300	30165*2	91.59	29854	98.97	428/2	109				
6BI	297:299	30096*2	93.04	29758	98.88	428/4	118				
6BII	298:300	30163*2	91.51	29795	98.78	427/5	207				
6BIII	299:300	30050*2	92.08	29730	98.94	428/4	66				
6MI	295:299	30864*2	92.74	30494	98.8	427/5	162				
6MII	296:299	30552*2	93.12	30183	98.79	427/5	154				
6MIII	299:297	30733*2	93.5	30388	98.88	426/6	195				
6TI	298:297	30581*2	93.11	30267	98.97	426/7	167				
6TII	293:299	30495*2	92.42	30034	98.49	427/4	163				
6TIII	294:299	30564*2	93.94	30201	98.81	428/3	86				
9BI	298:299	30806*2	92.33	30475	98.93	428/4	153				
9BII	299:299	30080*2	93.38	29717	98.79	428/4	180				
9BIII	300:299	29904*2	93.39	29504	98.66	428/3	62				
9MI	296:298	30864*2	92.2	30399	98.49	428/4	170				
9MII	297:296	30925*2	91.53	30571	98.86	427/6	238				
9MIII	297:298	30645*2	92.5	30304	98.89	428/1	49				
9TI	293:298	30474*2	91.42	29989	98.41	426/6	239				
9TII	294:298	30355*2	93.01	29936	98.62	428/4	200				
9TIII	295:298	30509*2	92.7	30155	98.84	429/1	60				
12BI	298:298	30338*2	91.67	29991	98.86	428/2	87				
12BII	299:298	30701*2	91.74	30298	98.69	428/2	130				
12BIII	300:298	30694*2	92.36	30182	98.33	427/5	59				
12MI	295:297	30577*2	92.36	30267	98.99	428/1	60				
12MII	296:297	30372*2	93.33	29990	98.74	429/1	64				
12MIII	297:297	30206*2	93.59	29890	98.95	428/2	72				
12TI	300:297	30570*2	92.34	30262	98.99	428/3	141				
12TII	293:297	30843*2	92.02	30418	98.62	428/2	102				
12TIII	294:297	30923*2	92.27	30620	99.02	428/1	38				
Average	293:300	30535*2	92.41	30169	98.80	427/3	117				

Table S2. Data Statistics of V3V4 sequence

T, M, and B represent the top, middle, and bottom silo positions, respectively; I, II, and III represent the repeat silos of the experiments. Read lengths are the range in each sample, the number of pairs of clean reads following removal of low quality reads. Tags are assembled from clean read pairs, and the tags were clustered to Operational Taxonomic Unit (OTU) by scripts in the program USEARCH (v7.0.1090) at a 97% threshold value

		F. graminearium	F. tricinctum	A. alternata	Xylariales sp.	B. albus	H. sinensis	D. zsoltiisis	B. cereus	B. flexus	P. fragi	P. veronii	P. viridiflava
Гii	Pearson r	1	0.84712	0.93888	0.91331	0.87259	0.8598	0.94651	-0.18241	-0.06082	-0.17302	0.14458	0.77929
F. graminearium	Sig.		2.21E-13	0	0	5.77E-15	3.93E-14	0	0.2304	0.69147	0.25571	0.34335	2.87E-10
T tricinature	Pearson r	0.84712	1	0.86276	0.88885	0.8053	0.7758	0.88585	-0.20459	-0.08198	-0.10351	0.28518	0.86444
F. tricinctum	Sig.	2.21E-13		2.55E-14	4.44E-16	2.56E-11	3.87E-10	6.66E-16	0.17762	0.59241	0.49862	0.05759	2.00E-14
A altomata	Pearson r	0.93888	0.86276	1	0.97012	0.9 2 041	0.90677	0.97879	-0.16199	-0.04334	-0.15631	0.17176	0.77819
A. utternutu	Sig.	0	2.55E-14		0	0	0	0	0.28773	0.7774	0.30517	0.25923	3.15E-10
Vulanialas on	Pearson r	0.91331	0.88885	0.97012	1	0.94827	0.90889	0.95853	-0.21306	-0.07348	-0.1484	0.23195	0.76075
Ayiuriules sp.	Sig.	0	4.44E-16	0		0	0	0	0.15995	0.63144	0.3306	0.12523	1.33E-09
B allows	Pearson r	0.87259	0.8053	0.92041	0.94827	1	0.98169	0.92592	-0.19056	-0.08372	-0.12896	0.31951	0.68972
D. utous	Sig.	5.77E-15	2.56E-11	0	0		0	0	0.20989	0.58454	0.39853	0.0324	1.60E-07
H sinonsis	Pearson r	0.8598	0.7758	0.90677	0.90889	0.98169	1	0.91179	-0.16291	-0.06251	-0.09659	0.31975	0.69216
11. Stitensis	Sig.	3.93E-14	3.87E-10	0	0	0		0	0.28495	0.68332	0.52792	0.03226	1.39E-07
D zaaltiisia	Pearson r	0.94651	0.88585	0.97879	0.95853	0.92592	0.91179	1	-0.17268	-0.05843	-0.13115	0.18877	0.82541
D. 2501111515	Sig.	0	6.66E-16	0	0	0	0		0.25667	0.70303	0.3905	0.21427	3.05E-12
R carous	Pearson r	-0.18241	-0.20459	-0.16199	-0.21306	-0.19056	-0.16291	-0.17268	1	0.20693	0.10402	-0.36187	-0.28489
D. cereus	Sig.	0.2304	0.17762	0.28773	0.15995	0.20989	0.28495	0.25667		0.1726	0.49652	0.01458	0.05785
R florus	Pearson r	-0.06082	-0.08198	-0.04334	-0.07348	-0.08372	-0.06251	-0.05843	0.20693	1	0.43683	-0.24038	-0.13559
D. flexus	Sig.	0.69147	0.59241	0.7774	0.63144	0.58454	0.68332	0.70303	0.1726		0.0027	0.1117	0.37451
D fuari	Pearson r	-0.17302	-0.10351	-0.15631	-0.1484	-0.12896	-0.09659	-0.13115	0.10402	0.43683	1	0.0962	-0.11291
F. Jrugi	Sig.	0.25571	0.49862	0.30517	0.3306	0.39853	0.52792	0.3905	0.49652	0.0027		0.52961	0.46022
D maranii	Pearson r	0.14458	0.28518	0.17176	0.23195	0.31951	0.31975	0.18877	-0.36187	-0.24038	0.0962	1	0.31185
1.0010111	Sig.	0.34335	0.05759	0.25923	0.12523	0.0324	0.03226	0.21427	0.01458	0.1117	0.52961		0.03703
P minidiflana	Pearson r	0.77929	0.86444	0.77819	0.76075	0.68972	0.69216	0.82541	-0.28489	-0.13559	-0.11291	0.31185	1
1.011111111100	Sig.	2.87E-10	2.00E-14	3.15E-10	1.33E-09	1.60E-07	1.39E-07	3.05E-12	0.05785	0.37451	0.46022	0.03703	

Table S3. The relationships of *Fusarium* sp. with other species

Static data of relative abundance of each fungal species at the top, middle, and bottom silo positions and different storage time points; analysis of their relationships; Pearson's r represents the correlation coefficient, Sig. indicates the significance level, yellow background indicates a significant correlation at *p* < 0.05 level.

		A. cibarius	A. flavus	A. penicillioides	R. taiwanensis	B. terrestris	G. pullulans	V. virens	B. cereus	B. flexus	P. fragi	P. veronii	P. viridiflava
A '7 '	Pearson r	1	0.47478	0.12905	-0.39622	-0.07679	0.21689	0.27066	-0.00141	-0.05298	-0.04451	0.04837	-0.10346
A. cibarius	Sig.		9.83E-04	0.3982	0.00705	0.61609	0.15241	0.07213	0.99266	0.72959	0.77157	0.75237	0.49885
A. A.	Pearson r	0.47478	1	0.13261	-0.27937	-0.05236	0.54666	0.52766	0.14974	-0.0025	-0.10076	-0.23991	-0.2362
A. fluous	Sig.	9.83E-04		0.38519	0.0631	0.73268	1.02E-04	1.96E-04	0.3262	0.98699	0.51017	0.11243	0.11826
1 nonicillicidos	Pearson r	0.12905	0.13261	1	-0.29848	0.15725	-0.03953	0.1367	0.02583	-0.05143	-0.18416	-0.2124	-0.22509
A. penicilioides	Sig.	0.3982	0.38519		0.04641	0.30224	0.79656	0.37056	0.86624	0.73725	0.22589	0.16128	0.13711
D taimananaia	Pearson r	-0.39622	-0.27937	-0.29848	1	-0.12669	-0.10177	-0.03587	-0.2681	-0.17781	-0.06562	0.37102	0.10461
K. tutwanensis	Sig.	0.00705	0.0631	0.04641		0.40693	0.5059	0.81503	0.07498	0.24259	0.66845	0.0121	0.49407
D. townsetsis	Pearson r	-0.07679	-0.05236	0.15725	-0.12669	1	-0.08084	-0.09191	0.12367	-0.04473	-0.05954	-0.16432	-0.14525
D. terrestris	Sig.	0.61609	0.73268	0.30224	0.40693		0.59759	0.54819	0.41831	0.77048	0.69766	0.28075	0.34109
C multulana	Pearson r	0.21689	0.54666	-0.03953	-0.10177	-0.08084	1	0.24724	0.0087	-0.0163	-0.01069	-0.16006	-0.15465
G. puttutuns	Sig.	0.15241	1.02E-04	0.79656	0.5059	0.59759		0.10154	0.95479	0.91535	0.94444	0.29357	0.31042
V minous	Pearson r	0.27066	0.52766	0.1367	-0.03587	-0.09191	0.24724	1	-0.12041	-0.02466	-0.11765	0.05352	-0.03526
v. otrens	Sig.	0.07213	1.96E-04	0.37056	0.81503	0.54819	0.10154		0.43075	0.87226	0.44149	0.72696	0.81811
P. compute	Pearson r	-0.00141	0.14974	0.02583	-0.2681	0.12367	0.0087	-0.12041	1	0.20693	0.10402	-0.36187	-0.28489
D. cereus	Sig.	0.99266	0.3262	0.86624	0.07498	0.41831	0.95479	0.43075		0.1726	0.49652	0.01458	0.05785
B florus	Pearson r	-0.05298	-0.0025	-0.05143	-0.17781	-0.04473	-0.0163	-0.02466	0.20693	1	0.43683	-0.24038	-0.13559
D. flexus	Sig.	0.72959	0.98699	0.73725	0.24259	0.77048	0.91535	0.87226	0.1726		0.0027	0.1117	0.37451
D fuqai	Pearson r	-0.04451	-0.10076	-0.18416	-0.06562	-0.05954	-0.01069	-0.11765	0.10402	0.43683	1	0.0962	-0.11291
r. jrugi	Sig.	0.77157	0.51017	0.22589	0.66845	0.69766	0.94444	0.44149	0.49652	0.0027		0.52961	0.46022
D maronii	Pearson r	0.04837	-0.23991	-0.2124	0.37102	-0.16432	-0.16006	0.05352	-0.36187	-0.24038	0.0962	1	0.31185
1.0010111	Sig.	0.75237	0.11243	0.16128	0.0121	0.28075	0.29357	0.72696	0.01458	0.1117	0.52961		0.03703
D minidiflare	Pearson r	-0.10346	-0.2362	-0.22509	0.10461	-0.14525	-0.15465	-0.03526	-0.28489	-0.13559	-0.11291	0.31185	1
1.011111111100	Sig.	0.49885	0.11826	0.13711	0.49407	0.34109	0.31042	0.81811	0.05785	0.37451	0.46022	0.03703	

Table S4. The relationships of Aspergillus sp. with other species

Static data of relative abundance of each fungal species at the top, middle, and bottom silo positions and different storage time points; analysis of their relationships; Pearson's r represents the correlation coefficient, Sig. indicates the significance level, yellow background indicates a significant correlation at p < 0.05 level.