Supplementary information for Sporocarp-associated fungal co-occurrence networks in a corn field revealed by long-read high-throughput sequencing

Running title: Sporocarp-associated fungal networks

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Table S1 The basic soil properties of the study site in this study.

	Mean ± SD	n
pH	5.61 ± 0.33	5
CEC (cmol/kg)	21.18 ± 1.41	5
Total carbon (g/kg)	11.65 ± 0.91	5
Total nitrogen (g/kg)	1.21 ± 0.08	5
Total phosphorus (g/kg)	0.531 ± 0.093	5
Total potassium (g/kg)	23.495 ± 0.322	5
SOC (g/kg)	10.99 ± 0.86	5
DOC (mg/kg)	30.72 ± 3.06	5
DON (mg/kg)	7.86 ± 2.67	5
Available phosphorus (mg/kg)	76.53 ± 87.85	5
Available potassium (mg/kg)	255.80 ± 67.86	5

Soil properties were measured in five independent plots (5 cores, 100m^2) within the area of 100m×100m.

Table S2 BLAST-based taxonomic affinity of the 10 most abundant OTUs detected in this study.

OTU ID	Scientific Name	Total Score	Coverage (%)	E value	Identity (%)	Accession number
OTU1	*Hebeloma* sp. dd08005	1336	88	0	99.60	FJ810132
	Agrocybe dura	1295	86	0	99.05	OP470472
	Agrocybe dura	1293	87	0	98.53	ON561563
	Agrocybe dura	1292	88	0	98.14	ON561565
	Agrocybe dura	1289	87	0	98.53	MT535732
	Agrocybe dura	1280	87	0	97.98	ON561493
	Agrocybe dura	1279	87	0	98.38	MT535724
	Agrocybe molesta	1277	86	0	98.51	OP643062
	Hebeloma sp.	1270	84	0	99.17	MZ997410
	Hebeloma sp.	1270	85	0	99.17	MW554016
OTU2	*Trichoderma* sp.	1274	100	0	100	MH550505
	Trichoderma sp.	1269	99	0	100	MH550506
	Trichoderma hamatum	1251	98	0	99.86	OP346774
	Trichoderma asperellum	7435	100	0	99.01	CP072832
	Trichoderma asperellum	1234	100	0	98.87	MH863553

	Trichoderma hamatum	1230	96	0	99.85	KM491888
	Trichoderma asperellum	18263	100	0	98.73	CP084949
	Trichoderma hamatum	1211	95	0	100	OM515061
	Trichoderma hamatum	1211	95	0	100	OM515060
	Trichoderma asperellum	1210	98	0	98.72	OP346771
OTU4	*Trichoderma* sp.	1304	100	0	99.86	OK584188
	Trichoderma harzianum	1296	100	0	99.72	OQ558874
	Trichoderma harzianum	1287	100	0	99.31	OQ789693
	Trichoderma harzianum	1284	100	0	99.17	OQ789694
	Trichoderma harzianum	1278	100	0	99.31	OQ789697
	Trichoderma harzianum	1272	100	0	98.90	OQ789692
	Trichoderma harzianum	1270	98	0	99.44	OP346777
	Trichoderma simmonsii	8823	100	0	99.04	CP075868
	Trichoderma sp.	1263	100	0	98.90	MF076625
	Trichoderma sp.	1258	96	0	99.72	OQ589890
OTU3	***Fusarium oxysporum*** Fo47	3520	100	0	100	CP052041
	Fusarium oxysporum	1173	100	0	100	MK828120

	Fusarium oxysporum	1173	100	0	100	MH864510
	Fusarium oxysporum	1173	100	0	100	MH864441
	Fusarium oxysporum f. sp. dianthi	1173	100	0	100	LT841236
	Fusarium oxysporum f. sp. dianthi	1173	100	0	100	LT841222
	Fusarium oxysporum f. sp. vasinfectum	11734	100	0	100	CP130301
	Fusarium oxysporum	1173	100	0	100	CP128294
	Fusarium oxysporum	1173	100	0	100	MW789355
	Fusarium oxysporum	1171	99	0	100	EU364842
OTU5	***Trichoderma virens***	9086	100	0	100	CP071110
	Trichoderma sp. H09	1299	100	0	100	HM000037
	Trichoderma virens	1291	100	0	99.86	ON399101
	Trichoderma virens FT-333	10332	100	0	99.86	CP071118
	Trichoderma virens	1277	98	0	99.86	OP346770
	Trichoderma hamatum	1237	100	0	98.47	MH864072
	Trichoderma pleuroticola	1230	100	0	97.66	MH864423
	Trichoderma harzianum	1225	100	0	97.80	OQ558874
	Trichoderma simmonsii	8533	100	0	97.66	CP075868

	Trichoderma harzianum	1223	100	0	97.66	OQ789697
OTU6	***Penicillium raperi***	1218	98	0	99.71	ON006844
	Penicillium raperi	1214	98	0	99.71	ON006848
	Penicillium raperi	1213	97	0	99.71	ON006861
	Penicillium raperi	1213	97	0	99.71	ON006778
	Penicillium raperi	1209	97	0	99.70	ON006862
	Penicillium raperi	1209	97	0	99.71	ON006799
	Penicillium raperi	1208	97	0	99.85	MK450712
	Penicillium simplicissimum	1208	100	0	98.99	MN029054
	Penicillium simplicissimum	1208	100	0	98.99	MH858780
	Penicillium raperi	1208	97	0	99.85	ON006807
OTU7	***Setophoma terrestris***	1129	98	0	99.22	ON006838
	Setophoma terrestris	1120	97	0	99.21	ON006839
	Setophoma terrestris	1107	96	0	99.20	ON006851
	Setophoma terrestris	1104	97	0	98.58	OM397067
	Setophoma terrestris	1104	97	0	98.58	OM397066
	Setophoma terrestris	1104	97	0	98.58	OM397065

	Setophoma terrestris	1104	97	0	98.58	OM397064
	Setophoma terrestris	1104	97	0	98.58	OM397063
	Setophoma terrestris	1104	97	0	98.58	OM397062
	Setophoma terrestris	1104	97	0	98.58	OM397061
OTU9	*Fusarium* sp.	1213	100	0	100	MH550473
	Fusarium oxysporum	1209	99	0	100	MT447552
	Fusarium oxysporum	1209	99	0	100	MT447549
	Fusarium oxysporum	1209	99	0	100	JN232136
	Fusarium solani	1206	99	0	99.85	JN232138
	Fusarium sp.	1205	99	0	99.85	MF281299
	Fusarium solani	1204	100	0	99.70	MH864512
	Fusarium solani	1202	99	0	99.70	JN232140
	Fusarium sp. SS-R3	1196	99	0	99.70	GU797142
	Fusarium solani	1189	99	0	99.40	JN232139
OTU11	***Linnemannia elongata***	1351	100	0	99.87	MT521755
	zygomycete sp. AM-2008a	1346	100	0	99.73	EU428773
	Linnemannia elongata	1344	100	0	99.73	MT521794

	Linnemannia elongata	1324	98	0	99.60	OM238146
	Linnemannia elongata	1292	100	0	98.40	MH047197
	fungal sp. SUN3	1242	100	0	96.67	JF439213
	Mortierella sp.	1233	100	0	96.69	MT521807
	Mucoromycota sp.	1219	90	0	99.85	MF281331
	Mucoromycota sp.	1214	90	0	99.71	MF281328
	Linnemannia elongata	1201	88	0	99.85	ON075155
OTU10	Chytridiaceae sp.	608	40	2E-168	99.71	MW472240
	Clydaea vesicula	502	69	6E-118	78.57	MT730721
	Clydaea vesicula	498	69	7E-117	78.39	EU352774
	Clydaea vesicula	496	69	2E-116	78.66	EU352773
	Lobulomycetales sp.	434	100	2E-116	71.76	OQ702874
	Lobulomycetales sp.	430	100	3E-115	71.65	OQ702870
	Maunachytrium keaense	391	64	3E-103	77.82	EF432822
	Clydaea vesicula	425	61	4E-95	77.98	NR_121339
	Lobulomycetales sp.	607	47	2E-72	94.27	OQ702922
	Lobulomycetales sp. AF017	511	39	3E-70	95.95	EF432821

The 10 dominant OTUs accounted for 94.3% of the sequences across all the samples. The information of 10 best hits were shown for each OTU. The assigned species names were written in bold font. Among them, OTU10 were only assigned to Lobulomycetales (order) according to the threshold of a previous study (Tedersoo et al., 2014). Scientific name, total score, coverage, E value, similarity identity and accession number were derived from the results of the blastn in NCBI. Before the blast, uncultured/environmental sample sequences were excluded.

Tedersoo, L. et al. Fungal biogeography. Global diversity and geography of soil fungi. Science 346, 1256688, doi:10.1126/science.1256688 (2014).

Table S3 Summarize of identified fungal OTUs and reads' number at the phylum, order and genus level.

	Fungal OTUs/Reads at phylum level	Fungal OTUs/Reads at order level	Fungal OTUs/Reads at genus level
Full length	**208/96885**	189/96693	**170/95860**
ITS Full	205/96861	**192/96795**	160/49106
ITS1	203/96849	191/96633	159/47786
ITS2	205/96862	192/96779	156/48718
18S V9	188/96573	160/95701	120/45053

Here, the OTU table was fixed with 213 fungal OTUs covering 96,897 reads. At each taxonomic level, the best assignment table was in bold. Full length: Full.length.assignments.txt, ITS full: ITS.full.assignments.txt, ITS1: ITS1.assignments.txt, ITS2: ITS2.assignments.txt, 18S V9: Output.18SV9.assignments.unite.txt. Please see the **Fig. S2 and S3** for details.

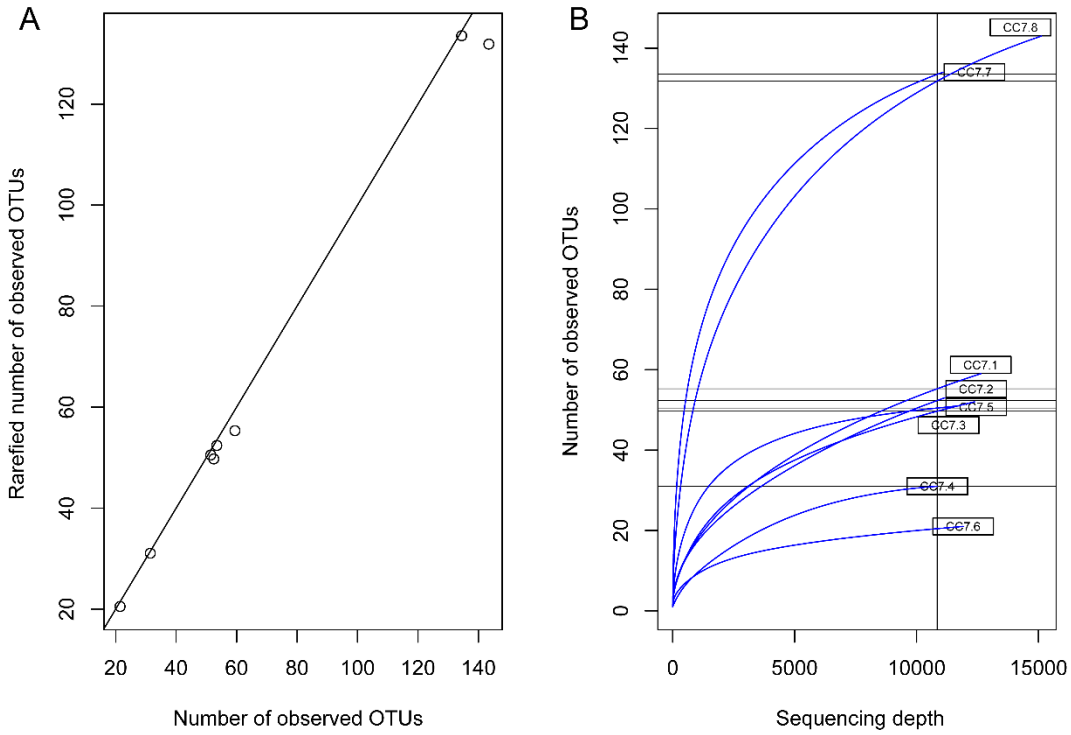

Fig. S1 The relationships between rarefied number of observed OTUs and number of observed OTUs (**A**) as well as the rarefaction curves of 8 samples (**B**). The rarefied number of observed OTUs was calculated at the minimum sequence number 10,854. CC7.1 and CC7.2 were the samples from mushroom cap, while CC7.3, CC7.4, CC7.5 and CC7.6 were the samples from rhizomorph. CC7.7 and CC7.8 were the samples of hyphosphere soil.

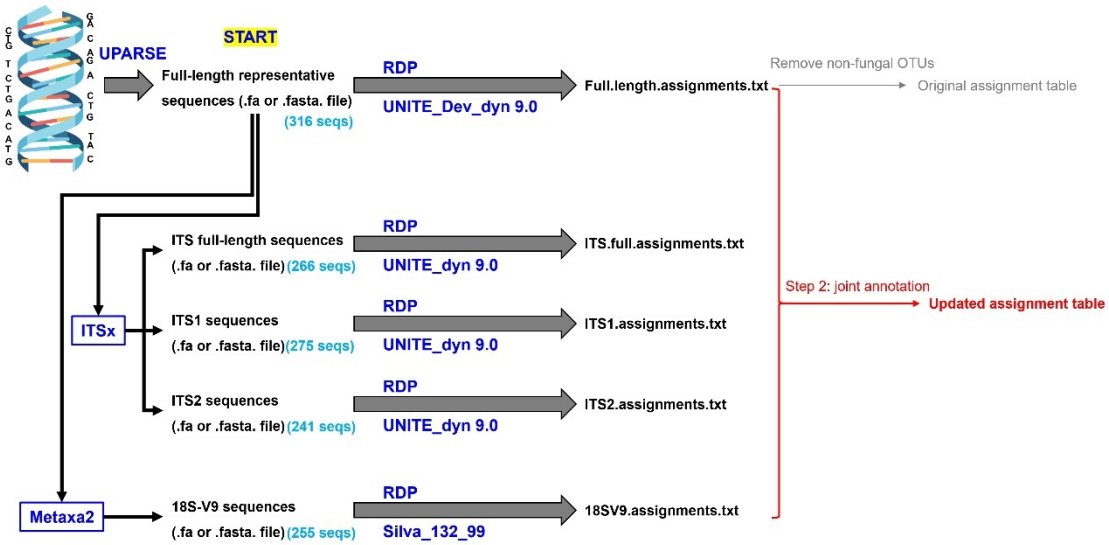

Fig. S2 Step 1 of the bioinformatic pipeline for higher taxonomic resolution: Production of five assignment tables. Ribosomal Database Project (RDP) Classifier was used to assign fungal taxonomy to each OTU (Wang et al., 2007). Readers can change it by other assignment methods, such as BLAST (Tedersoo et al., 2022) and sklearn-based taxonomy classifier (Pedregosa et al., 2011). The UNITE v.9.0 (Nilsson et al., 2019) and SILVA SSU 132 (Quast et al., 2013) were used as the reference database for fungal taxonomy. Readers can also change the reference database to suit their own needs.

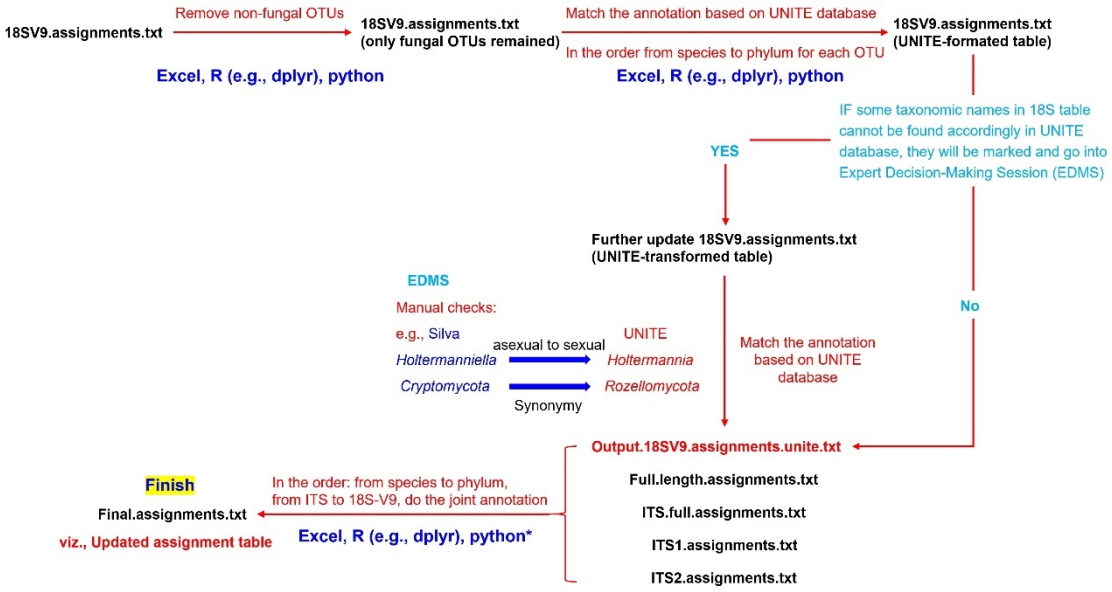

Fig. S3 Step 2 of the bioinformatic pipeline for higher taxonomic resolution: Joint annotation. The readers can deal with the delimited files by using Excel, R package "dplyr" or python codes as they like. The details are more intricate than we expect. Therefore, we caution readers to do the manual checks carefully in EDMS. *In the process of the merge of the five standardized assignment tables, we also provided an exemplar R script (**File S1**).

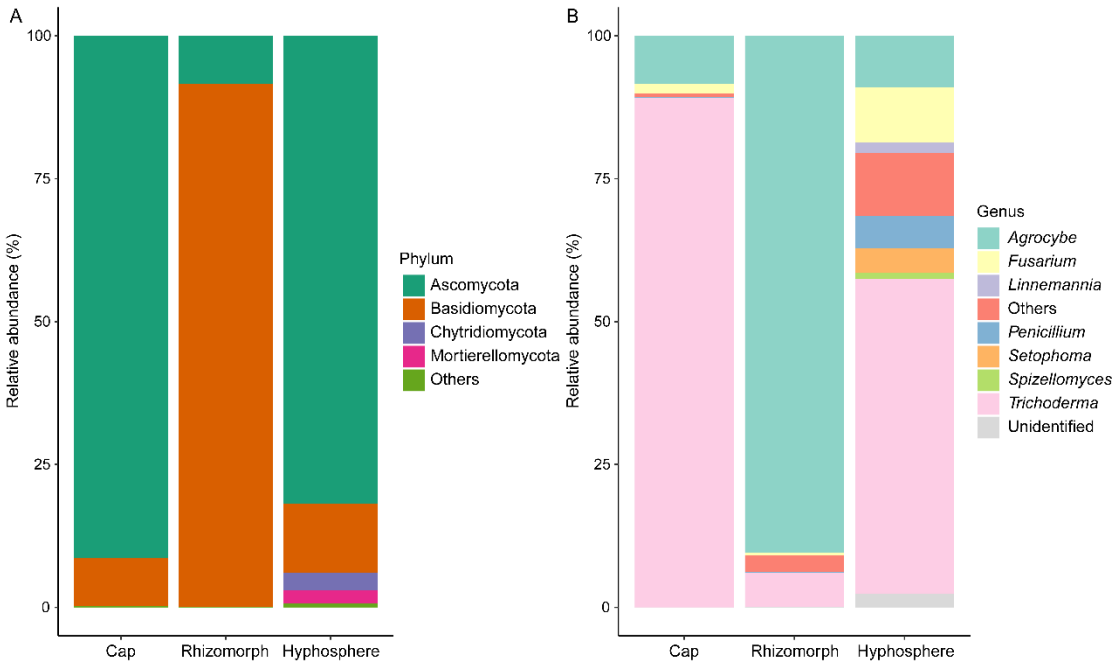

Fig. S4 Fungal taxonomic composition among the different habitats at the phylum (**A**) and genus (**B**) level. For clarity, the fungal phyla and genera whose relative abundance were less than 1% were grouped into 'Others'