

Supplementary Material

Characteristics of Soil Microbial Community Assembly Patterns in fields with Serious occurrence of Tobacco *Fusarium* Wilt Disease

Huidi Liu¹, Yongfeng Zhang², Hongchen Li³, Shilu Chen¹, Jingze Zhang^{4*} & Wei Ding^{1*}

¹ College of Plant Protection, Southwest university, Chongqing 400715, China;

² Shangluo Prefecture Branch of Shaanxi Tobacco Corporation, Shangluo 726099, China;

³ Sanmenxia Tobacco Corporation of Henan province, Sanmenxia 472000, China;

⁴ College of Plant Protection, Zhejiang University, Hangzhou 310000, China.

List of supporting information:

Fig.S1 Chao1 richness index and Shannon's diversity index of bacterial and fungal communities affected by FWD in the different sampling site.

Fig.S2 Principal coordinates analysis (PCoA) ordinations of Bray–Cutis dissimilarity matrices showing a significant association of the bacterial (up) and fungi (under) disease and health community composition in different sites.

Fig.S3 The percents of Proteobacteria (a), Basidiomycota (b) and *Fusarium* (c) for samples collected from healthy (H) and diseased (D) fields. The significance of the difference was determined by the t-test. ($P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***)

Fig.S4 The relationship between *Fusarium* (ZFOTU_585) and other microorganisms in the bacteria-fungi co-occurrence networks.

Table S1 Information of rhizosphere soil collection sample.

Table.S2 Dominant groups of diseased soil and healthy soil samples screened by LefSe analysis (LDA > 3.5 and remove unclassified microbiome)

Table.S3 PERMANOVA analysis using the bray-curtis distances for FWD and sampling site in beta diversity.

Table.S4 Topology properties of the intra- and inter-kingdom networks.

Table.S5 The taxonomic position of top 10 hubs in intra- and interkingdom networks

1 Supplementary figures

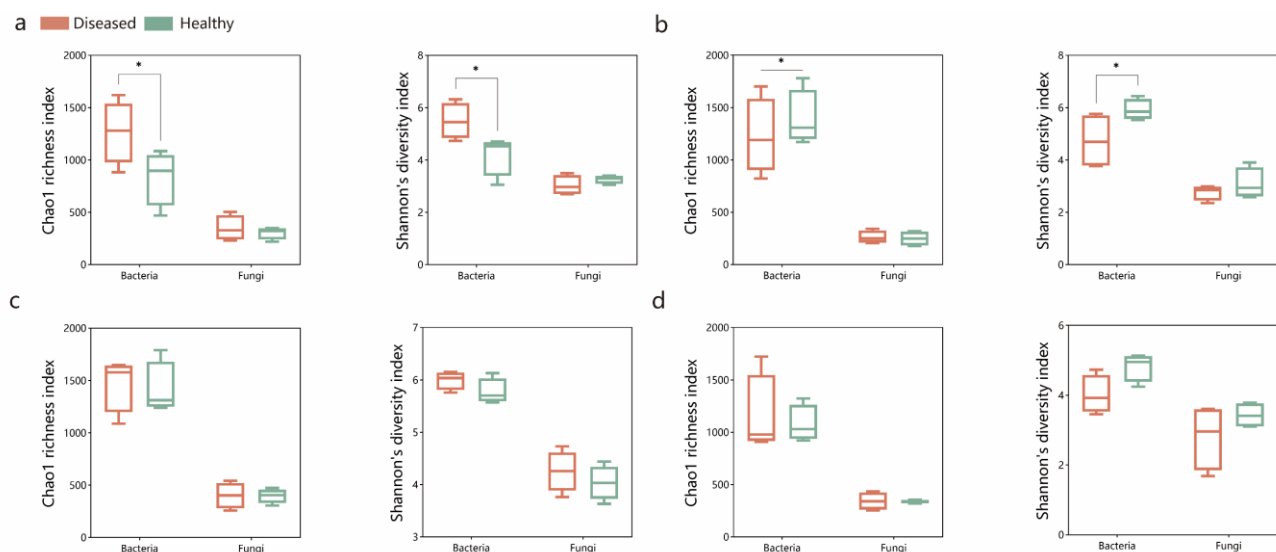


Fig.S1 Chao1 richness index and Shannon's diversity index of bacterial and fungal communities affected by FWD in the different sampling site. a. microbial α -diversity analysis in the Dragon King Ridge, Shangluo. (The Chao1 richness index and Shannon's diversity index of bacterial community in healthy plot was significantly higher than that in diseased plot) b. microbial α -diversity analysis in the Huamiao Village, Shangluo. (The Chao1 richness index and Shannon's diversity index of bacterial community in diseased plot was significantly higher than that in healthy plot)c. microbial α -diversity analysis in the Sanmenxia. d. microbial α -diversity analysis in the Neixiang.

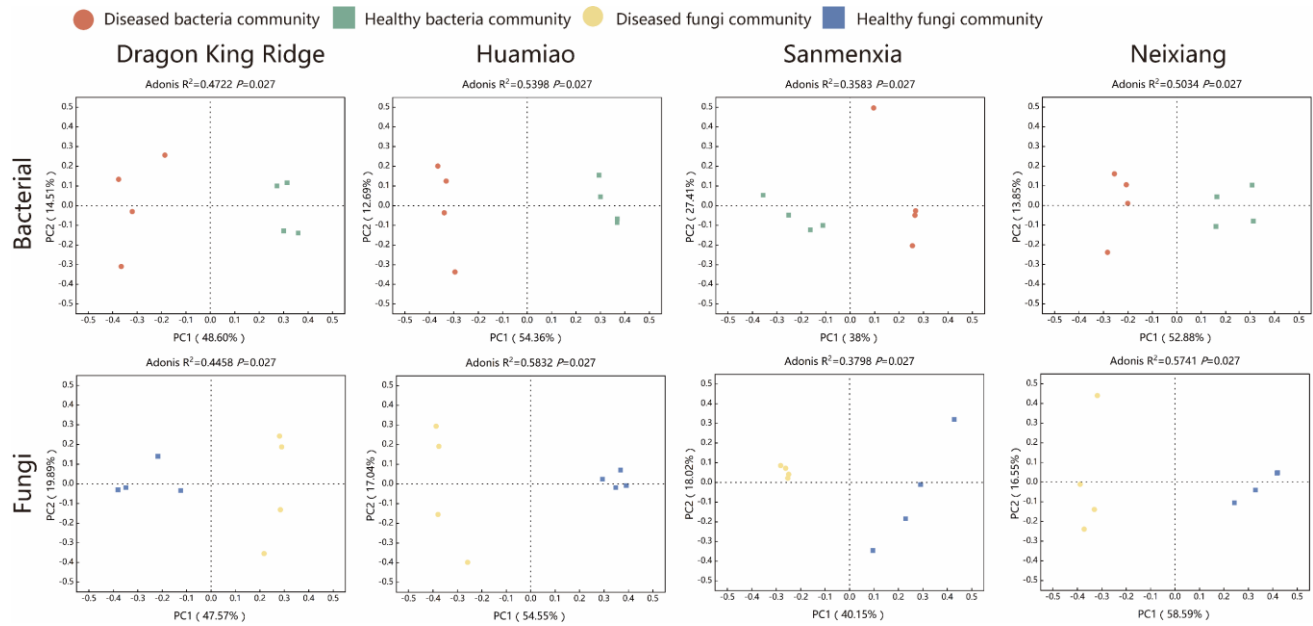


Fig.S2 Principal coordinates analysis (PCoA) ordinations of Bray–Curtis dissimilarity matrices showing a significant association of the bacterial (up) and fungi (under) disease and health community composition in different sites.

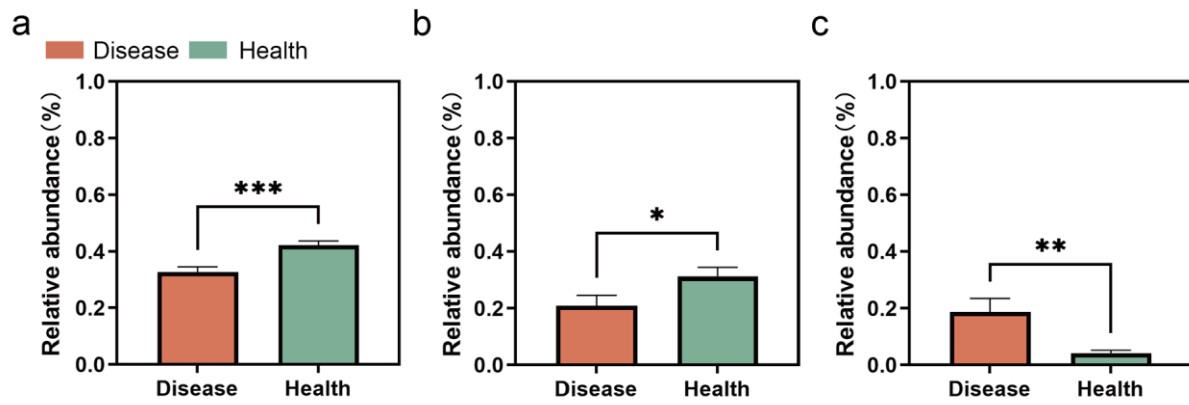


Fig.S3 The percents of Prteobacteria (a), Basidiomycota (b) and *Fusarium* (c) for samples collected form healthy (H) and diseased (D) fields. The significance of the difference was determined by the t-test. ($P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***)

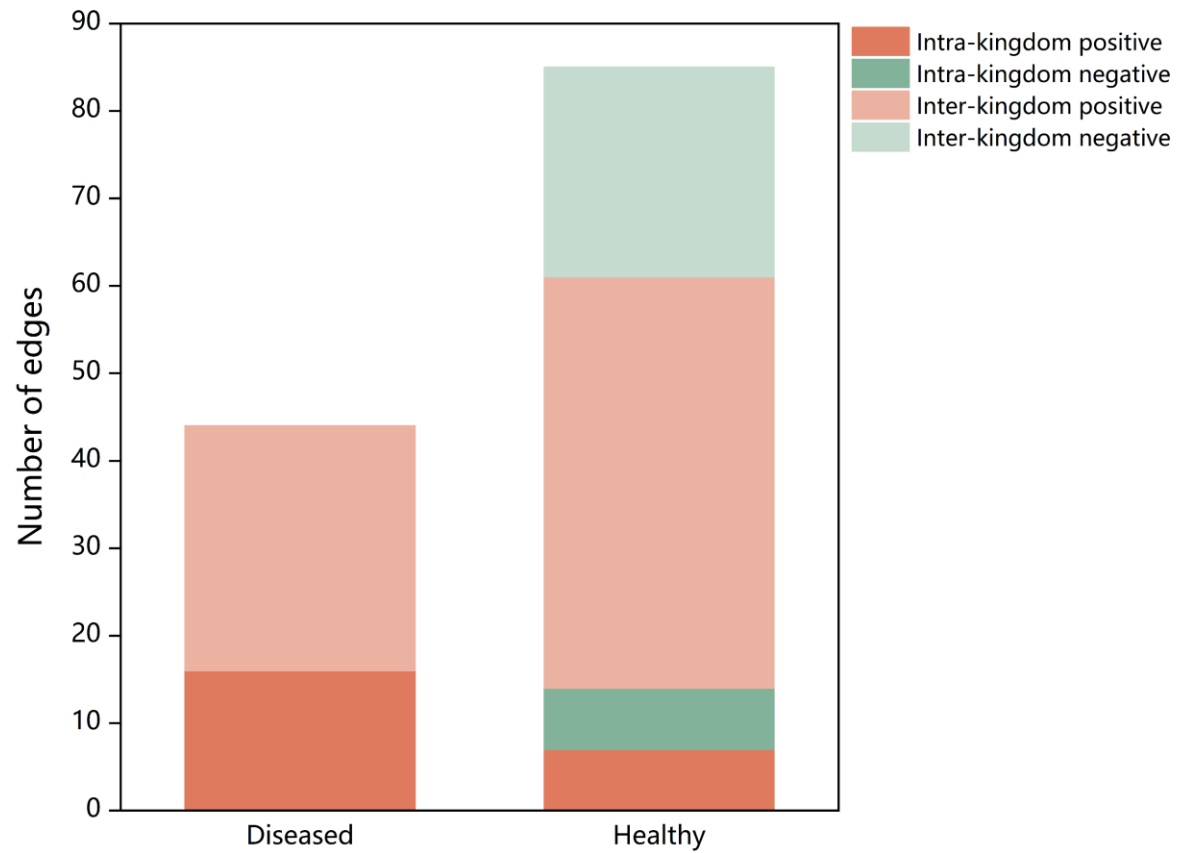


Fig.S4 The relationship between *Fusarium* (ZFOTU_585) and other microorganisms in the bacteria-fungi co-occurrence networks

2 Supplementary tables

Table S1 Origins and distribution of rhizosphere soil samples.

Name	Sample	Location	Latitude	Longitude	Altitude	DI ^a	DS ^b
SLD - LWL	Diseased	Shangluo	110°11'54.66"	34°11'46.10"	1070.4m	29.00 ±4.00	0.14± 0.03
SLH - LWL	Healthy	Shangluo	110°11'58.14"	34°11'44.60"	1078.5m		
SLD -HM	Diseased	Shangluo	110°12'35.11"	34°12'28.68"	1102.5m	40.00 ±8.22	0.20± 0.06
SLH -HM	Healthy	Shangluo	110°12'34.34"	34°12'29.28"	1109.2m		
SMXD	Diseased	Sanmenxia	111°0'16.72"	34°5'47.54"	872.6m	42.00 ±7.52	0.26± 0.04
SMXH	Healthy	Sanmenxia	111°0'13.15"	34°5'29.88"	802.5m		
NXD	Diseased	Nanyang	111°49'25.25"	33°8'50.92"	149.7m	26.00 ±5.79	0.16± 0.05
NXH	Healthy	Nanyang	111°49'3.42"	33°8'59.68"	149.7m		

^a Disease incidence, ^b Disease severity.

Table.S2 Dominant groups of diseased soil and healthy soil samples screened by LEfSe analysis

(LDA > 3.5 and remove unclassified microbiome)

Microbial communities	Species	Group	Mean	LDA value	<i>P</i> value
Bacterial community	Flavobacteriales	Disease	4.58	4.04	0.042
	Rhizobiales	Health	5.31	4.39	0.013
	Proteobacteria	Health	5.62	4.63	0.001
	Allorhizobium- Neorhizobium- Pararhizobium- Rhizobium	Health	5.04	4.18	0.046
	Sphingomonadales	Health	4.95	4.14	0.012
	Sphingomonadaceae	Health	4.95	4.14	0.012
	Sphingobium	Health	4.5	3.91	0.005
	Ensifer	Health	4.37	3.84	0.004
	Alphaproteobacteria	Health	5.49	4.57	0.002
	Ascomycota	Disease	5.84	4.8	0.012
Fungal community	Hypocreales	Disease	5.46	4.87	0.038
	Aspergillaceae	Disease	5.1	4.6	0.019
	Fusarium	Disease	5.27	4.86	0.000
	Penicillium	Disease	4.86	4.41	0.006
	Phaeosphaeriaceae	Disease	4.19	3.58	0.014
	Eurotiales	Disease	5.14	4.58	0.042
	Nectriaceae	Disease	5.43	4.94	0.000
	Plectosphaerella	Disease	4.76	4.18	0.018

Sordariomycetes	Disease	5.68	4.91	0.006
Mrakiaceae	Health	5.25	4.42	0.018
Tausonia	Health	5.25	4.42	0.018
Barnettozyma	Health	4.65	4.33	0.036
Basidiomycota	Health	5.49	4.69	0.005
Saccharomycetes	Health	4.67	4.34	0.007
Saccharomycetales	Health	4.67	4.34	0.007
Thielavia	Health	4.05	3.6	0.018
Leotiomycetes	Health	4.3	3.73	0.000
Piskurozymaceae	Health	5.01	4.31	0.004
Filobasidiales	Health	5.03	4.32	0.002
Tremellomycetes	Health	5.45	4.65	0.008
Solicoccozyma	Health	5.01	4.31	0.004
Cystofilobasidiales	Health	5.25	4.42	0.018
Phaffomycetaceae	Health	4.67	4.34	0.008

Table.S3 PERMANOVA analysis using the bray-curtis distances for FWD and sampling site in beta diversity. (PERMANOVA by adonis of all bacterial 16S and fungal ITS samples)

Microbial							
communitie s	Variables	Df ^a	SumsOfSqs ^b	MeanSqs ^c	F.Model	R ²	Pr(>F) ^d
Bacterial	sampling site	2	0.94	0.47	4.40	0.23	0.001
	FWD	1	0.20	0.20	1.86	0.05	0.071
	Residuals	28	2.98	0.11	-	0.72	-
Fungal	sampling site	2	1.47	0.73	4.91	0.23	0.001
	FWD	1	0.84	0.84	5.66	0.13	0.001
	Residuals	28	4.18	0.15	-	0.64	-

^a degrees of freedom, ^b sum of squares, ^c mean sum of squares, ^d p-values are based on 999 permutations with subsequent Bonferroni correction.

Table.S4 Topology properties of the intra- and inter-kingdom networks.

Name	Bacterial diseased	Bacterial healthy	fungal diseased	fungal healthy
nodes	647	702	193	213

edges	6831	12023	1472	2076
positive	6671	10308	1449	2008
negative	160	1715	23	68
Average degree	21.12	34.25	15.25	19.49
Average path length	4.48	3.50	4.33	4.06
Network diameter	13.30	13.95	11.37	11.17
Network density	0.03	0.05	0.079	0.09
Clustering coefficient	0.78	0.60	0.88	0.91

Table.S5 The taxonomic position of top 10 hubs in intra- and interkingdom networks.

ZOTU s in intra- kingd om	Taxonomic position
---------------------------------------	--------------------

netwo rks	
ZBOT U_70 1	Bacteria—Actinobacteriota—Thermoleophilia—Gaiellales--norank_o__Gaiellales-- norank_f__norank_o__Gaiellales--unclassified_g__norank_f__norank_o__Gaiellales
ZBOT U_83	Bacteria—Myxococcota--bacteriap25--norank_c__bacteriap25-- norank_o__norank_c__bacteriap25--norank_f__norank_o__norank_c__bacteriap25-- unclassified_g__norank_f__norank_o__norank_c__bacteriap25
ZBOT U_15 67	Bacteria—Proteobacteria—Gammaproteobacteria—Burkholderiales--Nitrosomonadaceae MND1--unclassified_g__MND1
ZBOT U_60 6	Bacteria—Actinobacteriota—Actinobacteria—Corynebacteriales—Nocardiaceae— Rhodococcus--unclassified_g__Rhodococcus
ZBOT U_20 750	Bacteria—Actinobacteriota—Rubrobacteria—Rubrobacterales—Rubrobacteriaceae— Rubrobacter--metagenome_g__Rubrobacter
ZBOT U_31 5	Bacteria—Actinobacteriota—Thermoleophilia—Gaiellales—Gaiellaceae—Gaiella-- unclassified_g__Gaiella

ZBOT U_34 18	Bacteria—Actinobacteriota—Acidimicrobiia--unclassified_c__Acidimicrobiia-- unclassified_c__Acidimicrobiia--unclassified_c__Acidimicrobiia-- unclassified_c__Acidimicrobiia-
ZBOT U_17 09	Bacteria—Actinobacteriota—Actinobacteria—Micromonosporales-- Micromonosporaceae--unclassified_f__Micromonosporaceae-- unclassified_f__Micromonosporaceae
ZBOT U_31 803	Bacteria—Actinobacteriota—Thermoleophilia—Gaiellales--norank_o__Gaiellales-- norank_f__norank_o__Gaiellales-- uncultured_microorganism_g__norank_f__norank_o__Gaiellales
ZBOT U_22 522	Bacteria—Bacteroidota—Bacteroidia—Chitinophagales—Chitinophagaceae-- unclassified_f__Chitinophagaceae--unclassified_f__Chitinophagaceae
ZBOT U_58 9	Bacteria—Acidobacteriota—Blastocatellia—Pyrinomonadales--Pyrinomonadaceae RB41--uncultured_Acidobacteriaceae_bacterium_g__RB41
ZBOT U_60 3	Bacteria—Acidobacteriota—Vicinamibacteria—Vicinamibacterales— Vicinamibacteraceae--norank_f__Vicinamibacteraceae-- unclassified_g__norank_f__Vicinamibacteraceae
ZBOT U_66 4	Bacteria—Bacteroidota—Bacteroidia—Cytophagales—Microscillaceae-- norank_f__Microscillaceae--unclassified_g__norank_f__Microscillaceae

ZBOT U_41 1	Bacteria—Proteobacteria—Gammaproteobacteria—Burkholderiales--SC-I-84-- norank_f__SC-I-84--unclassified_g__norank_f__SC-I-84
ZBOT U_46 6	Bacteria—Proteobacteria—Alphaproteobacteria—Rhizobiales--- Rhizobiales_Incertae_Sedis--Nordella unclassified_g__Nordella
ZBOT U_81 8	Bacteria—Planctomycetota—Phycisphaerae—Tepidisphaerales--WD2101_soil_group-- norank_f__WD2101_soil_group-- uncultured_planctomycete_g__norank_f__WD2101_soil_group
ZBOT U_60 7	Bacteria—Proteobacteria—Alphaproteobacteria—Rhizobiales---Beijerinckiaceae— Microvirga--unclassified_g__Microvirga
ZBOT U_48 8	Bacteria—Acidobacteriota—Blastocatellia—Pyrinomonadales—Pyrinomonadaceae-- RB41--unclassified_g__RB41
ZBOT U_41 7	Bacteria—Acidobacteriota—Blastocatellia—Blastocatellales—Blastocatellaceae-- unclassified_f__Blastocatellaceae---unclassified_f__Blastocatellaceae
ZBOT U_38 9	Bacteria—Actinobacteriota—Actinobacteria—Micrococcales—Microbacteriaceae— Agromyces--unclassified_g__Agromyces

ZFOT U_15 95	Fungi—Ascomycota—Eurotiomycetes—Eurotiales—Aspergillaceae—Penicillium-- unclassified_g__Penicillium
ZFOT U_22 77	Fungi—Ascomycota—Sordariomycetes—Sordariales—Chaetomiaceae—Chaetomium-- Chaetomium_sp
ZFOT U_22 52	Fungi—Ascomycota—Sordariomycetes—Hypocreales—Hypocreaceae—Trichoderma-- Trichoderma_polysporum
ZFOT U_22 47	Fungi—Ascomycota—Eurotiomycetes—Eurotiales—Aspergillaceae—Penicillium-- Penicillium_rubidurum
ZFOT U_22 67	Fungi—Ascomycota—Eurotiomycetes—Eurotiales—Aspergillaceae—Penicillium-- unclassified_g__Penicillium
ZFOT U_15 77	Fungi—Ascomycota—Eurotiomycetes—Eurotiales—Aspergillaceae—Penicillium-- Penicillium_ochrochloron
ZFOT U_17 93	Fungi—Ascomycota--Dothideomycetes --Botryosphaeriales—Botryosphaeriaceae— Macrophomina--Macrophomina_phaseolina

ZFOT U_22 57	Fungi—Ascomycota—Sordariomycetes—Sordariales—Lasiosphaeriaceae—Echria-- Echria_sp
ZFOT U_10 289	Fungi—Ascomycota—Eurotiomycetes—Chaetothyriales-- unclassified_o__Chaetothyriales--unclassified_o__Chaetothyriales-- unclassified_o__Chaetothyriales
ZFOT U_22 40	Fungi--Ascomycota—Sordariomycetes—Sordariales—Chaetomiaceae--- unclassified_f__Chaetomiaceae--unclassified_f__Chaetomiaceae
ZFOT U_45 08	Fungi—Ascomycota—Sordariomycetes—Hypocreales--unclassified_o__Hypocreales-- unclassified_o__Hypocreales--unclassified_o__Hypocreales
ZFOT U_22 90	Fungi—Ascomycota—Sordariomycetes—Hypocreales-- Hypocreales_fam_Incertae_sedis—Acremonium--Acremonium_acutatum
ZFOT U_44 91	Fungi—Ascomycota—Sordariomycetes—Hypocreales---Nectriaceae-- unclassified_f__Nectriaceae--Nectriaceae_sp
ZFOT U_22 635	Fungi—Mortierellomycota--Mortierellomycetes—Mortierellales—Mortierellaceae— Mortierella--Mortierella_simplex

ZFOT	
U_44	Fungi-- unclassified_k__Fungi
31	
ZFOT	
U_10	Fungi-- unclassified_k__Fungi
425	
ZFOT	
U_44	Fungi-- unclassified_k__Fungi
38	
ZFOT	
U_46	Fungi-- unclassified_k__Fungi
81	
ZFOT	
U_13	Fungi-- unclassified_k__Fungi
475	
ZFOT	
U_15	Fungi-- unclassified_k__Fungi
53	
ZOTU	
s in	Taxonomic position
interki	

ngdo m netwo rks	
ZBOT U_111	Bacteria-- Crenarchaeota --Nitrososphaeria—Nitrososphaerales— Nitrososphaeraceae--norank_f__Nitrososphaeraceae-- uncultured_euryarchaeote_g__norank_f__Nitrososphaeraceae
ZBOT U_22 522	Bacteria—Bacteroidota—Bacteroidia—Chitinophagales—Chitinophagaceae-- unclassified_f__Chitinophagaceae--unclassified_f__Chitinophagaceae
ZBOT U_60 6	Bacteria—Actinobacteriota—Actinobacteria—Corynebacteriales---Nocardiaceae— Rhodococcus--unclassified_g__Rhodococcus
ZBOT U_11 94	Bacteria—Acidobacteriota—Vicinamibacteria—Vicinamibacterales-- norank_o__Vicinamibacterales--norank_f__norank_o__Vicinamibacterales-- unclassified_g__norank_f__norank_o__Vicinamibacterales
ZBOT U_47 3	Bacteria—Bacteroidota—Bacteroidia—Chitinophagales—Chitinophagaceae--Puia unclassified_g__Puia

ZBOT U_20 750	Bacteria—Actinobacteriota—Rubrobacteria—Rubrobacterales—Rubrobacteriaceae— Rubrobacter--metagenome_g__Rubrobacter
ZBOT U_12 56	Bacteria-- Proteobacteria --Gammaproteobacteria—Burkholderiales— Oxalobacteraceae--unclassified_f__Oxalobacteraceae--unclassified_f__Oxalobacteraceae
ZBOT U_11 341	Bacteria—Acidobacteriota—Vicinamibacteria—Vicinamibacterales-- norank_o__Vicinamibacterales--norank_f__norank_o__Vicinamibacterales-- unclassified_g__norank_f__norank_o__Vicinamibacterales
ZBOT U_41 1	Bacteria-- Proteobacteria --Gammaproteobacteria—Burkholderiales--SC-I-84-- norank_f__SC-I-84--unclassified_g__norank_f__SC-I-84
ZBOT U_58 9	Bacteria—Acidobacteriota—Blastocatellia—Pyrinomonadales--Pyrinomonadaceae RB41--uncultured_Acidobacteriaceae_bacterium_g__RB41
ZBOT U_62 8	Bacteria—Acidobacteriota—Blastocatellia—Blastocatellales—Blastocatellaceae-- unclassified_f__Blastocatellaceae--unclassified_f__Blastocatellaceae
ZBOT U_60 7	Bacteria-- Proteobacteria --Alphaproteobacteria—Rhizobiales—Beijerinckiaceae— Microvirga--unclassified_g__Microvirga

ZBOT U_60 3	Bacteria--Proteobacteria--Deltaproteobacteria--Bdellovibrionales--Bdellovibrionaceae-- OM27 clade
ZBOT U_28 8	Bacteria--Proteobacteria--Deltaproteobacteria--Bdellovibrionales--Bdellovibrionaceae-- OM27 clade
ZBOT U_60 7	Bacteria--Proteobacteria--Deltaproteobacteria--Bdellovibrionales--Bdellovibrionaceae-- OM27 clade
ZBOT U_60 3	Bacteria—Acidobacteriota—Vicinamibacteria—Vicinamibacterales— Vicinamibacteraceae--norank_f__Vicinamibacteraceae-- unclassified_g__norank_f__Vicinamibacteraceae
ZBOT U_28 8	Bacteria—Actinobacteriota—Actinobacteria—Frankiales—Geodermatophilaceae-- unclassified_f__Geodermatophilaceae--unclassified_f__Geodermatophilaceae
ZBOT U_66 4	Bacteria—Bacteroidota—Bacteroidia—Cytophagales—Microscillaceae-- norank_f__Microscillaceae--unclassified_g__norank_f__Microscillaceae
ZBOT U_38 9	Bacteria—Actinobacteriota—Actinobacteria—Micrococcales—Microbacteriaceae— Agromyces--unclassified_g__Agromyces

ZBOT U_34 4	Bacteria—Acidobacteriota—Blastocatellia—Blastocatellales--Blastocatellaceae— Stenotrophobacter--uncultured_soil_bacterium_g__Stenotrophobacter
ZBOT U_22 2	Bacteria-- Proteobacteria --Gammaproteobacteria—Xanthomonadales— Xanthomonadaceae--unclassified_f__Xanthomonadaceae-- unclassified_f__Xanthomonadaceae
ZFOT U_58 5	Fungi--Ascomycota --Sordariomycetes--Hypocreales--Nectriaceae--Fusarium-- <i>Fusarium.nematophilum</i>
ZFOT U_43 04	Fungi—Mortierellomycota--Mortierellomycetes—Mortierellales—Mortierellaceae— Mortierella--Mortierella_alpina