

Chemical and Biological Technologies in Agriculture

Supplementary Material

The beneficial endophytic microbes enhanced tobacco defense system to resist bacterial wilt disease

Jiemeng Tao^{a,b}, Mengli Gu^{b,d}, Shizhou Yu^c, Jingjing Shi^b, Lingtong Cheng^b, Jingjing Jin^{a,b}, Peng Lu^{a,b}, Jianfeng Zhang^{a,b}, He Li^b, Peijian Cao^{a,b,d *}

^a Beijing Life Science Academy, Beijing 102200, China

^b China Tobacco Gene Research Center, Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China

^c Molecular Genetics Key Laboratory of China Tobacco, Guizhou Academy of Tobacco, Guiyang 550081, China

^d School of Agricultural Sciences, Zhengzhou University, Zhengzhou 450001, China

*To whom correspondence should be addressed.

Peijian Cao, peijiancao@163.com

SM: Figures

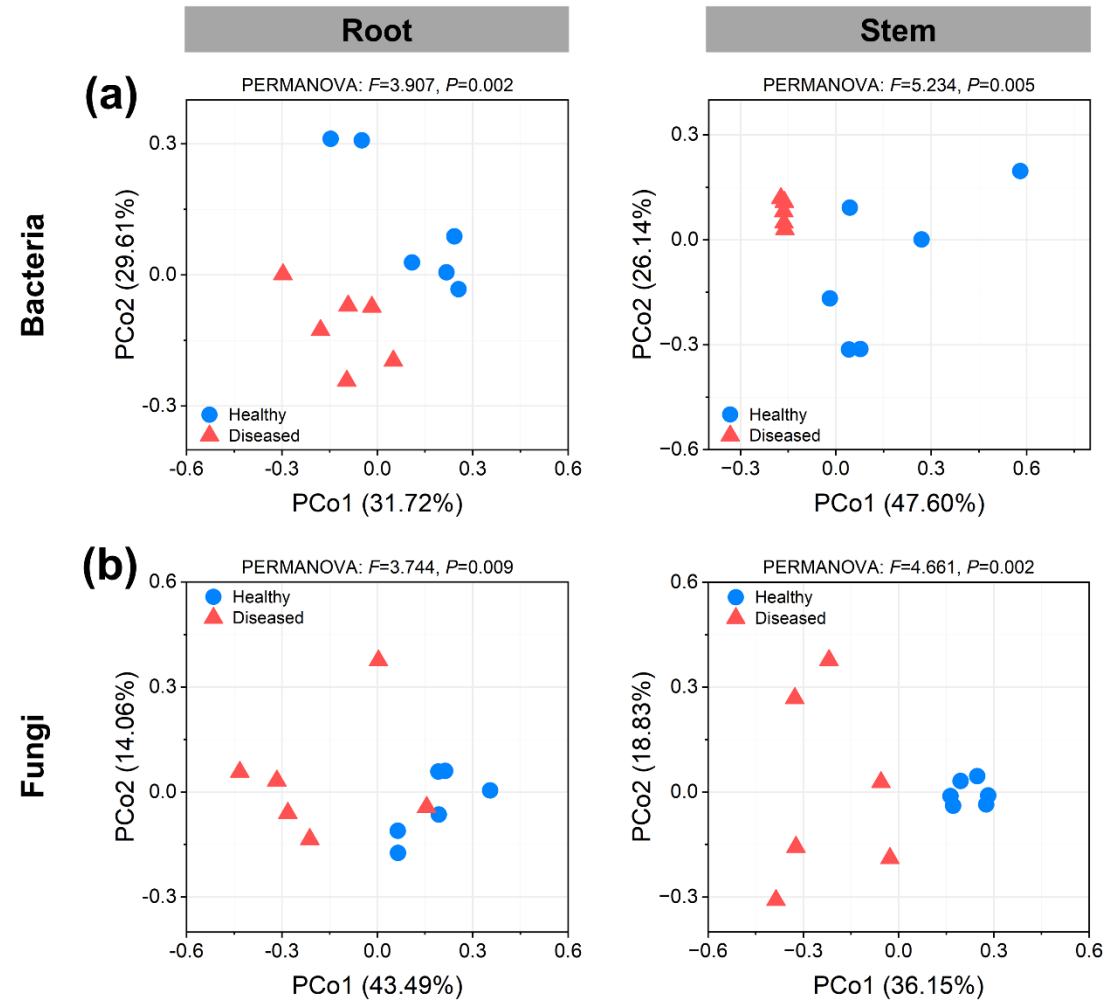


Figure S1. Principal coordinate analysis (PCoA) of Bray–Cutis dissimilarity matrices showing effects of bacterial wilt disease on the bacterial (a) and fungal (b) community structures of root and stem microbiomes.

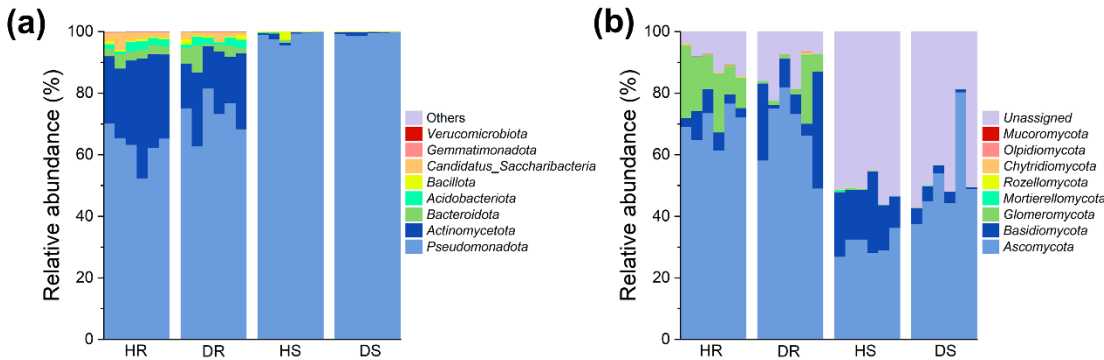


Figure S2. Stacked bar chart showing bacterial (a) and fungal (b) composition (at phylum level)

in root and stem microbiomes of healthy and diseased plants based on relative abundance data.

HR: Healthy root; DR: Diseased root; HS: Healthy stem; DS: Diseased stem.

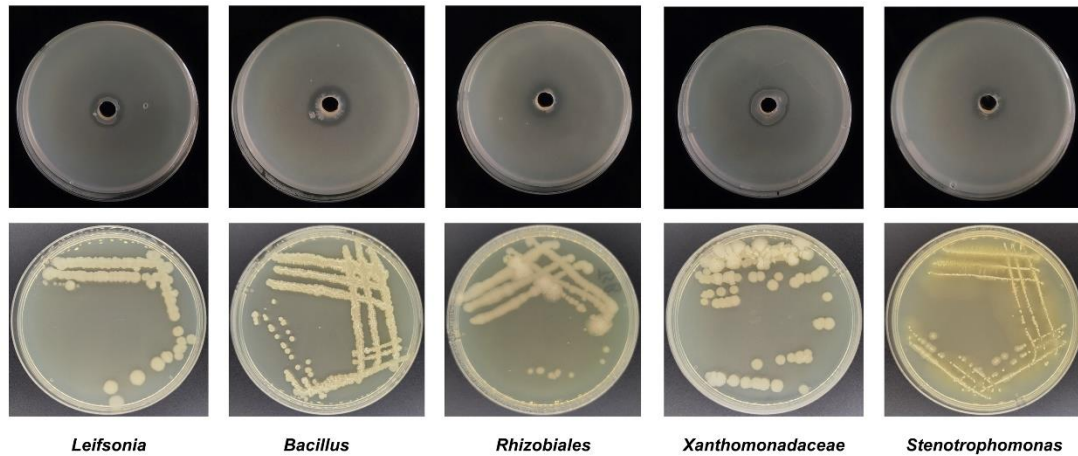


Figure S3. The antagonistic activities of five bacterial strains in plate confrontation experiments and their colonial morphology on plates.

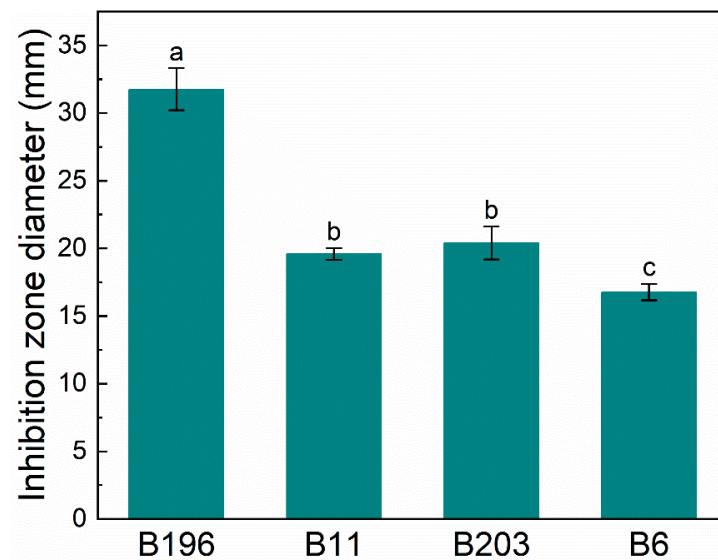


Figure S4. The inhibition zone diameters of *Burkholderia* sp. B196, *Paenibacillus* sp. B11, *Acidovorax* sp. B203 and *Sphingomonas* sp. B6 against pathogen *R. solanacearum*.

SM: Tables

Table S1 Primer sequences and PCR amplification conditions in this study.

Gene	Region	Primers	Sequences (5'-3')	PCR program
16s rRNA	V5-V7	799F	AACMGGATTAGATACCCCKG	2 min initial denaturation at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C, with a final 10 min elongation at 72 °C.
		1193R	ACGTCATCCCCACCTTCC	
ITS rNS	ITS1	ITS1F	CTTGGTCATTTAGAGGAAGTAA	2 min initial denaturation at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C, with a final 10 min elongation at 72 °C.
		ITS2	GCTGCGTTCTTCATCGATGC	

Table S2 The primer information for Real-Time PCR in this study.

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Efficiency (%)	Linear regression (r ²)	Slope
<i>EF1α</i>	CCCAAGAGGCCCTCAGACA	CACACGACCAACAGGGACAGT	99.51	0.9996	-3.3338
<i>GAPDH</i>	TGGGTGTCAACGAGAAGGAA	TCTGGGTGGCAGTAAGGGA	94.24	0.9924	-3.4681
<i>L25</i>	GCTTTCTTCGTCCCATCA	CCCCAAGTACCCTCGTAT	102.44	0.9941	-3.2648
<i>ACT</i>	TATCCCTAGTATTGTTGGC	CTGGGGTATTTAAAGTCTCA	104.92	0.9901	-3.2094
<i>NtAOC</i>	CGGCTCAACTGATTCTCTACAAC	CCTTTCTTTTCCTCTTCGTGTTTG	101.25	0.9894	-3.2924
<i>NtAOS</i>	CAATACGGAAGAGCCAAACGC	AACTCATCGGGTCGGTCAAA	97.93	0.9967	-3.3726
<i>NtCOI1</i>	GGATTGACTGATTGCGGAAGG	CCCCAGGCCGGACATAGAG	105.51	0.9816	-3.1966
<i>NtLOX</i>	ACGAAGGTGTTGAAGGCTCTGTC	ATGGTATCGCGGAAATGAGGTT	100.29	0.9956	-3.3150
<i>NtJAZ1</i>	GAGATTGTGGATTCCGGTCGAT	GCGGTGGATACCAAGGCTGA	103.15	0.9945	-3.2486
<i>NtNPR1</i>	TGATGGCACGTCTGAATTACCC	AACCGTTTTCCAAGTTCCACAGTT	94.23	0.9954	-3.4684
<i>NtPR1a</i>	CACATTTTCCACCAACACATTG	GAGGGCAGCCGTGCAA	98.50	0.9898	-3.3584
<i>NtPR5</i>	CGAGGTCAAAGCTGGTGGTTC	TGCATTGAATTCCGTGACATT	93.09	0.9927	-3.4994
<i>NtPAL</i>	GTCACACATTACCACAATCAG	GTGTTCCATAATAGCAGCAGCCT	101.58	0.9938	-3.2846

Table S3 PERMANOVA by adonis of all bacterial 16S and fungal ITS samples. PERMANOVA analysis using the Bray Curtis distances for compartment and bacterial wilt disease (BWD).

	Factor	Df	SumsOfSqs	MeanSqs	F.Model	R ²	Pr(>F)
Bacterial community	Compartment	1	3.718	3.718	23.365	0.619	0.001
	BWD	1	0.438	0.438	2.215	0.091	0.093
Fungal community	Compartment	1	0.636	0.636	2.643	0.107	0.032
	BWD	1	2.343	2.343	14.373	0.395	0.001

Table S4 Differentially abundant analysis showing the enriched bacterial ASVs in the root of diseased plants compared with that of healthy plants.

ID	Mean Diseased	Mean Healthy	log2Fold Change	log2 CPM	pvalue	FDR	Phylum	Genus
ASV_95	0.031	0.001	5.792	7.81	< 0.001	< 0.001	Proteobacteria	Pantoea
ASV_1	28.645	7.539	2.191	17.7	< 0.001	0.002	Proteobacteria	Ralstonia
ASV_649	0.044	0	7.038	8.33	< 0.001	0.01	Bacteroidetes	Moheibacter
ASV_40	0.017	0.002	3.33	7.21	< 0.001	0.029	Proteobacteria	Unassigned
ASV_1982	0.04	0.01	2.221	8.37	< 0.001	0.029	Proteobacteria	Ralstonia
ASV_2108	0.092	0.027	2.059	9.56	< 0.001	0.043	Proteobacteria	Ralstonia
ASV_110	0.025	0.005	2.482	7.73	< 0.001	0.045	Proteobacteria	Ralstonia
ASV_372	0.095	0.004	4.999	9.4	< 0.001	0.051	Bacteroidetes	Myroides
ASV_2172	0.026	0.007	2.198	7.85	< 0.001	0.054	Proteobacteria	Ralstonia
ASV_1101	0.009	0.002	2.453	6.62	< 0.001	0.057	Proteobacteria	Ralstonia
ASV_550	0.038	0.005	3.221	8.24	< 0.001	0.057	Proteobacteria	Burkholderia
ASV_447	0.128	0.035	2.106	9.98	0.001	0.069	Proteobacteria	Ralstonia
ASV_190	0.043	0.014	1.83	8.57	0.001	0.069	Proteobacteria	Buttiauxella
ASV_100	0.072	0.019	2.147	9.19	0.002	0.114	Proteobacteria	Unassigned
ASV_776	0.053	0.018	1.823	8.83	0.003	0.116	Proteobacteria	Ralstonia
ASV_79	0.385	0.079	2.613	11.5	0.003	0.121	Bacteroidetes	Chryseobacterium
ASV_533	0.034	0.013	1.658	8.27	0.003	0.121	Proteobacteria	Achromobacter
ASV_26	0.968	0.202	2.599	12.9	0.004	0.134	Bacteroidetes	Chryseobacterium
ASV_144	0.267	0.049	2.782	11	0.004	0.14	Proteobacteria	Luteimonas
ASV_435	0.082	0.009	3.419	9.27	0.006	0.159	Actinobacteria	Cellulomonas
ASV_590	0.033	0.011	1.811	8.22	0.006	0.159	Proteobacteria	Ralstonia
ASV_205	0.15	0.057	1.638	10.3	0.006	0.159	Proteobacteria	Acidovorax
ASV_326	0.003	0	3.252	5.73	0.006	0.159	Proteobacteria	Ralstonia
ASV_604	0.059	0.019	1.85	8.99	0.006	0.159	Proteobacteria	Bordetella
ASV_911	0.054	0.018	1.828	8.84	0.007	0.165	Proteobacteria	Bradyrhizobium
ASV_3396	0.008	0.002	2.01	6.48	0.009	0.191	Proteobacteria	Unassigned
ASV_286	0.113	0.019	2.876	9.77	0.01	0.191	Proteobacteria	Comamonas
ASV_427	0.056	0.012	2.534	8.85	0.011	0.222	Proteobacteria	Pseudomonas

ASV_3265	0.016	0.006	1.716	7.32	0.013	0.226	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_525	0.035	0.012	1.762	8.25	0.014	0.229	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_1876	0.02	0.007	1.656	7.62	0.015	0.239	<i>Proteobacteria</i>	<i>Bradyrhizobium</i>
ASV_493	0.043	0.017	1.667	8.62	0.016	0.248	<i>Proteobacteria</i>	<i>Xenophilus</i>
ASV_2448	0.005	0.001	2.487	6.11	0.018	0.258	<i>Proteobacteria</i>	<i>Sphingomonas</i>
ASV_825	0.032	0.009	2.115	8.16	0.019	0.261	<i>Firmicutes</i>	<i>Paenibacillus</i>
ASV_420	0.002	0	2.733	5.56	0.022	0.295	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_442	0.001	0	2.82	5.26	0.024	0.297	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_794	0.001	0	2.814	5.26	0.024	0.297	<i>Proteobacteria</i>	<i>Enterobacter</i>
ASV_140	0.003	0.001	2.054	5.75	0.026	0.299	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_3361	0.012	0.004	1.862	7.03	0.026	0.3	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_3318	0.037	0.014	1.615	8.37	0.027	0.301	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_409	0.037	0.012	1.933	8.34	0.029	0.319	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_1616	0.018	0.009	1.301	7.58	0.03	0.32	<i>Proteobacteria</i>	<i>Rhodopseudomonas</i>
ASV_616	0.061	0.022	1.794	9.08	0.031	0.324	<i>Proteobacteria</i>	<i>Bordetella</i>
ASV_779	0.023	0.011	1.369	7.87	0.032	0.324	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_624	0.015	0.005	1.863	7.3	0.032	0.324	<i>Proteobacteria</i>	<i>Franconibacter</i>
ASV_488	0.062	0.014	2.322	8.81	0.037	0.349	<i>Firmicutes</i>	<i>Paenibacillus</i>
ASV_2948	0.028	0.014	1.268	8.11	0.0399	0.350	<i>Actinobacteria</i>	<i>Amycolatopsis</i>
ASV_331	0.001	0	2.499	5.21	0.043	0.361	<i>Proteobacteria</i>	<i>Pantoea</i>
ASV_315	0.013	0.006	1.364	7.16	0.043	0.361	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_627	0.031	0.015	1.266	8.29	0.046	0.375	<i>Proteobacteria</i>	<i>Cupriavidus</i>
ASV_220	0.157	0.043	2.057	10.1	0.05	0.397	<i>Firmicutes</i>	<i>Paenibacillus</i>

Table S5 Differentially abundant analysis showing the enriched bacterial ASVs in the stem of diseased plants compared with that of healthy plants.

ID	Mean Diseased	Mean Healthy	log2Fold Change	log2CPM	pvalue	FDR	Phylum	Genus
ASV_446	0.117	0.01	4.003	9.658	< 0.001	0.028	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_442	0.066	0.006	3.688	8.903	0.003	0.055	<i>Proteobacteria</i>	<i>Unassigned</i>
ASV_1067	0.074	0.009	3.424	9.044	0.004	0.062	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_1617	0.059	0.009	3.192	8.781	0.01	0.113	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_497	0.049	0.008	2.988	8.567	0.013	0.136	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_1982	0.038	0.005	3.51	8.098	0.0131	0.136	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_596	0.049	0.008	2.954	8.566	0.018	0.172	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_1497	0.049	0.01	2.599	8.554	0.02	0.182	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_670	0.044	0.009	2.568	8.459	0.025	0.230	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_2948	0.001	0	3.241	5.217	0.03	0.255	<i>Actinobacteria</i>	<i>Amycolatopsis</i>
ASV_326	0.064	0.016	2.363	8.995	0.043	0.315	<i>Proteobacteria</i>	<i>Ralstonia</i>
ASV_138	0.177	0.042	2.424	10.387	0.046	0.332	<i>Proteobacteria</i>	<i>Unassigned</i>

Table S6 Differentially abundant analysis showing the enriched fungal ASVs in the root of diseased plants compared with that of healthy plants.

ID	Mean diseased	Mean healthy	log2FoldChange	log2CPM	pValue	FDR	Phylum	Genus
ASV_92	0.329	0.027	3.9	11.043	< 0.001	0.011	<i>Ascomycota</i>	<i>Macrophomina</i>
ASV_137	0.003	0	4.438	5.443	< 0.001	0.013	<i>Ascomycota</i>	<i>Periconia</i>
ASV_111	0.008	0	4.689	6.329	0.005	0.052	<i>Ascomycota</i>	Unassigned
ASV_267	0.044	0.003	4.625	8.743	0.005	0.052	<i>Ascomycota</i>	<i>Pseudallescheria</i>
ASV_90	0.013	0.003	2.102	6.906	0.027	0.143	<i>Ascomycota</i>	<i>Aspergillus</i>
ASV_331	0.001	0	3.526	5.14	0.0275	0.143	<i>Ascomycota</i>	<i>Melanospora</i>
ASV_11	0.889	0.228	2.431	12.805	0.039	0.176	<i>Ascomycota</i>	Unassigned
ASV_1	36.675	14.446	1.557	18.165	0.043	0.187	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_87	0.344	0	10.605	10.366	< 0.001	0.003	<i>Basidiomycota</i>	Unassigned
ASV_33	2.828	0.079	6.003	14.656	< 0.001	0.011	<i>Basidiomycota</i>	Unassigned
ASV_12	5.909	0.346	4.271	14.969	0.002	0.037	<i>Basidiomycota</i>	Unassigned
ASV_75	0.282	0.035	3.379	10.904	0.004	0.049	<i>Basidiomycota</i>	<i>Ceratobasidium</i>
ASV_47	0.07	0.007	3.387	8.776	0.008	0.068	<i>Basidiomycota</i>	Unassigned
ASV_290	0.038	0.002	4.28	8.087	0.015	0.094	<i>Basidiomycota</i>	<i>Psathyrella</i>
ASV_168	0.098	0.011	3.289	9.127	0.029	0.144	<i>Basidiomycota</i>	<i>Ceratobasidium</i>
ASV_188	0.007	0	5.607	6.048	< 0.001	0.003	Unassigned	Unassigned
ASV_155	0.106	0.003	4.832	8.796	0.002	0.035	Unassigned	Unassigned
ASV_241	0.007	0	4.955	5.694	0.003	0.043	Unassigned	Unassigned
ASV_120	0.148	0.026	2.531	9.742	0.009	0.075	Unassigned	Unassigned
ASV_103	0.008	0.001	2.574	6.257	0.012	0.089	Unassigned	Unassigned
ASV_278	0.001	0	3.509	5.14	0.013	0.090	Unassigned	Unassigned
ASV_36	0.017	0.004	2.21	7.156	0.03	0.144	Unassigned	Unassigned

Table S7 Differentially abundant analysis showing the enriched fungal ASVs in the stem of diseased plants compared with that of healthy plants.

ID	Mean diseased	Mean healthy	log2FoldChange	log2CPM	pValue	FDR	Phylum	Genus
ASV_896	0.171	0.007	6.25	10.549	< 0.001	0.000	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_15	7.253	0.478	5.589	15.939	< 0.001	0.001	Unassigned	Unassigned
ASV_68	0.011	0	6.772	6.585	< 0.001	0.001	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_1	15.317	3.697	3.752	17.174	< 0.001	0.001	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_69	0.623	0.068	5.521	13.126	< 0.001	0.001	<i>Ascomycota</i>	<i>Gibberella</i>
ASV_676	0.109	0.010	5.341	10.45	< 0.001	0.002	Unassigned	Unassigned
ASV_21	2.09	0.330	4.97	15.001	< 0.001	0.003	Unassigned	Unassigned

ASV_634	0.21	0.010	6.04	10.772	< 0.001	0.003	Unassigned	Unassigned
ASV_11	12.272	0.706	6.203	17.644	< 0.001	0.004	<i>Ascomycota</i>	Unassigned
ASV_458	0.073	0.013	4.278	9.698	< 0.001	0.006	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_306	0.119	0.006	5.74	9.851	< 0.001	0.009	<i>Ascomycota</i>	Unassigned
ASV_1485	0.054	0.005	5.467	9.708	< 0.001	0.010	<i>Ascomycota</i>	Unassigned
ASV_44	1.093	0.204	4.333	13.613	< 0.001	0.010	<i>Ascomycota</i>	<i>Colletotrichum</i>
ASV_914	0.022	0.002	5.214	8.381	< 0.001	0.017	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_4	10.641	3.117	3.796	16.963	0.001	0.018	<i>Ascomycota</i>	<i>Colletotrichum</i>
ASV_2	14.58	7.470	2.89	17.452	0.001	0.019	Unassigned	Unassigned
ASV_204	0.05	0.018	3.167	9.039	0.002	0.024	<i>Ascomycota</i>	<i>Phaeosphaeria</i>
ASV_6	0.006	0	5.641	5.814	0.002	0.025	<i>Ascomycota</i>	<i>Oidiodendron</i>
ASV_84	0.005	0.001	4.844	6.488	0.003	0.031	<i>Ascomycota</i>	<i>Macrophomina</i>
ASV_77	0.329	0.100	3.34	11.596	0.004	0.037	<i>Ascomycota</i>	Unassigned
ASV_241	0.041	0.001	6.324	7.978	0.007	0.055	Unassigned	Unassigned
ASV_10	0.004	0	4.899	5.401	0.007	0.055	<i>Ascomycota</i>	Unassigned
ASV_88	0.218	0.153	2.806	11.632	0.01	0.073	<i>Ascomycota</i>	Unassigned
ASV_267	0.001	0	3.611	4.881	0.011	0.073	<i>Ascomycota</i>	<i>Pseudallescheria</i>
ASV_1471	0.028	0.016	2.376	8.249	0.011	0.073	<i>Ascomycota</i>	<i>Fusarium</i>
ASV_207	0.042	0.019	3.419	9.178	0.012	0.076	<i>Ascomycota</i>	<i>Colletotrichum</i>
ASV_164	0.048	0.019	3.507	9.212	0.013	0.079	<i>Ascomycota</i>	<i>Colletotrichum</i>
ASV_16	3.811	2.512	2.149	15.245	0.015	0.080	<i>Ascomycota</i>	<i>Cercospora</i>
ASV_104	0.001	0	3.234	4.783	0.017	0.087	<i>Ascomycota</i>	Unassigned
ASV_120	0.001	0	3.234	4.783	0.017	0.087	Unassigned	Unassigned
ASV_30	0.001	0	3.456	4.846	0.019	0.091	<i>Basidiomycota</i>	Unassigned
ASV_199	0.056	0.035	2.836	9.684	0.021	0.098	<i>Ascomycota</i>	<i>Neosetophoma</i>
ASV_23	0.001	0	2.905	4.717	0.028	0.115	<i>Glomeromycota</i>	Unassigned
ASV_43	0.001	0	2.905	4.717	0.028	0.115	<i>Glomeromycota</i>	<i>Rhizophagus</i>
ASV_46	0.001	0	2.905	4.717	0.028	0.115	<i>Glomeromycota</i>	<i>Rhizophagus</i>
ASV_183	0.001	0	2.905	4.717	0.028	0.115	<i>Ascomycota</i>	Unassigned
ASV_92	0.003	0	3.285	5.321	0.03	0.120	<i>Ascomycota</i>	<i>Macrophomina</i>
ASV_305	0.029	0.017	2.661	8.514	0.031	0.138	<i>Ascomycota</i>	Unassigned
ASV_99	0.213	0.129	2.425	11.502	0.046	0.161	<i>Ascomycota</i>	<i>Diaporthe</i>
ASV_258	0.038	0.027	2.797	9.308	0.047	0.161	<i>Ascomycota</i>	<i>Colletotrichum</i>
ASV_12	0.492	0.326	2.157	12.116	0.05	0.163	<i>Basidiomycota</i>	Unassigned