

Appendix A Illumina MiSeq sequenced fungal data (at 97% sequence similarity) based on the ITS rRNA gene

Sample	Sequences	Bases (bp)	Average Length	Minimum length	Maximum length
RS_1	31160	8034681	258	202	444
RS_2	32368	8908033	275	216	399
RS_3	32503	8501690	262	216	393
CS_3	36825	9395336	255	216	430
CS_2	44117	11282986	256	216	444
CS_1	38345	9818320	256	216	432
RM_3	32516	8621864	265	217	410
RM_2	31400	8107406	258	216	432
RM_1	30650	7984903	261	216	388
CM_2	43690	11089943	254	217	432
CM_3	31220	8112754	260	216	437
CM_1	44348	11499957	259	216	446
RW_2	31310	8009132	256	216	436
RW_3	39124	10096318	258	216	365
RW_1	38648	9965262	258	216	404
CW_1	34095	8632680	253	216	409
CW_2	36787	9301790	253	216	456
CW_3	34555	8828194	255	216	426

Appendix B Relative abundance (%) of fungal phyla across all soil samples

Phylum	RS	CS	P (t test)	RM	CM	P (t test)	RW	CW	P (t test)
Ascomycota	43.63±14.77	56.42±4.18	0.222	69.74±8.16	61.52±11.70	0.450	66.31±6.61	62.67±11.54	0.862
Basidiomycota	28.32±13.39	1.72±1.30	0.080	10.43±7.76	19.54±3.16	0.219	9.71±3.50	9.16±6.18	0.899
Zygomycota	14.00±4.42	38.93±1.79	0.005	15.35±0.31	17.15±15.03	0.846	21.31±3.33	23.13±7.44	0.862
Chytridiomycota	0.21±0.18	0.025±0.01	0.169	0.95±0.58	0.22±0.09	0.219	0.49±0.68	0.24±0.28	0.862
Others	13.84±9.93	2.90±1.12	0.001	3.52±1.18	1.57±0.58	0.109	2.18±0.85	4.80±2.00	0.122

Means ± standard deviation by Student' t test ($p < 0.05$).

Appendix C Relative abundance (%) of fungal genera across all soil samples with relative abundance > 0.5% of total fungal sequences

Phylum	Genus	RS	CS	P (t test)
Ascomycota	<i>Antarctomyces</i>	9.99±17.17	0.00±0.00	0.371
	<i>Tetracladium</i>	5.27±1.71	5.14±1.15	0.922
	<i>Fusarium</i>	4.39±1.08	9.25±2.84	0.055
	<i>Penicillium</i>	3.53±0.27	1.13±0.18	0.000
	<i>Cistella</i>	0.98±1.58	0.02±0.00	0.352
	<i>Nectria</i>	0.87±0.20	0.69±0.14	0.319
	<i>Humicola</i>	0.76±0.29	2.19±0.47	0.008
	<i>Pseudogymnoascus</i>	0.52±0.22	0.18±0.25	0.158
	<i>Myrmecridium</i>	0.50±0.09	0.01±0.01	0.001
	<i>Trichoderma</i>	0.41±0.06	0.60±0.29	0.353
	<i>Purpureocillium</i>	0.36±0.20	5.72±1.64	0.006
	<i>Chloridium</i>	0.25±0.10	0.77±0.27	0.026
	<i>Acremonium</i>	0.23±0.09	1.16±0.55	0.044
	<i>Ilyonectria</i>	0.22±0.03	0.79±0.10	0.002
	<i>Alternaria</i>	0.16±0.05	0.49±0.11	0.008
	<i>Metacordyceps</i>	0.13±0.00	4.20±3.15	0.093
	<i>Clonostachys</i>	0.10±0.01	1.05±0.18	0.001
	<i>Metarhizium</i>	0.08±0.01	0.83±0.15	0.001
<i>Cadophora</i>	0.01±0.01	3.90±1.20	0.005	
Basidiomycota	<i>Guehomyces</i>	16.87±1.03	0.56±0.22	0.000
	<i>Typhula</i>	7.01±12.12	0.05±0.05	0.376
	<i>Ustilago</i>	1.85±0.17	0.01±0.00	<0.001
	<i>Mrakiella</i>	1.32±0.15	0.07±0.03	<0.001
	<i>Mrakia</i>	0.72±0.18	0.00±0.00	0.002
	<i>Entoloma</i>	0.01±0.01	0.51±0.85	0.360
Zygomycota	<i>Mortierella</i>	13.69±4.23	38.59±1.75	0.001
Phylum	Genus	RM	CM	P (t test)
Ascomycota	<i>Penicillium</i>	28.60±3.87	28.58±7.23	0.995
	<i>Fusarium</i>	8.41±1.86	7.46±0.67	0.440
	<i>Tetracladium</i>	2.38±0.57	1.20±0.95	0.126
	<i>Bipolaris</i>	1.90±0.38	0.08±0.03	0.001
	<i>Humicola</i>	1.43±0.43	0.74±0.09	0.056
	<i>Gibberella</i>	1.21±0.53	0.34±0.08	0.045
	<i>Myrmecridium</i>	0.91±0.47	0.31±0.18	0.108
	<i>Trichoderma</i>	0.81±0.14	2.23±0.65	0.018
	<i>Chloridium</i>	0.65±0.32	0.78±0.05	0.567
	<i>Cyphellophora</i>	0.60±0.62	0.02±0.02	0.188
	<i>Myrothecium</i>	0.57±0.27	0.19±0.02	0.075
	<i>Nectria</i>	0.56±0.04	0.36±0.07	0.015
	<i>Chaetomium</i>	0.50±0.41	0.16±0.07	0.230
<i>Exophiala</i>	0.46±0.11	0.80±0.25	0.082	

	<i>Phialocephala</i>	0.12±0.06	1.82±2.53	0.309
	<i>Talaromyces</i>	0.11±0.05	1.88±2.32	0.261
Basidiomycota	<i>Guehomyces</i>	6.32±2.29	17.90±3.14	0.006
	<i>Entoloma</i>	2.60±4.48	0.01±0.00	0.375
Zygomycota	<i>Mortierella</i>	15.24±0.31	17.09±15.04	0.839
Phylum	Genus	RW	CW	P (t test)
Ascomycota	<i>Alternaria</i>	13.01±1.38	3.88±2.35	0.005
	<i>Fusarium</i>	4.62±1.06	4.77±0.74	0.845
	<i>Cadophora</i>	4.29±1.91	0.12±0.02	0.022
	<i>Tetracladium</i>	3.49±0.68	2.33±0.81	0.149
	<i>Septoria</i>	2.37±4.10	0.00±0.00	0.373
	<i>Acremonium</i>	1.99±0.56	0.23±0.05	0.006
	<i>Gibberella</i>	1.87±0.47	3.38±0.94	0.072
	<i>Chloridium</i>	1.85±2.32	0.45±0.02	0.355
	<i>Monographella</i>	1.52±0.14	0.25±0.09	<0.001
	<i>Humicola</i>	1.44±0.43	2.18±0.58	0.151
	<i>Stagonosporopsis</i>	1.30±0.98	0.15±0.10	0.116
	<i>Mycosphaerella</i>	1.10±0.36	1.70±0.80	0.359
	<i>Bipolaris</i>	1.06±0.35	1.01±0.43	0.788
	<i>Nectria</i>	0.99±0.29	0.92±0.43	0.869
	<i>Penicillium</i>	0.76±0.19	0.46±0.05	0.038
	<i>Hydropisphaera</i>	0.75±0.02	0.05±0.04	<0.001
	<i>Microdochium</i>	0.60±0.21	2.28±0.20	<0.001
	<i>Clonostachys</i>	0.54±0.24	0.23±0.04	0.103
	<i>Myrmecridium</i>	0.29±0.16	1.40±0.88	0.096
	<i>Apodus</i>	0.26±0.33	0.50±0.30	0.424
<i>Leptosphaeria</i>	0.25±0.16	0.62±0.25	0.080	
<i>Sarocladium</i>	0.23±0.03	0.68±0.32	0.056	
<i>Myrothecium</i>	0.08±0.01	2.76±1.26	0.023	
<i>Lectera</i>	0.08±0.03	3.10±0.76	0.002	
<i>Articulospora</i>	0.08±0.05	3.32±3.11	0.146	
Basidiomycota	<i>Guehomyces</i>	5.86±2.89	2.42±0.40	0.112
	<i>Cryptococcus</i>	1.50±0.58	1.49±0.91	0.985
	<i>Conocybe</i>	0.59±1.01	0.00±0.00	0.374
	<i>Mrakiella</i>	0.09±0.02	0.64±0.45	0.090
Zygomycota	<i>Mortierella</i>	21.18±3.31	22.77±7.41	0.756

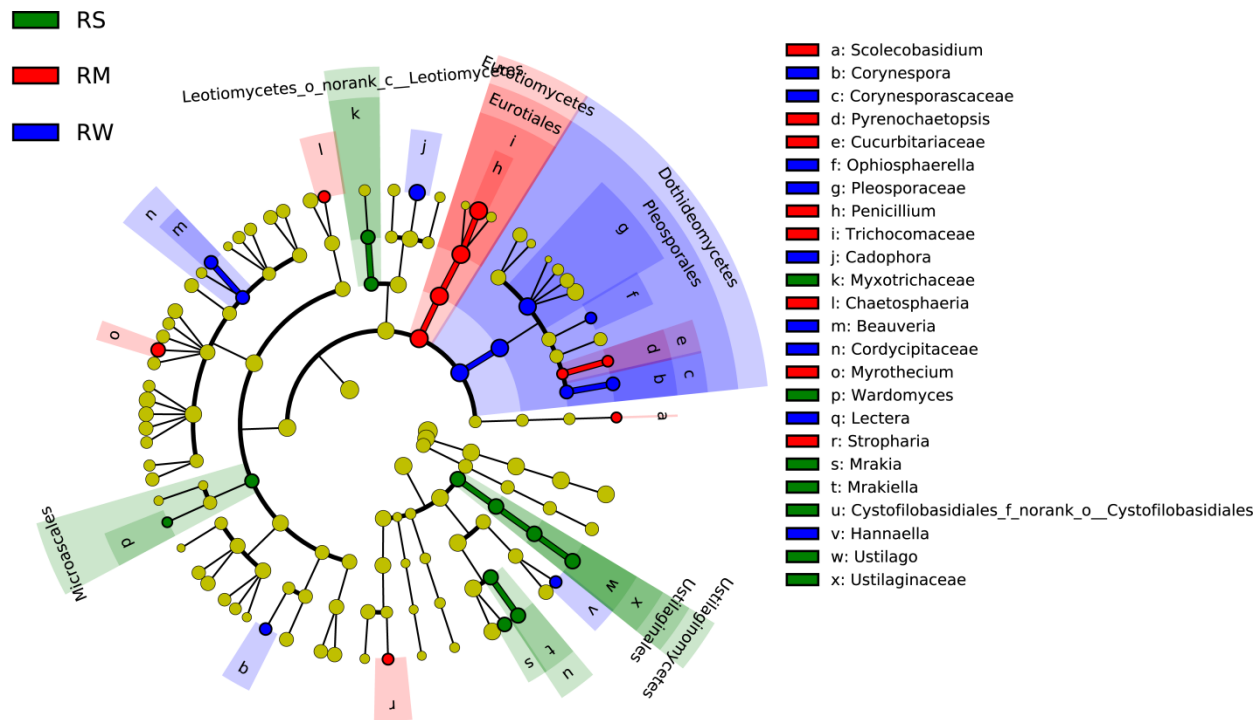
Means ± standard deviation by Student' t test (p < 0.05).

Phylum	Genus	CS	CM	CW	P
Ascomycota	<i>Fusarium</i>	9.25±2.84	7.46±0.67	4.77±0.74	0.026
	<i>Purpureocillium</i>	5.72±1.64	0.08±0.02	0.09±0.01	0.032
	<i>Tetracladium</i>	5.14±1.15	1.20±0.95	2.33±0.81	0.027
	<i>Metacordyceps</i>	4.20±3.15	0.01±0.00	0.04±0.02	0.142

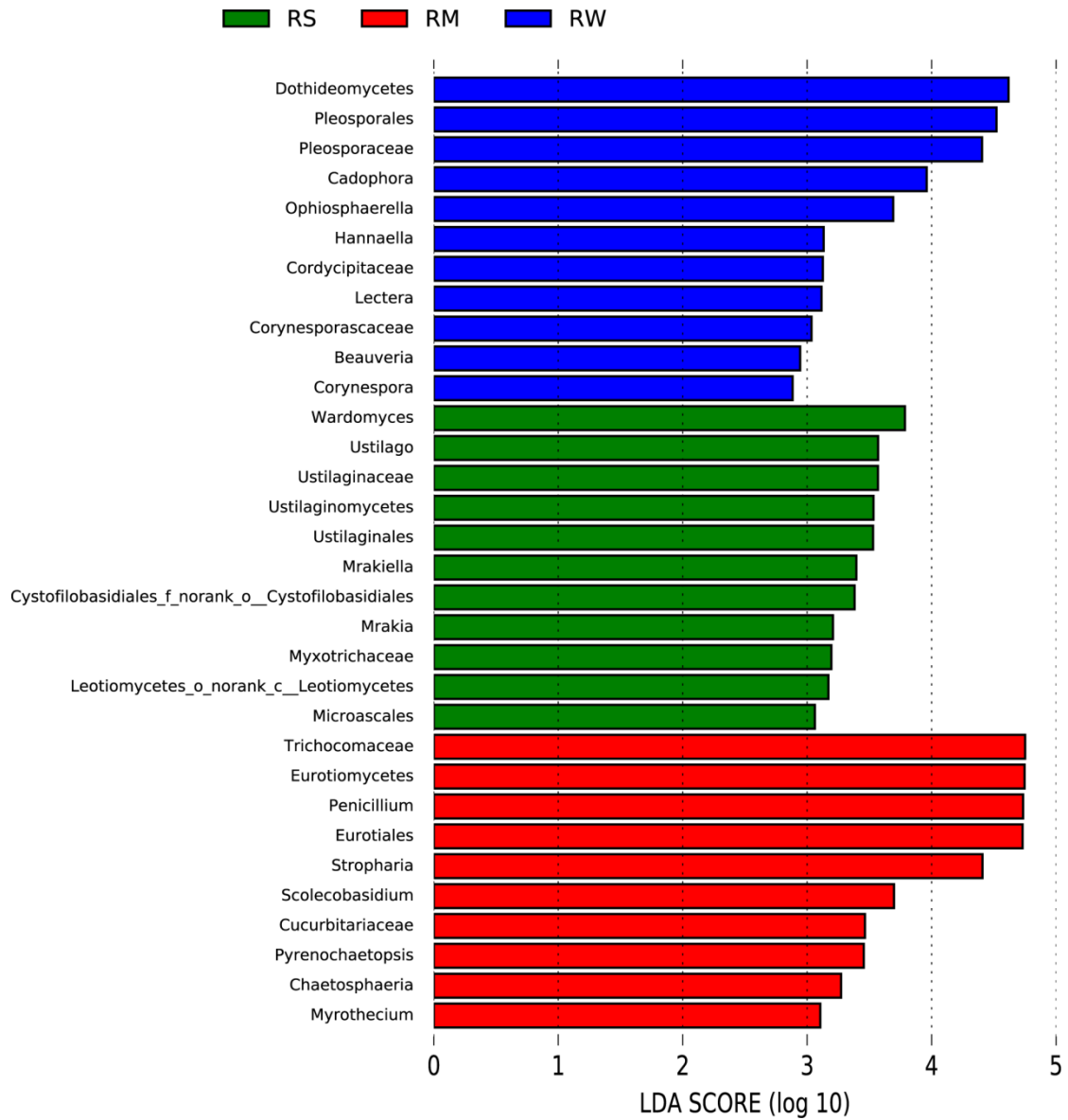
	<i>Cadophora</i>	3.90±1.20	0.04±0.02	0.12±0.02	0.010
	<i>Humicola</i>	2.19±0.47	0.74±0.09	2.18±0.58	0.021
	<i>Acremonium</i>	1.16±0.55	0.28±0.30	0.23±0.05	0.172
	<i>Penicillium</i>	1.13±0.18	28.58±7.23	0.46±0.05	0.010
	<i>Clonostachys</i>	1.05±0.18	0.15±0.03	0.23±0.04	0.005
	<i>Metarhizium</i>	0.83±0.15	0.04±0.03	0.29±0.07	0.004
	<i>Ilyonectria</i>	0.79±0.10	0.17±0.05	0.10±0.04	0.004
	<i>Chloridium</i>	0.77±0.27	0.78±0.05	0.45±0.02	0.008
	<i>Nectria</i>	0.69±0.14	0.36±0.07	0.92±0.43	0.051
	<i>Trichoderma</i>	0.60±0.29	2.23±0.65	0.23±0.02	0.037
	<i>Alternaria</i>	0.49±0.11	0.29±0.16	3.88±2.35	0.135
	<i>Gibberella</i>	0.36±0.12	1.21±0.08	3.38±0.94	0.024
	<i>Exophiala</i>	0.21±0.07	0.80±0.25	0.29±0.07	0.056
	<i>Microdochium</i>	0.17±0.08	0.28±0.10	2.28±0.20	0.000
	<i>Phialocephala</i>	0.15±0.07	1.82±2.53	0.02±0.00	0.077
	<i>Leptosphaeria</i>	0.08±0.05	0.14±0.02	0.62±0.25	0.072
	<i>Lectera</i>	0.06±0.02	0.00±0.00	3.10±0.76	0.018
	<i>Apodus</i>	0.04±0.05	0.08±0.06	0.50±0.30	0.150
	<i>Talaromyces</i>	0.03±0.01	1.88±2.32	0.10±0.03	0.096
	<i>Bipolaris</i>	0.02±0.00	1.90±0.03	1.01±0.43	0.052
	<i>Myrmecridium</i>	0.01±0.01	0.31±0.18	1.40±0.88	0.106
	<i>Articulospora</i>	0.00±0.00	0.02±0.02	3.32±3.11	0.297
	<i>Sarocladium</i>	0.00±0.00	0.08±0.02	0.68±0.32	0.063
Basidiomycota	<i>Guehomyces</i>	0.56±0.22	17.90±3.14	2.42±0.40	0.003
	<i>Entoloma</i>	0.51±0.85	0.01±0.00	0.00±0.00	0.162
	<i>Cryptococcus</i>	0.09±0.01	0.33±0.16	1.49±0.91	0.103
	<i>Mrakiella</i>	0.07±0.03	0.24±0.13	0.64±0.45	0.149
Zygomycota	<i>Mortierella</i>	38.59±1.75	17.09±15.04	22.77±7.41	0.075
Phylum	Genus	RS	RM	RW	P
Ascomycota	<i>Antarctomyces</i>	9.99±17.17	0.00±0.00	0.00±0.00	0.417
	<i>Tetracladium</i>	5.27±1.71	2.38±0.57	3.49±0.68	0.111
	<i>Fusarium</i>	4.39±1.08	8.41±1.86	4.62±1.06	0.094
	<i>Penicillium</i>	3.53±0.27	28.60±3.87	0.76±0.19	0.001
	<i>Cistella</i>	0.98±1.58	0.02±0.02	0.04±0.02	0.313
	<i>Nectria</i>	0.87±0.20	0.56±0.04	0.99±0.29	0.120
	<i>Humicola</i>	0.76±0.29	1.43±0.43	1.44±0.43	0.150
	<i>Pseudogymnoascus</i>	0.52±0.22	0.09±0.03	0.04±0.02	0.055
	<i>Myrmecridium</i>	0.50±0.09	0.91±0.47	0.29±0.16	0.182
	<i>Trichoderma</i>	0.41±0.06	0.81±0.14	0.41±0.28	0.056
	<i>Gibberella</i>	0.41±0.04	0.34±0.53	1.87±0.47	0.030
	<i>Purpureocillium</i>	0.36±0.20	0.38±0.03	0.45±0.25	0.896
	<i>Exophiala</i>	0.34±0.05	0.46±0.11	0.38±0.01	0.408
	<i>Microdochium</i>	0.31±0.05	0.37±0.16	0.60±0.21	0.189
	<i>Chloridium</i>	0.25±0.10	0.65±0.32	1.85±2.32	0.244

	<i>Acremonium</i>	0.23±0.09	0.39±0.03	1.99±0.56	0.039
	<i>Ilyonectria</i>	0.22±0.03	0.30±0.03	0.32±0.06	0.120
	<i>Alternaria</i>	0.16±0.05	0.28±0.10	13.01±1.38	0.001
	<i>Metacordyceps</i>	0.13±0.00	0.06±0.02	0.13±0.14	0.029
	<i>Clonostachys</i>	0.10±0.01	0.20±0.04	0.54±0.24	0.047
	<i>Hydropisphaera</i>	0.08±0.11	0.01±0.01	0.75±0.02	0.000
	<i>Metarhizium</i>	0.08±0.01	0.11±0.03	0.16±0.03	0.018
	<i>Monographella</i>	0.07±0.03	0.08±0.01	1.52±0.14	0.001
	<i>Bipolaris</i>	0.05±0.01	0.08±0.38	1.06±0.35	0.010
	<i>Myrothecium</i>	0.05±0.01	0.57±0.27	0.08±0.01	0.013
	<i>Phialocephala</i>	0.03±0.00	0.12±0.06	0.03±0.01	0.182
	<i>Cyphellophora</i>	0.03±0.02	0.60±0.62	0.04±0.03	0.364
	<i>Talaromyces</i>	0.02±0.00	0.11±0.05	0.49±0.25	0.089
	<i>Cadophora</i>	0.01±0.01	0.02±0.01	4.29±1.91	0.054
	<i>Chaetomium</i>	0.08±0.02	0.50±0.41	0.06±0.02	0.293
	<i>Septoria</i>	0.00±0.00	0.00±0.00	2.37±4.10	0.420
Basidiomycota	<i>Guehomyces</i>	16.87±1.03	6.32±2.29	5.86±2.89	0.006
	<i>Typhula</i>	7.01±12.12	0.04±0.05	0.01±0.01	0.548
	<i>Ustilago</i>	1.85±0.17	0.05±0.04	0.01±0.00	0.002
	<i>Mrakiella</i>	1.32±0.15	0.32±0.08	0.09±0.02	0.003
	<i>Mrakia</i>	0.72±0.18	0.02±0.00	0.05±0.03	0.026
	<i>Cryptococcus</i>	0.26±0.10	0.24±0.06	1.50±0.58	0.088
	<i>Entoloma</i>	0.01±0.01	2.60±4.48	0.04±0.05	0.474
	<i>Conocybe</i>	0.00±0.00	0.00±0.00	0.59±1.01	0.423
Zygomycota	<i>Mortierella</i>	13.69±4.23	15.24±0.31	21.18±3.31	0.151

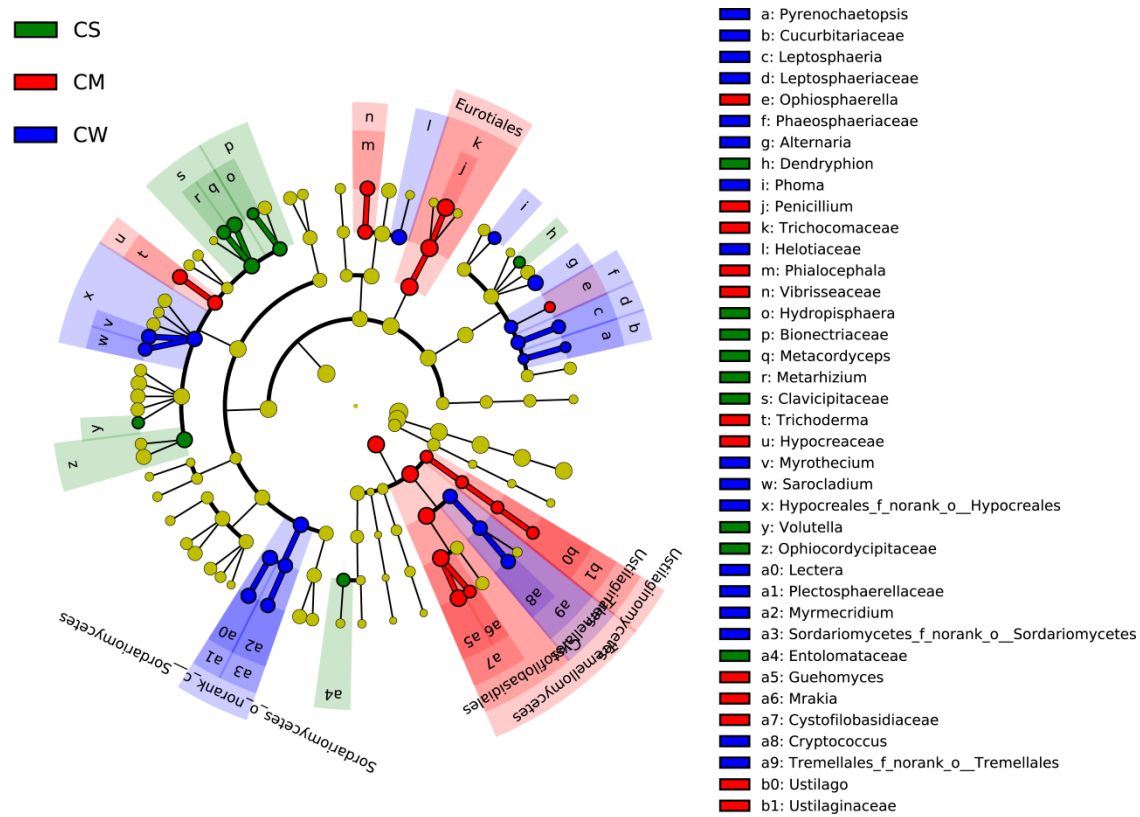
Means ± standard deviation by ANOVA ($p < 0.05$).



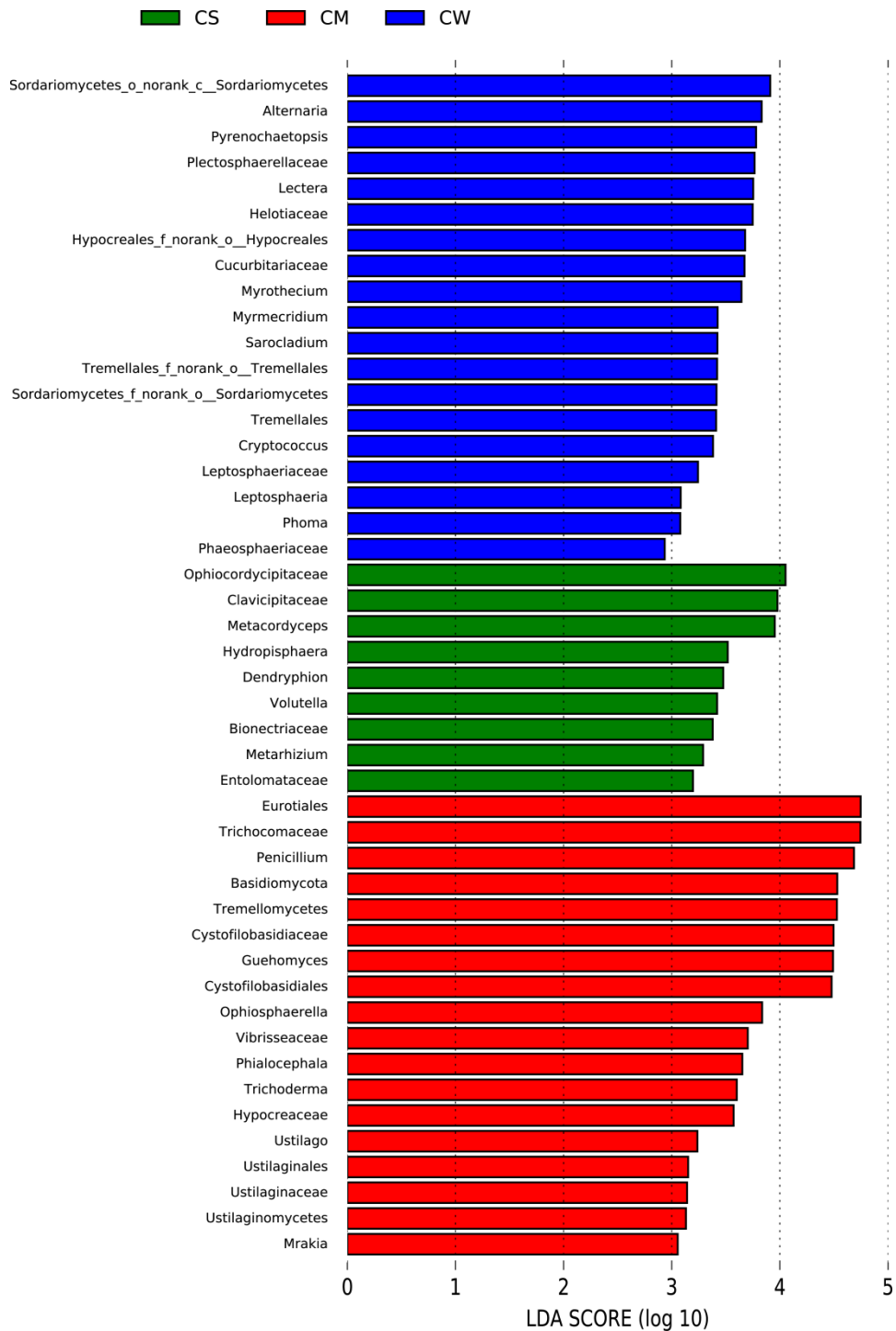
Appendix D Taxonomic cladogram produced from LEfSe analysis. The phylum, class, order, family, and genus levels are listed in order from inside to outside of the cladogram and the labels for levels of family and genus are abbreviated by a single letter. Green, red and blue showed taxa enriched in RS, RM and RW, respectively, whereas the yellow circles represented the taxa with no significant differences among three cropping systems.



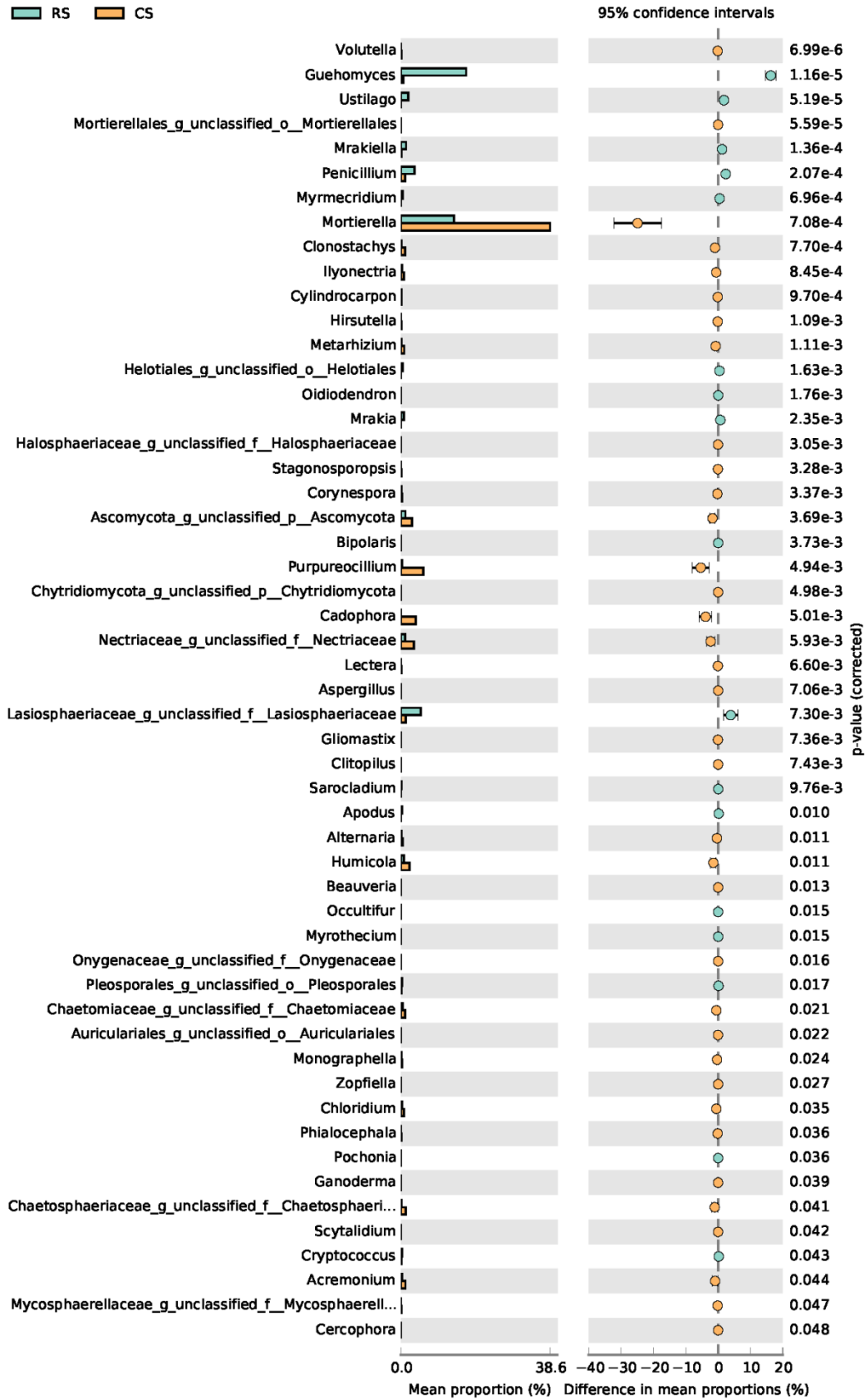
Appendix E Histogram of the linear discriminant analysis (LDA) scores. Taxa enriched in RS are shown in green with a positive LDA score, RM in red with positive LDA score and RW in blue with a positive LDA score.



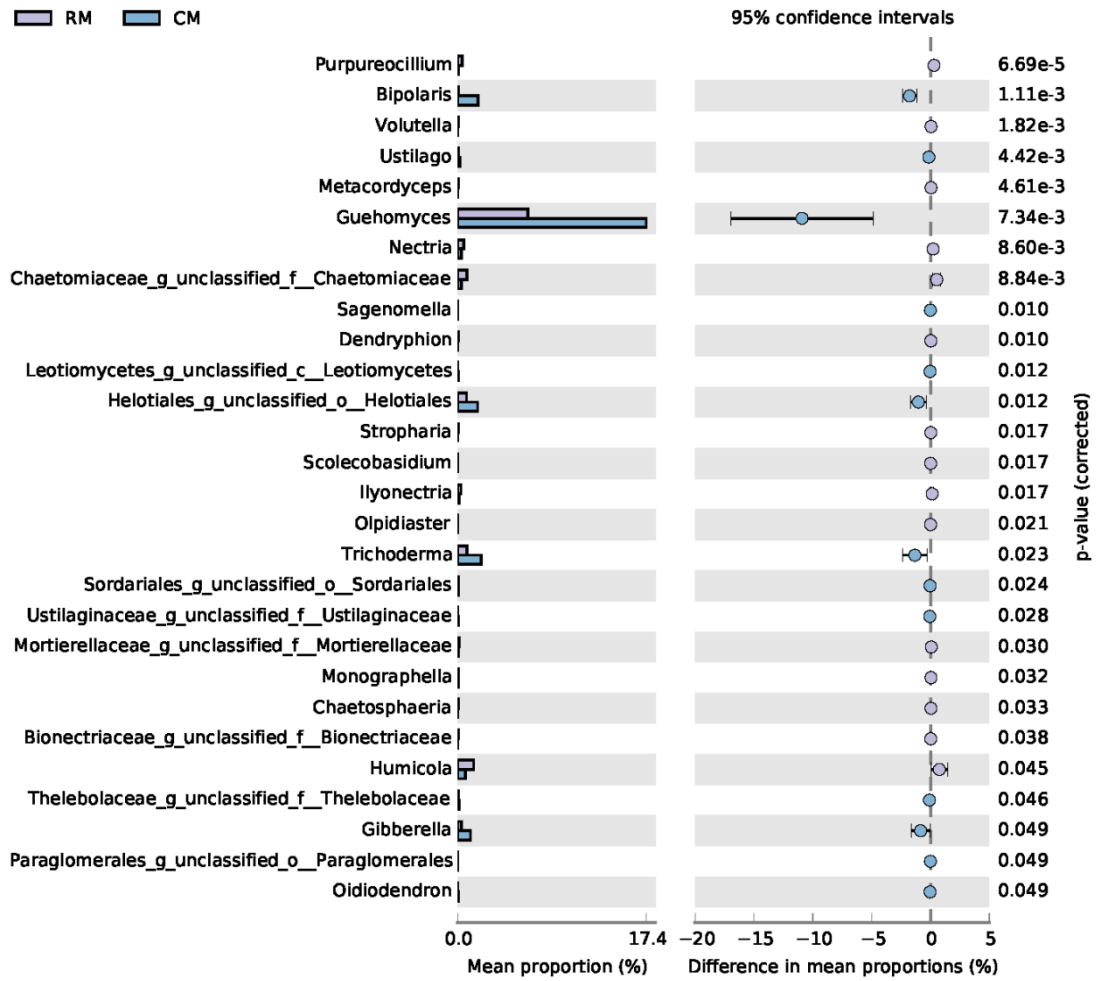
Appendix F Taxonomic cladogram produced from LefSe analysis. The phylum, class, order, family, and genus levels are listed in order from inside to outside of the cladogram and the labels for levels of family and genus are abbreviated by a single letter. Green, red and blue showed taxa enriched in CS, CM and CW, respectively, whereas the yellow circles represented the taxa with no significant differences among three cropping systems.



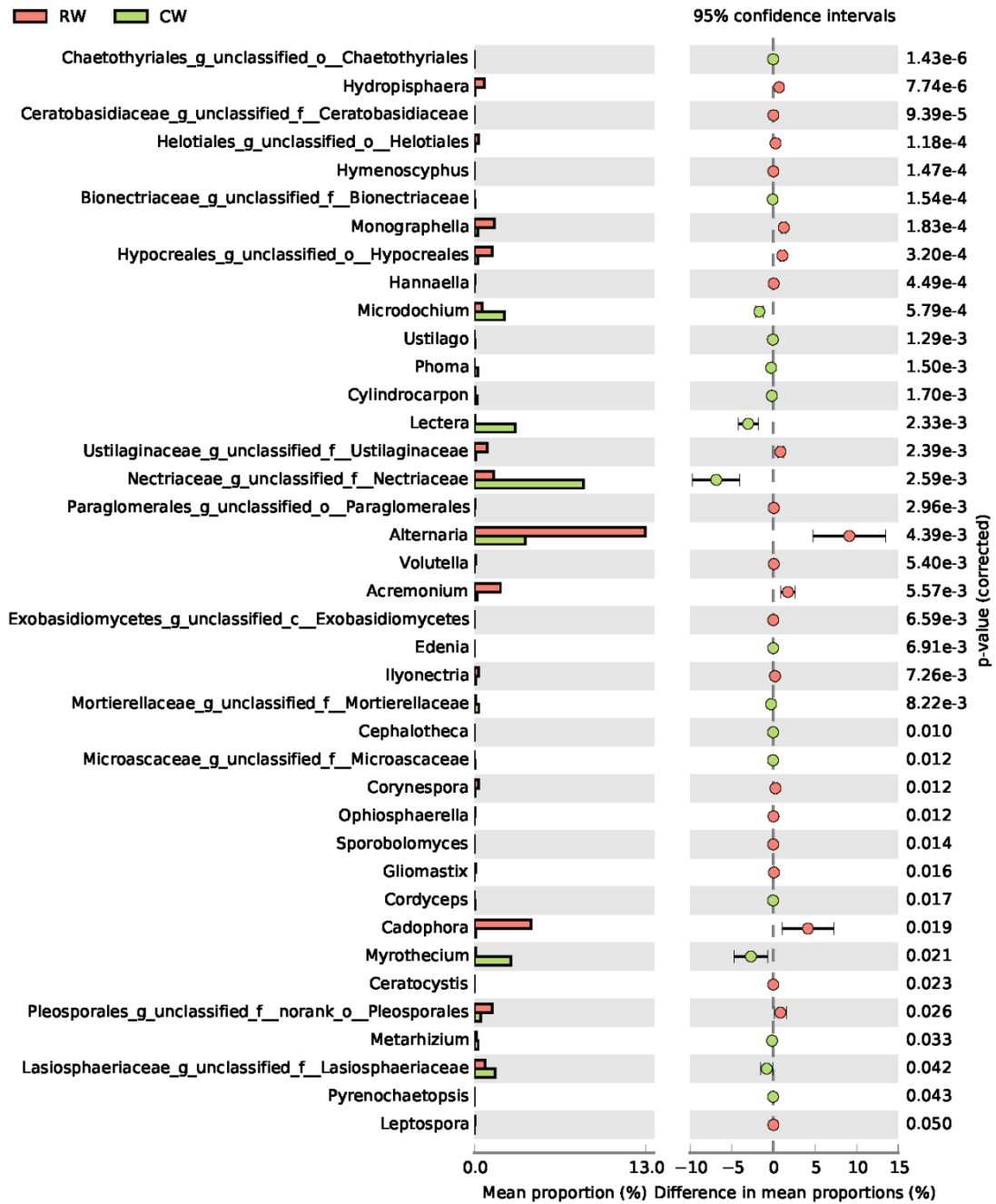
Appendix G Histogram of the linear discriminant analysis (LDA) scores. Taxa enriched in CS are shown in green with a positive LDA score, CM in red with positive LDA score and CW in blue with a positive LDA score.



Appendix H The variance of relative abundance of fungal genera in response to CS compared with RS at 95% confidence intervals.



Appendix I The variance of relative abundance of fungal genera in response to CM compared with RM at 95% confidence intervals.



Appendix J The variance of relative abundance of fungal genera in response to CW compared with RW at 95% confidence intervals.

Appendix K List of abbreviations

Soybean rotation	RS
Maize rotation	RM
Wheat rotation	RW
Long-term continuous soybean cropping	CS
Long-term continuous maize cropping	CM
Long-term continuous wheat cropping	CW
Quantitative real-time PCR	qPCR
Operational taxonomic units	OTUs
Principal coordinate analysis	PCoA
The similarity percentage analysis	SIMPER
Linear discriminant analysis	LDA
linear discriminant analysis effect size	LEfSe
