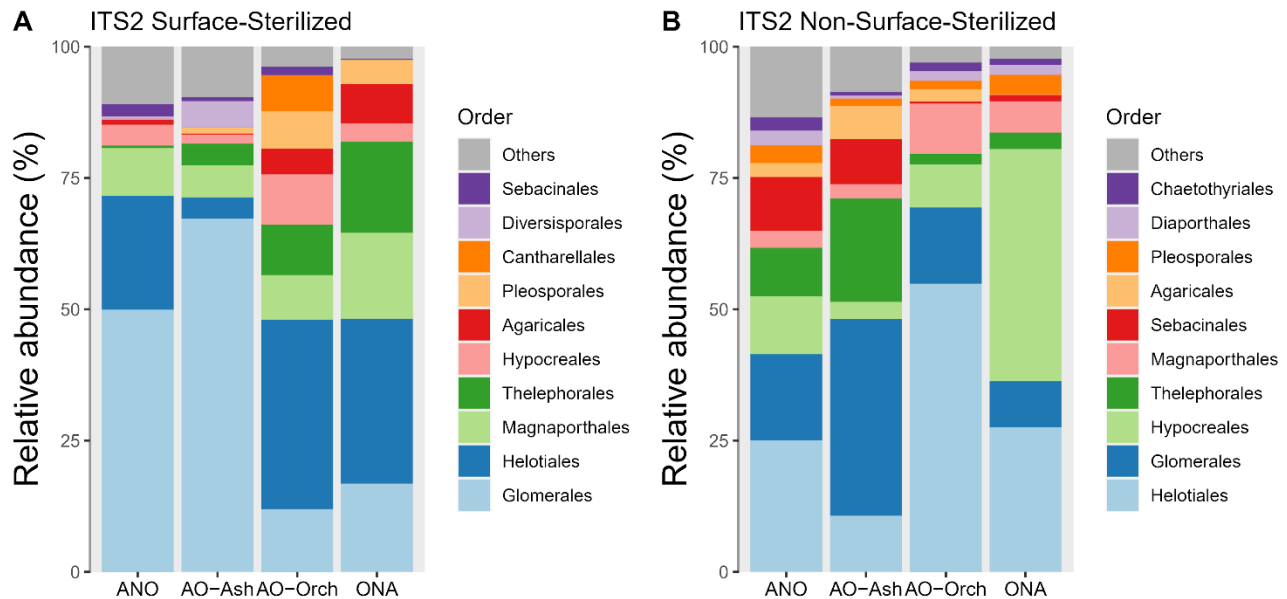
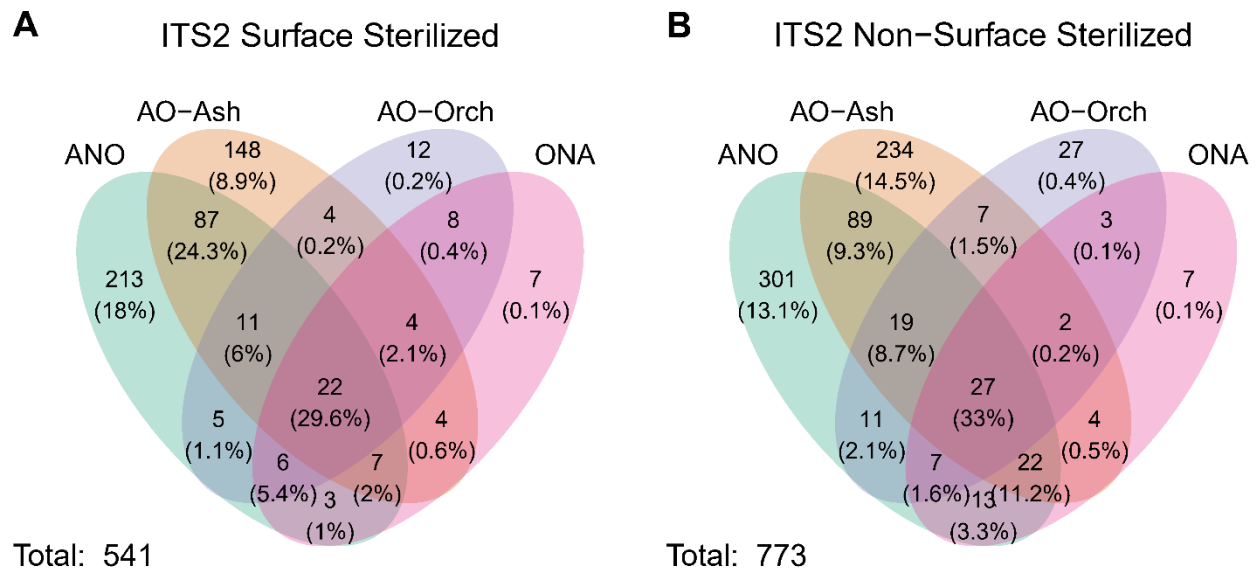


Supplementary Material

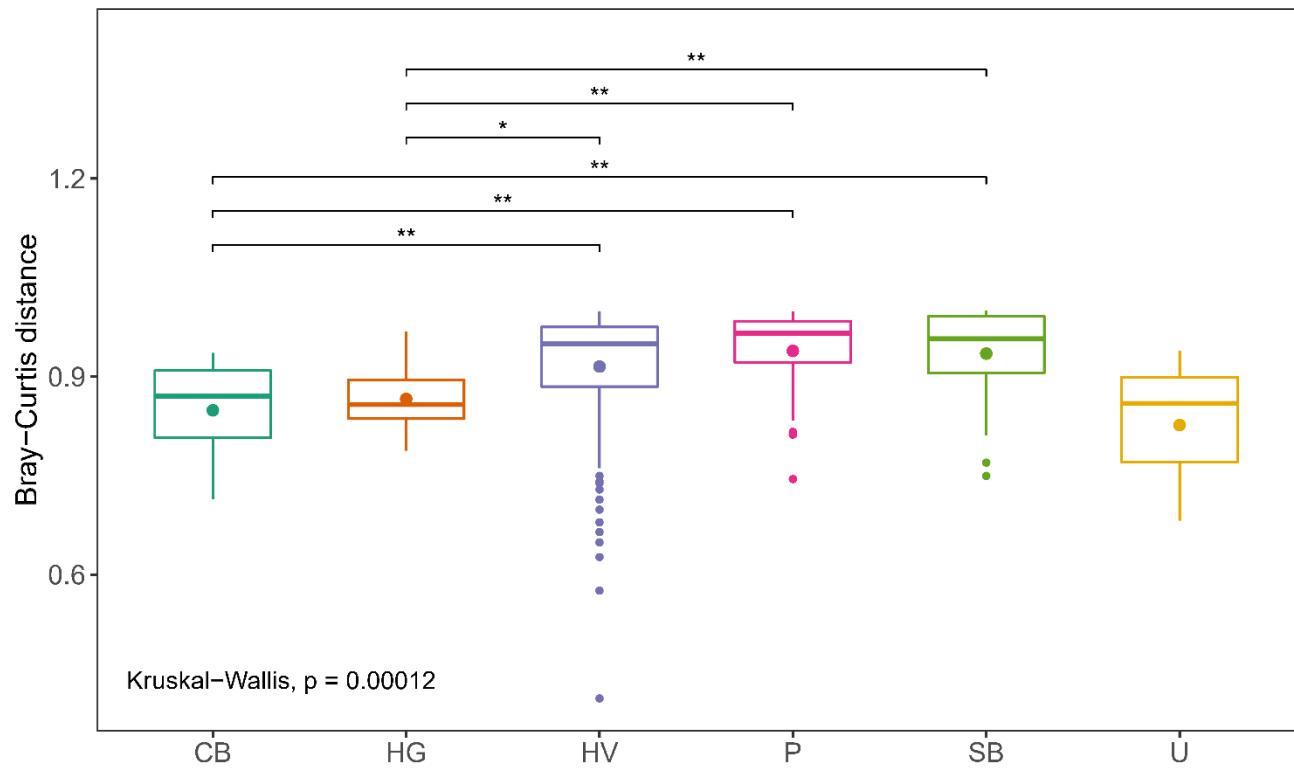
1 Supplementary Data



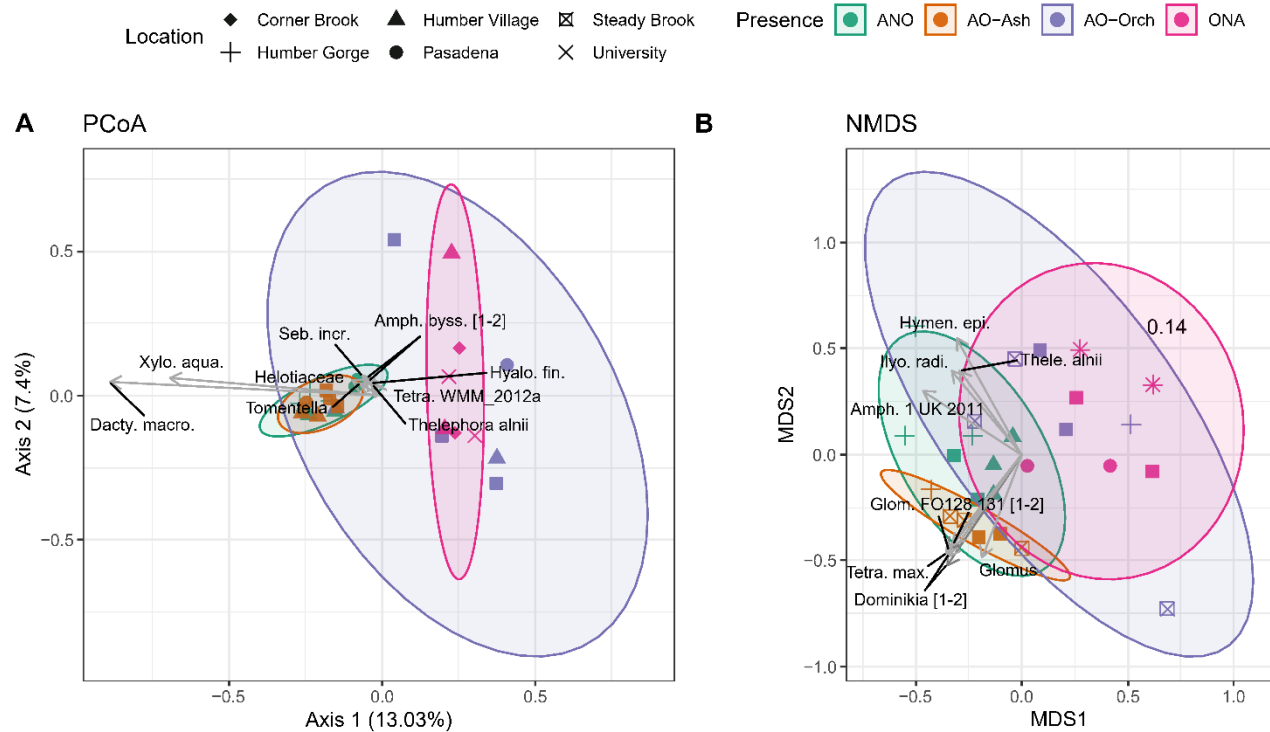
Supplementary Figure 1. Stacked relative abundances of (A) ITS2 surface-sterilized, and (B) ITS2 non-surface-sterilized ASVs found in showy lady's slipper orchid (*Cypripedium reginae*) and black ash (*Fraxinus nigra*) roots. Sample groups: ANO – ash, no orchid; AO-Ash – ash near orchid, AO-Orch – orchid near ash, and ONA – orchid, no ash.



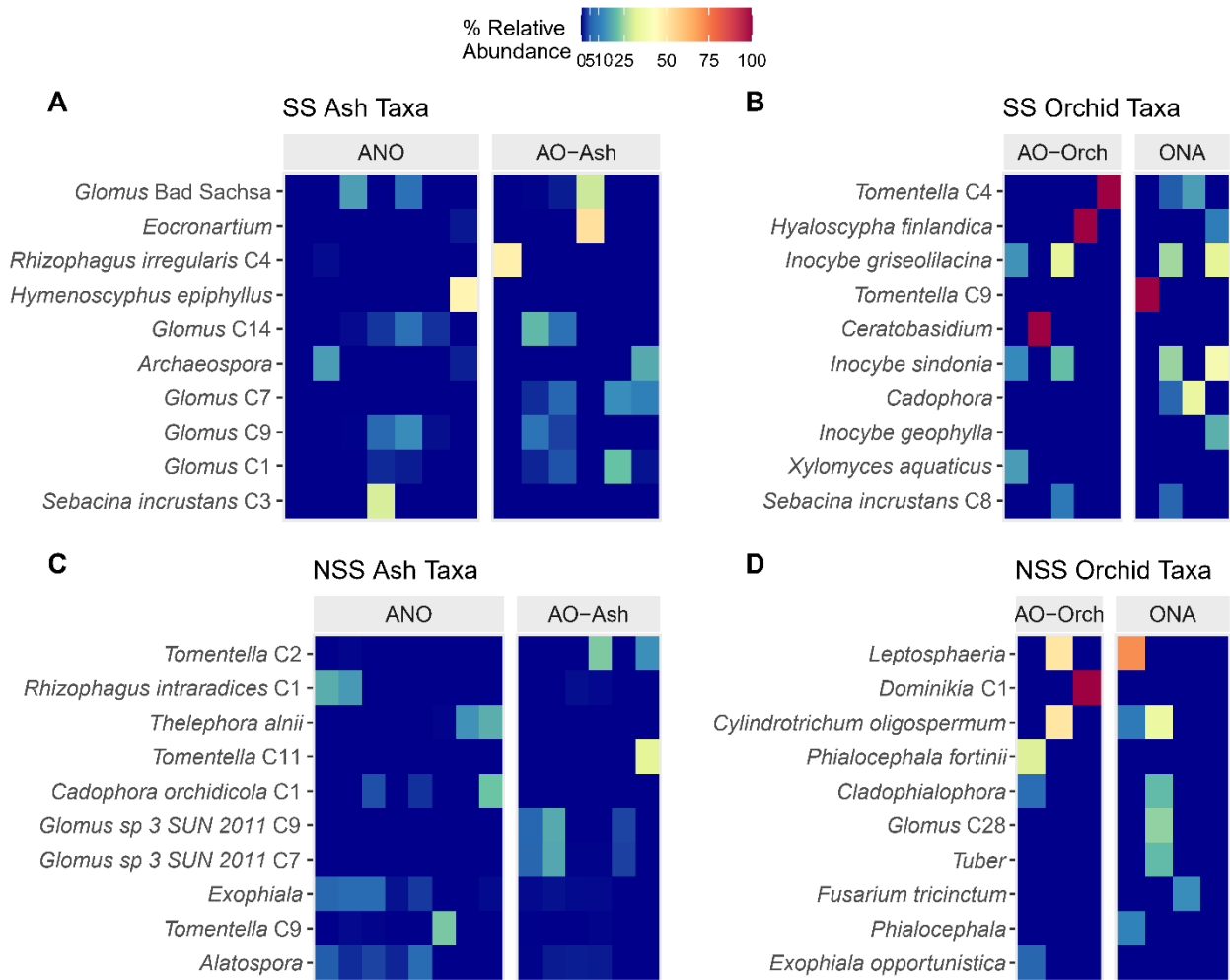
Supplementary Figure 2. A Venn diagram of ITS (A) surface-sterilized, and (B) non-surface-sterilized roots of showy lady's slipper orchid (*Cypripedium reginae*) and black ash (*Fraxinus nigra*) and fungal taxa. Sample groups: ANO – ash, no orchid; AO-Ash – ash near orchid, AO-Orch – orchid near ash, and ONA – orchid, no ash. Counts are distinct (unclustered) ASVs and percentages are the proportion of read counts from the total.



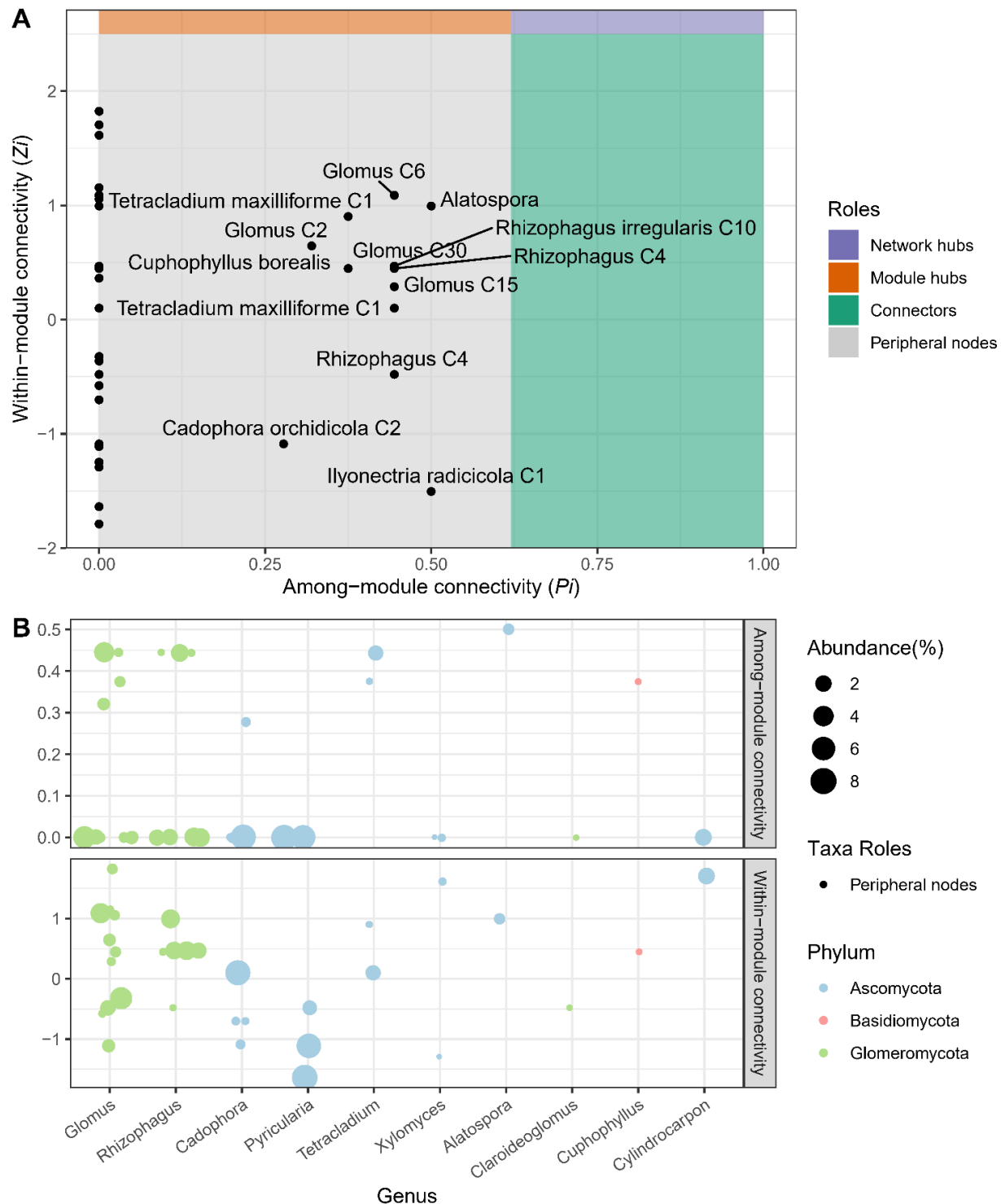
Supplementary Figure 3. A comparison of ITS2 ASV counts, from showy lady's slipper orchid (*Cypripedium reginae*) and black ash (*Fraxinus nigra*) roots, separated by location. Location codes: CB – Corner Brook, HG – Humber Gorge, HV – Humber Village, P – Pasadena, SB – Steady Brook, U – Grenfell Campus University. Dots within each boxplot represent group means. Significance codes: * as p -value < 0.05 , ** as p -value < 0.01 , *** as p -value < 0.001 , and **** as p -value < 0.0001 . P-values based on Dunn's test of multiple comparisons after Benjamini-Hotchberg p -value adjustment.



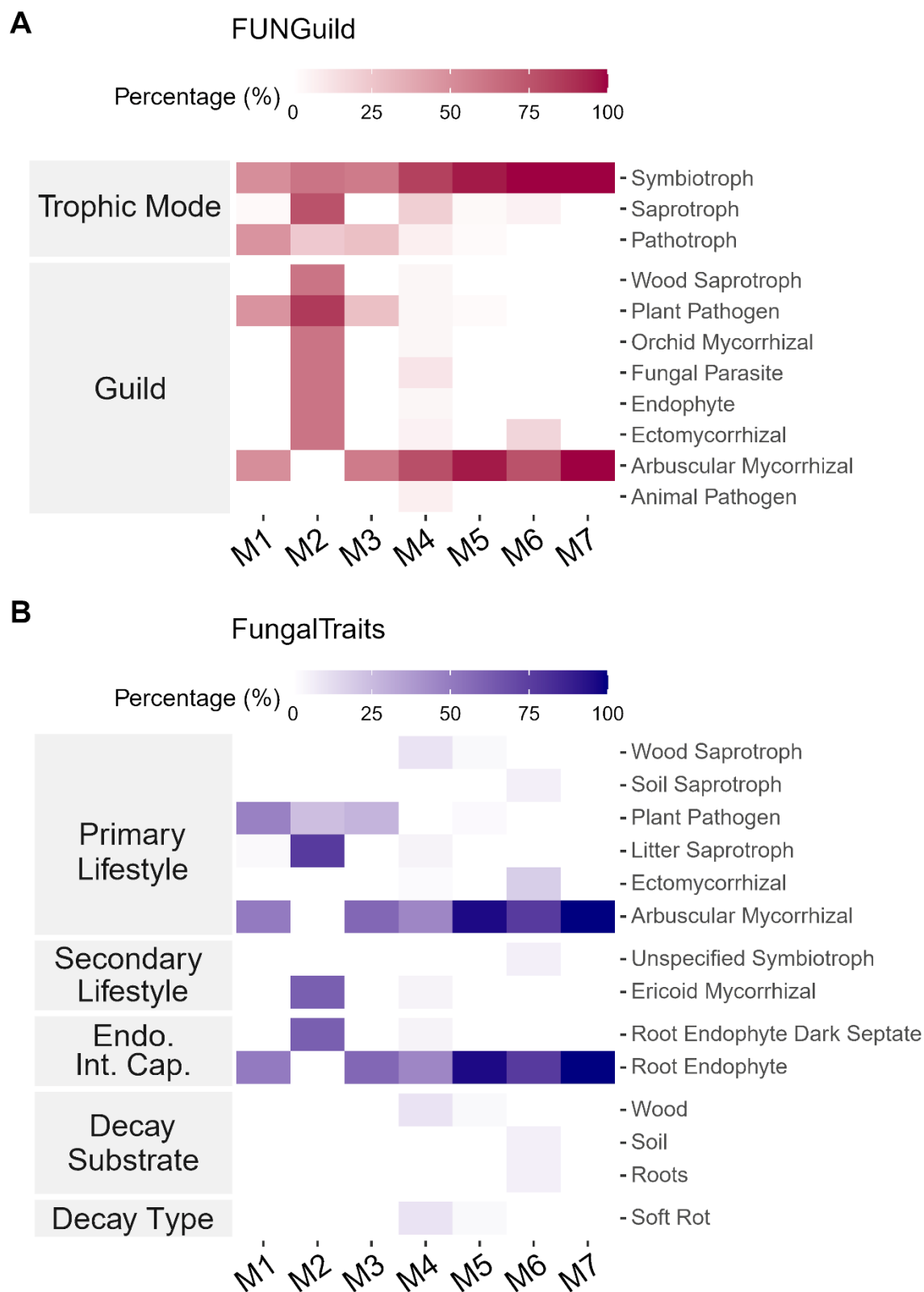
Supplementary Figure 4. (A) PCoA and (B) NMDS of non-surface-sterilized of showy lady's slipper orchid (*Cypripedium reginae*) and black ash (*Fraxinus nigra*) roots. All samples are identified by orchid-ash presence (ANO – ash, no orchid; AO-Ash – ash near orchid, AO-Orch – orchid near ash, and ONA – orchid, no ash) and location. PCoA sample distances calculated using Bray-Curtis. NMDS model stress values are included. Abbreviated ITS2 ASVs include: Amph. byss. – *Amphinema byssoides*, Dacty. macro. – *Dactylonectria macrodidyma*, Glom. – *Glomus*, Hyalo. fin. – *Hyaloscypha finlandica*, Hymen. epi. – *Hymenoscyphus epiphyllus*, Ilyo. radi. – *Ilyonectria radicola*, Seb. incr. – *Sebacina incrustans*. Tetra. max. – *Tetracladium maxilliforme*, Thele. alnii – *Thelephora alnii*, Xylo. aqua. – *Xylomyces aquaticus*. Where present, values in square brackets after each label indicate the number of clustered vectors (e.g., *Dominikia* [1-2] means there are two *Dominikia* vectors).



Supplementary Figure 5. Heatmap of the top 10 abundant fungal genera (ITS2) found unique to black ash (*Fraxinus nigra*) and showy lady's slipper orchid (*Cypripedium reginae*). Panels split samples by (A) surface-sterilized (SS) ash, (B) SS orchid, (C) non-surface-sterilized (NSS) ash, and (D) NSS orchid. Sample groups: ANO – ash, no orchid; AO-Ash – ash near orchid, AO-Orch – orchid near ash, and ONA – orchid, no ash. Identical names followed by the same clade (C) number signify ASVs that are within the same ML clade (<0.01 evolutionary distance, and likely belong to a single organism).



Supplementary Figure 6. Within-module (Z_i) and among-module (P_i) connectivity plot of surface-sterilized (SS) ITS2 ASVs shared between black ash (*Fraxinus nigra*) and showy lady's slipper orchid (*Cypripedium reginae*). (A) Z_i - P_i plot showing the distribution of ASVs based on topological roles, with labeled points highlighting ASVs with high relative Z_i and P_i scores and (B) distribution of all genera within the network. Abundance calculated within the 50 ASVs found in the trimmed SS dataset.



Supplementary Figure 7. Functional traits of weighted relative abundance taxa within each module (1-7) of the network analysis. Traits determined by (A) FUNGuild, and (B) FungalTraits. Endo. Int. Cap. – Endophytic Interaction Capability.

Supplementary Table 1. Sample metadata and grouping variables in analysis. Each sample was sequenced using ITS2 specific primers.

Sample	Presence	Plant	Location	Surface-Sterilized	Batch
ANOGH5	ANO	Ash	Humber Gorge	No	1
ANOGH3	ANO	Ash	Humber Gorge	No	1
ANOGH2	ANO	Ash	Humber Gorge	No	2
ANOHG1	ANO	Ash	Humber Gorge	Yes	1
ANOGH4	ANO	Ash	Humber Gorge	Yes	2
ANO5	ANO	Ash	Humber Village	No	1
ANO4	ANO	Ash	Humber Village	No	2
ANO1	ANO	Ash	Humber Village	Yes	1
ANO3	ANO	Ash	Humber Village	Yes	1
ANO2	ANO	Ash	Humber Village	Yes	2
ANOP2	ANO	Ash	Pasadena	No	1
ANOP1	ANO	Ash	Pasadena	No	2
ANOP3	ANO	Ash	Pasadena	No	2
ANOP5	ANO	Ash	Pasadena	Yes	1
ANOP6	ANO	Ash	Pasadena	Yes	2
AO10	AO-Ash	Ash	Humber Village	No	1
AO12	AO-Ash	Ash	Humber Village	No	2
AO4	AO-Ash	Ash	Humber Village	Yes	1
AO2	AO-Ash	Ash	Humber Village	Yes	2
AO9	AO-Ash	Ash	Humber Village	Yes	2
AOP1	AO-Ash	Ash	Pasadena	No	1
AOP2	AO-Ash	Ash	Pasadena	Yes	2
AOS3	AO-Ash	Ash	Steady Brook	No	1
AOS2	AO-Ash	Ash	Steady Brook	No	2
AOS4	AO-Ash	Ash	Steady Brook	No	2
AOS1	AO-Ash	Ash	Steady Brook	Yes	1
AOS5	AO-Ash	Ash	Steady Brook	Yes	1
AO10	AO-Orch	Orch	Humber Village	No	2
AO12	AO-Orch	Orch	Humber Village	No	1
AO4	AO-Orch	Orch	Humber Village	Yes	2
AO2	AO-Orch	Orch	Humber Village	Yes	1
AO9	AO-Orch	Orch	Humber Village	Yes	1
AOP1	AO-Orch	Orch	Pasadena	No	2
AOP2	AO-Orch	Orch	Pasadena	Yes	1
AOS3	AO-Orch	Orch	Steady Brook	No	2
AOS2	AO-Orch	Orch	Steady Brook	No	1
AOS4	AO-Orch	Orch	Steady Brook	No	1

AOS1	AO-Orch	Orch	Steady Brook	Yes	2
AOS5	AO-Orch	Orch	Steady Brook	Yes	2
ONAC1	ONA	Orch	Corner Brook	No	1
ONAC5	ONA	Orch	Corner Brook	No	1
ONAC3	ONA	Orch	Corner Brook	Yes	1
ONAC2	ONA	Orch	Corner Brook	Yes	2
ONAC4	ONA	Orch	Corner Brook	Yes	2
ONA6	ONA	Orch	Humber Village	No	1
ONA5	ONA	Orch	Humber Village	No	2
ONA4	ONA	Orch	Humber Village	Yes	1
ONA3	ONA	Orch	Humber Village	Yes	2
ONA7*	ONA	Orch	Humber Village	Yes	2
ONAU2	ONA	Orch	University	No	1
ONAU3	ONA	Orch	University	No	2
ONAU5*	ONA	Orch	University	No	2
ONAU1*	ONA	Orch	University	No	2
ONAU4	ONA	Orch	University	Yes	1

* samples were filtered prior to analysis due to low read count.

Supplementary Table 2. DADA2 settings, read retention after filtration and merging steps, and manual filtration steps for each primer. Singleton cutoff parameters removed amplified sequence variants (ASVs) that had fewer than 10 reads across all samples.

Primer	ITS2
Settings	
TruncQ	2
Max Errors	2,2
Min. Len.	50
TruncLen	-
Merged Length Trim	-
Chimera Detection	Consensus
dada2	
Input	1,304,351
Filtered	868,546
Denoised	858,984
Merged	798,665
Tabled	798,665
Non-Chimera	789,387
Final Reads	678,105
ASVs	1,114
Manual Filtration	
Rel. Abund. Cutoff	0.03%
Singleton Cutoff	10 reads
Total Reads	640,427
Total ASVs	1,012
Target Reads	630,513
Target ASVs	993

Supplementary Table 3. Alpha diversity measures of ITS2 amplified sequence variants (ASVs) from surface-sterilized and non-surface-sterilized black ash (*Fraxinus nigra*) and showy lady's slipper (*Cypripedium reginae*) roots. Orchid-ash presence groups: ANO – ash, no orchid; AO-Ash – ash near orchid, AO-Orch – orchid near ash, and ONA – orchid, no ash. DMRT – Duncan's Mean Range Test.

		Surface-sterilized			Non-Surface-Sterilized		
Measure	Presence	N	mean $\pm$ SD	DMRT	N	mean $\pm$ SD	DMRT
ITS2							
Observed	ANO	7	86.57 $\pm$ 11.89	a	8	92.13 $\pm$ 22.11	a
	AO-Ash	6	73 $\pm$ 22.81	a	6	109.83 $\pm$ 31.97	a
	AO-Orch	5	20.4 $\pm$ 14.81	b	6	21.5 $\pm$ 20.4	b
	ONA	6	16.33 $\pm$ 14.26	b	6	19.33 $\pm$ 20.1	b
Shannon (H)	ANO	7	3.33 $\pm$ 0.3	a	8	3.25 $\pm$ 0.51	a
	AO-Ash	6	3.17 $\pm$ 0.63	ab	6	3.68 $\pm$ 0.55	a
	AO-Orch	5	2.52 $\pm$ 0.65	bc	6	2.28 $\pm$ 0.6	b
	ONA	6	2.21 $\pm$ 0.86	c	6	2.16 $\pm$ 1.04	b
Simpson (D)	ANO	7	0.92 $\pm$ 0.03	a	8	0.9 $\pm$ 0.09	a
	AO-Ash	6	0.9 $\pm$ 0.06	ab	6	0.93 $\pm$ 0.08	a
	AO-Orch	5	0.88 $\pm$ 0.05	ab	6	0.83 $\pm$ 0.09	a
	ONA	6	0.83 $\pm$ 0.1	b	6	0.78 $\pm$ 0.21	a
InvSimpson (1/D)	ANO	7	15.72 $\pm$ 7.78	a	8	15.08 $\pm$ 8.49	ab
	AO-Ash	6	16.97 $\pm$ 14.51	a	6	27.33 $\pm$ 16.99	a
	AO-Orch	5	11.45 $\pm$ 8.43	a	6	8.41 $\pm$ 6.31	b
	ONA	6	9.59 $\pm$ 8.44	a	6	10.34 $\pm$ 12.32	b
Fisher	ANO	7	11.38 $\pm$ 1.59	a	8	12.19 $\pm$ 2.9	a
	AO-Ash	6	9.76 $\pm$ 3.09	a	6	15.14 $\pm$ 4.29	a
	AO-Orch	5	3.8 $\pm$ 2.82	b	6	3.67 $\pm$ 2.98	b
	ONA	6	3.19 $\pm$ 2.66	b	6	3.55 $\pm$ 3.88	b

Supplementary Table 4. Taxa shared between surface-sterilized roots of showy lady's slipper orchid (*Cypripedium reginae*) and black ash (*Fraxinus nigra*) growing within 15 m of one another (Orchid and Ash), and taxa unique to surface-sterilized roots of orchid or ash, based on identifications of ITS2 metabarcoding data. Symbols denote likely trophic modes for shared orchid and ash taxa based on FUNGuild and FungalTraits: * symbiotroph, † saprotroph, ‡ pathotroph. C1, etc. refers to clade number in maximum likelihood analysis.

Orchid and Ash	Orchid	Ash
<i>Alatospora</i> [†]	<i>Cadophora</i> C2	<i>Agrocybe erebia</i>
<i>Cadophora orchidicola</i> C1* [†]	<i>Ceratobasidium albasitensis</i>	<i>Alatospora acuminata</i>
<i>Cadophora orchidicola</i> C2* [†]	<i>Hyaloscypha finlandica</i>	<i>Cadophora</i> C1
<i>Claroideoglossum claroideum</i> *	<i>Glomus</i> sp Att565 7 C1	<i>Cadophora melinii</i>
<i>Cuphophyllus borealis</i> [†]	<i>Glomus versiforme</i> C1	<i>Calyptrina capula</i>
<i>Cylindrotrichum oligospermum</i> C9 [†]	<i>Inocybe geophylla</i>	<i>Ceratobasidium</i>
<i>Dactylonectria macrodidyma</i> C1‡	<i>Inocybe griseolilacina</i> C1	<i>Cistella</i>
<i>Dactylonectria pauciseptata</i> ‡	<i>Inocybe sindonia</i> C1	<i>Cistella caricis</i>
<i>Dominikia</i> C5*	<i>Laccaria laccata</i> var. <i>pallidifolia</i>	<i>Cladophialophora</i>
<i>Glomus</i> C1*	<i>Myrmecocystis microspora</i>	<i>Clathrosporium intricatum</i>
<i>Glomus</i> C2*	<i>Sebacina incrustans</i> C16	<i>Coprinopsis</i>
<i>Glomus</i> C3*	<i>Sebacina incrustans</i> C8	<i>Cryptosporiopsis radiculicola</i>
<i>Glomus</i> C6*	<i>Tomentella</i> C9	<i>Dimorphospora foliicola</i>
<i>Glomus</i> C15*	<i>Xylomyces aquaticus</i> C2	<i>Eocronartium</i>
<i>Glomus</i> C29*	<i>Zalerion arboricola</i>	<i>Exophiala</i>
<i>Glomus</i> C30*	unknown Eurotiales	<i>Exophiala equina</i>
<i>Glomus macrocarpum</i> C1*	unknown Helotiales	<i>Exophiala opportunistica</i>
<i>Glomus macrocarpum</i> C2*	unknown Eurotiomycetes	<i>Exophiala</i> sp KL 2011f
<i>Glomus macrocarpum</i> C5*	unknown Ascomycota	<i>Glutinoglossum heptaseptatum</i>
<i>Glomus macrocarpum</i> C11*		<i>Herpotrichia juniperi</i>
<i>Glomus</i> sp 3 SUN 2011 C2*		<i>Hymenoscyphus epiphyllus</i>
<i>Glomus</i> sp 3 SUN 2011 C11*		<i>Idriella</i>
<i>Ilyonectria radiculicola</i> C1 [†]		<i>Ilyonectria robusta</i>
<i>Inocybe ochroalba</i> *		<i>Minimelanolocus obscurus</i>
<i>Leptosphaeria</i> ^{††}		<i>Mirandina breviphora</i>
<i>Pyricularia</i> C1 [‡]		<i>Neonectria</i>
<i>Pyricularia</i> C2 [‡]		<i>Orbilina</i>
<i>Remispora stellata</i> C5 [†]		<i>Paraconiothyrium</i>
<i>Rhizophagus</i> C4*		<i>Phaeomoniella prunicola</i>
<i>Rhizophagus intraradices</i> C2*		<i>Phomopsis columnaris</i>
<i>Rhizophagus intraradices</i> C3*		<i>Podospora</i>
<i>Rhizophagus intraradices</i> C5*		<i>Podospora intestinacea</i>
<i>Rhizophagus irregularis</i> C2*		<i>Pyronemataceae</i> C2
<i>Rhizophagus irregularis</i> C10*		<i>Remispora stellata</i> C6
<i>Sebacina incrustans</i> C5*		<i>Remispora stellata</i> C7

<i>Spirosphaera cupreorufescens</i> C1 [†]		<i>Remispora/Cirrenalia</i> C8
<i>Tetracladium maxilliforme</i> C1 [†]		<i>Rhexocercosporidium panacis</i> C10
<i>Tetracladium</i> sp WMM_2012a C1 [†]		<i>Rhinoclatiella</i> sp YH 2009a
<i>Tomentella</i> C4*		<i>Sebacina incrustans</i> C1
<i>Tomentella</i> C24*		<i>Sebacina incrustans</i> C3
<i>Tomentella galzinii</i> C4*		<i>Sebacina incrustans</i> C4
<i>Varicosporium</i> [†]		<i>Sebacina incrustans</i> C12
<i>Xylomyces aquaticus</i> C1 ^{†‡}		<i>Sebacina incrustans</i> C15
unknown Helotiales		<i>Sebacina incrustans</i> C17
unknown Glomeromycota*		<i>Sebacina incrustans</i> C9
unknown Ascomycota		<i>Sebacina vermifera</i> C3
		<i>Septobasidium</i>
		<i>Spirosphaera cupreorufescens</i>
		<i>Tetracladium</i> C1
		<i>Tetracladium</i> C4
		<i>Tetracladium furcatum</i> C1
		<i>Tetracladium marchalianum</i>
		<i>Trichocladium opacum</i>
		<i>Veronaea botryosa</i>
		<i>Zalerion varium</i>
		<i>Zopfiella</i>
		unknown Halosphaeriaceae
		unknown Helotiaceae
		unknown Orbiliaceae
		unknown Thelephoraceae
		unknown Auriculariales inc. sed.
		unknown Diaporthales
		unknown Helotiales
		unknown Hypocreales
		unknown Leotiales
		unknown Pleosporales
		unknown Sebacinales
		unknown Agaricales
		unknown Microascales
		unknown Chaetosphaeriales
		unknown Agaricomycetes inc. sed.
		unknown Dothideomycetes
		unknown Sordariomycetes
		unknown Leotiomycetes
		unknown Basidiomycota
		unknown Ascomycota