

Supplementary Methods

Donor Fungus Sources

The source Clade-A fungi for *T. arizonensis* was originally from a laboratory colony of *Acromyrmex versicolor* collected in Tucson, Arizona (approximately 32°19' N, 110°54' W) in 2009¹, but had since been grown by a *T. arizonensis* colony. This source of Clade-A fungus was chosen for *T. arizonensis* due to *Acromyrmex versicolor* being a sympatric species that grows Clade-A fungi, making this source of fungi a potential means for *T. arizonensis* to encounter Clade-A fungus naturally^{2,3}. The source Clade-B fungi for *T. arizonensis* was donated from Clade-B fungus obtained from a mature laboratory colony of *T. arizonensis* collected at the Southwestern Research Station in 2010¹.

The source Clade-A fungi for *T. pomonae* came from a mature *T. arizonensis* laboratory colony that was growing fungus obtained from an *Acromyrmex versicolor* colony, while the source Clade-A fungi for *T. septentrionalis* came from an *Atta texana* colony collected near Tyler, Texas on April 22, 2021. This source of Clade-A fungus was chosen for *T. pomonae* due to *Acromyrmex versicolor* and *T. arizonensis* being the only co-occurring ants growing Clade-A fungus, while the source Clade-A fungus utilized for *T. septentrionalis* was chosen due to *Atta texana* being the only co-occurring ant species growing Clade-A fungus, making these sources of fungi the only means for these species to potentially encounter Clade-A fungus naturally²⁻⁴. The source Clade-B fungi for *T. pomonae* was donated from Clade-B4 fungus obtained from a mature laboratory colony of *T. arizonensis*, while the source Clade-B fungi for *T. septentrionalis* was donated from Clade-B4 fungus obtained from a colony of *T. septentrionalis* collected on the University of Texas at Tyler campus on April 5, 2021.

Subcolony Care

Each subcolony's nesting dish consisted of one smaller diameter, lidless, 9 mm tall Petri dish in a larger, 15 mm tall, non-vented Petri dish. A layer of moistened dental plaster was poured into the larger petri dish to keep the smaller petri dish in place and to act as a moisture reservoir for the ants. Subcolonies were visually monitored daily. The workers were provided fresh polenta and frass from eastern tent caterpillars (*Malacosoma americanum*) and forest tent caterpillars (*Malacosoma disstria*) every other day to allow the ants to collect these substrates *ad libitum* for their fungus gardens. While we did not monitor how much of the fungal substrate was consumed or the percentages of food provided, but all subcolonies received the same amount of food and the same type of food during each feeding day. Nest dishes were cleaned and maintained every other day to remove any unused substrate from the previous provision. Subcolonies were kept in saturated air at a temperature of 24–25 °C within an environmental chamber (VWR low temperature incubator, model number 10753-894), which are thought be ideal growing conditions for these ants ⁵. Subcolonies were then fed a 10% sucrose solution diet for a week to allow the workers to purge themselves of any residual fungi and acclimate to the new nest dishes ⁶. The 10% sucrose solution was replaced every two days.

Fungus Clade Confirmation

To validate whether each colony/subcolony was growing the intended fungal cultivar it was experimentally given, the DNA of the resulting fungus garden grown by each subcolony was extracted, sequenced, and compared to reference sequences. The fungal DNA was extracted from the gongylidia, or the nutrient filled hyphal tips of the fungus used as the food source for the ants ^{7,8}. To do this, a snip of gongylidia from the fungal garden was removed using ethanol–flame sterile techniques and placed into a 20% Chelex (Bio-Rad) solution prepared with 40uL of

Chelex beads and 160 uL ddH₂O. The resulting solutions were then vortexed, centrifuged, and heated in a thermocycler (first set at 60 °C for 90 minutes, then set at 99 °C for 15 minutes). The resulting supernatant was then used to make 1:10 dilutions of supernatant:ddH₂O for the following PCRs. Genotype determination was used by amplifying a fragment of the ribosomal gene internal transcribed spacer (ITS) region ⁹. The forward primer of this fragment was ITS5 (5' – GGAAGTAAAAGTCGTAACAAGG – 3') and the reverse primer was ITS4 (5' – TCCTCCGCTTATTGATATGC – 3') ¹⁰. The PCR for the ITS fragment had the following steps: 1) have a starting temperature of 94°C for two minutes, 2) denature the solution for 35 cycles at 94°C for 1 minute, anneal the solution at 51.5°C for 1 minute, and elongate the strands at 72°C for two minutes, then 3) hold at 72°C for five minutes. The resulting PCR products were sent to the DNA Sequencing Facility at the University of Texas at Austin for Sanger sequencing on an Applied Biosystems 3730 DNA Analyzer. The resulting ITS sequences were cleaned up and aligned in Geneious 10.1.2 ¹¹. Sequencing errors or misreads in the DNA sequences were manually corrected. Sequences were identified using BLAST at NCBI GenBank and personal databases of ITS sequences ¹².

Microbiome Sampling Details

Samples of a single worker from the collected *T. septentrionalis* and *T. pomonae* colonies were used as the source ants. Source samples of workers from each *T. septentrionalis* colony were taken three weeks after the subcolonies received their fungus donations. This delay was done to allow the microbiome of the ants to adjust to lab conditions and reduce the impact of transient environmental taxa between source and switch comparisons ¹³. As the *T. pomonae* colonies were collected in 2019 and had their microbiomes adjusted to the laboratory setting, their source samples were taken at the inception of the study. Five samples of small fungus

trimmings from the fungal gardens serving as the cultivar donation sources were collected at the inception of the experiment and act as the source fungus samples.

Samples of a single worker and small trimming of fungus from *T. septentrionalis* and *T. pomonae* subcolonies were taken monthly to ensure there was a sample prior to any unforeseen total collapse of the fungal gardens. At the time of a collapse, a single worker and small trimming of fungus were sampled for microbiome analysis, with all remaining ants and healthy fungus put in vials of 100% ethanol for future potential analyses. At the point where all Clade-A subcolonies collapsed, the remaining Clade-B subcolonies had a single worker and small trimming of fungus sampled for microbiome analysis, with all remaining ants and healthy fungus put in vials of 100% ethanol for future potential analyses. These final samples act as the post-donation samples for the ants and fungus. As no unforeseen total collapses occurred, all post-donation ant and fungus samples occurred utilizing the latter methods.

Bacterial Distribution

After the raw sequences were processed and filtered through Qiime2, the dataset consisted of 1,559,132 sequences, with 887,847 sequences associated with the 77 ant samples and 671,285 sequences associated with the 75 fungal samples. Rarefying the samples per the rarefaction thresholds reduced this down to 84,380 sequences, with 46,600 sequences associated with 76 ant samples and 37,780 sequences associated with 62 fungal samples. From these rarefied samples, 218 OTUs were detected, with 161 of these OTUs found within the ant samples and 117 OTUs found within the fungal samples.

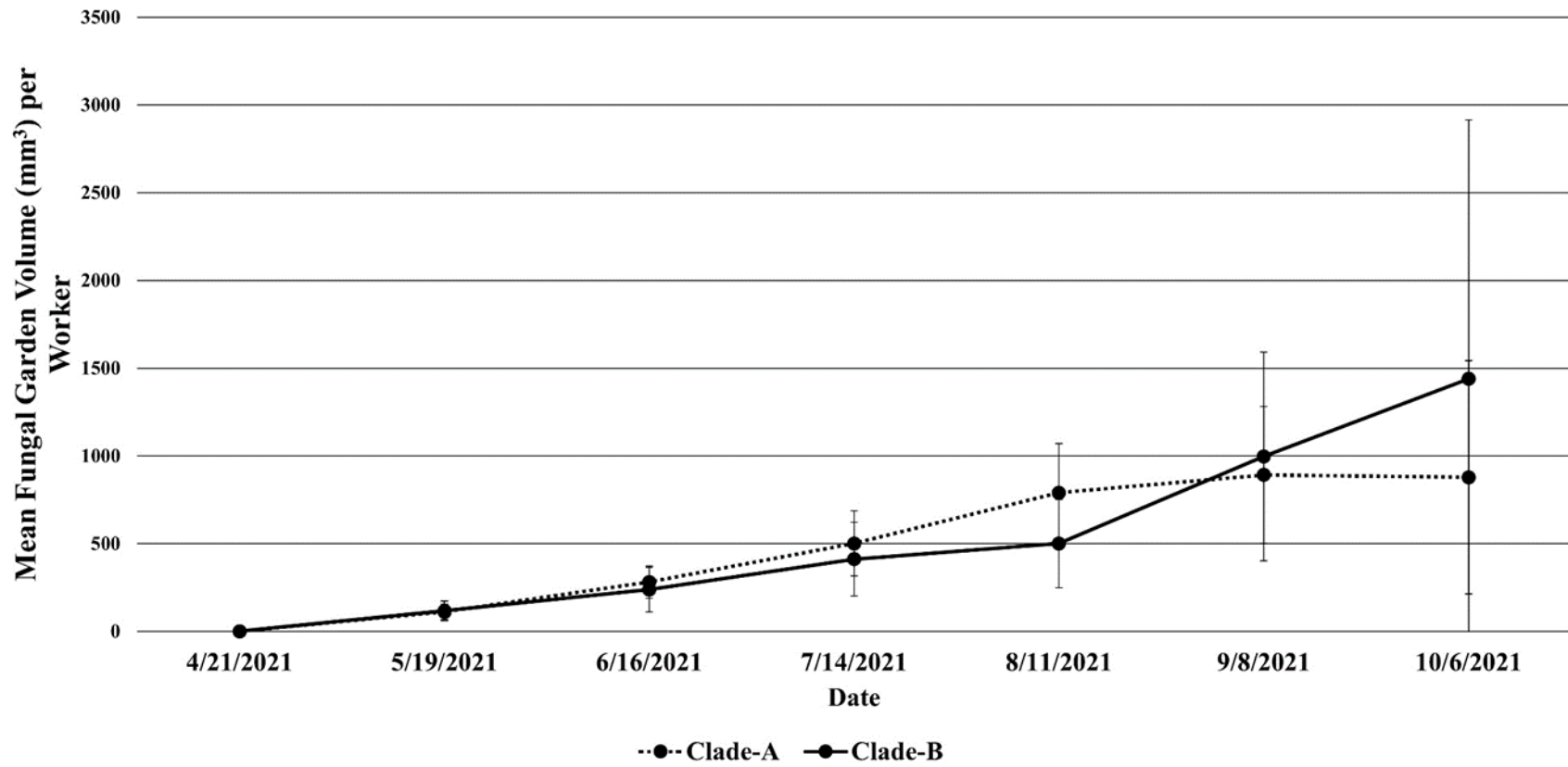


Figure S1. Mean fungal growth of *Trachymyrmex septentrionalis* subcolonies overtime Mean fungal garden volumes (mm³) per ant over time for both Clade-A (dysbiotic) and Clade-B (symbiotic) *T. septentrionalis* subcolonies. Error bars denote the 95% confidence interval around the mean. The repeated measures ANOVA found a significant effect of time on the volume of the fungal gardens as the fungal gardens grew over the course of the experiment ($F_{1.44,15.82} = 8.908$, $p = 0.005$). However, the repeated measures ANOVA did not find a significant effect of the fungal clade on the volume of the fungal gardens ($F_{1,11} = 0.046$, $p = 0.834$). The repeated measures ANOVA also did not find a significant interaction between the cultivar of fungus grown and the growth of the fungal garden over time ($F_{1.32,14.56} = 0.600$, $p = 0.496$).

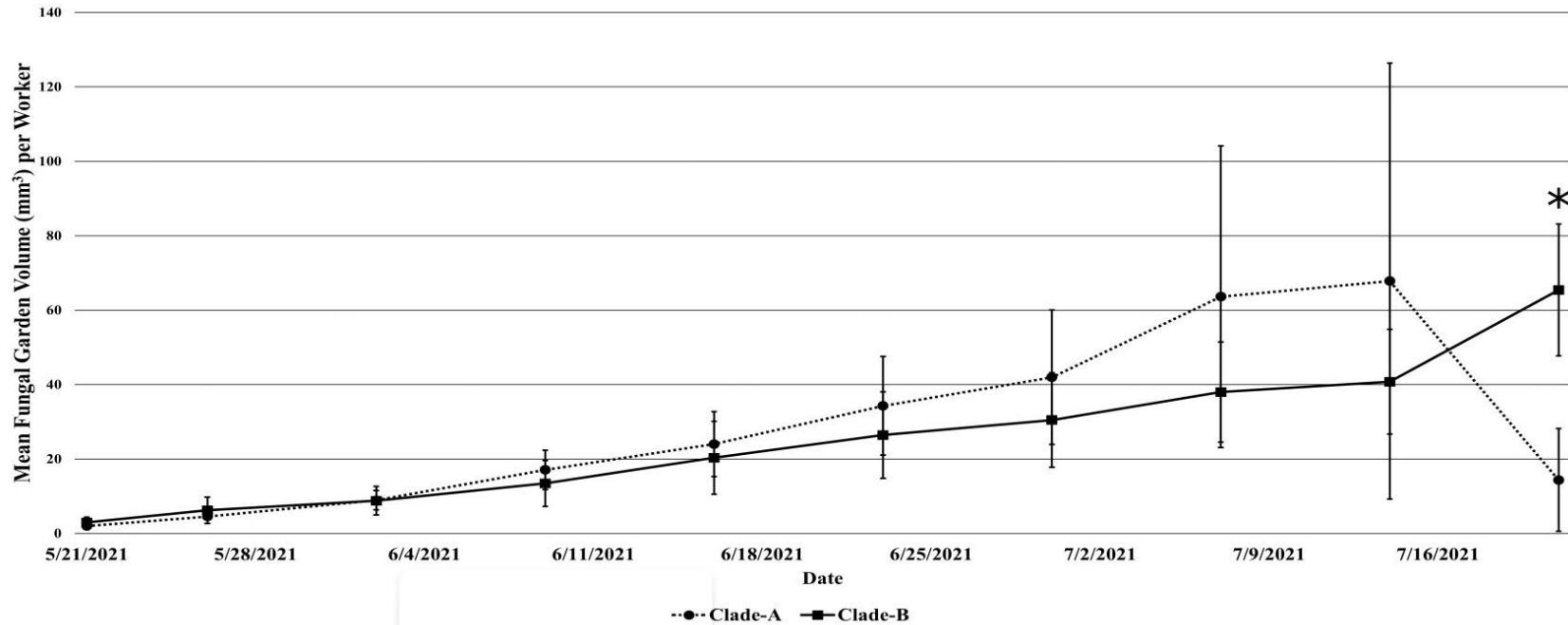


Figure S2. Mean fungal growth of *Trachymyrmex pomonae* subcolonies overtime Mean fungal garden volumes (mm³) per ant over time for both Clade-A (dysbiotic) and Clade-B (symbiotic) *T. pomonae* subcolonies. Error bars denote the 95% confidence interval around the mean. The repeated measures ANOVA found a significant effect of time on the volume of the fungal gardens ($F_{1,90,17.09} = 10.048$, $p = 0.001$), but not find a significant effect of the fungal clade on the volume of the fungal gardens ($F_{1,9} = 0.418$, $p = 0.534$). The repeated measures ANOVA did find a significant interaction between the cultivar of fungus grown and the growth of the fungal garden over time ($F_{1,94,17.49} = 3.763$, $p = 0.045$). Using pairwise ANOVAs, this interaction was caused by the collapse of the Clade-A fungal gardens, resulting in a potential significant difference in the fungal garden volumes on July 28, 2021 ($F_{1,9} = 28.6$, $p = 4.64e-3$). Symbols denote significant differences ($p < 0.05$) between the growths of Clade-A and Clade-B fungal gardens from the pairwise ANOVA, where the p-values were adjusted using Bonferroni correction.

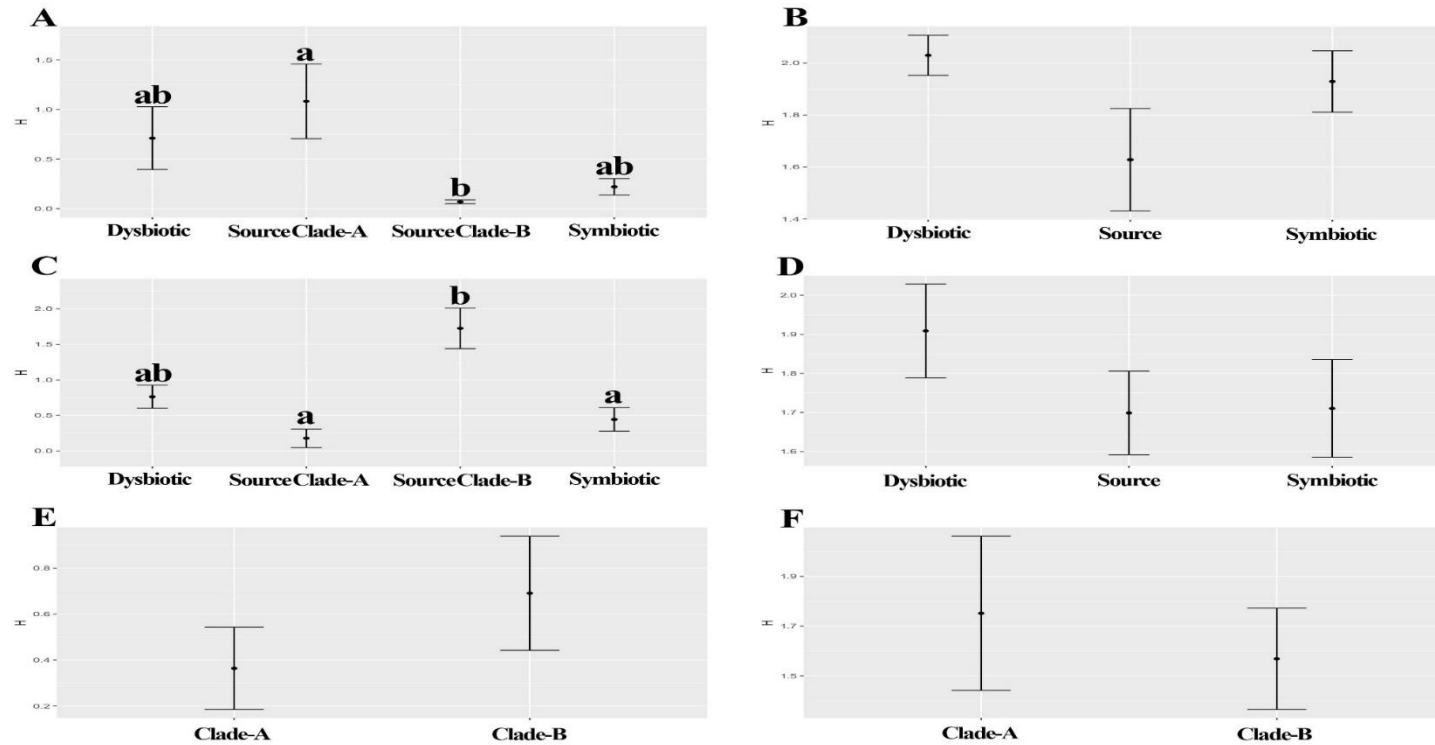


Figure S3. Shannon's diversity index (H) plots (A) *Trachymyrmex septentrionalis* fungal gardens, (B) *T. septentrionalis* ants, (C) *T. pomonae* fungal gardens, (D) *T. pomonae* ants, (E) *T. arizonensis* fungal gardens, and (F) *T. arizonensis* ants. All sample types were compared using Kruskal-Wallis tests to test for significant differences. Sample types containing different letters were found to be significantly different (Bonferroni adjusted p-value < 0.05) when tested with a Dunn's test (post-hoc test). Error bars denote the standard error around the mean. The mean Shannon's diversity indexes between the *T. septentrionalis* fungal gardens were significantly different (Kruskal-Wallis: $\chi^2 = 9.6797$, df = 3, p = 0.02149), which was driven by source Clade-B fungi having a significantly lower Shannon's index than source Clade-A fungi (Dunn's: Z = 2.956, padj = 0.019). The mean Shannon's diversity indexes between the *T. pomonae* fungal gardens were significantly different (Kruskal-Wallis: $\chi^2 = 10.357$, df = 3, p = 0.01577), which was driven by source Clade-B fungi having a more diverse community of OTUs than that of the source Clade-A fungi (Dunn's: Z = -2.869, padj = 0.025) and that of the symbiotic fungi (Dunn's: Z = 2.638, padj = 0.050).

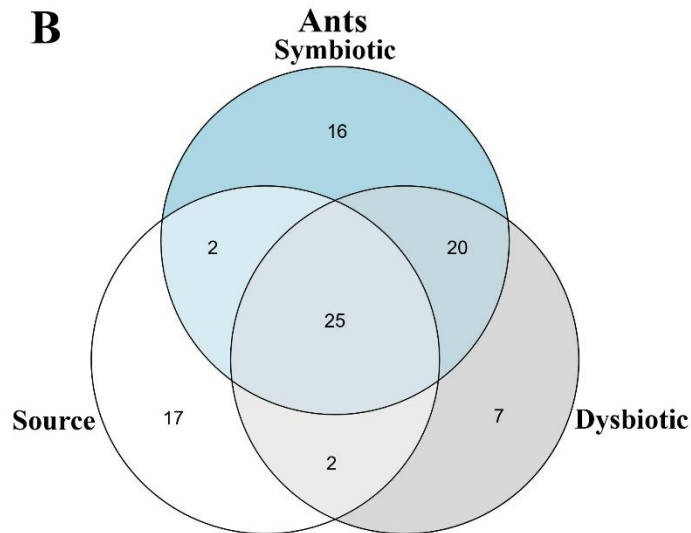
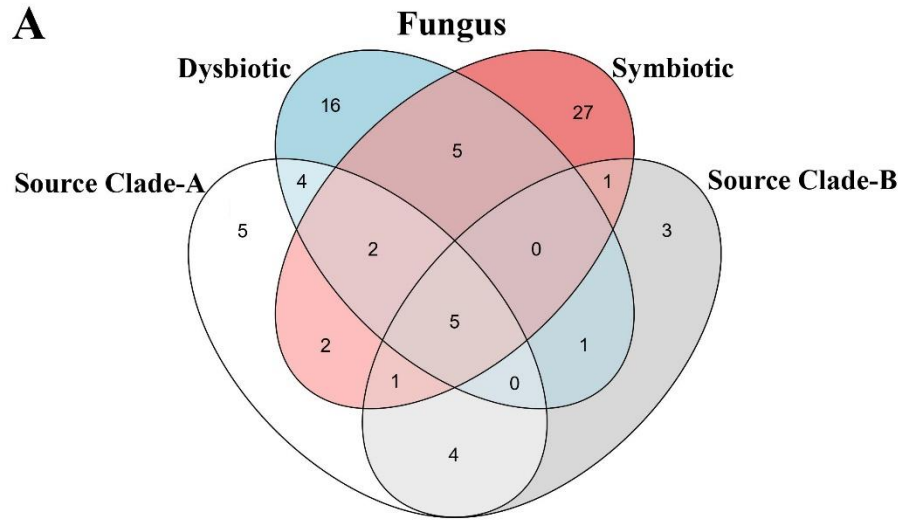


Figure S4. Venn diagrams of OTUs shared between *Trachymyrmex septentrionalis* associated ants and fungal gardens Venn diagrams illustrating shared OTUs between *T. septentrionalis* (A) associated fungi and (B) ant samples. Of the 76 bacterial OTUs found in the *T. septentrionalis* fungus gardens, five were found across all sample types. However, only *Mesoplasma* OTU 1 was found in abundances greater than 1% across each sample type. While dysbiotic gardens and symbiotic gardens additionally shared another five more OTUs, none were in abundances greater than 1%. Of the 89 OTUs found in all the *T. septentrionalis* ant microbiomes, 25 were found across each sample type. 11 of these OTUs were found in abundances greater than 1% and constitute the majority of the sequences found for each experimental group.

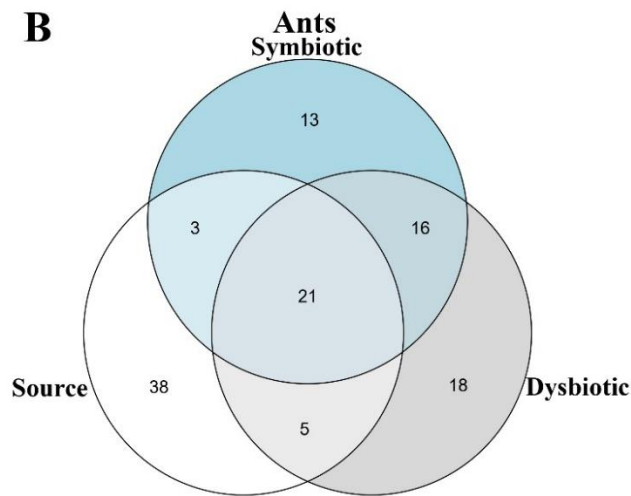
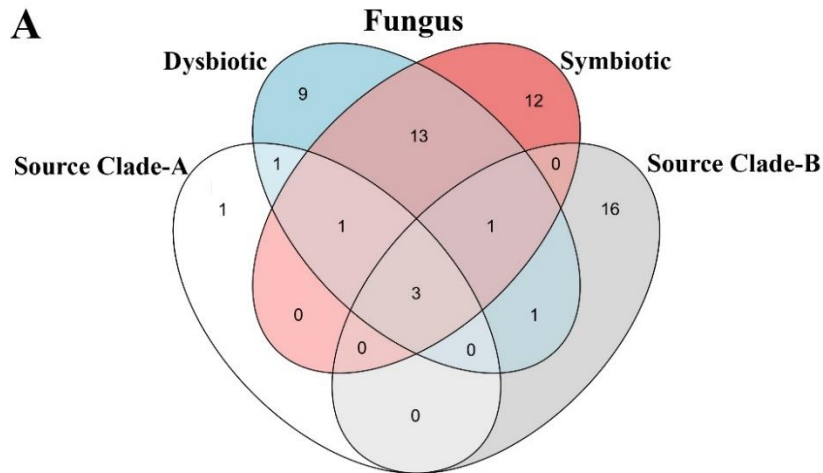


Figure S5. Venn diagrams of OTUs shared between *Trachymyrmex pomonae* associated ants and fungal gardens Venn diagrams illustrating shared OTUs between *T. pomonae* (A) associated fungi and (B) ant samples. Of the 58 OTUs found in the *T. pomonae* fungal microbiomes, only 3 were found within each sample type, but none of these OTUs were in percentages greater than 1% across all sample types. Notably, the dysbiotic Clade-A and symbiotic Clade-B samples uniquely shared thirteen OTUs, with *Spiroplasma* OTU 1, *Serratia marcescens* OTU 1, Unclassified Enterobacterales OTU 1, *Stenotrophomonas* OTU 1, and *Stenotrophomonas nitritireducens* OTU 1 being found in greater than 1% abundances in both sample types. Of the 114 OTUs found in the *T. pomonae* ant microbiomes, 21 were found within samples of each sample type. Of these 21 OTUs, 7 were found in abundances greater than 1% for each sample type. Dysbiotic ants growing Clade-A fungi and symbiotic ants growing Clade-B fungi also uniquely shared 16 OTUs, of which only 2 were found in abundances greater than 1% for each sample type.

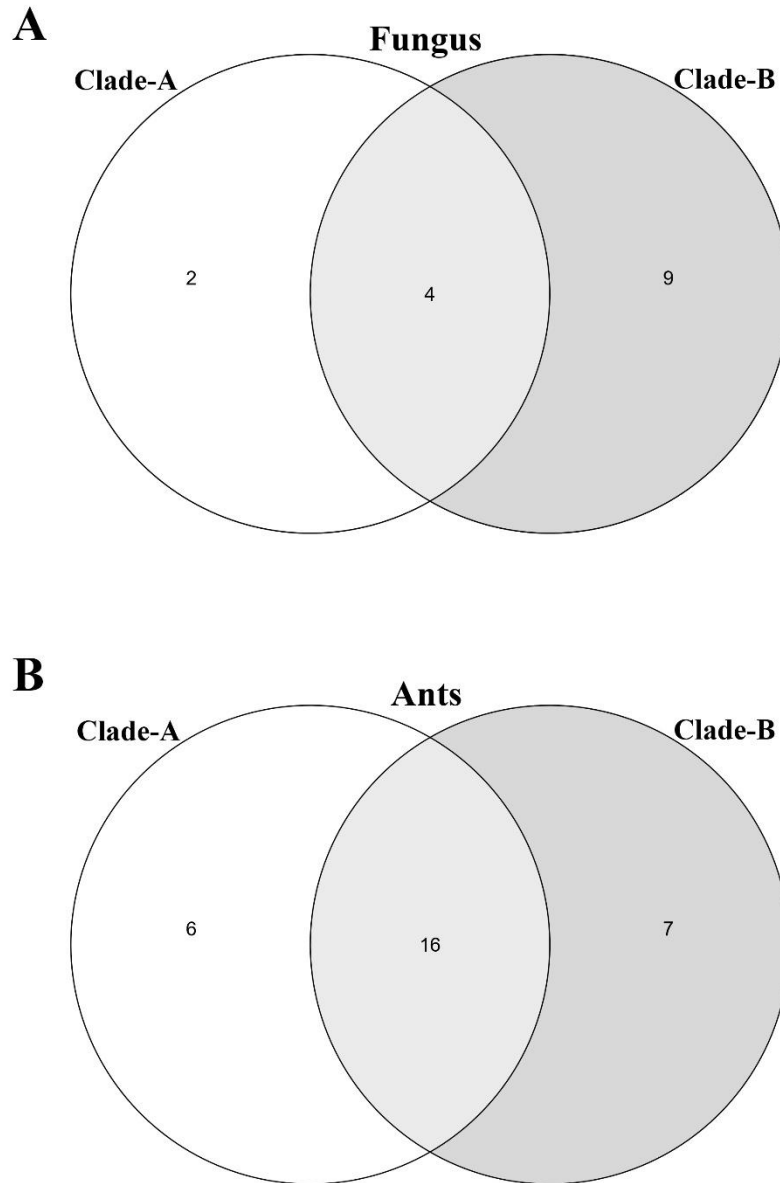


Figure S6. Venn diagrams of OTUs shared between *Trachymyrmex arizonensis* associated ants and fungal gardens Venn diagrams illustrating shared OTUs between *T. arizonensis* (A) associated fungi and (B) ant samples. Of the 29 OTUs found in the *T. arizonensis* ant microbiomes, 16 OTUs were found within both sample types, with only 11 found in abundances greater than 1% for each sample type. These 11 OTUs constitute the majority of the sequences found for both sample types. Of the 15 OTUs found in the *T. arizonensis* fungi microbiomes, 4 were found within both sample types. Only *Spiroplasma* OTU 1 and Unclassified Rhizobiaceae OTU 1 were found in abundances greater than 1% for each sample type, but these were the majority of the sequences found for both sample types.

Table S1. Number of workers utilized for each subcolony Initial number of workers placed in each *T. septentrionalis* (TS) or *T. pomonae* (TP) subcolony. Clade–A subcolonies are identified by “At” present at the end of the subcolony identifier for *T. septentrionalis* subcolonies, while “Ac” is used for *T. pomonae*. Clade–B subcolonies are identified with “B” present at the end of the subcolony identifier for both species.

Subcolony Species	Subcolony Identifier	Initial Number of Workers
TS	BB210408–1B	20
TS	BB210408–2B	20
TS	KB210408–1B	20
TS	KB210408–2B	20
TS	KB210408–3B	10
TS	BB210409–1B	20
TS	BB210409–2B	20
TS	BB210409–3B	15
TS	KB210409–1B	20
TS	KB210409–2B	20
TS	KB210409–3B	20
TS	KB210409–4B	20
TS	BB210408–1At	20
TS	BB210408–2At	20
TS	KB210408–1At	20
TS	KB210408–2At	20
TS	KB210408–3At	10
TS	BB210409–1At	20
TS	BB210409–2At	20
TS	BB210409–3At	15
TS	KB210409–1At	20
TS	KB210409–2At	20

TS	KB210409-3At	20
TS	KB210409-4At	20
TP	JNS190802-6B	20
TP	JNS190802-2B	20
TP	JNS190802-9B	20
TP	KK190802-1B	20
TP	CR190803-1BB	10
TP	JNS190802-3B	20
TP	CVB190812-2B	20
TP	JNS190802-4B	15
TP	JNS190802-8B	5
TP	JNS190801-1B	15
TP	JNS190802-6Ac	20
TP	JNS190802-2Ac	20
TP	JNS190802-9Ac	20
TP	KK190802-1Ac	20
TP	CR190803-1BAc	10
TP	JNS190802-3Ac	20
TP	CVB190812-2Ac	20
TP	JNS190806-4Ac	15
TP	JNS190802-8Ac	5
TP	JNS190801-1Ac	15

Table S2. Mean percentage of each major OTU comprising each fungus sample type OTUs with mean sample type abundance percentages greater than 1%, along with their corresponding mean percentage and 95% confidence interval (%), for source Clade-A, dysbiotic (grown Clade-A gardens), source Clade-B, and symbiotic (grown Clade-B gardens) fungus for *T. septentrionalis* and *T. pomonae*. Additionally, mean sample type abundance percentages of *T. arizonensis* Clade-A and Clade-B fungi are presented.

<i>T. septentrionalis</i>							
Source Clade-A		Dysbiotic		Source Clade-B		Symbiotic	
OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI
<i>Pantoea</i> OTU 1	65.29 ± 27.78	<i>Mesoplasma</i> OTU 1	51.39 ± 33.50	<i>Mesoplasma</i> OTU 1	99.00 ± 0.55	<i>Mesoplasma</i> OTU 1	95.38 ± 4.06
<i>Pseudomonas</i> OTU 1	8.86 ± 17.36	<i>Serratia marcescens</i> OTU 1	15.88 ± 26.78				
<i>Mesoplasma</i> OTU 1	8.19 ± 9.02	Unclassified Enterobacterales OTU 1	10.76 ± 17.70				
<i>Erwinia</i> OTU 2	5.95 ± 11.67	<i>Pantoea rwandensis</i> OTU 1	5.84 ± 11.39				
<i>Raoultella</i> OTU 1	3.67 ± 7.19	<i>Pantoea</i> OTU 1	4.02 ± 7.37				
<i>Ewingella americana</i> OTU 1	1.48 ± 2.89	<i>Sphingobacterium</i> OTU 1	3.65 ± 6.84				
Unclassified Burkholderiaceae OTU 1	1.14 ± 1.28	Unclassified Erwiniaceae OTU 1	2.88 ± 3.60				
<i>Luteimonas</i> OTU 1	1.05 ± 0.34	Unclassified Enterobacteriaceae OTU 1	1.41 ± 2.76				
		<i>Erwinia</i> OTU 2	1.06 ± 2.08				
<i>T. pomonae</i>							
Source Clade-A		Dysbiotic		Source Clade-B		Symbiotic	
OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI
Unclassified Rhizobiaceae OTU 1	95.86 ± 6.45	<i>Serratia marcescens</i> OTU 1	22.71 ± 21.39	<i>Pseudonocardia</i> OTU 1	24.21 ± 37.15	<i>Spiroplasma</i> OTU 1	35.06 ± 31.15
<i>Erwinia</i> OTU 1	1.51 ± 2.97	<i>Spiroplasma</i> OTU 1	17.74 ± 20.39	<i>Variovorax</i> OTU 1	17.28 ± 17.17	<i>Gluconobacter</i> OTU 1	15.47 ± 22.38
<i>Turicibacter</i> OTU 1	1.38 ± 2.71	Unclassified Enterobacterales OTU 1	13.42 ± 15.19	<i>Wolbachia</i> OTU 1	12.54 ± 12.34	<i>Stenotrophomonas</i> OTU 1	10.82 ± 19.33

		Unclassified Rhizobiaceae OTU 1	12.34 ± 19.32	<i>Cutibacterium acnes</i> OTU 1	9.21 ± 13.90	<i>Rosenbergiella</i> OTU 1	10.41 ± 20.40
		<i>Lactococcus garvieae</i> OTU 1	10.26 ± 14.54	<i>Paracoccus</i> OTU 2	8.16 ± 15.99	<i>Pantoea</i> OTU 1	9.24 ± 18.11
		<i>Acinetobacter</i> OTU 1	9.89 ± 18.09	<i>Aerococcus</i> OTU 1	4.65 ± 5.07	<i>Serratia marcescens</i> OTU 1	9.06 ± 16.74
		<i>Tatumella ptyseos</i> OTU 1	3.03 ± 5.65	<i>Bacillus</i> OTU 1	3.07 ± 6.02	Unclassified Enterobacterales OTU 1	5.44 ± 6.86
		<i>Pseudomonas aeruginosa</i> OTU 1	2.32 ± 3.14	<i>Staphylococcus hominis</i> OTU 1	2.98 ± 5.85	<i>Erwinia</i> OTU 1	1.17 ± 2.29
		<i>Stenotrophomonas</i> OTU 1	2.00 ± 2.56	<i>Turicibacter</i> OTU 1	2.54 ± 4.99	<i>Stenotrophomonas nitritireducens</i> OTU 1	1.05 ± 1.94
		<i>Erwinia</i> OTU 1	1.50 ± 2.94	<i>Pandoraea norimbergensis</i> OTU 1	2.37 ± 2.54		
		Unclassified Erwiniaceae OTU 1	1.47 ± 2.89	<i>Staphylococcus xylosus</i> OTU 1	2.28 ± 4.47		
		<i>Stenotrophomonas nitritireducens</i> OTU 1	1.39 ± 2.68	<i>Streptococcus</i> OTU 1	1.67 ± 3.27		
				<i>Paracoccus</i> OTU 1	1.58 ± 3.09		
				<i>Spiroplasma</i> OTU 1	1.49 ± 2.92		
				<i>Sedimentibacter</i> OTU 1	1.40 ± 2.75		
				<i>Stappia</i> OTU 1	1.23 ± 2.41		
T. arizonensis							
Clade-A				Clade-B			
OTU	Mean Percentage ± 95% CI			OTU	Mean Percentage ± 95% CI		
Unclassified Rhizobiaceae OTU 1	88.60 ± 13.52			<i>Spiroplasma</i> OTU 1	45.38 ± 36.50		
<i>Spiroplasma</i> OTU 1	4.95 ± 9.70			Unclassified Rhizobiaceae OTU 1	43.24 ± 31.54		
<i>Arthrobacter agilis</i> OTU 1	2.5 ± 4.90			<i>Pullulanibacillus camelliae</i> OTU 1	4.92 ± 8.47		
Unclassified Bacteria	2.05 ± 2.44			<i>Wolbachia</i> OTU 1	1.22 ± 1.86		
<i>Erwinia</i> OTU 1	1.38 ± 2.69			<i>Rosenbergiella</i> OTU 1	1.20 ± 2.35		
				<i>Rosenbergiella</i> OTU 2	1.12 ± 2.20		

Table S3. Indicator species analysis on fungal gardens for each ant species The OTUs with significant (p-value < 0.05) indicator values (IVs) when comparing the microbiomes of source Clade-A, dysbiosis (grown Clade-A fungi), source Clade-B, and symbiosis (grown Clade-B fungi) fungus samples using an indicator species analysis (ISA) for *T. septentrionalis* and *T. pomonae*. Additionally, results comparing *T. arizonensis* Clade-A and Clade-B fungi are presented. Each analysis utilized 9,999 permutations. OTUs found to be the indicator of a combination of multiple sample types are indicated with superscripts (^a) and are listed with each sample type. While *T. arizonensis* had no indicator taxa, *T. pomonae* and *T. septentrionalis* had indicator taxa; with notable taxa being Unclassified Rhizobiaceae OTU 1 being associated with source Clade-A *T. pomonae*, *Pantoea* OTU 1 associated with source Clade-A *T. septentrionalis*, and *Mesoplasma* OTU 1 associated with both source Clade-B and symbiosis *T. septentrionalis*.

<i>T. septentrionalis</i>							
Source Clade-A		Dysbiosis		Source Clade-B		Symbiosis	
OTU	IV, p-value	OTU	IV, p-value	OTU	IV, p-value	OTU	IV, p-value
<i>Pantoea</i> OTU 1	0.928, 0.0004	No indicator species	-	<i>Mesoplasma</i> OTU 1 ^a	0.788, 0.0027	<i>Mesoplasma</i> OTU 1 ^a	0.788, 0.0027
<i>Luteimonas</i> OTU 1	0.903, 0.0006						
<i>Solirubrobacter</i> OTU 1	0.884, 0.0004						
<i>Naumannella</i> <i>huperziae</i> OTU 1	0.813, 0.0010						
<i>Aeromicrobium</i> OTU 1	0.731, 0.0006						
Unclassified Burkholderiaceae OTU 1	0.701, 0.0006						
<i>Niabella</i> OTU 1	0.620, 0.0174						
<i>Pseudomonas</i> OTU 1	0.520, 0.0373						
<i>T. pomonae</i>							
Source Clade-A		Dysbiosis		Source Clade-B		Symbiosis	
OTU	IV, p-value	OTU	IV, p-value	OTU	IV, p-value	OTU	IV, p-value
Unclassified Rhizobiaceae OTU 1	0.929, 0.0004	No indicator species	-	<i>Wolbachia</i> OTU 1	0.773, 0.0092	No indicator species	-

				<i>Variovorax</i> OTU 1	0.770, 0.0077		
				<i>Pandora</i> <i>norimbergensis</i> OTU 1	0.745, 0.0092		
				<i>Aerococcus</i> OTU 1	0.740, 0.0077		
				<i>Cutibacterium</i> <i>acnes</i> OTU 1	0.622, 0.0103		
				<i>Pseudonocardia</i> OTU 1	0.616, 0.0044		
<i>T. arizonensis</i>							
Clade-A				Clade-B			
OTU		IV, p-value		OTU		IV, p-value	
No indicator species		-		No indicator species		-	

Table S4. PERMANOVA results for ant comparisons Results of PERMANOVA tests between the different sample types for *T. septentrionalis* and *T. pomonae* ants. Every PERMANOVA test utilized Bray Curtis distances and had 9,999 permutations. Bolded statistics mean the bacterial microbiomes of the sample types were found to be significantly different, or $p < 0.05$.

<i>T. septentrionalis</i>			
	Source	Dysbiotic	Symbiotic
Source			
Dysbiotic	F = 3.89, R² = 0.15, p = 0.012		
Symbiotic	F = 2.19, R ² = 0.09, p = 0.087	F = 4.77, R² = 0.18, p = 0.001	
<i>T. pomonae</i>			
	Source	Dysbiotic	Symbiotic
Source			
Dysbiotic	F = 1.83, R² = 0.09, p = 0.024		
Symbiotic	F = 2.04, R² = 0.10, p = 0.040	F = 1.22, R ² = 0.06, p = 0.235	

Table S5. Mean percentage of each major OTU comprising each ant sample type OTUs with mean sample type abundance percentages greater than 1%, along with their corresponding mean percentage and 95% confidence interval (%), for source, dysbiotic (ants provided Clade-A fungus), and symbiotic (ants provided Clade-B fungus) ants for *T. septentrionalis* and *T. pomonae*. Additionally, mean sample type abundance percentages of *T. arizonensis* ants growing Clade-A and Clade-B fungi are presented.

<i>T. septentrionalis</i>					
Source		Dysbiotic		Symbiotic	
OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI
<i>Mesoplasma</i> OTU 1	37.69 ± 22.43	<i>Luteimonas</i> OTU 1	22.70 ± 9.04	<i>Mesoplasma</i> OTU 1	36.83 ± 13.04
<i>Solirubrobacter</i> OTU 1	12.43 ± 4.91	<i>Solirubrobacter</i> OTU 1	13.24 ± 4.82	<i>Luteimonas</i> OTU 1	13.83 ± 5.67
<i>Luteimonas</i> OTU 1	7.87 ± 5.08	Unclassified Burkholderiaceae OTU 1	12.30 ± 3.79	<i>Solirubrobacter</i> OTU 1	7.88 ± 3.31
Unclassified Burkholderiaceae OTU 1	7.51 ± 3.36	<i>Solirubrobacter</i> OTU 2	6.24 ± 2.42	Unclassified Burkholderiaceae OTU 1	6.92 ± 2.53
<i>Solirubrobacter</i> OTU 2	6.83 ± 3.09	<i>Pseudomonas aeruginosa</i> OTU 1	5.67 ± 10.98	<i>Aeromicrobium</i> OTU 1	4.35 ± 2.37
Unclassified Intrasporangiaceae OTU 1	5.38 ± 2.83	<i>Mesoplasma</i> OTU 1	5.38 ± 4.66	<i>Niabella</i> OTU 1	4.00 ± 2.39
<i>Aeromicrobium</i> OTU 1	5.04 ± 4.68	<i>Microlunatus</i> OTU 1	3.74 ± 1.43	<i>Olivibacter</i> OTU 1	3.81 ± 6.95
<i>Naumannella huperzia</i> OTU 1	4.63 ± 1.94	<i>Serratia marcescens</i> OTU 1	3.73 ± 4.89	<i>Solirubrobacter</i> OTU 2	2.71 ± 1.01
<i>Microlunatus</i> OTU 1	4.29 ± 2.01	Unclassified Intrasporangiaceae OTU 1	3.39 ± 2.42	<i>Flavobacterium</i> OTU 1	1.62 ± 3.17
<i>Agrococcus terreus</i> OTU 1	3.83 ± 2.20	<i>Nocardioides</i> OTU 1	3.18 ± 1.82	<i>Naumannella huperzia</i> OTU 1	1.61 ± 0.60
<i>Niabella</i> OTU 1	1.24 ± 1.58	<i>Niabella</i> OTU 1	3.08 ± 2.48	<i>Microlunatus</i> OTU 1	1.60 ± 0.39
		<i>Aeromicrobium</i> OTU 1	2.76 ± 1.57	<i>Serratia marcescens</i> OTU 1	1.44 ± 1.23
		<i>Naumannella huperzia</i> OTU 1	2.11 ± 0.65	Unclassified Intrasporangiaceae OTU 1	1.31 ± 0.74
		<i>Luteipulveratus mongoliensis</i> OTU 1	2.08 ± 1.66	<i>Olivibacter domesticus</i> OTU 1	1.24 ± 2.20

		<i>Pantoea</i> OTU 1	1.96 ± 3.32	<i>Ochrobactrum</i> OTU 1	1.17 ± 1.97
		<i>Sphingobacterium</i> OTU 1	1.24 ± 2.25		
		Unclassified Erwinaceae OTU 1	1.23 ± 1.93		
		<i>Pantoea rwandensis</i> OTU 1	1.21 ± 1.93		
		<i>Agrococcus terreus</i> OTU 1	1.15 ± 0.50		
<i>T. pomonae</i>					
Source		Dysbiotic		Symbiotic	
OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI
Unclassified Burkholderiaceae OTU 1	31.97 ± 11.49	<i>Solirubrobacter</i> OTU 2	18.11 ± 7.37	<i>Solirubrobacter</i> OTU 2	32.03 ± 10.26
<i>Solirubrobacter</i> OTU 1	16.37 ± 7.06	Unclassified Burkholderiaceae OTU 1	16.61 ± 7.09	Unclassified Burkholderiaceae OTU 1	20.37 ± 8.26
<i>Solirubrobacter</i> OTU 2	15.05 ± 4.95	<i>Agrococcus terreus</i> OTU 1	9.45 ± 4.68	<i>Solirubrobacter</i> OTU 1	11.08 ± 3.27
<i>Agrococcus terreus</i> OTU 1	8.08 ± 4.44	<i>Spiroplasma</i> OTU 1	8.97 ± 11.70	<i>Agrococcus terreus</i> OTU 1	8.05 ± 3.52
<i>Pseudonocardia</i> OTU 1	7.32 ± 12.86	<i>Solirubrobacter</i> OTU 1	7.82 ± 3.03	<i>Stenotrophomonas nitritireducens</i> OTU 1	6.37 ± 12.48
<i>Naumannella huperzia</i> OTU 1	6.11 ± 5.66	<i>Pseudomonas aeruginosa</i> OTU 1	7.71 ± 13.88	<i>Nocardioides</i> OTU 1	4.00 ± 1.75
<i>Mesoplasma</i> OTU 1	4.13 ± 8.10	<i>Acinetobacter</i> OTU 1	5.42 ± 8.78	<i>Naumannella huperzia</i> OTU 1	3.74 ± 2.71
<i>Nocardioides</i> OTU 1	2.66 ± 1.56	<i>Nocardioides</i> OTU 1	4.11 ± 2.10	<i>Rosenbergiella</i> OTU 1	2.79 ± 5.47
<i>Ponticoccus</i> OTU 1	1.16 ± 0.90	Unclassified Rhizobiaceae OTU 1	3.11 ± 5.22	<i>Spiroplasma</i> OTU 1	2.05 ± 3.07
		<i>Naumannella huperzia</i> OTU 1	3.00 ± 2.34	<i>Ponticoccus</i> OTU 1	1.50 ± 0.47
		<i>Paracoccus</i> OTU 1	2.03 ± 3.75	<i>Acinetobacter</i> OTU 1	1.37 ± 2.17
		<i>Serratia marcescens</i> OTU 1	1.76 ± 1.90	<i>Stenotrophomonas</i> OTU 1	1.26 ± 2.42
		<i>Ponticoccus</i> OTU 1	1.00 ± 0.61		
<i>T. arizonensis</i>					
Clade-A			Clade-B		

OTU	Mean Percentage ± 95% CI	OTU	Mean Percentage ± 95% CI
<i>Wolbachia</i> OTU 1	33.96 ± 32.52	<i>Wolbachia</i> OTU 1	32.78 ± 26.16
Unclassified Burkholderiaceae OTU 1	10.94 ± 5.66	Unclassified Burkholderiaceae OTU 1	15.50 ± 8.95
Unclassified Rhizobiaceae OTU 1	10.12 ± 17.72	Unclassified Rhizobiaceae OTU 1	14.00 ± 26.57
<i>Solirubrobacter</i> OTU 2	6.66 ± 4.93	<i>Solirubrobacter</i> OTU 2	8.90 ± 7.92
Unclassified Intrasporangiaceae OTU 1	6.54 ± 4.61	<i>Agrococcus terreus</i> OTU 1	6.40 ± 1.72
<i>Agrococcus terreus</i> OTU 1	4.92 ± 3.59	<i>Ponticoccus</i> OTU 1	5.46 ± 6.07
<i>Ponticoccus</i> OTU 1	4.78 ± 4.27	Unclassified Intrasporangiaceae OTU 1	4.54 ± 3.66
<i>Solirubrobacter</i> OTU 1	3.02 ± 3.64	<i>Niabella</i> OTU 1	3.34 ± 2.33
<i>Naumannella huperziae</i> OTU 1	2.80 ± 2.59	Unclassified Propionibacteriaceae OTU 1	2.26 ± 1.99
Unclassified Propionibacteriaceae OTU 1	2.70 ± 3.00	<i>Aeromicrobium</i> OTU 2	1.60 ± 2.31
<i>Nocardioides</i> OTU 2	2.40 ± 2.43	<i>Naumannella huperziae</i> OTU 1	1.20 ± 1.25
<i>Pseudonocardia</i> OTU 1	2.24 ± 2.69	<i>Solirubrobacter</i> OTU 1	1.14 ± 2.23
<i>Microbacterium</i> OTU 1	2.16 ± 4.23		
<i>Agrococcus</i> OTU 1	1.60 ± 2.12		
<i>Aeromicrobium</i> OTU 2	1.42 ± 1.32		
<i>Mesorhizobium</i> OTU 1	1.00 ± 1.96		
<i>Luteimonas</i> OTU 1	1.00 ± 1.35		

Table S6. Indicator species analysis for each ant species The OTUs with significant (p-value < 0.05) indicator values (IVs) when comparing the microbiomes of source, dysbiosis (Clade-A provided ants), and symbiosis (Clade-B provided ants) ant samples using an indicator species analysis (ISA) for *T. septentrionalis* and *T. pomonae*. Additionally, results comparing *T. arizonensis* ants growing Clade-A and Clade-B fungi are presented. Each analysis utilized 9,999 permutations. OTUs found to be the indicator of a combination of two out the three sample types are indicated with superscripts (^a, ^b, ^c) and are listed with each sample type. While *T. arizonensis* had no indicator taxa, *T. pomonae* and *T. septentrionalis* had indicator taxa for each sample type; with notable taxa being *Pseudonocardia* OTU 1 being associated with source *T. pomonae*, *Serratia marcescens* OTU 1 associated with dysbiosis *T. pomonae*, and *Mesoplasma* OTU 1 associated with both source and symbiosis *T. septentrionalis*.

<i>T. septentrionalis</i>					
Source		Dysbiosis		Symbiosis	
OTU	IV, p-value	OTU	IV, p-value	OTU	IV, p-value
<i>Naumannella huperzia</i> OTU 1	0.530, 0.0034	<i>Nocardioides</i> OTU 1	0.443, 0.0193	<i>Mesoplasma</i> OTU 1 ^a	0.504, 0.0057
<i>Agrococcus terreus</i> OTU 1	0.518, 0.0018	<i>Luteimonas</i> OTU 1	0.428, 0.0240	<i>Stenotrophomonas</i> OTU 1	0.471, 0.0158
<i>Ponticoccus</i> OTU 1	0.515, 0.0028	<i>Microlunatus</i> OTU 1 ^b	0.422, 0.0307	<i>Sphingobacterium siyangense</i> OTU 1	0.363, 0.0259
<i>Mesoplasma</i> OTU 1 ^a	0.504, 0.0057	<i>Solirubrobacter</i> OTU 2 ^c	0.413, 0.0361		
<i>Microlunatus</i> OTU 1 ^b	0.422, 0.0307	Unclassified Burkholderiaceae OTU 1	0.397, 0.0467		
<i>Solirubrobacter</i> OTU 2 ^c	0.413, 0.0361	<i>Pseudomonas aeruginosa</i> OTU 1	0.241, 0.0215		
<i>T. pomonae</i>					
Source		Dysbiosis		Symbiosis	
OTU	IV, p-value	OTU	IV, p-value	OTU	IV, p-value
<i>Flavobacterium</i> OTU 1	0.516, 0.0222	<i>Pseudonocardia</i> OTU 2	0.522, 0.0232	<i>Solirubrobacter</i> OTU 2	0.517, 0.0091
<i>Shinella</i> OTU 1	0.496, 0.0231	<i>Ochrobactrum</i> OTU 1	0.514, 0.0097		
<i>Pseudonocardia</i> OTU 1	0.282, 0.0134	<i>Serratia marcescens</i> OTU 1	0.405, 0.0376		
		<i>Chryseobacterium indologenes</i> OTU 1	0.405, 0.0223		
<i>T. arizonensis</i>					

Clade-A		Clade-B	
OTU	IV, p-value	OTU	IV, p-value
No indicator species	-	No indicator species	-

Supplemental References

1. Seal, J. N., Schiøtt, M. & Mueller, U. G. Ant-fungus species combinations engineer physiological activity of fungus gardens. *J. Exp. Biol.* **217**, 2540–2547 (2014).
2. Rabeling, C., Cover, S. P., Johnson, R. A. & Mueller, U. G. A review of the North American species of the fungus-gardening ant genus *Trachymyrmex* (Hymenoptera: Formicidae). *Zootaxa* 1–53 (2007). doi:10.5281/zenodo.180014
3. Mueller, U. G. *et al.* Phylogenetic patterns of ant–fungus associations indicate that farming strategies, not only a superior fungal cultivar, explain the ecological success of leafcutter ants. *Mol. Ecol.* **27**, 2414–2434 (2018).
4. Wheeler, W. M. The fungus–growing ants of North America. *Bull. Am. Museum Nat. Hist.* **23**, 669–807 (1907).
5. Bollazzi, M. & Roces, F. Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. *Insectes Soc.* **49**, 153–157 (2002).
6. Kooij, P. W., Pullens, J. W. M., Boomsma, J. J. & Schiøtt, M. Ant mediated redistribution of a xyloglucanase enzyme in fungus gardens of *Acromyrmex echinator*. *BMC Microbiol.* **16**, 81 (2016).
7. Rodrigues, A., Mueller, U. G., Ishak, H. D., Bacci, M. & Pagnocca, F. C. Ecology of microfungal communities in gardens of fungus-growing ants (Hymenoptera: Formicidae): A year-long survey of three species of attine ants in Central Texas. *FEMS Microbiol. Ecol.* **78**, 244–255 (2011).
8. Seal, J. N., Gus, J. & Mueller, U. G. Fungus-gardening ants prefer native fungal species: Do ants control their crops? *Behav. Ecol.* **23**, 1250–1256 (2012).
9. Kellner, K. *et al.* Co-evolutionary patterns and diversification of ant-fungus associations in the asexual fungus-farming ant *Mycocepurus smithii* in Panama. *J. Evol. Biol.* **26**, 1353–1362 (2013).
10. Mueller, U. G., Rehner, S. A. & Schultz, T. R. The Evolution of Agriculture in Ants. *Science* **281**, 2034–2038 (1998).
11. Kearse, M. *et al.* Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
12. Luiso, J., Kellner, K., Matthews, A. E., Mueller, U. G. & Seal, J. N. High diversity and multiple invasions to North America by fungi grown by the northern-most *Trachymyrmex* and *Mycetomoellerius* ant species. *Fungal Ecol.* **44**, 100878 (2020).

13. Rowan, C. D. Comparative microbiome analysis of the fungus gardening ant species *Trachymyrmex arizonensis*. (University of Texas at Tyler, 2021).