Appendix A. Apigo and Oono (2018) Host Specificity in Foliar Fungal Endophytes

Appendix A1. Bioinformatic pipeline using USEARCH (version 9.2.64; Edgar 2010), BLAST+ (Camacho et al. 2008), MEGAN (Huson et al. 2007) and QIIME (Caporaso et al. 2010). Raw sequences can be downloaded from GenBank with Sequence Read Archive (SRA) BioProject Accession Number: PRJNA356423.

Raw Sequence Processing with USEARCH

Raw forward and reverse reads were merged within the same samples. Merged sequences were relabeled with unique numeric identifiers and the sample ID. Merged sequences shorter than 100 base pairs were excluded from downstream processing.

```
usearch -fastq_mergepairs *_R1*.fastq -relabel @ -fastq_minmergelen 100
-fastqout reads.fq
```

Merged sequences were filtered relative to a maximum expected error score of 1.0.

```
usearch -fastq_filter merged.fq -fastq_maxee 1.0 -fastaout filtered.fa
```

Filtered sequences were 'deprelicated' to isolate only unique sequences (i.e., 100% similarity).

```
usearch -fastx_uniques filtered.fa -fastaout uniques.fa –sizeout
```

Unique sequences were run against the UNOISE algorithm (Edgar 2016) to generate 100% OTUs (ZOTUs) with a minimum read abundance of one. Sequences with sequencing error, chimeric or PhiX sequences were removed.

```
usearch -unoise uniques.fa -tabbedout out.txt -fastaout denoised.fa
```

ZOTUs were sorted by length for input into the –cluster_smallmem command.

```
usearch -sortbylength denoised.fa -fastaout seqs_sorted.fasta -
minseqlength 64
```

Sorted 100% OTUs were clustered into 97% OTUs.

```
usearch -cluster_smallmem seqs_sorted.fasta -id 0.97 -centroids
97_OTUs.fa
```

Merged reads were then mapped to 97% OTUs.

```
usearch -usearch_global merged.fq -db 97_OTUs.fa -strand plus -id 0.97
-otutabout otu_table.txt
```

Taxonomic Assignment with BLAST+

OTUs were queried against the GenBank nucleotide database (as of November 2017) with BLAST+ on the Knot Cluster at the University of California, Santa Barbara.

```
blastn -db ntfiles/nt -query 97_OTUs.fasta -outfmt 5 -out
fungal_otus_97.xml -evalue 0.001 -num_threads 12
```

Taxonomic Filtering with MEGAN and QIIME

All 97% OTUs (3,790) were parsed through MEGAN with default parameters for a total of 1,679 OTUs identified within the fungal subtree. All other OTUs (2,111) classified as bacteria, unclassified archaea, Viridiplantae, no hits and not assigned were excluded from all analyses. Non-fungal OTUs were removed from the OTU tables with the filter_samples_from_otu_table.py command in QIIME. Reads from replicate plant species across quadrats were concatenated (see Appendix A2). Sequencing depth per sample was rarefied to 13,322 reads.

References

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335–336

Edgar RC (2016) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing [Internet]. doi:10.1101/081257

Gobet A, Quince C, Ramette A (2010) Multivariate Cutoff Level Analysis (MultiCoLA) of large community data sets. Nucleic Acids Res 38: e155

Huson DH, Auch AF, Qi J, Schuster SC (2007) MEGAN analysis of metagenomic data. Genome Res 17: 377–386

Appendix A2. Key for SRA sample IDs to plant species.

SRA BioProject Accession Number: PRJNA356423		
Full SRA Sample ID	Abbreviated SRA ID	Plant Host Species
James_San_Jacinto_Quadrat3_Understory7	JSJ_Q3_U7	Abies concolor
James_San_Jacinto_Quadrat4_Canopy1	JSJ_Q4_C1	Abies concolor
James_San_Jacinto_Quadrat1_Understory5	JSJ_Q1_U5	Acmisponheermannii var. heermannii
James_San_Jacinto_Quadrat2_Understory7	JSJ_Q2_U7	Acmisponheermannii var. heermannii
James_San_Jacinto_Quadrat4_Understory10	JSJ_Q4_U10	Acmisponheermannii var. heermannii
James_San_Jacinto_Quadrat4_Understory2	JSJ_Q4_U2	Acmisponheermannii var. heermannii
James_San_Jacinto_Quadrat5_Understory4	JSJ_Q5_U4	Acmisponheermannii var. heermannii
James_San_Jacinto_Quadrat5_Canopy3	JSJ_Q5_C3	Alnus rhombifolia
James_San_Jacinto_Quadrat1_Understory7	JSJ_Q1_U7	Angelica tomentosa
James_San_Jacinto_Quadrat1_Understory9	JSJ_Q1_U9	Angelica tomentosa
James_San_Jacinto_Quadrat5_Understory15	JSJ_Q5_U15	Aquilegia formosa
James_San_Jacinto_Quadrat4_Canopy4	JSJ_Q4_C4	Arctostaphylos pungens
James_San_Jacinto_Quadrat1_Understory10	JSJ_Q1_U10	Artemisia dracunculus
James_San_Jacinto_Quadrat1_Understory11	JSJ_Q1_U11	Artemisia dracunculus
James_San_Jacinto_Quadrat5_Understory5	JSJ_Q5_U5	Artemisia dracunculus
James_San_Jacinto_Quadrat3_Understory3	JSJ_Q3_U3	Bromus carinatus var. carinatus
James_San_Jacinto_Quadrat2_Canopy2	JSJ_Q2_C2	Calocedrus decurrens
James_San_Jacinto_Quadrat3_Canopy3	JSJ_Q3_C3	Calocedrus decurrens
James_San_Jacinto_Quadrat4_Canopy3	JSJ_Q4_C3	Calocedrus decurrens
James_San_Jacinto_Quadrat1_Understory14	JSJ_Q1_U14	Carex fracta
James_San_Jacinto_Quadrat2_Understory2	JSJ_Q2_U2	Carex fracta
James_San_Jacinto_Quadrat5_Understory1	JSJ_Q5_U1	Carex fracta

James_San_Jacinto_Quadrat1_Canopy4	JSJ_Q1_C4	Ceanothus palmeri
James_San_Jacinto_Quadrat1_Understory8	JSJ_Q1_U8	Elymus elymoides
James_San_Jacinto_Quadrat4_Understory7	JSJ_Q4_U7	Elymus elymoides
James_San_Jacinto_Quadrat3_Understory1	JSJ_Q3_U1	Erigeron foliosus var. foliosus
James_San_Jacinto_Quadrat4_Understory5	JSJ_Q4_U5	Erigeron foliosus var. foliosus
James_San_Jacinto_Quadrat4_Understory9	JSJ_Q4_U9	Eriogeron foliosus var. foliosus
James_San_Jacinto_Quadrat1_Understory13	JSJ_Q1_U13	Eriogonum wrightii var. membranaceum
James_San_Jacinto_Quadrat2_Understory5	JSJ_Q2_U5	Eriogonum wrightii var. membranaceum
James_San_Jacinto_Quadrat4_Understory14	JSJ_Q4_U14	Galium angustifolium
James_San_Jacinto_Quadrat1_Understory4	JSJ_Q1_U4	Galium angustifolium
James_San_Jacinto_Quadrat2_Understory6	JSJ_Q2_U6	Galium angustifolium
James_San_Jacinto_Quadrat3_Understory5	JSJ_Q3_U5	Galium angustifolium
James_San_Jacinto_Quadrat4_Understory3	JSJ_Q4_U3	Galium angustifolium
James_San_Jacinto_Quadrat4_Understory8	JSJ_Q4_U8	Galium angustifolium
James_San_Jacinto_Quadrat5_Understory9	JSJ_Q5_U9	Galium angustifolium
James_San_Jacinto_Quadrat5_Understory11	JSJ_Q5_U11	Lepidiumvirginicum
James_San_Jacinto_Quadrat5_Understory13	JSJ_Q5_U13	Leptosiphon ciliates
James_San_Jacinto_Quadrat1_Understory12	JSJ_Q1_U12	Lonicera subspicata var. denudata
James_San_Jacinto_Quadrat5_Understory2	JSJ_Q5_U2	Lonicera subspicata var. denudata
James_San_Jacinto_Quadrat3_Understory4	JSJ_Q3_U4	Lupinus latifolius
James_San_Jacinto_Quadrat5_Understory8	JSJ_Q5_U8	Muhlenbergia rigens
James_San_Jacinto_Quadrat5_Understory10	JSJ_Q5_U10	Unknown herbaceous species
James_San_Jacinto_Quadrat2_Understory1	JSJ_Q2_U1	Penstemon labrosus
James_San_Jacinto_Quadrat3_Canopy4	JSJ_Q3_C4	Pinus lambertiana
James_San_Jacinto_Quadrat4_Canopy6	JSJ_Q4_C6	Pinus lambertiana
James_San_Jacinto_Quadrat1_Canopy3	JSJ_Q1_C3	Pinus ponderosa
James_San_Jacinto_Quadrat2_Canopy1	JSJ_Q2_C1	Pinus ponderosa
James_San_Jacinto_Quadrat3_Canopy5	JSJ_Q3_C5	Pinus ponderosa
James_San_Jacinto_Quadrat4_Canopy7	JSJ_Q4_C7	Pinus ponderosa
James_San_Jacinto_Quadrat5_Canopy1	JSJ_Q5_C1	Pinus ponderosa
James_San_Jacinto_Quadrat1_Understory1	JSJ_Q1_U1	Pteridium aquilinum var. pubescens
James_San_Jacinto_Quadrat2_Understory4	JSJ_Q2_U4	Pteridium aquilinum var. pubescens
James_San_Jacinto_Quadrat5_Understory14	JSJ_Q5_U14	Pteridium aquilinum var. pubescens
James_San_Jacinto_Quadrat2_Canopy6	JSJ_Q2_C6	Quercus chrysolepis
James_San_Jacinto_Quadrat3_Canopy2	JSJ_Q3_C2	Quercus chrysolepis
James_San_Jacinto_Quadrat4_Canopy2	JSJ_Q4_C2	Quercus chrysolepis
James_San_Jacinto_Quadrat1_Canopy1	JSJ_Q1_C1	Quercus kelloggii
James_San_Jacinto_Quadrat2_Canopy3	JSJ_Q2_C3	Quercus kelloggii
James_San_Jacinto_Quadrat4_Canopy5	JSJ_Q4_C5	Quercus kelloggii

James_San_Jacinto_Quadrat5_Canopy2	JSJ_Q5_C2	Quercus kelloggii
James_San_Jacinto_Quadrat1_Canopy5	JSJ_Q1_C5	Quercus wislizenii
James_San_Jacinto_Quadrat2_Canopy5	JSJ_Q2_C5	Quercus wislizenii
James_San_Jacinto_Quadrat3_Canopy1	JSJ_Q3_C1	Quercus wislizenii
James_San_Jacinto_Quadrat5_Understory7	JSJ_Q5_U7	Rhododendron occidentale
James_San_Jacinto_Quadrat5_Understory6	JSJ_Q5_U6	Ribes nevadense
James_San_Jacinto_Quadrat5_Understory12	JSJ_Q5_U12	Salix lasiolepis
James_San_Jacinto_Quadrat3_Understory2	JSJ_Q3_U2	Saltugilia splendens ssp. splendens
James_San_Jacinto_Quadrat1_Understory6	JSJ_Q1_U6	Scutellaria siphocampyloides
James_San_Jacinto_Quadrat2_Understory8	JSJ_Q2_U8	Scutellaria siphocampyloides
James_San_Jacinto_Quadrat4_Understory4	JSJ_Q4_U4	Scutellaria siphocampyloides
James_San_Jacinto_Quadrat2_Understory11	JSJ_Q2_U11	Sisymbrium altissimum
James_San_Jacinto_Quadrat1_Understory3	JSJ_Q1_U3	Thalictrum fendleri var. fendleri
James_San_Jacinto_Quadrat5_Understory3	JSJ_Q5_U3	Thalictrum fendleri var. fendleri
James_San_Jacinto_Quadrat4_Understory1	JSJ_Q4_U1	Unknown Grass
James_San_Jacinto_Quadrat2_Understory9	JSJ_Q2_U9	Wyethia ovata
James_San_Jacinto_Quadrat4_Understory13	JSJ_Q4_U13	Wyethia ovata