**Supplemental Methods**

Sample Processing for metabarcoding

We hand-homogenized unsieved soil samples from the northern and southern plots of each tree. Using soil sieves and tap water, we washed away the soil and picked out fine roots of woody plants, examined them under a stereoscope for evidence of ectomycorrhizal colonization, then combined subsamples from the north and south plots into a single sample, before drying them at 52°C for 24 h.  We stored dried root samples at room temperature during shipping and then froze them at -20°C following arrival. We ground dried root samples cryogenically with liquid nitrogen in a mortar and pestle.

DNA Extraction

We extracted DNA of roots and soil samples with the Qiagen PowerSoil Kit (QIAGEN 2023) following the protocol as written, but reducing the amount of input material to 50 mg for roots and to 150 mg for soil, and performed 1 min of bead beating in the lysis buffer with a BioSpec Products MiniBeadBeater-16 followed by a 10 min incubation at room temperature. Duplicate extractions were performed on root samples and single extractions on soil samples for a total of 72 DNA extractions.

We prepared amplicon sequencing libraries using degenerate primer mixes ITS3NGS and ITS4NGR to amplify ITS2 (described in Cregger et al 2018; White et al. 1990; Tedersoo et al. 2014), primers 515F and 806R to amplify the V4 region of 16S, and primers WANDA and AML2 to amplify the SSU. These primer sets targeted total fungi, total prokaryotes, and Glomeromycota fungi (i.e., AMF) respectively. We carried out PCR reactions using a 3-step protocol, with the first step using unmodified primers to enrich target DNA, the second step using modified primers with an adapter region and frameshift section, and the third step using a universal forward primer and a sample-specific barcoded reverse primer. We incorporated an ITS peptide nucleic acid blocker to block the amplification of plant ITS2 sequences, and the pPNA and mPNA peptide nucleic acid blockers were used to block the amplification of plant plastid 16S and mitochondrial 16S, respectively. We used the Phusion Hot Start II High Fidelity polymerase for PCR reactions. Cycle conditions are listed in the supplement. After the final PCR step, we checked amplicons on a 1% agarose gel and then cleaned up small fragments using the Omega Mag-Bind Bead Cleanup kit with a 0.9 ratio of beads:DNA. Following cleanup, we quantified amplicons using the Qubit HS dsDNA kit. We then pooled amplicons for the three loci at equal concentrations into a single library for sequencing, and the final library was again bead cleaned to adjust the DNA to the final concentration for sequencing. We performed sequencing at the Duke University Sequencing Core on an Illumina MiSeq using the V3 300 bp PE kit.

Microbial Community Bioinformatics and Annotation

Amplicon sequencing reads for 16S, ITS2, and 18S were processed using QIIME2 (Bolyen et al., 2019), with methodology tailored to each locus, as described below. Amplicon sequence variants (ASVs) were used as the unit of analysis for 16S and 18S data, while 97% operational taxonomic units (OTUs) were employed for ITS2 to account for the differing evolutionary rates between these loci (Estensmo et al., 2021). For consistency, we refer to all resulting data tables as “feature tables” and to ASVs and OTUs collectively as “taxa.”

Paired-end 16S rRNA sequences were imported into QIIME2 (Bolyen et al., 2019), where primers and adapter sequences were trimmed using Cutadapt (Martin, 2011). ASVs were generated using DADA2 (Callahan et al., 2016) and taxonomically classified against the SILVA ribosomal database (Quast et al., 2012) using the classify-sklearn command in QIIME2 (Bokulich et al., 2018; Pedregosa et al., 2011). ASVs identified as mitochondrial or chloroplast sequences were removed from downstream analysis.

Additionally, ITS2 paired-end reads were merged using PEAR (Zhang et al., 2014), and the ITS2 region was extracted using ITSxpress (Rivers et al., 2018). The processed sequences were imported into QIIME2 (Bolyen et al., 2019) and denoised with DADA2 (Callahan et al., 2016). The resulting sequences were clustered into 97% OTUs using VSEARCH (Rognes et al., 2016). Taxonomy was assigned to ITS2 representative sequences using the classify-sklearn command in QIIME2 (Bokulich et al., 2018; Pedregosa et al., 2011) with the UNITE fungal ITS database (Abarenkov et al., 2024). Fungi of interest, including introduced ectomycorrhizal fungi and other abundant OTUs, were manually verified using BLAST searches (Altschul et al., 1990) against the NCBI Core Nucleotide Database. Fungal guild annotation was done with a combination of FungalTraits and FunGuild (Nguyen et al., 2016; Põlme et al., 2020).

Furthermore, raw paired-end 18S sequences were provided demultiplexed and underwent quality control in QIIME2 v.2023.5 (Bolyen et al., 2019). Primer regions and low-quality sequences were trimmed, and chimeras were filtered using DADA2 (Callahan et al., 2016), resulting in 1,364 ASVs from 716,988 total sequences. Taxonomy was assigned to representative sequences using the MaarjAM database (Öpik et al., 2010) and the feature-classifier plugin in QIIME2 (Bolyen et al., 2019; Bokulich et al., 2018). To identify ASVs belonging to the Glomeromycotina (the subphylum of arbuscular mycorrhizal fungi), sequence similarity searches were conducted at 95%, 90%, and 80% similarity thresholds while maintaining query coverage >90% and a BLAST e-value <1e-50, following methods similar to Kajihara et al. (2022). This yielded 622 ASVs at 95%, 658 ASVs at 90%, and 57 ASVs at 80% sequence similarity. Additionally, unassigned sequences were compared to Glomeromycotina reference sequences from the NCBI Nucleotide database at >80% sequence similarity, identifying six additional ASVs. All ASVs were merged into a single table using the QIIME2 feature-table plugin.

Finally, feature tables and taxonomic annotations for all loci were imported into RStudio for downstream analysis. Feature tables and taxonomic annotations are available in Supplemental File S2. Datasets were rarefied to even sequencing depths of 2,053 for 18S, 24,860 for 16S, and 70,218 for ITS2. After rarefaction, technical replicates from the same sample were pooled by summing their sequence abundances. This final dataset included 1,281 taxa for 18S, 18,775 taxa for 16S, and 4,159 taxa for ITS2.

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**Supplemental Figures**

* Table of top 10-20 taxa? genera? in pine and eucalypt samples – **Jake needs to make this**
* Rarefaction Curves – **Jake needs to make this**
* Ecoplate functional diversity figure
* ~~Effect sizes figure for all non-significant variable~~ – we made this into a table that is in S2
* Stacked barplots of Suilloids, other ECM – **done**, see below
* Spatial autocorrelation figures – get from Elena
* Ergoesterol~ECM abundance\*tree species scatterplot and ANOVA table – **done and in google doc** - maybe we should consider just pasting a p-value and r2 onto the plot instead of including full anova table?
* 18S PcoA – **done**
* dbRDA for 18S, 16S, ITS - **done**

A graph of different colored bars

Description automatically generated with medium confidence

**Supplemental Fig S\_\_.** Relative ITS2 sequence abundance of the introduced northern hemisphere fungal genera *Phialocephala*, *Rhizopogon*, and *Suillus* and “other ECM” which includes a mix of native ectomycorrhizal fungi, introduced ectomycorrhizal fungi, and fungi for which provenance could not be determined. The “other” category includes fungi with other lifestyles (a mix of free living saprotrophs, endophytes, plant pathogens, lichenized fungi, etc)